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Distinct functions of TIR1 and AFB1 receptors in auxin signaling

Dear Editor,

Auxin is the major plant hormone regulating growth and development (Friml, 2022). Forward genetic approaches have identified major components of auxin signaling and established the canonical mechanism mediating transcriptional and thus developmental reprogramming in *Arabidopsis thaliana*. In this textbook view, TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN-SIGNALING F-BOX (AFB) proteins are auxin receptors, which act as F-box subunits determining the substrate specificity of the Skp1–Cullin1–F box protein (SCF) type E3 ubiquitin ligase complex. Auxin acts as a "molecular glue," increasing the affinity between TIR1/AFBs and the Auxin/Indole-3-Acetic Acid (Aux/IAA) repressors. Subsequently, Aux/IAAs are ubiquitinated and degraded, thus releasing auxin transcription factors from their repression and making them free to mediate transcription of auxin response genes (Yu et al., 2022). Nonetheless, accumulating evidence suggests the existence of rapid, non-transcriptional responses downstream of TIR1/AFBs such as auxin-induced cytosolic calcium (Ca^{2+}) transients, plasma membrane depolarization, and apoplast alkalinization, all converging on the process of root growth inhibition and root gravitropism (Li et al., 2022). Particularly, these rapid responses are mostly contributed by predominantly cytosolic AFB1, while the long-term growth responses are mediated by mainly nuclear TIR1 and AFB2–AFB5 (Prigge et al., 2020; Li et al., 2021; Serre et al., 2021). How AFB1 conducts auxin-triggered rapid responses and how it is different from TIR1 and AFB2–AFB5 remains elusive. Here, we compare the roles of TIR1 and AFB1 in transcriptional and rapid responses by modulating their subcellular localization in *Arabidopsis* and by testing their ability to mediate transcriptional responses when part of the minimal auxin circuit is reconstituted in yeast.

One prominent difference between TIR1 and AFB1 is their subcellular localization. TIR1 primarily localizes to the nucleus while AFB1 to the cytoplasm (Figure 1A and Supplemental Figure 1) (Prigge et al., 2020). To test whether their specific localization is a necessary prerequisite for their function in either transcriptional or rapid responses, we fused *Venus* report gene combined with nuclear exporting signal (NES) or nuclear localization signal (NLS) at the C termini of *TIR1* (*TIR1–NES–Venus*) and *AFB1* (*AFB1–NLS–Venus*), respectively. We showed that the majority of TIR1–NES–Venus is shifted to cytosol, while AFB1–NLS–Venus mostly concentrates in the nucleus (Figure 1B and Supplemental Figure 1).

To characterize the importance of nuclear versus cytosolic localization of TIR1 and AFB1 in auxin-mediated transcription, we tested if they can rescue the mutant phenotype in a sustained root growth inhibition after auxin treatment for 6 days. We introduced the cytosolic-localized *TIR1–NES–Venus* into a *tir1* mutant background and found that all *TIR1* constructs were able to completely restore auxin sensitivity of root growth (Figures 1C and 1D and Supplemental Figures 1 and 2). This can be explained by either of the following: (i) the residual TIR1 present in the nucleus is sufficient to conduct full transcriptional activity, or (ii) cytosolic TIR1 may still degrade Aux/IAAs, releasing the ARFs from their inhibition. Besides, we introduced the nuclear-localized *AFB1–NLS–Venus* into *tir1 afb2* mutants but did not observe any rescue of the auxin-insensitive phenotype in root growth inhibition, root gravitropism, lateral root formation, and root hair elongation (Figures 1C and 1D and Supplemental Figures 1 and 2). This implies that AFB1, even when localized to the nucleus, cannot functionally replace TIR1 for its transcriptional regulation and related development.

The predominantly cytosolic AFB1 seems to be the major receptor for the rapid auxin effects (Prigge et al., 2020). Therefore, we introduced our mistargeted TIR1 and AFB1 versions into the *afb1* mutant background (Figures 1A and 1B and Supplemental Figure 1) and tested their effect in auxin-induced rapid root growth inhibition in a microfluidic vRootchip system. AFB1, when targeted to the nucleus, could no longer mediate the rapid auxin effect on root growth (Figure 1E). On the other hand, TIR1, despite being present in the cytosol, could not rescue the *afb1* mutant (Figure 1F). This reveals that cytosolic AFB1 is necessary for its function but that cytosolic TIR1 cannot replace or supplement the AFB1 function.

