SCF$^{TIR1/AFB}$ auxin signaling for bending termination during shoot gravitropism

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One-sentence summary: TIR1/AFB signaling is required and sufficient for auxin-mediated PIN3 re-polarization and shoot gravitropic bending termination.

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 re-establishment of the symmetrical auxin distribution due to auxin-mediated re-establishment 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: (i) the nuclear auxin receptors TIR1/AFB, which mediate both transcriptional and non-transcriptional responses (Salehin et al., 2015; Fendrych et al., 2016, 2018); and (ii) the AUXIN BINDING PROTEIN1 (ABP1) pathway with an unclear function (Gao et al., 2015; Grones et al., 2015). While $abp1$ mutant showed a normal hypocotyl gravitropic response (Supplemental Fig. 1B), the $tir1$ $afb2$ $afb3$ triple hypocotyls were hyperbending (Fig. 1A), suggesting a defect in the
termination response. Application of PEO-IAA, which specifically interferes with auxin binding to TIR1 and inactivates 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 co-receptor (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. 1C), 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 the gravity-induced PIN3 polarization to the lower side of the cell (Fukaki et al., 1998; Rakusová et al., 2011). Bending termination involves the re-establishment 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 amyloplasts sedimentation in hypocotyl endodermal cells (Supplemental Fig. 2). Next, we analyzed PIN3 polarization. Without gravity stimulation, PIN3-GFP is distributed symmetrically at both inner and outer sides of hypocotyl endodermal cells in the wild-type (Rakusová et al 2011), or in HS::axr3-1 hypocotyls with or without heat shock induction (Supplemental Fig. 3A, B). After 2 hours or 6 hours gravistimulation, PIN3-GFP was polarized, as manifested by a stronger PIN3-GFP signal at lower sides of endodermal cells in wild-type and HS::axr3-1 hypocotyls with or without heat shock induction (Supplemental Fig. 3C-H). Similarly, inhibition of TIR1/AFB auxin perception by PEO-IAA significantly affected the transcriptional auxin signaling in hypocotyls (Supplemental Fig. 4A-B), but did not affect gravity-induced PIN3 polarization (Supplemental Fig. 4C-H). Thus, steady-state PIN3 localization and gravity-induced PIN3 polarization does not strongly depend on the TIR1/AFB signaling pathway.

We then investigated the involvement of TIR1/AFB pathway in the PIN3 repolarization at later stages of gravitropic response (Rakusová et al., 2016). After 24 hours of gravity stimulation, PIN3-GFP repolarized to inner side of endodermal cells at the bottom side of the wild-type hypocotyl (Figure 1D, 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 strong signal at the lower side of hypocotyl endodermal cells (Fig. 1E - H). As expected, we observed a normal PIN3-GFP polarization in the non-induced HS::axr3-1 hypocotyls (Supplemental Fig. 5A, B). These observations revealed an involvement of TIR1/AFB auxin signaling in the re-establishment of symmetric PIN3 distribution during hypocotyl bending termination.

Exogenous auxin application also induces PIN3 inner-lateralization, similarly as observed at later stages of gravitropic response. As shown previously (Rakusová et al., 2016, 2019), PIN3-GFP relocated to inner side of endodermal cells after 4 hours of auxin (NAA) treatment (Fig. 2A, B, H). When 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. 2C, H). Inactivation of 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 hours of NAA incubation (Supplemental Fig. 6A, B, E); whereas it disappeared in HS::axr3-1 hypocotyls without heat shock induction (Supplemental Fig. 6C, D, 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/concave-TIR1 perception system (Uchida et al., 2018). For the concave TIR1 (ccvTIR1) and control TIR1 (cTIR1) auxin perception system, ccvTIR1 is less sensitive to natural IAA, but binds to the 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. 7A-H), and the PIN3-GFP localization in ccvTIR1 hypocotyls was normal (Fig. 2D, 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. 8A, B, D); however, in the ccvTIR1 hypocotyls, the effect was less pronounced (Fig. 2E, I). By contrast, cvxIAA did not induce PIN3-GFP repolarization to inner side of endodermal cells in the wild type (Fig. 2F, J) or cTIR1 hypocotyls (Supplemental Fig. 8C, D), although it did induce strong PIN3-GFP repolarization to the inner side of endodermal cells in ccvTIR hypocotyls (Fig. 2G, 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 re-establishment of symmetric PIN3 polarization during gravitropic response, leading to shoot overbending. Similarly, TIR1/AFB signaling is required for auxin-mediated PIN3 re-polarization. Furthermore, activation of TIR1 pathway using synthetic cvxIAA-ccvTIR1 pair is sufficient to induce PIN3 re-polarization. Collectively, these observations reveal the essential role of SCF$^{\text{TIR1/AFB}}$ auxin signaling pathway in mediating auxin feedback on auxin transport directionality for bending termination during plant adaptive development.

