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PIN transcriptional regulation shapes root system architecture

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Abstract

Regulation of auxin distribution by PIN transporters is key in the dynamic modulation of root growth and branching. Three novel papers shed light on an intricate network through which several hormones and transcriptional regulators collectively fine-tune the transcriptional level of these auxin transporters in the root.

Main text

Root system fulfils major nutritional and mechanical functions, and the dynamic elaboration of its architecture is paramount to its efficiency and to plant adaptation to environmental constraints [1]. Root system architecture results from three parameters: branching rate, growth rate, and growth orientation, especially in response to gravity. Two phytohormones, auxin and cytokinins, predominantly modulate these three processes through a complex network of interactions [2,3]. Polar auxin transport controlled by the plasma membrane-localized PIN-FORMED (PIN) family of auxin efflux carriers regulates root tropism and branching. The dynamic modulation of *PIN* expression and polar distribution produces auxin-signalling gradients that act as positional information coordinating cell behaviour at the tissue scale [4]. So far, the regulation of PIN polar distribution received most attention [5]. Three novel papers [6,7,8] shed light on the gene regulatory network that fine-tunes the transcriptional level of PINs in roots to modulate root system architecture (Figure 1).

Auxins and cytokinins are prominent players in the complex hormonal cross-talk regulating plant cell behaviour. Transcriptional gene regulation by cytokinins is mediated by two families of transcription factors, namely the type-B ARABIDOPSIS

RESPONSE REGULATORS (ARRs) and the CYTOKININ RESPONSE FACTORS (CRFs) [3]. The report by Šimášková *et al.* [7] reveals that cytokinins induce *PIN* expression through the CRFs to modulate root system architecture. A promoter deletion identified domains in the promoters of *PIN7* and *PIN1* called PCRE7 and PCRE1 respectively, responsible for their cytokinin-inducibility. A yeast one-hybrid (Y1H) screen using PCRE7 as a DNA-bait, combined with chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) and a protoplast-based luciferase assay demonstrate that the CRF2 and CRF6 transcription factors are positive regulators of *PIN7* and *PIN1* expression. Although CRF3 is able to bind to the PCRE7 fragment, it does not alter its transcription, but might compete with other CRF factors when co-expressed. An analysis of *PIN7* and *PIN1* expression in gain-of-function and loss-of-function *crf* mutants confirms the relevance of these interactions *in planta,* although the resulting phenotypes suggest complex interactions are at work. The triple *crf2,3,6* mutant phenocopies auxin transport defective mutants confirming that PINs are targets of these transcription factors. Hence, this study reveals for the first time the transcriptional complex that directly control PIN transcription in response to cytokinins and could fine tune root development in response to environmental signals.

Gravitropism relies on gravity perception in the root tip by columella cells that strongly express *PIN3* and *PIN7* and redistribute shoot-derived auxin sideward to the neighbouring lateral root cap cells [9]. Upon gravistimulation, PIN3 and PIN7 re-localize to the lower cell membranes thus increasing auxin fluxes to the lower side of the root and causing differential growth in the elongation zone and reorientation of the root toward the gravity vector. Importantly, this mechanism of gravity-directed root tropism through PIN-mediated auxin redistribution differs in lateral roots since *PIN3* is not

63 expressed from mature lateral root columella cells. In their study, Wang *et al.* [8]
64 identify two novel regulators of *PIN3* and *PIN7* involved in root gravitropism, The *flp-1*
65 loss-of-function mutant displays a decreased gravitropic response in the primary root
66 and an increased response in young LR, whereas in the *myb88* mutant background, only
67 mature LR showed an increased gravitropic response. *FOUR LIPS* (*FLP*/*MYB124*) and
68 *MYB88*, encode closely related MYB transcription factors acting together during
69 stomatal development. These two genes are also expressed in roots: *FLP* is expressed in
70 the primary root columella cells and in the tip of young LR, whereas *MYB88* is expressed
71 exclusively in the tip of mature LR. However, both *FLP* and *MYB88* are able to rescue the
72 *flp-1* primary root gravitropic defects when expressed under the control of the *FLP*
73 promoter, indicating that they share common targets. Y1H, ChIP-qPCR and
74 electrophoretic mobility shift assays (EMSA) indicate that *PIN3* and *PIN7* are direct
75 targets of FLP and MYB88. Although PIN3 and PIN7 are both involved in root
76 gravitropism, their expression patterns differ in primary and lateral roots. Expression
77 and genetic complementation analyses indicate that FLP predominantly controls *PIN3*
78 and *PIN7* expression in the primary root tip and in young LRs, whereas MYB88 controls
79 *PIN7* expression in mature LRs. How these molecular pathways precisely fit into the
80 regulation of gravitropism in primary and lateral roots remains to be elucidated.
81 Importantly, although changes on overall *PIN3* expression was not detected upon
82 gravistimulation, FLP was shown to be required for auxin-mediated enhancement of
83 *PIN3* expression in the primary root.

