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Report

A Signaling Module Controlling the Stem Cell Niche in *Arabidopsis* Root Meristems

Yvonne Stahl,[1] René H. Wink,[1] Gwyneth C. Ingram,[2] and Rüdiger Simon[1,*]
[1]Institut für Genetik
Heinrich-Heine Universität
Universitätsstrasse 1
D-40225 Düsseldorf
Germany
[2]Institute of Molecular Plant Sciences
University of Edinburgh
Mayfield Road
Edinburgh EH9 3JR
UK

Summary

The niches of the *Arabidopsis* shoot and root meristems, the organizing center (OC) and the quiescent center (QC), orchestrate the fine balance of stem cell maintenance and the provision of differentiating descendants. They express the functionally related homeobox genes *WUSCHEL* (*WUS*) and *WOX5*, respectively, that promote stem cell fate in adjacent cells [1]. Shoot stem cells signal back to the OC by secreting the CLAVATA3 (CLV3) dodecapeptide [2], which represses *WUS* expression [3]. However, the signals controlling homeostasis of the root stem cell system are not identified to date. Here we show that the differentiating descendants of distal root stem cells express CLE40, a peptide closely related to CLV3. Reducing CLE40 levels delays differentiation and allows stem cell proliferation. Conversely, increased CLE40 levels drastically alter the expression domain of *WOX5* and promote stem cell differentiation. We report that the receptor kinase ACR4, previously shown to control cell proliferation [4], is an essential component, and also a target, of *CLE40* signaling. Our results reveal how, in contrast to the shoot system, signals originating from differentiated cells, but not the stem cells, determine the size and position of the root niche.

Results and Discussion

CLE40 Expression Pattern and Mutant Phenotypes

CLE40, the closest homolog of the stem cell restricting signal *CLV3*, is expressed in specific root tissues, indicating a role in controlling root cell fates. Stem cells on the proximal (toward the shoot) site of the quiescent center (QC) generate vasculature and pericycle (Figure 1A), lateral stem cells give rise to endodermis, cortex, epidermis, and lateral root cap, and distal columella stem cells (CSC) generate the protective cap of columella cells (CC) with distinct starch granules that sense the gravitational field. After expression in the entire globular stage embryo, *CLE40* becomes successively restricted to the basal regions of the embryo that form the root meristem and the vasculature. After germination, *CLE40* remains expressed only in the differentiation zone of the stele that forms the inner layers of the root and in differentiating CCs (Figures 1B–1G). We identified two loss-of-function alleles of *CLE40* to investigate the role of *CLE40* as a potential signal for intercellular communication in the root (see Figure S1 available online). *cle40* mutant roots are shorter [5] and root tips appeared irregularly shaped, indicating that *CLE40* function is required for organized cell divisions in the root meristem. Because *CLE40* is normally expressed in CCs, we analyzed development of the distal meristem in detail (Figure 2). Wild-type roots carry mostly one (at D1 position) or, after a recent cell division, two layers of CSCs distal to the QC (at D1 and D2 positions) which lack stainable starch granules (Figure 2A; Table S1). By day 5, additional CSCs in more distal positions (D2) were found in 58% of the *cle40* roots, but in only 33% of the wild-type meristems, suggesting that differentiation of CSC daughters into CCs was significantly delayed when *CLE40* was lacking (Figure 2B; Table S1).

CLE40 Peptide Promotes Differentiation in the Distal Root Meristem in a Dose-Dependent Manner

We asked whether differentiation toward CC fate depends on the dosage of CLE40 peptide (CLE40p). Previous studies have shown that synthetic CLE peptides can activate CLE-dependent signaling pathways in shoot and root development [6–8]. Growing *cle40* mutant roots on medium containing 1 μM synthetic CLE40p largely suppressed the formation of extra CSCs and restored organized cell file formation. However, further increasing the CLE40 dosage by CLE40p treatment of wild-type roots, carrying two functional copies of the *CLE40* gene, resulted in ectopic starch granule accumulation also in the D1 layer, indicating loss of CSC identity (Figures 2C and 2D). Differentiation of D1 cells was also triggered by CLV3p, which is closely related to CLE40p, but not by the less similar TDIFp, which controls differentiation of xylem cells [7] (Table S1). Together, this indicated that cell identities in the distal meristem are regulated by a signaling pathway that is governed by the dosage of CLE40p; reduction of CLE40 activity permits stem cell proliferation, whereas increased CLE40 levels promote differentiation of distal cells.

