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Article Addendum

Localized auxin biosynthesis and postembryonic root development in Arabidopsis

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Auxin is a phytohormone essential for plant development. Due to the high redundancy in auxin biosynthesis, the role of auxin biosynthesis in embryogenesis and seedling development, vascular and flower development, shade avoidance and ethylene response were revealed only recently. We previously reported that a vitamin B_6 biosynthesis mutant *pdx1* exhibits a short-root phenotype with reduced meristematic zone and short mature cells. By reciprocal grafting, we now have found that the *pdx1* short root is caused by a root locally generated signal. The mutant root tips are defective in callus induction and have reduced *DR5::GUS* activity, but maintain relatively normal auxin response. Genetic analysis indicates that *pdx1* mutant could suppress the root hair and root growth phenotypes of the auxin overproduction mutant *yucca* on medium supplemented with tryptophan (Trp), suggesting that the conversion from Trp to auxin is impaired in *pdx1* roots. Here we present data showing that *pdx1* mutant is more tolerant to 5-methyl anthranilate, an analogue of the Trp biosynthetic intermediate anthranilate, demonstrating that *pdx1* is also defective in the conversion from anthranilate to auxin precursor tryptophan. Our data suggest that locally synthesized auxin may play an important role in the postembryonic root growth.

The plant hormone auxin modulates many aspects of growth and development including cell division and cell expansion, leaf initiation, root development, embryo and fruit development, pattern formation, tropism, apical dominance and vascular tissue differentiation.[1-3] Indole-3-acetic acid (IAA) is the major naturally occurring auxin. IAA can be synthesized in cotyledons, leaves and roots, with young developing leaves having the highest capacity.[4,5] Auxin most often acts in tissues or cells remote from its synthetic sites, and thus depends on non-polar phloem transport as well as a highly regulated intercellular polar transport system for its distribution.[2]

The importance of local auxin biosynthesis in plant growth and development has been masked by observations that impaired long-distance auxin transport can result in severe growth or developmental defects.[3,6] Furthermore, a few mutants with reduced free IAA contents display phenotypes similar to those caused by impaired long-distance auxin transport. These phenotypes include defective vascular tissues and flower development, short primary roots and reduced apical dominance, or impaired shade avoidance and ethylene response.[7-15] Since these phenotypes most often could not be rescued by exogenous auxin application, it is difficult to attribute such defects to altered local auxin biosynthesis. By complementing double, triple or quadruple mutants of four Arabidopsis shoot-abundant auxin biosynthesis *YUCCA* genes with specific *YUCCA* promoters driven bacterial auxin biosynthesis *iaaM* gene, Cheng et al. provided unambiguous evidence that auxin biosynthesis is indispensable for embryo, flower and vascular tissue development.[8,13] Importantly, it is clear that auxin synthesized by YUCCAs is not functionally interchangeable among different organs, supporting the notion that auxin synthesized by YUCCAs mainly functions locally or in a short range.[6,8,13]

The central role of auxin in root meristem patterning and maintenance is well documented,[1,2,16] but the source of such IAA is still unclear. When ^{14}C-labeled IAA was applied to the five-day-old pea apical bud, the radioactivity could be detected in lateral root primordia but not the apical region of primary roots.[17] Moreover, removal of the shoot only slightly affected elongation of the primary root, and localized application of auxin polar transport inhibitor naphthylphthalamic acid (NPA) at the primary root tip exerted more profound inhibitory effect on root elongation than at any other site.[18] These results suggest that auxin generated near the root tip may play a more important role in primary root growth than that transported from the shoot. In line with this notion, Arabidopsis roots have been shown to harbor multiple auxin biosynthesis sites including root tips and the region upward from the tip.[4]

