$SCF^{TIR1/AFB}$ Auxin Signaling for Bending Termination during Shoot Gravitropism[1][OPEN]

Dear Editor,

Gravitropism is a plant adaptive response that involves asymmetric auxin distribution (Friml et al., 2002; Rakusová et al., 2015; Su et al., 2017). The auxin asymmetry leading to the shoot and root bending is initiated by the gravity-induced subcellular relocalization of PIN auxin transporters (Friml et al., 2002; Kleine-Vehn et al., 2010; Rakusová et al., 2011). Bending termination is much less well characterized, although it depends on the reestablishment of the symmetrical auxin distribution due to auxin-mediated reestablishment of the symmetric PIN localization (Supplemental Fig. S1A; Rakusová et al., 2016, 2019). Which auxin signaling pathway mediates this auxin feedback on PIN repolarization and bending termination remains unknown.

To evaluate which auxin signaling machinery mediates auxin feedback on PIN3 repolarization for bending termination, we examined two best-characterized auxin perception pathways: (1) the nuclear TIR1/AFB auxin receptors, which mediate both transcriptional and nontranscriptional responses (Salehin et al., 2015; Fendrych et al., 2016, 2018); and (2) the AUXIN BINDING PROTEIN1 (ABP1) pathway, the function of which is unclear (Gao et al., 2015; Grones et al., 2015). While *abp1* mutant showed a normal hypocotyl gravitropic response (Supplemental Fig. S1B), the *tir1 afb2 afb3* triple hypocotyls were hyperbending (Fig. 1A), suggesting a defect in the termination response. Application of α-(Phenylethyl-2-one)-indole-3-acetic acid (PEO-IAA), which specifically interferes with auxin binding to TIR1 and inactivates the TIR1 pathway (Hayashi et al., 2008), also triggered hypocotyl hyperbending (Fig. 1B). The *HS::axr3-1* mutant carries a mutation in the DII domain of the IAA17/AXR3 protein, a TIR1 coreceptor (Calderón Villalobos et al., 2012), and is conditionally expressed under a heat shock-inducible promoter (Knox et al., 2003). Whereas the *HS::axr3-1* hypocotyls without heat shock induction displayed a normal gravitropic response (Supplemental Fig. S1C), *HS::axr3-1* hypocotyls were hyperbending after heat shock induction (Fig. 1C). These data collectively suggest that TIR1/AFB pathway is required for hypocotyl bending termination.

Hypocotyl gravitropic bending is initiated by the sedimentation of amyloplasts in hypocotyl endodermal cells followed by gravity-induced PIN3 polarization to the lower side of the cell (Fukaki et al., 1998; Rakusová et al., 2011). Bending termination involves the reestablishment of auxin-induced symmetrical PIN3 subcellular distribution at later stages (Supplemental Fig. S1A; Rakusová et al., 2016, 2019). Therefore, we investigated these processes under conditions of compromised TIR1/AFB auxin signaling. Disruption of the TIR1/AFB pathway did not have any obvious effect on amyloplast sedimentation in hypocotyl endodermal cells (Supplemental Fig. S2). Next, we analyzed PIN3 polarization. PIN3-GFP is distributed symmetrically at both the inner and outer sides of hypocotyl endodermal cells in the wild type without gravity stimulation, (Rakusová et al., 2011), or in *HS::axr3-1* hypocotyls with or without heat shock induction (Supplemental Fig. S3, A and B). After 2 h or 6 h gravistimulation, PIN3-GFP was polarized, as manifested by a stronger PIN3-GFP signal at the lower sides of endodermal cells in wild-type and *HS::axr3-1* hypocotyls with or without heat shock induction (Supplemental Fig. S3, C–H). Similarly, inhibition of TIR1/AFB auxin perception by PEO-IAA significantly affected the transcriptional auxin signaling in hypocotyls (Supplemental Fig. S4, A and B), but did not affect gravity-induced PIN3 polarization (Supplemental Fig. S4, C–H). Thus, steady-state PIN3 localization and gravity-induced PIN3 polarization do not strongly depend on the TIR1/AFB signaling pathway.