The observations that nuclear AFB1 cannot functionally replace TIR1 and that cytosolic TIR1 cannot functionally replace AFB1 show that TIR1 and AFB1 have distinct functional properties unrelated to their subcellular localization. To confirm this, we made use of the minimal auxin signaling pathway reconstructed in yeast (Pierre-Jerome et al., 2014). In this system, only TIR1, not AFB1, regardless of their subcellular localization, was able to mediate the auxin effect on transcription as monitored by the fluorescence intensity of P3_Venus transcriptional auxin reporter (Figure 1G).

To understand why AFB1 cannot mediate transcriptional signaling, we tested its ability to form an SCF complex using the yeast two-hybrid approach. Only TIR1, not AFB1, was able to interact with CUL1 (Cullin1), the key component of the ubiquitin ligase complex (Figure 1H). This is consistent with the available coimmunoprecipitation/mass spectrometry data, where all SCF components were detected to interact with TIR1; however, for AFB1, no or only an extremely weak interaction with CUL1 was detected (Supplemental Figure 3) (Yu et al., 2015; Li et al., 2021). The reason why AFB1 does not interact with CUL1 might be the natural mutation of the glutamic 8 site in AFB1 (Yu et al., 2015). The absence of an interaction with the SCF components will

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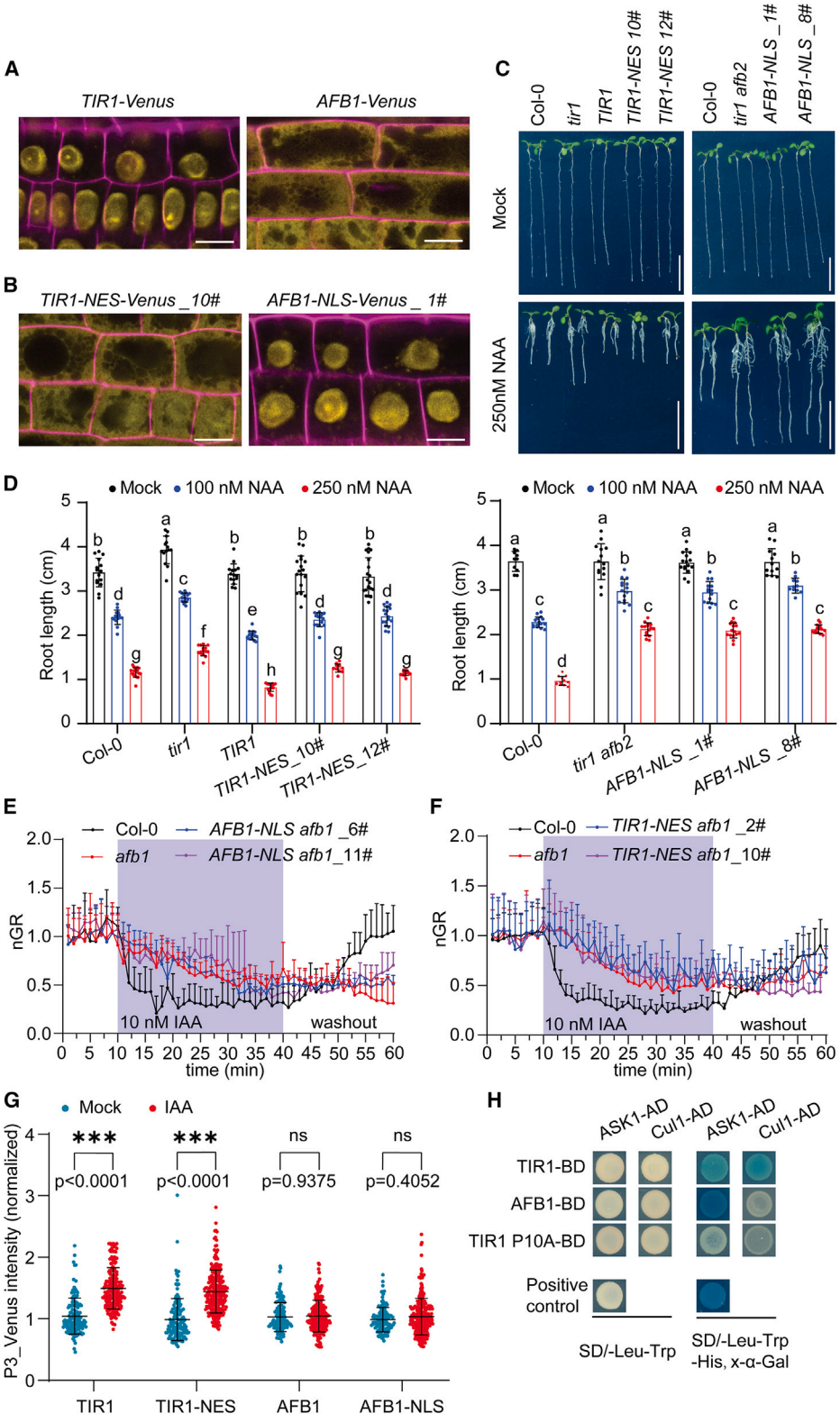

