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Article Addendum

A Role for KNAT Class II Genes in Root Development

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Addendum to:

A Map of KNAT Gene Expression in the Arabidopsis Root

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ABSTRACT

Homeodomain proteins set up domains of gene expression during the development of animal and plant body plans. In plants, homeodomain proteins of the KNOX class I family have been shown to play a role in shoot apical meristem development. Recently, we have investigated the role of the *Arabidopsis thaliana KNOX* class II genes *KNAT3*, *KNAT4* and *KNAT5* in root development. These genes showed root domain and cell type specific expression patterns, and their expression was regulated by hormones that influence root growth. Moreover, sub-cellular localization of the KNAT proteins exhibited regulation, suggesting that post-transcriptional control contributes to KNOX class II protein activity. Our data provide a survey of *KNAT* gene expression in the root and indicate that the investigated *KNAT* genes might play distinct roles during root development.

Homeodomain proteins have been shown to be key regulators of animal development.[1] They are transcription factors that can act as combinatorial switches to turn the expression of cascades of genes on and off. As the DNA binding affinity of homeodomain proteins alone is generally weak, protein-protein interaction of homeodomain proteins are a crucial aspect of their function. By interacting with different protein partners, this characteristic of homeodomain proteins makes it possible for them to be involved in a series of developmental processes.

In plants, homeodomain proteins emerge to function in a similar way. Among the first plant homeodomain proteins to be identified were the KNOX and BELL proteins,[2,3] both members of the TALE (three amino acid loop extension) class.[4] It has been shown that TALE proteins physically interact with each other and with members of the OVATE protein family in *Arabidopsis thaliana.*[5-10] These interactions have been shown to be important for localisation of homeodomain proteins to the nucleus and for DNA binding specificity. The complexity of the interaction network suggests functional redundancy within the TALE protein family and the possibility for compensatory interactions within the regulatory network.[8]

The *Arabidopsis* genome contains eight *KNOX* genes. The class I *KNOX* genes (*STM1*, *BP/KNAT1*, *KNAT2*, and *KNAT6*) are all expressed in the shoot meristem and play a role in shoot development.[11-18] Genetic interactions of the proteins with other members of the TALE family have been shown.[6,7,9,10] The class II genes (*KNAT3*, *KNAT4*, *KNAT5*, and *KNAT7*) have broader expression patterns.[19,20] Consistent with its expression in stele tissue,[21] *KNAT7* has recently been shown to play a role in xylem formation.[22] The functions of *KNAT3*, *KNAT4* and *KNAT5* have not been established so far.

As a first step to understand the role of these genes, we have examined the regulation of their expression in the *Arabidopsis* root.[23] A role for the class I gene *KNAT6* in lateral root development was demonstrated,[24] and expression of *KNAT* class II genes in the *Arabidopsis* root has been reported.[19,21,25]

The *Arabidopsis* root can be divided into several zones (Fig. 1e).[26] In the meristematic zone at the tip of the root, cells proliferate. The adjacent elongation zone consists of a distal elongation zone (or transition zone) in which cells cease to divide and gain the competence for rapid elongation, and a proximal elongation zone in which cells elongate rapidly before they enter the differentiation zone.[27] In the mature root zone, lateral roots develop from pericycle cells that regain meristematic activity. In a cross section through a mature *Arabidopsis* root, single layers of pericycle, endodermis, cortex and epidermis cells can be seen surrounding the central vascular tissue (Fig. 1a).

KNAT3, *4* and *5* promoter driven β-glucuronidase gene (*GUS*) expression showed root zone and cell type specific GUS activity. While *KNAT4* and *KNAT5* promoters exhibited activity from the beginning of the distal elongation zone (Fig. 1g and h), the *KNAT3* promoter was active only in the mature root zone (Fig. 1f). In cross sections through the