We then investigated the involvement of the TIR1/AFB pathway in PIN3 repolarization at later stages of the gravitropic response (Rakusová et al., 2016). After 24 h of gravity stimulation, PIN3-GFP repolarized to the inner side of endodermal cells at the bottom side of the wild-type hypocotyl (Fig. 1, D and G; Rakusová et al., 2016, 2019). By contrast, when the TIR1/AFB pathway was inactivated by PEO-IAA, or in the heat shock-induced *HS::axr3-1* hypocotyls, we observed persistence of PIN3-GFP asymmetry, with a strong signal at the lower side of hypocotyl endodermal cells (Fig. 1, E–H). As expected, we observed a normal PIN3-GFP polarization in the noninduced *HS::axr3-1* hypocotyls (Supplemental Fig. S5, A and B). These observations revealed an involvement of TIR1/AFB

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[2]Author for contact: jiri.friml@ist.ac.at.

[3]Senior author.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Jiří Friml (jiri.friml@ist.ac.at).

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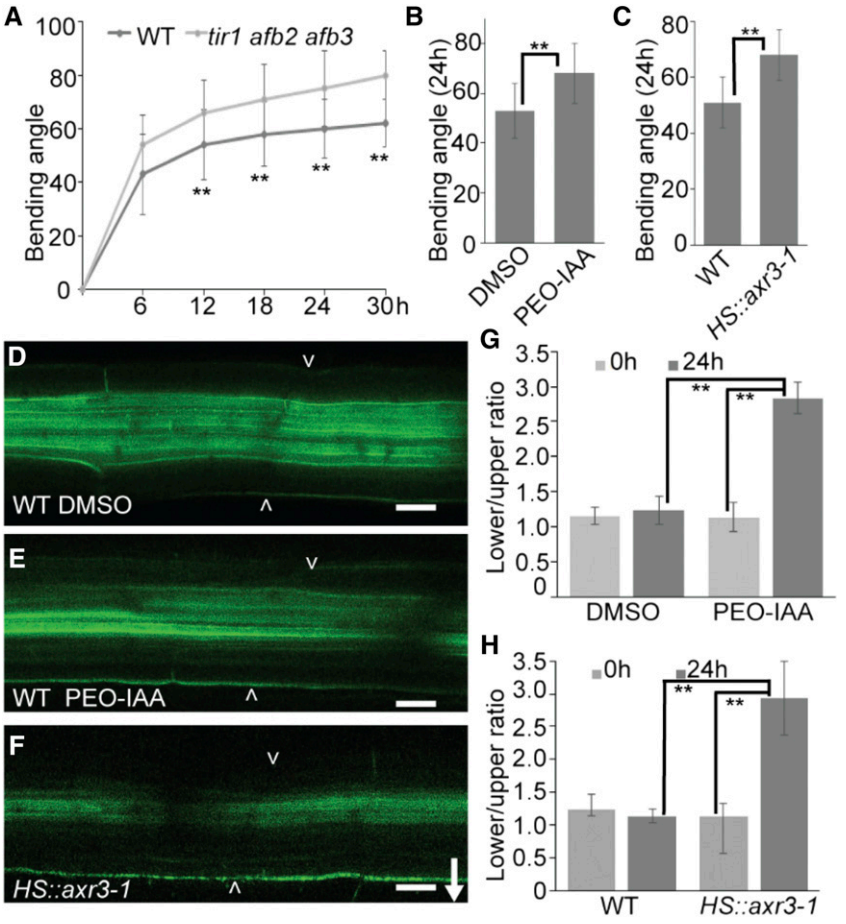

