

# Auxin gradient is crucial for the maintenance of root distal stem cell identity in *Arabidopsis*

Huiyu Tian<sup>†</sup>, Tiantian Niu<sup>†</sup>, Qianqian Yu, Taiyong Quan, and Zhaojun Ding\*

The Key Laboratory of Plant Cell Engineering and Germplasm Innovation; Ministry of Education; School of Life Sciences; Shandong University; Jinan, PR China

<sup>†</sup>These authors contributed equally to this work.

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The plant hormone auxin plays a critical role in the maintenance of root stem cell niches in *Arabidopsis*. We have recently reported that WUSCHEL-RELATED HOMEBOX 5 (WOX5) transcription factor modulates free auxin production in the quiescent center (QC) of the root and its expression is inhibited in a feedback-dependent manner by canonical auxin signaling that involves indole-3-acetic acid 17 (IAA17) auxin response repressor. WOX5-IAA17 feedback circuit assures the maintenance of auxin response maximum in the root tip and thereby contributes to the maintenance of distal stem cell (DSC) populations. Here, we provide evidence to show that an optimal auxin maximum in QC guided auxin signaling gradient in root tips is crucial for maintaining root DSC identity.

The small signaling molecule auxin is a versatile regulator of plant growth and development including root patterning.<sup>1-3</sup> In the *Arabidopsis* root, a gradient-guided auxin maximum is maintained by both polar auxin transport and local auxin biosynthesis. The acropetal and basipetal auxin flows in the root are largely coordinated by PIN proteins that determine the direction of polar auxin transport, and by AUX/LAX auxin influx and PGP/MDR/ABCB auxin efflux carriers.<sup>4,5</sup> Besides polar auxin transport, we recently found that the WUSCHEL-RELATED HOMEBOX 5 (WOX5) transcription factor that is specifically expressed in the quiescent center (QC) is also involved in the establishment of the root-associated auxin maximum via modulating free IAA production.<sup>6</sup> This root-associated auxin maximum guides root growth and development by modulating cell division, cell expansion and stem cell differentiation.<sup>5,7,8</sup>

Previous studies based on the DR5::GUS auxin signaling response reporter suggested that the auxin maximum is mainly located in the root columella cells.<sup>7,9</sup> However, direct auxin measurements<sup>10</sup> and theoretical predictions<sup>11,12</sup> suggested that the highest auxin content should be spatially restricted to the QC. When examining carefully the auxin response in root tips with the alternative DR5rev::GFP reporter, we found that the auxin signaling maximum is indeed in the QC of the root apex, and there is a clear auxin signaling gradient between QC cells and root apex cells below the QC (Fig. 1).

Our recent investigations showed that locally suppressed auxin signaling in the QC via driving the inactivated version of IAA17<sup>13</sup> under the QC-specific WOX5 promoter strongly enhances the root DSC differentiation.<sup>6</sup> To determine whether

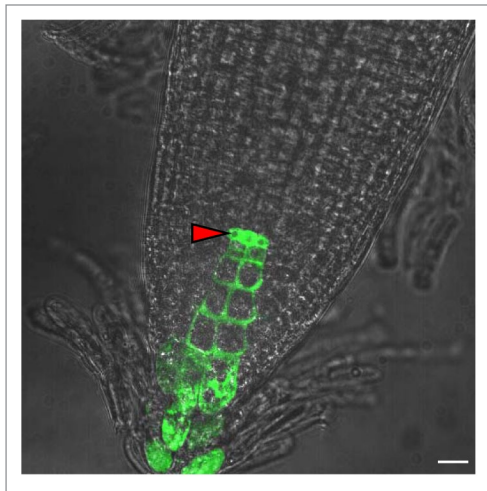
the QC-associated auxin signaling maximum is sufficient to maintain the root DSC identity, we generated WOX5-VP-IAA17mImII lines, which have locally activated auxin signaling in the QC via driving the activated version of IAA17<sup>13</sup> under the QC-specific WOX5 promoter for our studies. In these lines, compared with the wild-type control, the DR5rev::GFP signals in the QC were highly increased, whereas the signal in root apex cells below the QC was similar (Fig. 2A and B). Surprisingly, we found that WOX5-VP-IAA17mImII lines also showed highly enhanced root DSC differentiation phenotypes (Fig. 2C–D, 93/109), similar to that in WOX5-HA-IAA17mImII lines, which exhibit the absence of auxin signaling maximum in the root QC.<sup>6</sup> Our investigations showed that both increasing and decreasing auxin signaling in the root QC disturb the root DSC maintenance. Therefore, the auxin gradient between QC cells and root apex cells below the QC might be crucial for maintaining the root DSC identity.

To confirm this observation, we generated ACL5::WOX5 lines in which WOX5 is ectopically expressed in distal root cap cells under the ACL5 promoter (Fig. 3A), thus possibly altering auxin gradient between the QC and the root apex cells below the QC via WOX5 modulated free IAA production.<sup>6</sup> In ACL5::WOX5 lines, the QC and DSC cells in roots are actively dividing (Fig. 3B–D, 62/112) and the root DSC differentiation was enhanced compared with the wild-type control (Fig. 3D, 65/121). Although we couldn't observe the clear difference in the auxin gradient between QC cells and root apex cells below the QC with DR5rev::GFP as a reporter (Fig. 3E and F), the defective phenotypes of root stem cell niches in ACL5::WOX5 lines

\*Correspondence to Zhaojun Ding; Email: dingzhaojun@sdu.edu.cn

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**Figure 1.** The auxin gradient in the root apex shown by the auxin signaling reporter DR5rev::GFP. The auxin signaling maximum is located into the QC marked with red arrows. The scale bar represents 20  $\mu\text{m}$ .

might result from the disturbed auxin gradient, which couldn't be distinguished with DR5rev::GFP as a reporter.

