Prohibitin 3 gives birth to a new lateral root primordium

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Li S, Li Q, Tian X, Mu L, Ji M, Wang X, Li N, Liu F, Shu J, Crawford NM, Wang Y. 2022. PHB3 regulates lateral root primordia formation via NO-mediated degradation of AUXIN/INDOLE-3-ACETIC ACID proteins. Journal of Experimental Botany 73, 4034–4045.

Plant lateral roots (LRs) initiate when a small group of pericycle cells are primed to undergo cell division to form LR primordia (LRPs). This process involves a complex gene regulatory network. In Arabidopsis, an auxin-dependent AUX/IAA14/SOLITARY-ROOT (Okushima et al., 2007), IAA18/POTENT (Perianez-Rodriguez et al., 2021), and IAA28, interact with AUXIN RESPONSE FACTOR7 (ARF7) and ARF19, to regulate the founder cell specification and LR initiation. Downstream of auxin signaling, MEMBRANE-ASSOCIATED KINASE REGULATOR4 (MAKR4) (Xuan et al., 2015), and the transcription factors (TFs) GATA23 (De Rybel et al., 2010) and LATERAL ORGAN BOUNDARIES-DOMAIN 16/ASYMMETRIC LEAVES2-LIKE 18 (LBD16/ASL18) (Goh et al., 2012), are induced by auxin to promote LR initiation. However, it was unknown whether auxin signaling is modulated during LR initiation.

In this issue, Li et al. (2022) identified prohibitin 3 (PHB3) as a new regulator of LR initiation. PHB has previously been known as a tumor suppressor in mammals, and is also involved in plant stress responses (Wang et al., 2020). NO may also act as a signal molecule to promote LR formation (Correa-Aragunde et al., 2006; Schlicht et al., 2013), though the mechanism remains unclear. In this study, the authors revealed that PHB3 causes accumulation of endogenous NO, which leads to the degradation of IAA14 and IAA28, thereby inducing the expression of GATA23 and LBD16 to activate LR initiation.

PHB3 affects the accumulation of nitric oxide to activate LR initiation

The authors first focused on the regulation by PHB3 of LRP development. LR density was significantly lower in phb3 mutants due to the reduced LRP number and density. Histological analysis showed that PHB3 was strongly expressed in stage 1 of LRPs. These results suggest that PHB3 is required...
for LR initiation. Gravistimulation assays showed that PHB3 may also regulate LRP development. The new LRPs induced by 18 h gravistimulation were predominantly inhibited or delayed at stage I in the phb3 mutants while LRPs in the wild type were mainly at the second, third, and fourth stages.

A previous study has raised the possibility that NO and auxin are involved in the inhibition of LRP formation in phb3 mutants (Wang et al., 2010). Exogenous IAA or SNAP (S-nitroso-N-acetylpenicillamine; an NO donor) treatments significantly induced the expression of GATA23 and LBD16, and affected LR density, which indicated that IAA and NO promote LR initiation. Interestingly, treatments with 1-naphthylphthalamic acid (NPA; an auxin transport inhibitor), cPTIO (an NO scavenger), and their combination caused a similar inhibitory effect on LR formation, indicating that NO and IAA might function in the same pathway to regulate LR formation. Application of IAA did not restore LR density in the cPTIO-treated seedlings, indicating that NO is required for the auxin-induced LR development. The fluorescence of the NO indicator DAF-FM DA showed that NO was mainly distributed around LRPs and induced by IAA in the wild type while in phb3 mutants the induction was much lower. By RNA-seq analysis, differentially expressed genes in the wild type and phb3 were clustered in three Gene Ontology (GO) terms: root development; auxin biosynthesis and response; and NO response. Therefore, PHB3 is involved in regulating NO- and auxin-induced LR formation.

PHB3 regulates the degradation of IAA proteins through endogenous NO accumulation

RNA-seq and GO analysis revealed that the expression of LR-related genes and auxin metabolic and biosynthetic pathways were altered by NO treatment, strongly indicating that the NO regulation of LR formation may involve auxin signaling pathways. Notably, almost all the genes up-regulated by NO treatment were down-regulated in the phb3 mutant, and vice versa. These observations suggest that PHB3 and NO activate the same signaling components for LR formation. NO was previously reported to regulate root development. The expression of GATA23 and LBD16 could rescue the mutant phenotype of phb3. These results confirmed that GATA23 and LBD16 work downstream of PHB3 and NO to modulate LR formation.

The expression of GATA23 and LBD16 is negatively regulated by IAA28 and IAA14. The expression of IAA28 and IAA14 was also changed in phb3 mutants, which indicated the PHB3 modulation of IAA28 and IAA14 transcripts. Protein degradation assays revealed that IAA28–green fluorescent protein (GFP) and IAA14–GFP signals disappeared within 5 min of IAA treatment in wild-type roots, while the signals persisted in phb3 roots even after 10 min of IAA treatment, and the degradation could be restored by NO supply. These results indicate that NO functions in the degradation of IAA28 and IAA14 and that PHB3 regulates LR initiation by modulating NO-mediated AUX/IAA degradation.

Perspectives

These findings reveal a novel ‘PHB3–NO’ signaling module regulating LR initiation through modulation of the canonical AUX/IAA-mediated auxin signaling cascade (Box 1).

**Box 1. The molecular network of PHB3 and NO in regulating LR initiation**

Prohibitin 3 (PHB3) induces nitric oxide (NO) accumulation to promote lateral root (LR) initiation. During LR initiation, a pair of xylem pole pericycle cells are primed by auxin signaling and specified as founder cells that undergo asymmetric cell division to develop as a stage I LR primordium (LRP). This process is activated by an auxin/indole-3-acetic acid (AUX/IAA)–AUXIN RESPONSE FACTOR (ARF)-dependent auxin signaling cascade. PHB3 accumulates NO in pericycle cells and LRPs, and NO in turn triggers the degradation of AUX/IAA28 and IAA14 and the activation of ARFs, thereby inducing the expression of transcription factor genes GATA23 and LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16) to promote LR initiation and LRP development.
Remarkably, the cyclic degradation and accumulation of IAA proteins create oscillating signals in the oscillation zone, which in turn triggers pre-branch site formation (Kircher and Schopfer, 2018; Xuan et al., 2020). This process occurs even preceding LR initiation. Considering the strong regulation by ‘PHB3–NO’ on AUX/IAA protein degradation, it will be interesting to probe the role of PHB and NO signals in oscillation signals and periodic pre-branch site formation. Furthermore, LR development is influenced by environmental signals (Lavenus et al., 2013; Motte et al., 2019; Duan et al., 2021). PHB3 and NO both interact with reactive oxygen species to mediate plant development responses to environmental stresses (Scheler et al., 2013; Kong et al., 2018), thus it raises the question of whether the PHB3–NO signal module may serve as an intermediate that adapts LR development patterns to the ever-changing environmental conditions.

Keywords: Auxin, founder cell, lateral root, nitric oxide, PHB3.

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