

Article Addendum

Is the Arabidopsis root niche protected by sequestration of the CLE40 signal by its putative receptor ACR4?

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A tight but also dynamic regulation is necessary to control the size of stem cell populations in response to internal and external cues. The stem cells of the Arabidopsis shoot and root meristems are governed by the niche cells of the organizing centre (OC) and the quiescent centre (QC), respectively. The well characterized *CLV3/WUS* negative feedback loop adjusts homeostasis of the stem cell population in the shoot. Here, the *CLAVATA3 (CLV3)* dodecapeptide, expressed by the stem cells, signals to repress *WUSCHEL (WUS)*, which is expressed in the subjacent OC cells, and in turn activates *CLV3* expression non-cell autonomously. However, a similar signaling module controlling the root stem cell population was as yet unknown. In the June issue of *Current Biology* we report on such a signaling module comprising *CLE40* (a *CLV3* homologue) that acts via the receptor kinase *Arabidopsis Crinkly4 (ACR4)* to repress the *WUS* homologue *WOX5* which maintains distal root stem cells. Furthermore, we showed that *CLE40* peptide (*CLE40p*) treatment upregulates *ACR4* expression. In this Addendum, we are further elaborating our hypothesis in which the upregulation of *ACR4* as a consequence of ectopic *CLE40p* builds a protective barrier for the QC niche cells.

Intercellular signaling processes mediated by small peptide ligands play important roles in plant development. The stem cells in the Arabidopsis shoot apical meristem express and secrete the *CLV3* dodecapeptide and this signal is transmitted by the membrane localized leucine rich repeat (LRR) receptor kinase *CLAVATA1 (CLV1)*, and independently by *CORYNE (CRN)* and *CLAVATA2 (CLV2)* to the subjacent niche cells of the OC. This

signaling eventually leads to the downregulation of the homeodomain transcription factor *WUS* which is expressed in the OC. *WUS* in turn promotes stem cell fate, and thereby also *CLV3* expression, in the overlying stem cells thus leading to a negative feedback loop adjusting stem cell homeostasis in the shoot.¹⁻⁴ In Arabidopsis, 31 members of the *CLAVATA3/ENDOSPERM SURROUNDING REGION (CLE)* family, carrying a conserved 14 aa C-terminal CLE motif, are known.⁵ It was shown in previous studies that overexpression of *CLE* genes or addition of synthetic peptides can activate *CLE*-dependent signaling pathways and it was proposed that they could act through *CLV*-related LRR receptor-like kinases. Two classes of peptides are distinguishable by their effect on the root and shoot meristems. Class A peptides, e.g., *CLV3p*, *CLE19p* and *CLE40p*, promote cell differentiation resulting in reduced root meristem size and a short root phenotype.⁶⁻⁹ Class B peptides, e.g., *CLE41 (TDIF)* do not reduce root size, but instead promote proliferation of vascular cells.⁹

The *WUS* homologue *WUSCHEL-RELATED HOMEBOX5 (WOX5)* is expressed in the QC of the Arabidopsis root, which represents the equivalent to the OC of the shoot apical meristem harboring the niche cells of the root.¹⁰ The stem cells of the root, the so called initial cells, are surrounding the QC and give rise to all the different root tissues (labeled in Fig. 1). In *wox5* mutants the columella stem cells (CSCs) differentiate which is visible by their accumulation of starch granules. *WOX5* was shown to be necessary and sufficient for distal (towards the root tip) root stem cell maintenance. *WUS* and *WOX5* can functionally replace each other if expressed in the appropriate domains, demonstrating their close homology.¹⁰

Mutants in the receptor-like kinase *ACR4* were recently shown to carry additional layers of columella stem cells (CSCs), arguing for a negative regulation of distal root stem cell fate by *ACR4*.¹¹

CLE40 is the closest homolog of *CLV3* and was shown to be able to substitute *CLV3* in the shoot; *cle40* mutants were previously described to display a short root phenotype.¹²

In our recent publication,¹³ we describe two new mutant alleles of *cle40* focusing on their distal meristem phenotypes. We show that *CLE40* expression starts during embryogenesis and gets gradually confined to the future distal root meristem and the stele. *cle40* mutants show, like *acr4* mutants, additional CSCs. Addition of

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the synthetic CLE40p or the related CLV3p (but not TDIFp), can rescue this phenotype in *cle40* mutants, but importantly not in *acr4* mutants. Furthermore, in *cle40* and *acr4* mutant roots the *WOX5* expression domains are equally enlarged laterally. Therefore CLE40 can negatively regulate *WOX5* expression, resembling the *CLV3/WUS* feedback regulation in the shoot; however, the CLE40 signal in the root originates from differentiated instead of stem cells.