Figure 1. Hypocotyl gravitropic bending termination depends on TIR1/AFB signaling. A, Bending kinetics of wild-type (WT) and *tir1 afb2 afb3* hypocotyls. B, Bending angle of dimethyl sulfoxide- (DMSO) or 10 μM PEO-IAA-treated wild-type hypocotyls after 24 h gravistimulation. C, Bending angle of heat shock-induced *HS::axr3-1* hypocotyls after 24 h gravistimulation. D to F, PIN3-GFP localization after 24 h gravistimulation in wild-type hypocotyls upon DMSO treatment (D) and 10 μM PEO-IAA treatment (E) and in heat shock-induced *HS::axr3-1* hypocotyls (F). Arrowheads depict PIN3-GFP at the outer side of endodermal cells, and the arrow in F indicates the gravity direction and hence the lower and upper sides of the hypocotyl. Scale bars = 20 μm. G and H, Quantification of PIN3-GFP intensity in PEO-IAA-treated wild-type hypocotyls (G) and heat shock-induced *HS::axr3-1* hypocotyls (H) after 24 h gravistimulation. The ratio was calculated by dividing the PIN3-GFP intensity at the outer side of endodermal cells between the lower and upper sides of hypocotyls. Data and error bars represent the means ± SD. $n = 30$ to 40 for the bending assay and 15 for PIN3-GFP intensity quantification; $^{**}P < 0.05$, determined by Student's *t* test.

auxin signaling in the reestablishment of symmetric PIN3 distribution during hypocotyl bending termination.

Exogenous auxin application also induces PIN3 inner-lateralization, similar to our observations at later stages of the gravitropic response. As shown previously (Rakusová et al., 2016, 2019), PIN3-GFP relocated to the inner side of endodermal cells after 4 h of auxin (1-naphthaleneacetic acid [NAA]) treatment (Fig. 2, A, B, and H). When the TIR1/AFB pathway was inactivated by applying PEO-IAA, this relocation did not happen, as evidenced by a strong PIN3-GFP signal at the outer side of endodermal cells (Fig. 2, C and H). Inactivation of the TIR1/AFB pathway in the *HS::axr3-1* hypocotyls yielded the same result: in the heat shock-induced hypocotyls, we observed a persisting PIN3-GFP signal at the outer side of endodermal cells after 4 h of NAA incubation (Supplemental Fig. S6, A, B, and E), whereas the signal disappeared in *HS::axr3-1* hypocotyls without heat shock induction (Supplemental Fig. S6, C, D, and F). This shows a requirement for the TIR1/AFB pathway in auxin-induced PIN3 relocation.

To test whether activation of TIR1/AFB is sufficient to mediate PIN3 relocation, we used an engineered convex-IAA (cvxIAA)/concave-TIR1 (ccvTIR1) perception system (Uchida et al., 2018). For the ccvTIR1 and control TIR1 (cTIR1) auxin perception system, ccvTIR1 is less sensitive to natural IAA, but binds to synthetic cvxIAA, thus activating the auxin response. Whereas cTIR1 is unable to bind to cvxIAA, and thus does not activate the auxin response, it responds normally to natural IAA. The *ccvTIR1* and *cTIR1* hypocotyls showed a normal gravity response and gravity-induced PIN3 polarization (Supplemental Fig. S7, A–H), and the PIN3-GFP localization in *ccvTIR1* hypocotyls was normal (Fig. 2, D and I). IAA treatment induced PIN3-GFP repolarization to the inner side of endodermal cells in wild-type hypocotyls (Fig. 2I; Rakusová et al., 2016), as well as in *cTIR1* hypocotyls (Supplemental Fig. S8, A, B, and D); however, in the *ccvTIR1* hypocotyls, the effect was less pronounced (Fig. 2, E and I). By contrast, cvxIAA did not induce PIN3-GFP repolarization to the inner side of endodermal cells in the wild type (Fig. 2, F and J) or *cTIR1* hypocotyls (Supplemental Fig. S8, C and D), although it did induce strong PIN3-GFP repolarization to the inner side of endodermal cells in *ccvTIR* hypocotyls (Fig. 2, G and J). These results show that a specific activation of the TIR1/AFB pathway is sufficient to repolarize PIN3 in hypocotyl endodermis (Fig. 2K).