Figure 1. Distinct functions of TIR1 and AFB1 receptors in auxin signaling.

(A and B) Confocal images of *Arabidopsis* root epidermis cells expressing *TIR1–Venus*, *AFB1–Venus*, *TIR1–NES–Venus*, and *AFB1–NLS–Venus* as indicated. Venus signal is shown in yellow in all panels. Cell wall stained with propidium iodide is shown in purple. Scale bars, 10 μm.

(C and D) Evaluation of the roles of TIR1–NES–Venus and AFB1–NLS–Venus in auxin-mediated long-term root growth inhibition. The indicated *TIR1*-related constructs were transformed into *tir1*, and the *AFB1*-related constructs were transformed into *tir1 afb2*. Primary root length in the 6-day-old

(legend continued on next page)

prevent AFB1 from conducting E3 ubiquitin ligase activity, thus failing to mediate Aux/IAA degradation (Yu et al., 2015) and transcriptional regulation (Figure 1G). This also explains why AFB1, even when artificially targeted to the nucleus, still cannot replace the TIR1 function.

Our observations also imply that CUL1 is not essential for rapid auxin responses. This requires further clarification on the role of SCF components as well as Aux/IAA ubiquitination and degradation in rapid auxin responses. A recent study revealed the novel function of TIR1/AFBs in producing 3′,5′-cyclic adenosine monophosphate (cAMP), a prominent second messenger in animals. Though this activity of TIR1 specifically seems not important for rapid auxin responses (Qi et al., 2022), it is still possible that AFB1-mediated cAMP production in the cytosol is. However, whether and how the adenylate cyclase activity of AFB1 contributes to rapid auxin responses remain unknown.

In summary, we demonstrated that TIR1 and AFB1 have distinct functions, with the predominantly nuclear TIR1 mediating slow responses and cytosolic AFB1 conducting rapid responses. This functional divergence is not, however, simply due to the differential subcellular localization of these auxin receptors. The function of TIR1 in mediating slow/transcriptional responses seems to be independent of its predominant localization. In contrast, the function of AFB1 in rapid responses necessitates both its localization in the cytosol and the specific AFB1 protein properties themselves. Furthermore, cytosolic AFB1 mediates rapid auxin responses without forming SCF machinery, leaving the mechanism of AFB1-mediated rapid responses an exciting topic for future investigations.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

H.C., L.L., and J.F. designed the studies. H.C. and M.Z. performed the experiment. H.C., L.Q., and J.F. wrote the manuscript.

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REFERENCES

Friml, J. (2022). Fourteen Stations of Auxin. Cold Spring Harbor Perspect. Biol. **14**, a039859. https://doi.org/10.1101/cshperspect.a039859.

