

previously considered, playing a regulatory role in growth and development [11]. These new plant studies also support this notion. Furthermore, a plastid-specific NAT has recently been identified [12], and chloroplastic proteins are Nt-acetylated post-translationally, following a transit-peptide cleavage step [4]. This implies that compartmentalisation of NAT-activity is functionally important. There has been growing interest in the study of Nt-modifications in plants in recent years, particularly in relation to the N-end rule pathway of proteolysis [2]. It will now be important to determine whether the recently identified Ac/N-end rule pathway functions in plants. Homologues of the relevant E3 ligases are present in plant genomes [2], and the effects of NAT activity on SNC1 turnover [and a second NLR, RESISTANCE TO *P. syringae* pv *maculicola* 1 (RPM1)] also support this proposition [10]. Another pertinent question is how ABA- and pathogen-associated stress signals impact on NAT function. Effects on transcription and protein-depletion are implicated by Linster *et al.*, but it is also possible that these signals may modulate NAT enzymatic activity in other ways, for example via post-translational modifications.

It is becoming increasingly apparent that what was previously considered a constitutive and inert modification actually has a great deal of functional significance, at protein-specific and proteome-wide levels. The scene is now set for further studies into the complexity and functional relevance of this widespread but enigmatic modification in plants and beyond.

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Spotlight

WOX5 is Shining in the Root Stem Cell Niche

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The *WUS-RELATED HOMEBOX 5 (WOX5)* gene is expressed in the quiescent center (QC) to regulate the columella stem cell (CSC) identity. Three recent reports not only show how *WOX5* is controlled but also highlight the key role of *WOX5* in root stem cell niche maintenance.

WOX5 is a Root Stem Cell Organizer

In higher plants, the root is an important organ system for nutrient uptake, anchorage, and storage. Its growth depends on the continuous division of cells in the root meristem. At the tip of root apical meristem, a pool of stem cells, which are defined by the ability to renew themselves and contribute undifferentiated daughter cells to produce new tissues, surround a small group of organizing cells, the quiescent center (QC) [1]. Laser ablation experiments showed that root stem cells rapidly differentiate if the QC cells are ablated, indicating the crucial role of QC to maintain root stem cell identity [2].

The homeobox gene *WUSCHEL-RELATED HOMEBOX 5 (WOX5)* was reported to be specifically expressed in QC. Loss of *WOX5* function in the root stem cell niche causes terminal differentiation of CSCs [3]. A pathway involving the signaling peptide CLAVATA3/EMBRYOSURROUNDING REGION 40 (CLE40), the receptor-like kinases ARABIDOPSIS CRINKLY4 (ACR4)/CLAVATA1 (CLV1) was reported to maintain the columella stem cells (CSCs) through negative regulation of *WOX5* in the root apical meristem [4–6]. Although the CLE40-ACR4/CLV1-*WOX5* module is well characterized, the molecular mechanisms by which *WOX5* promotes stem cell fate and controls QC division remain obscure. By identifying the downstream targets and upstream regulators of *WOX5* in *Arabidopsis*, three recent reports [7–9] provide novel mechanistic insights into the regulation of *WOX5* and its action modes in the stem cell niche [7–9].

Effector Genes Mediates *WOX5* Function in the Stem Cell Niche

Using a *WOX5* translational reporter line (*pWOX5:WOX5-GFP*) which could completely rescue the CSC defective phenotype of the *wox5-1* mutant, Pi *et al.* showed that *WOX5* protein accumulates

not only in the QC but also in the CSCs [7]. This finding indicates that the mobile WOX5 protein may be at least part of the long-sought after QC signals that are controlling CSCs, a mechanism that has been hypothesized from laser ablation experiments [2]. By employing mRNA profiling assays, the authors demonstrate that *CDF4*, which encodes a group II Dof transcription factor and promotes root stem cell differentiation, is one of the WOX5 targets. Using *in vitro* and *in vivo* assays, the authors demonstrated the direct binding of WOX5 to the *CDF4* promoter. Next, using an immunoprecipitation step coupled with a mass spectrometry approach and an *in vitro* assay, Pi *et al.* revealed that WOX5 could interact with all TPL family members, suggesting that WOX5 acts together with TPL/TPR to repress the downstream transcription factors [7]. It has been reported that TPL/TPR co-repressors and their interaction proteins typically possess a conserved motif, named ETHYLENE RESPONSE FACTOR (ERF)-associated amphiphilic repression (EAR) domains. Intriguingly, the interaction of WOX5 with TPR1 requires the WUS box, rather than the EAR domain [7]. Similarly, the WUS box rather than the EAR domain is critical for WUS-mediated transcriptional activation and repression, although the WUS box and the EAR domain are both transcriptional repression domains. Nevertheless, it will be interesting to elucidate the functions of the EAR domain in the WOX5 protein. TPL/TPR co-repressors can interact with histone deacetylases in both animals and plants, and the authors showed that HAD19, belonging to Class I RPD3 histone deacetylase genes, reduced H3K9 and H3K14 acetylation at the *CDF4* locus. Consistently, *tpl-1* and *had19-3* mutants have a similar CSC defect phenotype as that for *wox5-1* [7]. These results indicate that WOX5 maintains stem cell niche identity through WOX5-TPL/TPR-HDA19 complex-mediated histone deacetylation and repression of *CDF4* which encodes a differentiation factor.