In conclusion, we demonstrated that genetic or chemical interference with TIR1/AFB signaling interferes with auxin-mediated reestablishment of symmetric

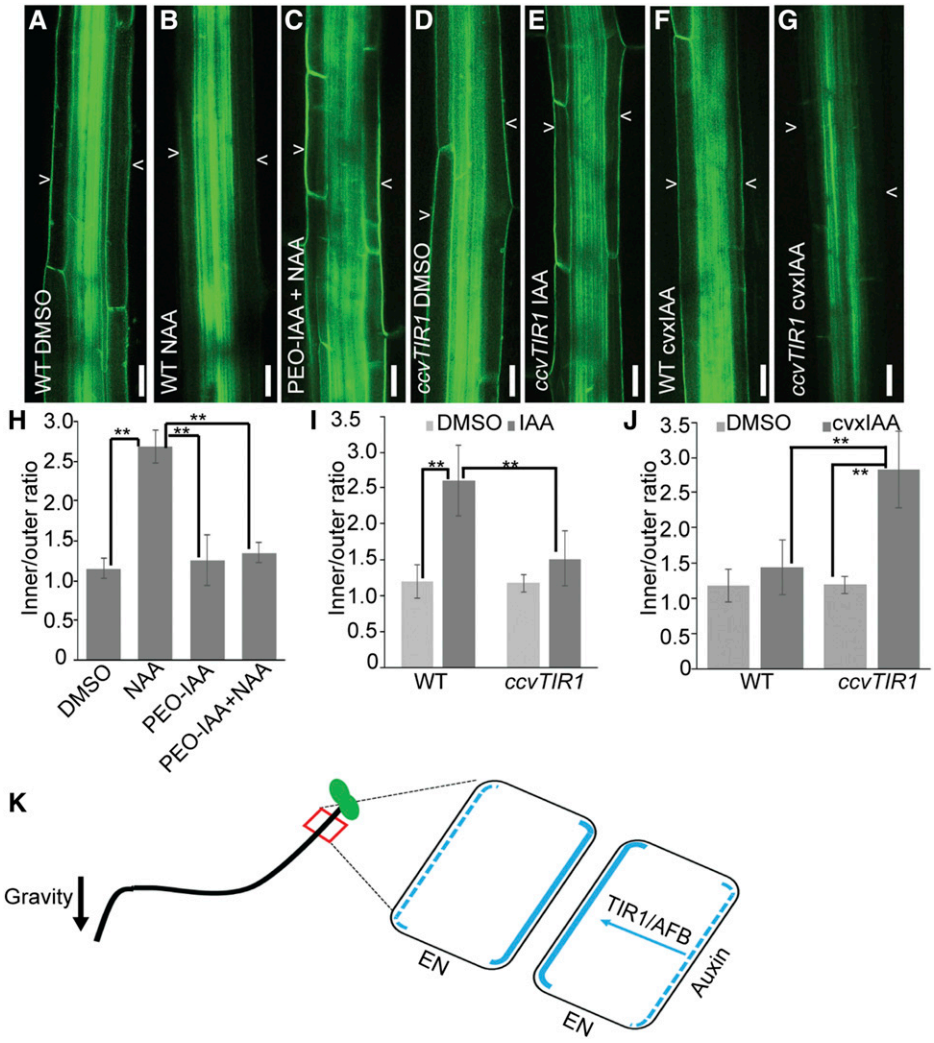

Figure 2. TIR1/AFB signaling mediates auxin feedback on PIN3 repolarization. A to G, PIN3-GFP localization in dimethyl sulfoxide (DMSO)-treated wild-type (WT) hypocotyls (A), 10 μM NAA-treated wild-type hypocotyls (B), 10 μM PEO-IAA and 10 μM NAA co-treated wild-type hypocotyls (C), DMSO-treated *ccvTIR1* hypocotyls (D), 10 μM IAA-treated *ccvTIR1* hypocotyls (E), 10 μM cvxIAA-treated wild-type hypocotyls (F), and 10 μM cvxIAA-treated *ccvTIR1* hypocotyls (G). Arrowheads depict PIN3-GFP at outer side of endodermal cells. Scale bars = 20 μm. H to J, Quantification of PIN3-GFP intensity in wild-type hypocotyls treated with PEO-IAA (H), IAA-treated *ccvTIR1* hypocotyls (I), and cvxIAA-treated *ccvTIR1* hypocotyls (J). The ratio was calculated by dividing the PIN3-GFP intensity at inner and outer side of hypocotyl endodermal cells. Data and error bars represent the means ± SD. $n = 15$. $^{**}P < 0.05$, determined by Student's test. K, Schematic diagram of auxin receptor TIR1/AFB-mediated PIN3 repolarization for hypocotyl bending termination. At a later stage of shoot gravitropism (24 h), TIR1/AFB mediates auxin perception and facilitates the repolarization of PIN3 to the inner side of endodermal (EN) cells at the lower hypocotyl side, to equalize auxin distribution and thus terminate hypocotyl bending. Blue lines indicate PIN3 distribution at EN cells; the blue arrow indicates auxin-TIR1/AFB-mediated PIN3 repolarization from the outer side (blue dashed line) to the inner side (blue solid line) at the lower-side hypocotyl EN cells; and the black arrow indicates gravity direction.

