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LETTER TO THE EDITOR

CRE/LOX-based analysis of cell lineage during root formation and regeneration in *Arabidopsis*

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Abstract The root system of *Arabidopsis thaliana* comprises primary, lateral, and adventitious roots. Different types of roots are formed by diverse inductive cues and developmental programs. Here, we adopted the CRE/LOX system to trace cell lineage during the three types of root formation under the control of the promoter of *WUSCHEL-RELATED HOMEOBOX5*. The results show that the cells forming adventitious roots during de novo root regeneration from detached leaves and lateral roots from the primary root are descendants of the *WOX5*-expressing root primordium. During the post-embryonic growth of the primary root, some vascular and root cap cells are descendants of the *WOX5*-expressing stem cell niche in the root apical meristem. Overall, our data suggest that the CRE/LOX system is a useful tool to trace cell lineage in different types of root organogenesis.

Keywords Root regeneration, Adventitious root, Lateral root, CRE/LOX, *WOX5*, *Arabidopsis thaliana*

DEAR EDITOR,

During postembryonic development, *Arabidopsis thaliana* can form different types of roots. The pericycle-derived acropetal lateral roots (hereafter referred to as lateral roots) are formed from an existing root (e.g. the primary root) and the adventitious roots are mainly formed from non-root organs. The formation of both lateral and adventitious roots requires the establishment of root founder cells and then the division of root founder cells initiates the root primordium. During adventitious rooting from detached leaves (known as de novo root regeneration, Fig. 1A), wound-induced auxin accumulation promotes cell fate transition from a regeneration-competent cell (i.e. procambium and some vascular parenchyma cells) into an adventitious root founder cell, and then the division of the adventitious root founder cell initiates the adventitious root primordium (Liu et al. 2014; Hu and Xu 2016; Xu 2018). During lateral rooting from the primary root, root cap-derived auxin flux activates several pairs of xylem-pole pericycle cells to become the lateral root founder cells, and then asymmetric division of those lateral root founder cells initiates the lateral root primordium (Xuan et al. 2015, 2016; Du and Scheres 2018; Motte et al. 2019; Banda et al. 2019).

Here, we adopted the CRE/LOX system (Metzger et al. 1995; Brocard et al. 1998; Smetana et al. 2019) to trace the cell lineage during adventitious, lateral and primary root formation under the control of the *WUSCHEL-RELATED HOMEOBOX5* (*WOX5*) promoter ($WOX5_{pro}$), because *WOX5* is expressed in both adventitious and lateral root primordia and in the stem cell niche in the root apical meristem (Sarkar et al. 2007; Liu et al. 2014; Hu and Xu 2016; Du and Scheres 2017). Two constructs were generated in the $WOX5_{pro}$-driven CRE/LOX system

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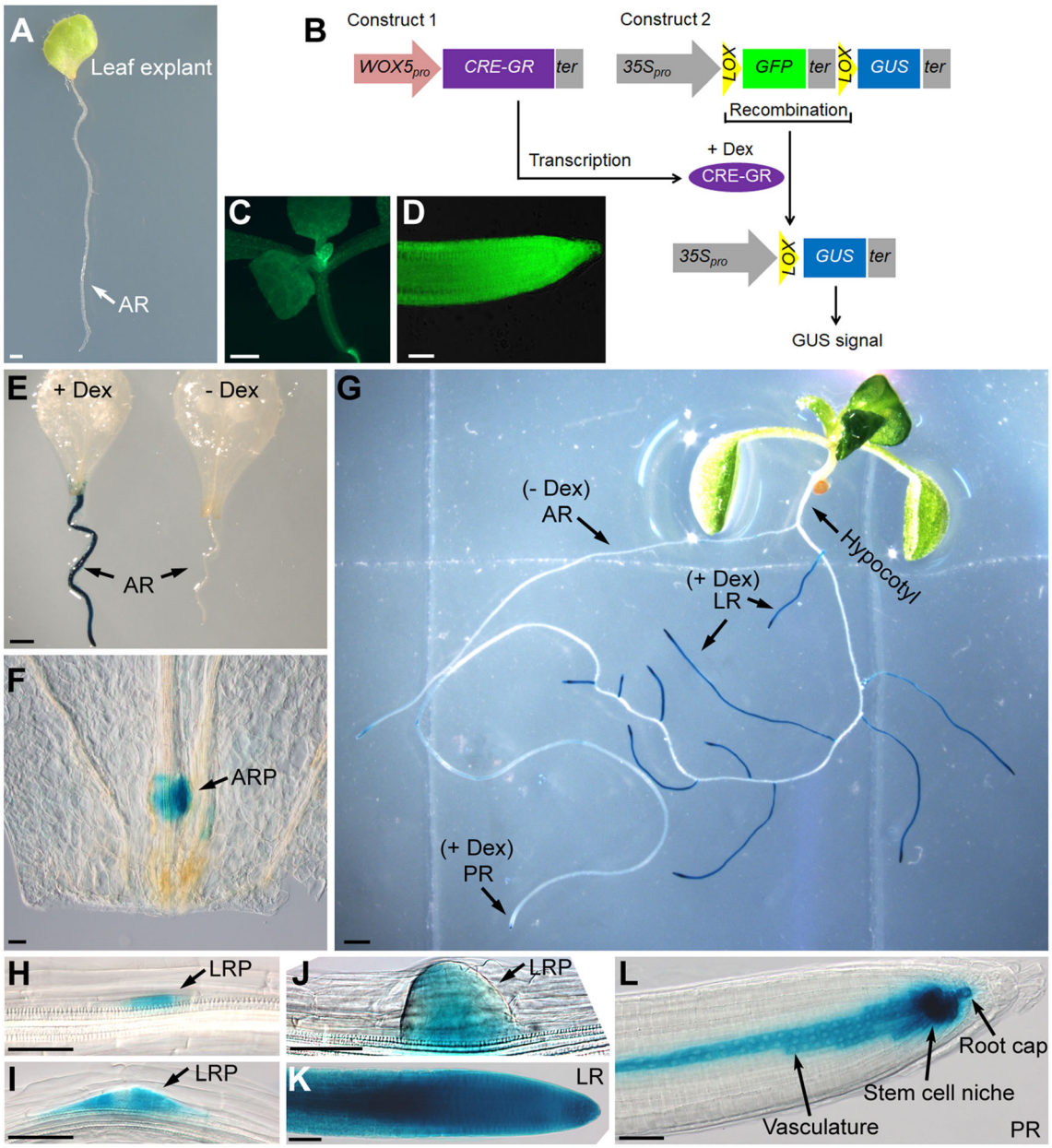