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Many steps of tryptophan synthesis and its conversion to auxin involve transamination reactions, which require the vitamin B_6 pyridoxal 5-phosphate (PLP) as a cofactor. We previously reported that the Arabidopsis mutant *pdx1* that is defective in vitamin B_6 biosynthesis displays dramatically reduced primary root growth with smaller meristematic zone and shorter mature cortical cells.[19] In the current investigation, we found that the root tips of *pdx1* have reduced cell division capability and reduced *DR5::GUS* activity, although the induction of this reporter gene by exogenous auxin was not changed. Reciprocal grafting indicates that the short-root phenotype of *pdx1* is caused by a root local rather than shoot generated factor(s). Importantly, *pdx1* suppresses *yucca* mutant, an auxin overproducer, in root hair proliferation although it fails to suppress the hypocotyl elongation phenotype.[20] Our work thus demonstrated that *pdx1* has impaired root local auxin biosynthesis from tryptophan. To test whether the synthesis of tryptophan is also affected in *pdx1* mutant, we planted *pdx1* together with wild-type seeds on Murashige and Skoog (MS) medium supplemented with 5-mehtyl-anthranilate (5-MA), an analogue of the Trp biosynthetic intermediate anthranilate.[21] Although *pdx1* seedlings grew poorly under the control conditions, the growth of wild-type seedlings was more inhibited than that of the *pdx1* seedlings on 10 μM 5-MA media (Fig. 1A–D). Compared with the elongated primary root on MS, wild-type seedlings showed very limited root growth on 5-MA (Fig. 1E). The relatively increased tolerance to 5-MA of *pdx1* thus indicates that the *pdx1* mutant may be defective in Trp biosynthesis, although amino acid analysis of the bulked seedlings did not find clear changes in Trp levels in the mutants (our unpublished data).

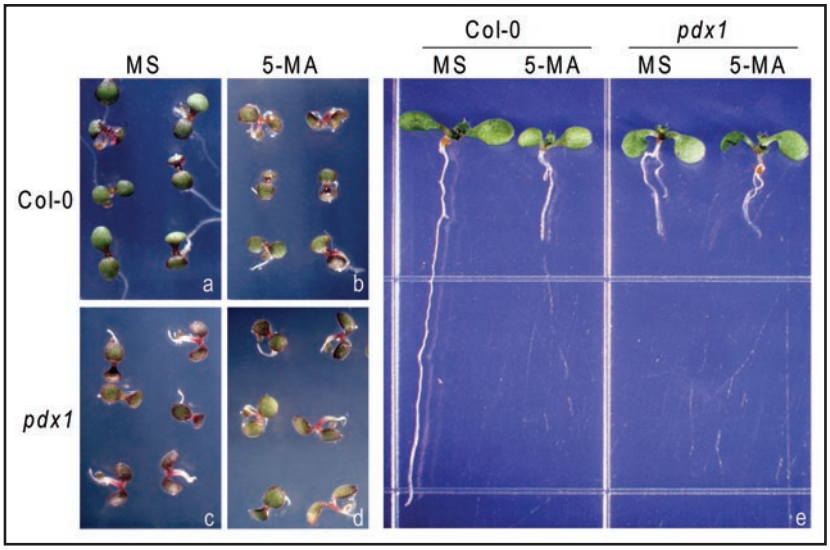

Figure 1. The *pdx1* mutant seedlings are relatively less sensitive to toxic 5-methyl anthranilate (5-MA). (A and C) Five-day-old seedlings of the wild type (Col-0) (A) or *pdx1* (C) on MS medium. (B and D) Five-day-old seedlings of the wild type (B) or *pdx1* (D) on MS medium supplemented with 10 μM 5-MA. (E) Eight-day-old seedlings of the wild type or *pdx1* on MS medium without or with 10 μM 5-MA supplement. Sterilized seeds were planted directly on the indicated medium and after two days of cold treatment, the plates were incubated under continuous light at 22–24°C before taking pictures.

We reported that PDX1 is required for tolerance to oxidative stresses in Arabidopsis.[19] Interestingly, redox homeostasis appears to play a critical role in Arabidopsis root development. The glutathione-deficient mutant *root meristemless1* (*rml1*) and the vitamin C-deficient mutant *vitamin C1* (*vtc1*) both have similar stunted roots.[22,23] Nonetheless, *pdx1* is not rescued by either glutathione or vitamin C[19] suggesting that the *pdx1* short-root phenotype may not be resulted from a general reduction of antioxidative capacity. Interestingly, ascorbate oxidase is found to be highly expressed in the maize root quiescent center.[24] This enzyme can oxidatively decarboxylate auxin in vitro, suggesting that the quiescent center may be a site for metabolizing auxin to control its homeostasis.[25] It is therefore likely that the reduced auxin level in *pdx1* root tips could be partially caused by increased auxin catabolism resulted from reduced vitamin B_6 level. We thus conducted experiments to test this possibility. A quiescent center-specific promoter *WOX5* driven bacterial auxin biosynthetic gene *iaaH*[26] was introduced into *pdx1* mutant. The transgenic seeds were planted on media supplemented with different concentrations of indoleacetamide (IAM), the substrate of iaaH protein. Although promotion of lateral root growth was observed at higher IAM concentrations, which indicates increased tryptophan-independent auxin production from the transgene, no change in root elongation was observed between *pdx1* with or without the *WOX5::iaaH* transgene at any concentration of IAM tested (data not shown), suggesting that the *pdx1* short-root phenotype may not be due to increased auxin catabolism.