The QC is maintained at the G1/S cell cycle checkpoint and thus divides infrequently. The *wox5-1* mutant displays ectopic QC divisions [7]. However, how WOX5 is involved in the control of the G1/S cell cycle checkpoint was not clear so far. Now Forzani *et al.* identified *CYCD3;3*, which is involved in cell cycle regulation, as another effector gene of WOX5, providing new insights about the WOX5-mediated G1 to S phase transition. Because D-type cyclins are essential for the G1-to-S transition, ectopic expression of *CYCD3;3* (in the QC driven by the WOX5 promoter), resulted in an increased frequency of cell division. In addition, *CYCD3;3* expression was detected in the QC of the *wox5-1* mutant but not in the wild-type control, suggesting that WOX5 excludes *CYCD3;3* expression from the QC [8]. Furthermore, using chromatin immunoprecipitation (ChIP)-quantitative PCR (qPCR), Forzani *et al.* also demonstrated direct binding of WOX5 with the *CYCD3;3* promoter [8]. However, the mechanism of WOX5-mediated repression of *CYCD3;3* remains elusive. It is still an open question, whether the mechanism might involve histone deacetylation similar to the repression of *CDF4*.

Precise Control of WOX5 Through Epigenetic Modifications

It has been reported that auxin acts through *AUXIN RESPONSE FACTOR 10* (*ARF10*) and *ARF16* to repress and confine WOX5 expression in the QC cells and thereby promote the differentiation of CSC daughter cells [10]. However, the upstream regulatory mechanisms controlling WOX5 expression was largely unknown. Recently, Zhang *et al.* found that the REPRESSOR OF WUSCHEL1 (*ROW1*), also called BARD1, directly binds to the WOX5 promoter region and restricts its transcription to the QC [9]. Genetic analysis indicate that WOX5 acts downstream of *ROW1*, and down-regulation of WOX5 expression partially rescues the stem cell niche defect in *row1* [9]. Furthermore, ectopic expression of *ROW1* using the WOX5 promoter

represses WOX5 transcription. Results from ChIP-qPCR showed that *ROW1* specifically binds trimethylated histone H3 lysine 4 (H3K4me3) in the proximal WOX5 promoter region [9]. Previous studies have suggested that *ROW1* represses *WUS* transcription through interacting and inhibiting the chromatin remodeling complex *SYD*, an SWI/SNF chromatin remodeling ATPase, which is essential for *WUS* expression [11]. Yang *et al.* reported that the *Arabidopsis* SWI2/SNF2 chromatin remodeling ATPase *BRAHMA* directly targets *PIN* genes to control root stem cell niche identity via regulating auxin transport [12]. Therefore, it will be interesting to elucidate whether SWI2/SNF2 chromatin remodeling factors are involved in the epigenetic modification of WOX5 expression.

Concluding Remarks and Open Questions

In summary, three recent studies [7–9] not only represent a significant step toward understanding the regulation of WOX5 expression and its action mechanisms in the root but also uncover the central role of WOX5 in the maintenance of CSC identity (Figure 1). Nevertheless, there are some open questions still awaiting to be addressed. For example, how is the movement of WOX5 from the QC into the CSCs controlled? Which of the WOX5 targets are involved in the inhibition of cell differentiation in CSCs? In addition, because *CDF4* is a key factor in stem cell differentiation, the identification of *CDF4* downstream targets will help us understanding how the WOX5-*CDF4* pathway functions in the root. It is also interesting to further dissect the interaction between CLE40-*ACR4/CLV1* module and *CDF4* in controlling root stem cell niche identity. While *WUS* is regulated by *CLV3*-mediated feedback signals in the shoot apical meristem, no feedback regulation was demonstrated for CLE40 peptide toward WOX5. Therefore, it will be interesting to investigate if one or more additional unknown peptides are responsible for the feedback regulation of WOX5 in root development.

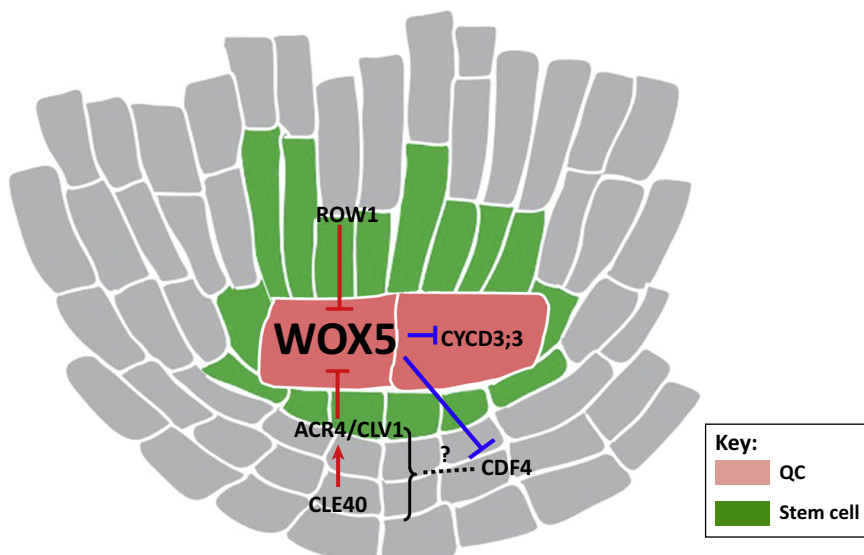


Figure 1. Maintenance of the Root Stem Cell Niche. *WOX5* is expressed in the QC (pink) and inhibits stem cells differentiation (green). The *CLE40* peptide from differentiated CCs signals via its receptors (*ACR4/CLV1*) that are expressed below the QC, to inhibit the *WOX5* activity and to promote root stem cell differentiation. Stem cell fate is also controlled by *ROW1* signaling via the repression of *WOX5*. *WOX5* silences the differentiation factor *CDF4* in the regulation of stem cell maintenance through intercellular movement, and also suppresses *CYCD3;3* activity to maintain quiescence of the QC.

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