Fig. 1 CRE/LOX analysis of cell lineage in root formation. **A** Adventitious root formation from detached Arabidopsis leaves cultured on B5 medium for 10 d. **B** Schematic of analyses using CRE/LOX system under the control of *WOX5*$_{pro}$ to trace cell lineage in (**C**–**L**). Two independent lines were analyzed and showed the same results. Representative results are shown in (**C**–**L**). **C**, **D** GFP signals in seedling (**C**) and root (**D**) of F_1 population. **E**, **F** GUS staining in *WOX5*$_{pro}$-driven CRE/LOX analysis, showing positive GUS signal in the adventitious root (**E**) and adventitious root primordium (**F**) from detached leaves. **G**–**K** GUS staining in *WOX5*$_{pro}$-driven CRE/LOX analysis, showing positive GUS signal in lateral roots (**G**, **K**) and lateral root primordium at different stages (**H**–**J**) from the primary root. **L** GUS staining in *WOX5*$_{pro}$-driven CRE/LOX analysis, showing primary root. *AR* adventitious root, *ARP* adventitious root primordium, *LR* lateral root, *LRP* lateral root primordium, *PR* primary root. For adventitious rooting (**E**, **F**), seedlings were germinated on Dex-free 1/2 MS medium for 12 days, and two leaves (first leaf pair) from the same plant were detached. One of them were cultured on B5 medium without Dex treatment (− Dex) for 7 days (**E**), and the other leaf were cultured on B5 medium with 10 μM Dex treatment (+ Dex) for 7 days (**E**) or 4 days (**F**). For lateral and primary rooting (**G**–**L**), seedlings were germinated and grown vertically on Dex-free medium for 5 days, then transferred to medium containing 10 μM Dex for further growth. Adventitious root from hypocotyl was initiated before Dex treatment (− Dex) as a negative control, and lateral roots were initiated after Dex treatment (+ Dex) (**G**). Scale bars, 1 mm in (**A**, **C**, **E**, **G**) and 50 μm in (**D**, **F**, **H**–**L**)

in this study (Fig. 1B). The first construct consisted of the *CRE* gene fused with a dexamethasone (DEX)-induced nuclear localization domain *GLUCOCORTICOID RECEPTOR* (*GR*) (Aoyama and Chua 1997) inserted

between *WOX5*$_{pro}$ and the terminator (ter). The second construct comprised the *35S* promoter and the *β-GLUCURONIDASE* (*GUS*) reporter gene, between which the fused gene *LOX2272-GREEN FLUORESCENT PROTEIN* (*GFP*)-*ter-LOX2272* was inserted to block the expression of *GUS* driven by the 35S promoter. The two constructs were each transformed into the wild-type *Arabidopsis* Columbia-0 (Col-0) background. The T_1 generations containing each construct were obtained and crossed to obtain the F_1 population, which harbored the two constructs in every single plant and could be used to trace cell lineage during the rooting process. The GFP signal was confirmed in the seedlings (Fig. 1C) and roots (Fig. 1D) of the F_1 population before DEX treatment. During rooting, *WOX5*$_{pro}$ was activated and the *CRE-GR* gene was transcribed. At this time, DEX treatment allowed the CRE-GR protein into the nuclei, allowing the recombination of the two *LOX2272* sequences to form a single *LOX2272* sequence to replace the *LOX2272-GFP-ter-LOX2272* fragment and activate *GUS* expression. Therefore, tissues with GUS signals could be identified as descendants of the cell(s) that had expressed *WOX5*.