CLE40 Regulates *WOX5* Expression and Distal Cell Fates

WOX5 acts from the QC to maintain the distal stem cell population [1], and can functionally replace *WUS*. Similarly, *CLE40* can replace *CLV3* if expressed from the shoot stem cell domain [5]. Together, this suggests that pathways controlling stem cell fate in shoot and root are at least partially conserved at the molecular level. In *wox5* mutants the distal root meristem appears disorganized, and D1 cells lose CSC identity and differentiate as CCs [1] (Figure 3A). Ectopic expression of *WOX5* inhibits the differentiation of CSC daughters, resulting in amplification of distal cell layers that maintain CSC identity [1]. During wild-type development, *WOX5* activity in the QC may generate a short-ranging signal that suffices to confer CSC identity to D1, but not to D2, cells. Because *CLE40* appears to regulate the distal stem cell domain antagonistically to *WOX5*, we tested whether *WOX5* expression is subject to regulation by *CLE40*. In 67% of *cle40* mutant roots, the

*Correspondence: ruediger.simon@uni-duesseldorf.de

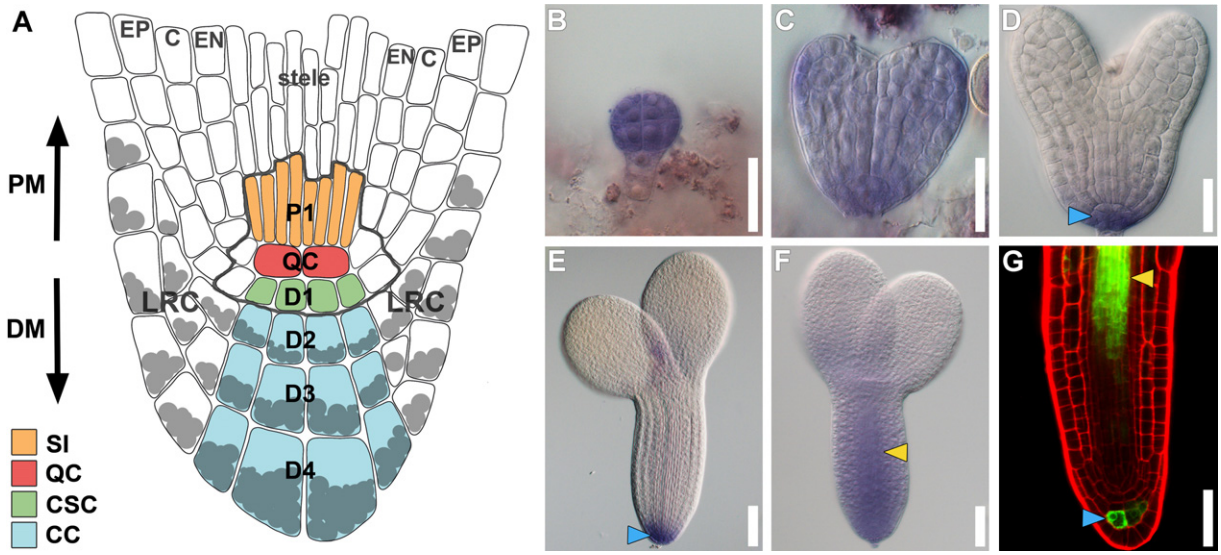

Figure 1. *CLE40* Is Expressed in the Embryo and Differentiated Root Cells

(A) Diagram illustrating root cell positions from proximal (P1) to distal (D1–D4). Color codes indicate cell fates. Stem cells surrounding the QC are outlined in gray. SI, stele initials; QC, quiescent center; CSC, columella stem cell; CC, columella cell; DM, distal meristem; PM, proximal meristem; LRC, lateral root cap; EP, epidermis; C, cortex; EN, endodermis. Gray dots, starch granules.