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**Supplemental Data**

**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 non-induced HS::axr3-1 hypocotyls after 24 hours 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.

FIGURE LEGENDS

Figure 1. Hypocotyl gravitropic bending termination depends on TIR1/AFB signaling.

(A) Bending kinetics of wild type and tir1 afb2 afb3 hypocotyls.

(B) Bending angle of DMSO or 10 µM PEO-IAA treated wild type hypocotyls after 24 hours gravistimulation.

(C) Bending angle of heat shock induced HS::axr3-1 hypocotyls after 24 hours gravistimulation.

(D - F) PIN3-GFP localization after 24 hours gravistimulation. Wild type hypocotyls upon DMSO treatment (D) and 10 µM PEO-IAA treatment (E), heat shock induced HS::axr3-1 hypocotyls (F).

(G - H) Quantification of PIN3-GFP intensity. PEO-IAA treated wild type hypocotyls after 24 hours gravistimulation (G); heat shock induced HS::axr3-1 hypocotyls after 24 hours gravistimulation (H). The ratio was calculated by dividing the PIN3-GFP intensity at outer side of endodermal cells between lower and upper side of hypocotyls. Data and error bars represent the mean ± SD. n = 30 - 40 for bending assay, n = 15 for PIN3-GFP intensity quantification. ** P < 0.05 determined by Student’s t-test. Arrowheads depict PIN3-GFP at outer side of endodermal cells, arrow indicates the gravity direction and hence determines lower and upper side of hypocotyl. Scale bar = 20 µm.

Figure 2. TIR1/AFB signaling mediates auxin feedback on PIN3 repolarization.
(A - G) PIN3-GFP localization in DMSO treated wild type 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 cvvTIR1 hypocotyls (D), 10 µM IAA treated cvvTIR1 hypocotyls (E), 10 µM cvxIAA treated wild type hypocotyls (F), 10 µM cvxIAA treated cvvTIR1 hypocotyls (G).

(H - J) Quantification of PIN3-GFP intensity. Wild type hypocotyls treated with PEO-IAA (H); IAA treated cvvTIR1 hypocotyls (I); cvxIAA treated cvvTIR1 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 mean ± SD. N = 15, ** P < 0.05 determined by Student’s test. Arrowheads depict PIN3-GFP at outer side of endodermal cells. Scale bar = 20 µm.

(K) Schematic diagram of auxin receptor TIR1/AFB mediated PIN3 repolarization for hypocotyl bending termination. At later stage of shoot gravitropism (24 hours), TIR1/AFB mediates auxin perception facilitates the repolarization of PIN3 to inner side of endodermal cells at the lower hypocotyl side, to equalize auxin distribution and thus terminate the hypocotyl bending. EN: endodermal cells; blue lines indicate PIN3 distribution at endodermal cells; blue arrow indicates auxin-TIR1/AFB mediated PIN3 repolarization from the outer side (blue dashed line) to inner side (blue solid line) at lower side hypocotyl endodermal cells; black arrow indicates gravity direction.

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