The results showed that both adventitious roots from detached leaves (Fig. 1E, F) and lateral roots from the primary root (Fig. 1G–K) were positively GUS-stained. Positive GUS staining could be observed in the adventitious (Fig. 1F) and lateral (Fig. 1H–J) root primordia as well as mature adventitious (Fig. 1E) and lateral (Fig. 1K) roots. This suggests that both adventitious and lateral rooting require the *WOX5*-mediated root primordium stage, and the cells forming adventitious or lateral roots are descendants of the root primordium.

In addition, we found that in the *WOX5*$_{pro}$-driven CRE/LOX system, the primary root had GUS signals in the root apical meristem, involving some cells of the stem cell niche, the vascular region, and the root cap (Fig. 1L). However, there were no GUS signals in most of the cells in mature regions of primary roots (Fig. 1G, L). Because DEX treatment was supplied from 5 days after germination, this result indicates that the *WOX5*-mediated stem cell niche contributes to the cell lineage of some special types of cells, e.g. vascular and root cap cells, but not to the cell lineage of many other cell types in the differentiated region of the primary root in our culture conditions and at this post-embryonic stage of the rooting process.

Understanding the cell lineage in organ initiation is essentially important in plant development. In this study, we provide direct evidence for the cell lineage of adventitious and lateral roots and the root apical meristem of the primary root. Both adventitious and lateral root organogenesis experience the *WOX5*-expressing root primordium stage. The stem cell niche contributes to certain tissues during cell differentiation in the root apical meristem during post-embryonic development. Using different cell-type-specific promoters, the CRE/LOX system will be a useful tool for further detailed analyses of different types of root formation in diverse species in the future (Bellini et al. 2014; Verstraeten et al. 2014; Ge et al. 2016; Liu and Xu 2018; Yu et al. 2020).

MATERIALS AND METHODS

Plant materials and GUS staining

For de novo root regeneration, *Arabidopsis* seeds were germinated and grown on 1/2 Murashige and Skoog basal (MS) medium with 1% (w/v) sucrose at 22 °C under a 16-h light/8-h dark photoperiod for 12 days. The first pair of rosette leaves from 12-days-old seedlings was cut at the junction of the blade and petiole, and the detached blades were cultured on B5 medium with 3% sucrose at 22 °C under dark conditions. For lateral root analysis, the *Arabidopsis* seeds were germinated and grown vertically on 1/2 MS medium with 1% (w/v) sucrose at 22 °C under a 16-h light/8-h dark photoperiod. Staining to detect GUS was performed as previously described (He et al. 2012). GFP signals were observed using Nikon SMZ1500 and Nikon C2 microscopes.

CRE/LOX system

Cell lineage was traced using the CRE/LOX system (Metzger et al. 1995; Brocard et al. 1998) under the control of *WOX5*$_{pro}$. Two constructs were used. In the first construct, the *WOX5*$_{pro}$*:CRE-GR* fragment was cloned into pBI101 to replace the *GUS* gene. In the second construct, the fused *LOX2272-GFP-ter-LOX2272-GUS-ter* fragment was cloned into the pCAMBIA1300 vector. The following primers were used for molecular cloning: 5′-ACGCGTCGACTCAGAGACCAAATTATTTTGG-3′ and 5′-GGTACCGTTCAGATGTAAAGTCCTCAACTG-3′ for *WOX5*$_{pro}$; 5′-CCCGGGTACCATGTCCAATTTACTGACCGT-3′ and 5′-GGATCCATCGCCATCTTCCAGCAG-3′ for *-CRE*; 5′-AGATCTATGATTCAGCAAGCCACAGCAGG-3′ and 5′-GGATCCTCACTTTTGATGAAACAG-3′ for *GR*; 5′-CCCGGGTACCATGGTGAG-3′ and 5′-GAGCTCTCACAATTCATCATGCTTGTACAGCTCGTCC-3′ for *GFP*; and 5′-CCCGGGTGGTCAGTCCCTTATGTTACGTCCTGTAGAAACC-3′ and 5′-GCCAGGAGAGTTGTTGATTCATTGTTTGCCTCCCTG-3′ for *GUS*.Transgenic plants were obtained by *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis* wild-type Col-0.

Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative under the following accession number: *WOX5* (AT3G11260).

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Author contributions Both authors conceived the study and analyzed the data. NZ performed experiments. LX wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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