(B–F) *CLE40* expression (purple-blue) in embryos. Arrowheads, root progenitor cells (blue) and stele (yellow). High probe concentrations (F) detect *CLE40* expression also in the stele.

(G) *pCLE40:CLE40-GFP* expression (green) in the stele and CC of a lateral root.

The scale bars represent 25 μm in (B)–(D) and 50 μm in (E)–(G).

WOX5 expression domain expanded from the QC into the adjacent lateral stem cells (Figures 2F, 2G, and 2M; Figures S2 and S3; Tables S2 and S3), suggesting that *CLE40* is required to spatially confine *WOX5* during normal development. Treatment of *cle40* roots with CLE40p caused a near total restoration of the wild-type expression pattern of *WOX5* (Figures 2I and 2M). The presence of supernumerary CSCs at the D2 position in *cle40* mutants may thus be caused by increased or ectopic *WOX5* expression.

When wild-type roots were treated with CLE40p, *WOX5* expression was reduced in the QC and shifted to a more proximal position, indicating that the position of the *WOX5* expression domain along the proximo-distal axis of the root is controlled by CLE40p levels (Figure 2H). To elucidate whether *WOX5* is functional at the new, more apical position, we used the enhancer trap line *QC184* that is normally expressed in the QC in a *WOX5*-dependent manner [1] (Figures 2E and 2J; Table S4). In root meristems growing on CLE40p, the *QC184* expression domain was similarly displaced from the QC and coincided with the new proximal *WOX5* domain, showing that *WOX5* expression, but not *WOX5* function, is regulated by *CLE40* (Figures 2E and 2J). Cells at the D1 position differentiate toward CC in these plants, indicating that *WOX5* signaling from the more proximal location is insufficient for stem cell maintenance at D1.

Phenotypically, CLE40p-treated roots strongly resemble *wox5* mutants, suggesting that *WOX5* is a major target for repression by *CLE40*. However, when *wox5* mutant roots were grown on CLE40p medium, we observed a further proximal shift of CC identity, so that cells at the QC position accumulated starch granules (Figures 3A, 3D, and 3G). This indicated that *CLE40* signaling also interferes with the activity of another, *WOX5*-independent pathway that acts in parallel to *WOX5* and promotes distal stem cell maintenance.

CLV2 Is Not Required for Distal Stem Cell Regulation by *CLE40*

The observation that exogenous application of CLE40p repressed *WOX5* expression in the QC, but still permitted de novo *WOX5* expression in a proximal region, reveals that CLE40p is differentially perceived along the root axis, which may reflect the differential expression of the corresponding receptor protein(s). In several shoot tissues, CLE peptides have been found to signal via transmembrane receptors carrying extracellular LRR domains [9–11]. Shoot stem cells of *Arabidopsis* secrete the CLV3 peptide, which is perceived by the CLV1 and CLV2/CRN receptors on subjacent cells [12] and downregulates expression of *WUS*, a transcription factor that in turn non-cell-autonomously promotes stem cell fate [13]. In root tissues, external application of different CLE peptides, including CLE40p, showed that the LRR receptor protein CLV2 is required for overall growth restriction and meristem arrest [6]. However, the role of *CLV2* in root development has remained unclear, because *clv2* mutant roots appear aphenotypic and show a normal pattern of cell differentiation in the distal meristem (Figure 3B). Interestingly, *clv2* roots grow to normal length in the presence of CLE40p, but still show differentiation of D1 cells toward CC (Figures 3E and 3G). This indicates that exogenous CLE peptide applications require *CLV2* function in the proximal meristem to repress overall root growth, but not to perceive the *CLE40* signal in the QC or the distal meristem.

ACR4 Perceives the CLE40 Signal

Roots mutant for *ACR4*, encoding a receptor-like kinase of the *CRINKLY4* family, carry additional CSCs at the D2 position, revealing that *ACR4*, like *CLE40*, controls cell fate in the columella lineage [4] (Figures 3C and 3G). In *acr4* mutant roots, the number of *WOX5*-expressing cells increased, and the