84 Another study by Chen *et al.* [6] reports the regulation of *PIN3* by FLP in early stages of
85 LR development. In Arabidopsis, LRs initiate from xylem-pole pericycle cells some of
86 which are primed in the basal meristem to become LR founder cells [10]. Auxin

accumulation in LR founder cells triggers asymmetric divisions through an AUXIN RESPONSE FACTOR 7 (ARF7)- dependent auxin-signalling pathway to generate a stage I LR primordium [10]. Chen *et al.* [6] identify FLP as a direct target of ARF7. The *flp* loss-of-function mutant shows a reduced number of lateral roots, and this phenotype is enhanced in *flp myb88* double mutant. A more detailed analysis revealed that this phenotype is not due to a defect in pericycle cells priming (founder cells formation marked by *DR5-GFP* expression), but rather to a defect in lateral root initiation (indicated by the asymmetric division of founder cells). Genetic analyses indicate that *PIN3* and *FLP*/*MYB88* act in the same pathway. Indeed, the authors confirmed that *PIN3* is a direct target of both ARF7 and FLP, and these three factors are co-expressed in stage I LR primordia. Complementation of the *pin3* mutant by *PIN3* expression under the *PIN3* promoter featuring either wild-type or mutated ARF7 and FLP binding sites further demonstrate that *PIN3* regulation by ARF7 and FLP are both functionally important for LR formation. Mathematical modelling suggests that this coherent feed-forward loop topology could maintain a high level of *PIN3* expression after auxin level drops. In other words, this particular topology could help LR founder cells reach a critical auxin level threshold to induce lateral root initiation. However, previous studies have shown that PIN3 role during LRI is dependent on its expression in the endodermis rather than in the pericycle [11]. As FLP does no seem to be expressed in the endodermis [6], it raises the question of the origin of the LR initiation phenotype of the *flp* mutant and the regulatory network controlling *PIN3* expression in the endodermis remains to be identified.

In conclusion, these three recent studies identify several major transcriptional regulators of *PIN* genes expression downstream of auxin and cytokinins in the root.

Together, they shed light on the complex integration of several cues to fine-tune a common output, the transcriptional level of auxin-transport PIN proteins, to regulate root system architecture. Additional levels of crosstalk are still waiting to be unravelled, such as, for example, the auxin-inducibility of FLP expression. Systems biology approaches will help to integrate these data and analyze the emerging properties of such an intricate network.

Kommentar [l1]: ? FLP est une cible direct de ARF7 d'apres Chen et al.

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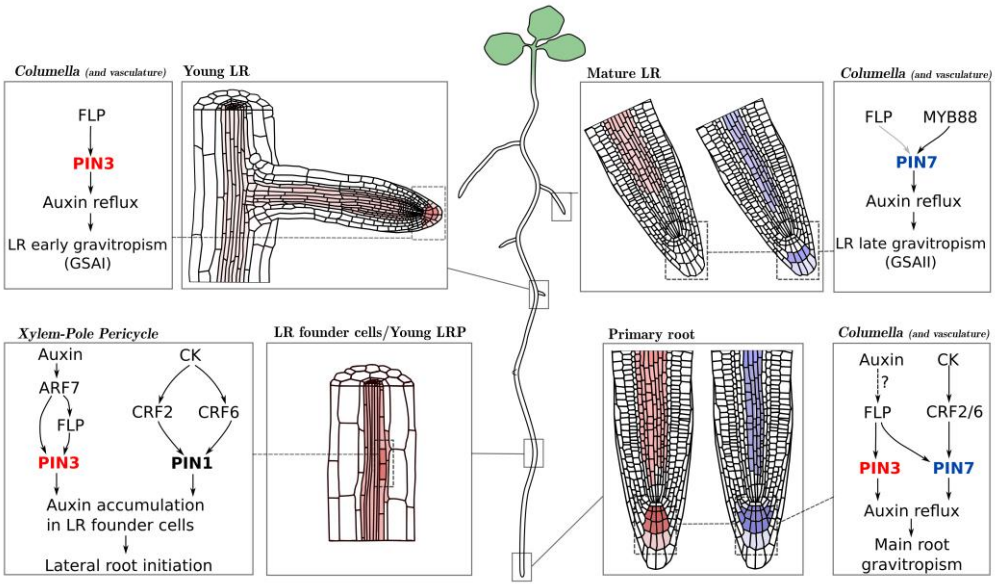

Figure 1. Transcriptional Regulation of PIN Genes in the Root Tip and Lateral Root Primordia in Arabidopsis thaliana. Red colour indicates PIN3 expression while blue colour corresponds PIN7 expression.