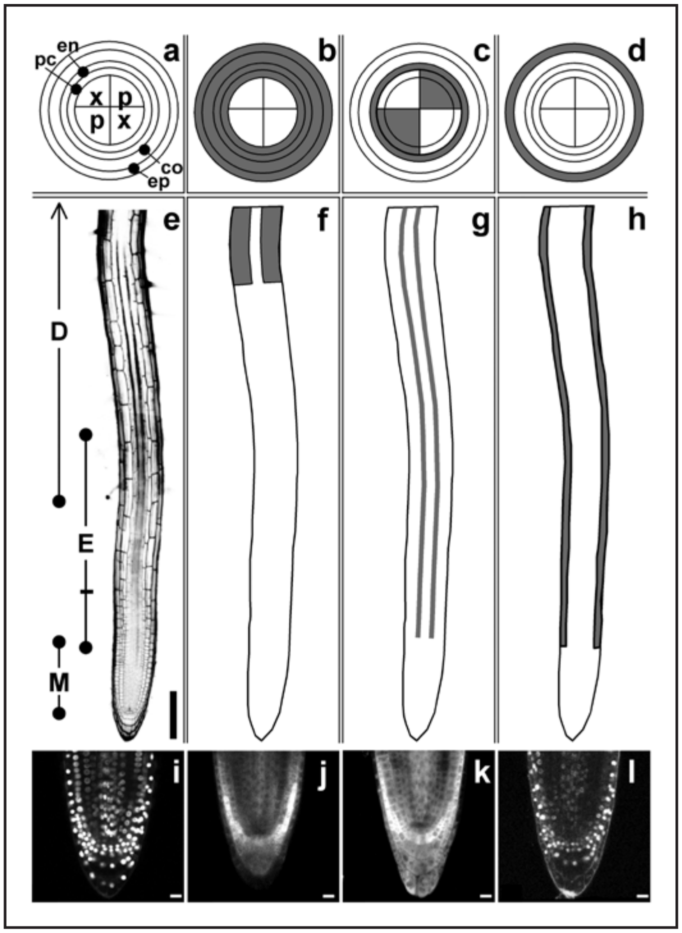

Figure 1. Expression of *KNAT3*, *KNAT4* and *KNAT5* in the *Arabidopsis* root. (a to d) Schematics showing expression (dark grey) in cross sections of the mature root. (a) Diagram of a cross section through an Arabidopsis root. Single layers of pericycle (pc), endodermis (en), cortex (co) and epidermis (ep) cells surround a central vascular bundle consisting of xylem (x) and phloem (p) cells. (b) *KNAT3* expression. (c) *KNAT4* expression. (d) *KNAT5* expression. (e to h) Schematics showing expression (dark grey) along the longitudinal axis of the root tip. (e) Confocal microscope image of a longitudinal section through an *Arabidopsis* root tip showing the meristematic zone (M), the elongation zone (E), comprising a distal elongation zone (distal of horizontal line) and a zone of rapid cell elongation (proximal of horizontal line), and the differentiation zone (D). (f) Expression of *KNAT3*. (g) Expression of *KNAT4*. (h) Expression of *KNAT5*. (i to l) Sub-cellular localization of KNAT proteins in the root meristem. (i) Nuclear localization of a HISTONE-YFP fusion used as control. (j) Cytoplasmic localization of KNAT3-YFP. (k) Cytoplasmic localization of KNAT4-YFP. (l) Nuclear localization of KNAT5-YFP. Scale bars: 100 mm in (e), 10 mm in (i to l).

mature root zone, *KNAT3* expression was strong in pericycle, endodermis and cortex, and weaker in the epidermis (Fig. 1b). *KNAT4* expression was seen in phloem and pericycle cells located above the phloem poles. In the mature root, the strongest *KNAT4* expression was detected in the endodermis (Fig. 1c). *KNAT5* promoter activity was generally specific to epidermal cells (Fig. 1d). Occasionally, weak *KNAT5* promoter activity was also detected in the cortex and stele of the mature root zone. In lateral root primordia the investigated *KNAT* promoters also showed zone specific expression patterns.

Cytokinins and ethylene affect the balance of cell elongation and differentiation in the root.[28,29] As the *KNAT* promoters were active in distinct zones of the root, we wanted to determine if these hormones had an effect on the patterning of *KNAT* gene expression. While *KNAT4* promoter activity was not affected by the hormone treatments, we found that ethylene increased the domain of *KNAT5* promoter activity and cytokinin drastically decreased the activity of the *KNAT3* promoter. Therefore, while the morphology of roots grown on ethylene or cytokinin was similar, these hormones exhibited opposing effects on the domains of *KNAT* class II promoter activity.

We also investigated the sub-cellular localization of the KNAT proteins in root cells. Consistent with their predicted function as transcriptional regulators, fusions of the KNAT proteins with YFP were all nuclear localized in mature root cells. In the root meristem, however, KNAT3- and KNAT4-YFP fusions were clearly localized in the cytoplasm (Fig. 1j and k). This suggests that a regulatory mechanism exists that prevents KNAT3 and KNAT4 from regulating transcription in meristematic root cells.

It is particularly intriguing to speculate on a role for *KNAT3* and *KNAT4* in lateral root development. Both genes are expressed in pericycle cells in the mature part of the root where lateral roots are initiated, but their expression is downregulated (*KNAT3*) or absent (*KNAT4*) in pericycle cells that form lateral root primordia. Moreover, KNAT3 and KNAT4 are excluded from the nuclei of meristematic cells, suggesting that their activity might interfere with meristematic cell fate. These results are consistent with a role of KNAT3 and KNAT4 as negative regulators of lateral root formation.

Taken together, the complex regulation of *KNAT* class II gene expression in the *Arabidopsis* root suggests that these genes have distinct functions during root development. The lack of altered root phenotypes in overexpression lines and in single and double knockout lines for *KNAT3*, *KNAT4*, and *KNAT5* points towards functional redundancy of these genes in the root. Their overlapping expression patterns in some cell types of the root could allow for protein-protein interaction within the class II proteins. Moreover, the *KNAT* class I genes *BP/KNAT1*, *KNAT2*, and *KNAT6,* and several of the *BELL* and *OVATE* genes are also expressed in the root.[21,23,24,30] A more detailed study of the expression of these genes will identify possible interaction partners for KNAT class II proteins in the root. This will bring us closer towards understanding the role of these proteins in root development.

In addition to their expression in the root, *KNAT3*, *KNAT4* and *KNAT5* are also expressed in the shoot. The role of these genes in the shoot is currently being investigated.

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