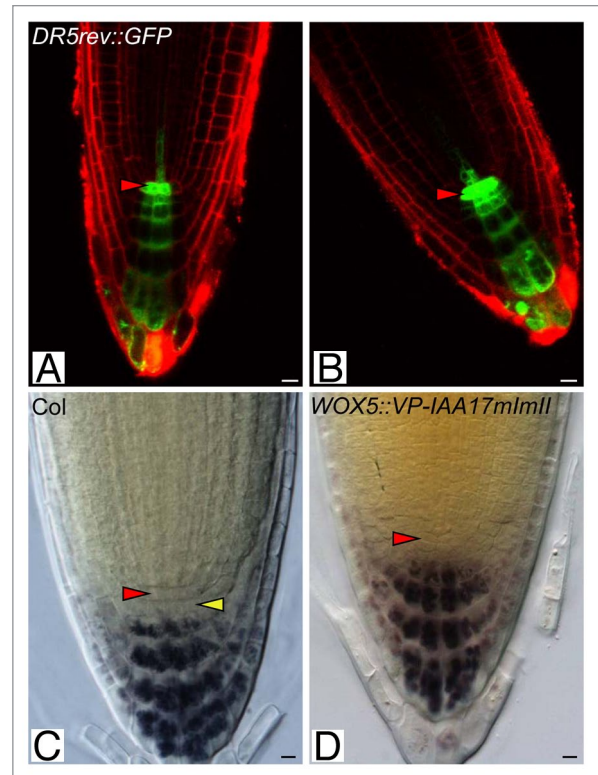
In summary, our investigations show that either increasing or decreasing auxin signaling in root QC cells will disturb root DSC maintenance, suggesting that an optimal auxin maximum is required in the QC for the maintenance of the root DSC identity. The graded auxin response between QC cells and root apex cells below the QC in root tips is crucial for maintaining the root DSC identity.

#### Disclosure of Potential Conflicts of Interest

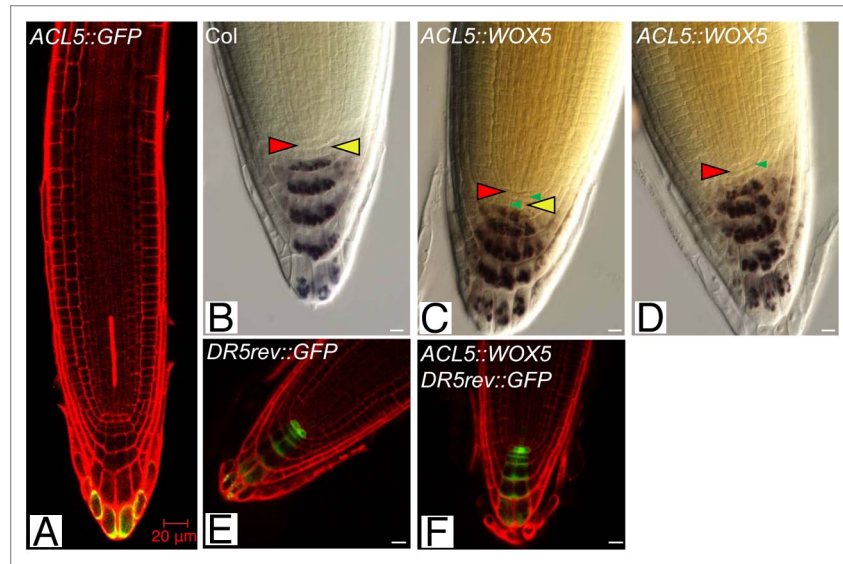
No potential conflicts of interest were disclosed.

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**Figure 2.** Increased auxin signaling maximum in the root QC results in enhanced root DSC differentiation. (A–B) The auxin gradient in root apex, which is shown by auxin signaling reporter DR5rev::GFP. Compared with the wild type control (A), the auxin signaling maximum is strongly increased in WOX5-VP-IAA17mImlII lines, which have QC specifically expressed activated version of IAA17 (B). Red arrows represent the auxin maximum in the QC. The scale bar represents 20  $\mu\text{m}$ . (C–D) Differentiation status of root DSCs (yellow arrowhead) below the QC (red arrowhead) in 4-d-old seedlings as inferred from the lugol staining. Wild-type roots show typically 1 tier of root DSCs (C), WOX5-VP-IAA17mImlII (D) shows complete root DSC differentiation. The scale bar represents 20  $\mu\text{m}$ .



**Figure 3.** Ectopic expression of WOX5 causes QC division and promotes root DSC differentiations in root tips. **(A)** ACL5::GFP expression patterns in the root apex. The scale bar represents 20  $\mu$ m. **(B-D)** Differentiation status of root DSCs (yellow arrowhead) below the QC (red arrowhead) in 4-d-old seedlings as inferred from the lugol staining. Wild-type roots show typically 1 tier of root DS cells **(A)**, ACL5::WOX5 lines show complete root DSC differentiation **(C)** or active cell divisions in the QC and root DSCs **(B-C)**. The scale bar represents 20  $\mu$ m. The green arrows indicate the ectopic cell division in the QC or stem cells. **(E-F)** The auxin gradient in the root apex which is shown by the auxin signaling reporter DR5rev::GFP. The auxin signaling gradient in ACL5::WOX5 lines **(E-F)** is not changed compared with the wild-type control **(D)**. The scale bar represents 20  $\mu$ m.

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