Role of PINOID-mediated COP1 phosphorylation in *Arabidopsis* photomorphogenesis is overemphasized

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Etiolated *Arabidopsis* seedlings have long hypocotyls and closed yellow cotyledons (skotomorphogenesis); however, light-grown plants exhibit short hypocotyls and opened green cotyledons (photomorphogenesis) (1). It has been revealed that E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) is a central negative regulator for plant photomorphogenesis. COP1 directly targets a plethora of transcription factors for degradation in darkness, while light suppresses COP1 activity to stabilize these transcription factors (2). Many of these transcription factors are able to associate with light-responsive promoters to trigger a proportion of transcriptomic alternations and then establish photomorphogenesis (3).

Lin et al. (4) argue that they find Ser/Thr kinase PINOID (PID) directly phosphorylates COP1 and inhibits COP1 activity during photomorphogenesis. Although we appreciate this interesting finding, we have several concerns on the interpretation of their genetic data and particularly whether the PID-mediated COP1 phosphorylation matters in photomorphogenesis.

The authors identified one mutation (*pid-15*) that successfully reverses the short hypocotyl of *cop1-6* (*cop1* weak allele) back to normal (4). They further demonstrated that PID directly phosphorylates COP1 at Ser20.

It was explained that in *cop1-6*, PID phosphorylates the residue COP1 protein and inhibits its activity, while in *cop1-6 pid-15* double mutant, loss of *PID* dephosphorylates COP1 and reactivates the residue COP1 activity, which completely recovers the normal etiolation growth (4). If it stands, we will assume that the PID-mediated COP1 phosphorylation is critical for COP1 activity. However, either YFP tag-fused phosphorylation dead mimic (YFP-COP1 S20A) or phosphorylation active mimic (YFP-COP1 S20D) completely rescues *cop1-6* mutants. Although the authors argue that YFP-COP1 S20D has weaker activity than YFP-COP1 S20A, the hypocotyl lengths of YFP-COP1 S20D/*cop1-6* are already equal to the wild-type plants. This result indicates that the PID-mediated COP1 phosphorylation at Ser20 is dispensable for COP1 activity. In addition, the abundance of COP1 target protein HY5 was not changed in *PID* overexpression lines, suggesting that enhancing PID levels did not significantly reduce COP1 E3 ligase activity.

Although we question the biological consequence of PID-mediated COP1 phosphorylation in photomorphogenesis, we do not doubt that *pid* suppresses *cop1* phenotype and PID–COP1 physical interactions. In contrast, to put *COP1* downstream of *PID*, we propose that *PID* might act downstream of *COP1* based on the authors' observations (4). The *pid-15* mutants have a normal etiolation growth phenotype, while *pid-15 cop1-6* resembles *pid-15*. PID is not likely to be a degradation target for COP1, because PID protein levels are the same in Col-0 and *cop1-6* plants. However, the authors do not examine PID kinase activity in *cop1* mutants and even do not mention too much on the biological function of PID. PID phosphorylates auxin efflux carriers and modulates auxin homeostasis (5–7). Since auxin is crucial for hypocotyl elongation, we suggest that it is required to reexamine the impact of COP1–PID interaction and check whether COP1 affects PID kinase activity and auxin flow.

In summary, we do not believe that *pid-15* suppression of *cop1-6* is caused by PID-mediated COP1 phosphorylation. Instead, it is urged to pursue other possibilities, like whether COP1 affects PID kinase activity on auxin efflux proteins.

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