In light of these results we suggest ACR4 (a member of the *CRINKLY4* receptor-like kinase family) as the receptor for the CLE40 signal. We propose a signaling module with CLE40 as a secreted peptide ligand originating from differentiated root cells to act via the ACR4 receptor to regulate *WOX5* expression levels and it's expression domain in order to maintain distal root stem cells.

Addition of synthetic CLE40 or CLV3 peptides promotes differentiation of CSC cells towards CC cells (observable by starch accumulation in CSC cells). Intriguingly, the expression of *WOX5* in the QC position is gradually lost and *WOX5* expression emerges instead at more proximal positions.

In the root meristem *ACR4* is expressed, as visualized by a *pACR4:ACR4-GFP* reporter line,¹⁴ mainly in the CSCs and at much lower levels in the CC cells, the root cap and QC in between the CLE40 and *WOX5* expressing cells of the columella and QC cells, respectively. *ACR4* expression levels are strongly upregulated after CLE40p or CLV3p treatment (but not after TDIFp treatment) and the *ACR4* expression now systematically includes the QC position. This parallels the proximal displacement of the *WOX5* expression domain upon CLE40p or CLV3p treatment. These observations indicate a dual role for ACR4 so that the receptor is not only involved in transmitting the CLE40 signal but is also positively regulated by its peptide ligand. Notably, the *WOX5* expression is still proximally adjacent to the *ACR4* expression now in QC position; therefore CLE40 is also able to control the proximo-distal axis of *WOX5* expression.

The adjacent and partly overlapping expression domains of *CLE40*, *ACR4* and *WOX5* (see Fig. 1) and their changes upon CLE40p or CLV3p treatment lead us to the hypothesis that by upregulation of the receptor ACR4 the plant seeks to protect the *WOX5* expression in the niche cells of the QC thereby maintaining the surrounding stem cells. *CLE40* is expressed in the differentiated columella cells of the distal root meristem and overlaps there with weak *ACR4* expression. As CLE40p is presumably secreted to the extracellular space it would be capable of spreading into the surrounding cells. The range of the intercellular movement of the CLE40 signal could be restricted via ligand sequestration by the ACR4 receptor as is also thought for CLV3 and CLV1 in the shoot. A protective barrier of *ACR4* expressing CSCs could act to dynamically buffer alterations in ligand abundance during changes in requirement of differentiated cells in different developmental stages of the plant. ACR4 would not only relay the CLE40 signal into the receiving cells to restrict *WOX5* expression but also sequester its own ligand to protect the QC from too much CLE40p; which in turn would allow *WOX5* expression in the QC to maintain the surrounding stem cells (see Fig. 1).

Future analyses of *ACR4* and *CLE40* expression and localization dynamics might give further insight into this sequestration model.

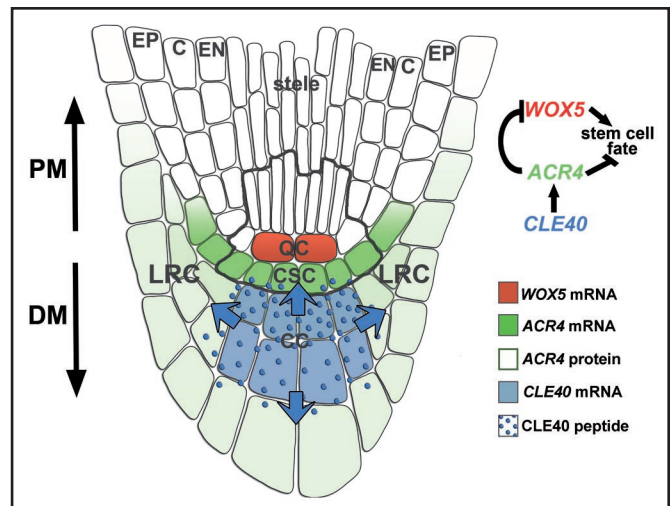


Figure 1. Model of CLE40 sequestration in the distal root meristem by its receptor ACR4 allows *WOX5* expression in the QC and thereby confers stem cell fate in the surrounding cells. Schematic representation of the Arabidopsis root meristem. Color codes indicate mRNA and protein abundance of *WOX5* (red), *ACR4* (green and light green) and *CLE40* (blue). Arrows indicate CLE40p movement into surrounding cells. The stem cells surrounding the QC are outlined by a grey line. DM, distal meristem; PM, proximal meristem; QC, quiescent centre; CSC, columella stem cells; CC, columella cells; LRC, lateral root cap; Ep, epidermis; C, cortex; EN, endodermis.

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