LETTER

REPLY TO JIN AND ZHU: PINOID-mediated COP1 phosphorylation matters in photomorphogenesis in *Arabidopsis*

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We thank Jin and Zhu for their comments (1). Our study in PNAS focuses on the molecular role for PINOID (PID) and PID-mediated CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) phosphorylation in the regulation of seedling development in *Arabidopsis* (2). Not only hypocotyl elongation but also apical hook maintenance and cotyledon aperture are phenotypic readouts for photomorphogenesis in *Arabidopsis* (3, 4). Transgenic and phenotypic analyses clearly suggest that phosphomimic COP1 (YFP-COP1 S20D) possesses weaker activity in maintaining the apical hook and promoting hypocotyl elongation in *Arabidopsis* (2). Extensive studies have uncovered that multiple factors and regulators contribute to precise modulation of COP1 abundance and activity (5). It is therefore reasonable that PID-mediated phosphorylation of COP1 does not cause a dramatic effect on seedling development. As we assume in the paper (2), the nonaltered ELONGATED HYPOCOTYL 5 (HY5) protein abundance and HY5-regulated gene expression in dark-grown *PID* overexpressors are likely due to the already extremely low HY5 level, as COP1 is enriched in the nucleus and directly targets HY5 for ubiquitination and degradation in darkness (6).

In the various light conditions tested, *pid-15 cop1-6* exhibits intermediated hypocotyl length, indicating that COP1 and PID may work synergistically in regulating photomorphogenesis in the light (2). In darkness, *pid-15* is epistatic to *cop1-6*, but we cannot rule out the possibility that *PID* might act upstream of *COP1*, as *cop1-6* is a weak allele, not a null mutant (7). PID directly interacts with and targets COP1 for phosphorylation (2). Thus, our biochemical findings support that PID acts upstream of COP1. The *cop1-6* produces a functional COP1-6 mutant protein with five novel amino acids (Cys-Leu-Val-Leu-Trp) inserted in-frame between Glu301 and Phe302 of the wild-type protein at a lower level (7, 8). Suppression of constitutively photomorphogenic phenotype of *cop1-6* is indeed caused by the disruption of PID as demonstrated by the complementation assays (2). PID likely has no effect on the COP1 abundance and its nucleocytoplasmic partitioning. However, phosphomimic COP1 exhibits weaker activity in repressing enlargement of the apical hook unfolding angle and promoting hypocotyl growth in *Arabidopsis* (2). Thus, it appears that suppression of *cop1-6* by *pid-15* might, at least in part, be attributed to the loss of PID-mediated phosphorylation of COP1. We also clearly point out that "PID-mediated phosphorylation is only partially responsible for the repression of COP1 activity..." in our discussion (2).

COP1 does not regulate PID abundance in plants (2). Despite extensive efforts, we have been unable to show that COP1 affects PID activity. PID is a key player in auxin signaling and targets auxin efflux carriers for phosphorylation (9–11). Our data clearly demonstrate that PID is a positive regulator of photomorphogenesis (2). Together, these facts indicate that cross-talk between light and auxin signaling and the interplay between PID and COP1 might play a critical role in directional auxin transport, auxin distribution, auxin biosynthesis, or auxin signaling pathways. We agree with Jin and Zhu (1) in their perspective that the role of PID–COP1 in the integration of light and auxin signaling awaits further detailed investigation.

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