Taken together, in addition to auxin transport; temporally, spatially or developmentally coordinated local auxin biosynthesis defines the plant growth and its response to environmental changes.[8,14,15]

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References

1. Woodward AW, Bartel B. Auxin: regulation, action and interaction. Ann Bot 2005; 95:707-35.
2. Teale WD, Paponov IA, Palme K. Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 2006; 7:847-59.
3. Chandler JW. Local auxin production: a small contribution to a big field. Bioessays 2009; 31:60-70.
4. Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G. Sites and regulation of auxin biosynthesis in Arabidopsis roots. Plant Cell 2005; 17:1090-104.
5. Ljung K, Hull AK, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G. Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana.* Plant Mol Biol 2002; 49:249-72.
6. Zhao Y. The role of local biosynthesis of auxin and cytokinin in plant development. Current Opinion in Plant Biology 2008; 11:16-22.
7. Takase T, Nakazawa M, Ishikawa A, Kawashima M, Ichikawa T, Takahashi N, et al. *ydk1--D*, an auxin-responsive *GH3* mutant that is involved in hypocotyl and root elongation. Plant J 2004; 37:471-83.
8. Cheng Y, Dai X, Zhao Y. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes Dev 2006; 20:1790-9.

9. Tobena-Santamaria R, Bliek M, Ljung K, Sandberg G, Mol JN, Souer E, Koes R. FLOOZY of petunia is a flavin mono-oxygenase-like protein required for the specification of leaf and flower architecture. Genes Dev 2002; 16:753-63.
10. Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JR, et al. Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev 2002; 16:3100-12.
11. Symons GM, Ross JJ, Murfet IC. The *bushy* pea mutant is IAA-deficient. Physiol Plant 2002; 116:389-97.
12. LeClere S, Rampey RA, Bartel B. *IAR4*, a gene required for auxin conjugate sensitivity in Arabidopsis, encodes a pyruvate dehydrogenase E1α homolog. Plant Physiol 2004; 135:989-99.
13. Cheng Y, Dai X, Zhao Y. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. Plant Cell 2007; 19:2430-9.
14. Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie D-Y, Dolezal K, et al. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 2008; 133:177-91.
15. Tao Y, Ferrer J-L, Ljung K, Pojer F, Hong F, Long JA, et al. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell 2008; 133:164-76.
16. Jiang K, Feldman LJ. Regulation of root apical meristem development. Annu Rev Cell Dev Biol 2005; 21:485-509.
17. Rowntree RA, Morris DA. Accumulation of ^{14}C from exogenous labelled auxin in lateral root primordia of intact pea seedlings (*Pisum sativum L.*). Planta 1979; 144:463-6.
18. Reed RC, Brady SR, Muday GK. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. Plant Physiol 1998; 118:1369-78.
19. Chen H, Xiong L. Pyridoxine is required for post-embryonic root development and tolerance to osmotic and oxidative stresses. Plant J 2005; 44:396-408.
20. Chen H, Xiong L. The short-rooted vitamin B_6-deficient mutant *pdx1* has impaired local auxin biosynthesis. Planta 2009; 229:1303-10.
21. Last RL, Bissinger PH, Mahoney DJ, Radwanski ER, Fink GR. Tryptophan mutants in Arabidopsis: the consequences of duplicated tryptophan synthase β genes. Plant Cell 1991; 3:345-58.
22. Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, et al. The *ROOT MERISTEMLESS1/CADMIUM SENSITIVE2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. Plant Cell 2000; 12:97-110.
23. Lukowitz W, Nickle TC, Meinke DW, Last RL, Conklin PL, Somerville CR. Arabidopsis *cyt1* mutants are deficient in a mannose-1-phosphate guanylyltransferase and point to a requirement of N-linked glycosylation for cellulose biosynthesis. Proc Natl Acad Sci USA 2001; 98:2262-7.
24. Kerk N, Feldman L. A biochemical model for the initiation and maintenance of the quiescent center: implications for organization of root meristems. Development 1995; 121:2825-33.
25. Kerk NM, Jiang K, Feldman LJ. Auxin metabolism in the root apical meristem. Plant Physiol 2000; 122:925-32.
26. Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, et al. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 2005; 433:39-44.