Li, L., Gallei, M., and Friml, J. (2022). Bending to auxin: fast acid growth for tropisms. Trends Plant Sci. **27**:440–449. https://doi.org/10.1016/j.tplants.2021.11.006.

Li, L., Verstraeten, I., Roosjen, M., Takahashi, K., Rodriguez, L., Merrin, J., Chen, J., Shabala, L., Smet, W., Ren, H., et al. (2021). Cell surface and intracellular auxin signalling for H(+) fluxes in root growth. Nature **599**:273–277. https://doi.org/10.1038/s41586-021-04037-6.

Pierre-Jerome, E., Jang, S.S., Havens, K.A., Nemhauser, J.L., and Klavins, E. (2014). Recapitulation of the forward nuclear auxin response pathway in yeast. Proc. Natl. Acad. Sci. USA **111**:9407–9412. https://doi.org/10.1073/pnas.1324147111.

Prigge, M.J., Platre, M., Kadakia, N., Zhang, Y., Greenham, K., Szutu, W., Pandey, B.K., Bhosale, R.A., Bennett, M.J., Busch, W., et al. (2020). Genetic analysis of the Arabidopsis TIR1/AFB auxin receptors reveals both overlapping and specialized functions. Elife **9**, e54740. https://doi.org/10.7554/eLife.54740.

Qi, L., Kwiatkowski, M., Chen, H., Hoermayer, L., Sinclair, S., Zou, M., Del Genio, C.I., Kubeš, M.F., Napier, R., Jaworski, K., et al. (2022). Adenylate cyclase activity of TIR1/AFB auxin receptors in plants. Nature **611**:133–138. https://doi.org/10.1038/s41586-022-05369-7.

Serre, N.B.C., Kralík, D., Yun, P., Slouka, Z., Shabala, S., and Fendrych, M. (2021). AFB1 controls rapid auxin signalling through membrane depolarization in Arabidopsis thaliana root. Native Plants **7**:1229–1238. https://doi.org/10.1038/s41477-021-00969-z.

Yu, H., Zhang, Y., Moss, B.L., Bargmann, B.O.R., Wang, R., Prigge, M., Nemhauser, J.L., and Estelle, M. (2015). Untethering the TIR1 auxin receptor from the SCF complex increases its stability and inhibits auxin response. Native Plants **1**, 14030. https://doi.org/10.1038/nplants.2014.30.

Yu, Z., Zhang, F., Friml, J., and Ding, Z. (2022). Auxin signaling: Research advances over the past 30 years. J. Integr. Plant Biol. **64**:371–392. https://doi.org/10.1111/jipb.13225.

seedlings of different genotypes grown on mock or 250 nM NAA plate. Scale bars, 1 cm, n ≥ 10 **(D)**. Lowercase letters indicate significant difference, two-way ANOVA test, p ≤ 0.01. Error bar, ±SD.

(E and F) Evaluation of the roles of TIR1–NES–Venus and AFB1–NLS–Venus in in auxin-mediated rapid root growth inhibition using microfluidic vRootchip. The constructs *TIR1–NES–Venus* and *AFB1–NLS–Venus* were transformed into an *afb1-3* mutant. Root growth rate of the indicated genotypes was normalized to the respective average root growth rate within 10 min before IAA application. The imaging interval is 1 min. Error bar, +SD.

(G) Differential effects of TIR1, TIR1–NES, AFB1, and AFB1–NLS in auxin-mediated transcriptional responses in the minimal auxin signaling pathway reconstructed in *Saccharomyces cerevisiae*. Fluorescence intensity was quantified with the images captured after the yeast cells were treated with 10 μM IAA for 6 h. Error bar, ±SD, t test, ***p ≤ 0.001, NS, not significant (p > 0.05).

(H) Interactions of different SCF components in yeast two-hybrid assay. The yeast transformants were plated on SD/-Leu-Trp-His drop-out medium with 4 mg/mL x-α-galactrosidase and were cultured for 3 days to assess the protein-protein interactions. TIR1 P10A acts as a negative control here. α-Galactosidase activity manifested as blue color indicates the interaction.