PIN3 polarization during the gravitropic response, leading to shoot overbending. Similarly, TIR1/AFB signaling is required for auxin-mediated PIN3 repolarization. Furthermore, activation of the TIR1 pathway using the synthetic cvxIAA-ccvTIR1 pair is sufficient to induce PIN3 repolarization. Collectively, these observations reveal the essential role of the $SCF^{TIR1/AFB}$ auxin signaling pathway in mediating auxin feedback on auxin transport directionality for bending termination during plant adaptive development.

Supplemental Data

The following supplemental materials are available.

Supplemental Methods.

Supplemental Figure S1. ABP1 is not involved in hypocotyl gravitropic bending termination.

Supplemental Figure S2. Modification of the TIR1/AFB pathway does not affect amyloplast sedimentation in Arabidopsis hypocotyl endodermal cells.

Supplemental Figure S3. Auxin-induced AUX/IAA protein degradation is not required for gravity-induced PIN3 polarization.

Supplemental Figure S4. Compromised TIR1/AFB signaling does not affect gravity-induced PIN3 polarization.

Supplemental Figure S5. Normal PIN3-GFP repolarization in noninduced *HS::axr3-1* hypocotyls after 24 h gravity stimulation.

Supplemental Figure S6. Auxin-induced AUX/IAA protein degradation is required for auxin-mediated PIN3 repolarization.

Supplemental Figure S7. Normal gravity response and gravity-induced PIN3 polarization in *ccvTIR1* and *cTIR1* hypocotyls.

Supplemental Figure S8. Normal auxin-induced PIN3 repolarization in the *cTIR1* mutant.

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Huibin Han
Institute of Science and Technology (IST), 3400 Klosterneuburg, Austria

Hana Rakusová
Institute of Science and Technology (IST), 3400 Klosterneuburg, Austria

Inge Verstraeten
ORCID ID: 0000-0001-7241-2328
Institute of Science and Technology (IST), 3400 Klosterneuburg, Austria

Yuzhou Zhang
Institute of Science and Technology (IST), 3400 Klosterneuburg, Austria

Jiří Friml[2,3]
ORCID ID: 0000-0002-8302-7596
Institute of Science and Technology (IST), 3400 Klosterneuburg, Austria

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