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ABSTRACT BOOK

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IDENTIFICATION OF NOVEL CYTOKININ TRANSPORTERS IN ARABIDOPSIS THALIANA

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ABSTRACT

Cytokinins are essential throughout the plant life for growth, development and stress adaptations. Therefore, cytokinin levels and response are tightly regulated. While cytokinin biosynthesis, metabolism, perception, and signaling are highly studied, our knowledge of cytokinin movement and localization is limited as only a few cytokinin transporters were found. Identification of novel transporters activities is a challenging task due to the multi-gene families and vast redundancy, masking the effect of single gene knockouts. In order to overcome this difficulty, we used a transportome, multi-targeted forward-genetic screen based on artificial microRNAs (amiRNAs). We generated a library of 3,000 T2 amiRNA lines expressing 1,777 amiRNAs targeting subclades in transporter families and identified mutants showing diverse cytokinin related-phenotypes. Biochemical transport assays showed significant cytokinin transport in Xenopus laevis oocytes. Knockout of multiple Purine Permease (PUP) cytokinin transporters revealed various and opposing plant growth and development phenotypes. PUP miss-expression lines showed abnormal distribution of cytokinin response (TCS:Venus) accompanied by sever defected development. While several PUP transporters were found to localize to the plasma membrane, some were localized to the vacuole, suggesting for different mechanisms and specificity.

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ANALYSIS OF THE MOLECULAR COMPONENTS OF AUXIN TRANSPORT THAT CONTROLS LATERAL ROOT SPACING IN ARABIDOPSIS

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ABSTRACT

Auxin is a key hormone in many plant developmental programs, including lateral root development, therefore, plants have established intricate mechanisms to control auxin distribution within tissues. AUX1 is central to the major auxin uptake mechanism in the inward radial auxin transport route that controls prebranch site formation. However, the efflux components remain elusive and current model contains important inaccuracies at the level of the auxin efflux components in the root meristem.

We performed a multi-targeted gene-silencing strategy to overcome the potential genetically functional redundancy among ABCBs and identified an uncharacterized subgroup of ABCBs mediating the inward radial auxin transport controls lateral root formation. Oscillatory auxin signal visualized by DR5::LUC indicated that the lateral root defect of silencing lines of subgroup ABCBs is rather derived from an impaired auxin pulse amplitude than from an impaired frequency of auxin pulses. We corroborated the results of the silencing approaches genetically by a multiplexed sgRNA CRISPR/Cas9 and recovered a genetically inheritable large deletion of 59kb, which corresponds to a abcb15, b16, b17, b18, b22 quintuple loss-of-function mutant. Moreover, for ABCB15, B16, B17, B18 and B22 the coding region was fused to YFP and overexpressed in N. bethamiana leaves, showing plasma membrane localization. Overexpression of these constructs stimulated IAA efflux from protoplasts at rates comparable to those of ABCB1, suggesting that subgroup III ABCBs define a new group of plasma membrane localized auxin transporters.

In conclusion, our results reveal that ABCB-mediated auxin transport in specific root tissues controls lateral root spacing.

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A MECHANISM FOR XYLEM LOADING OF CYTOKININ MEDIATED BY ABCG14

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ABSTRACT

Roots and shoots communicate each other to coordinate plant growth in response to environmental changes. Cytokinins (CKs), a class of phytohormones translocated via phloem and xylem, play a key role in the communication. ABCG14 is a factor required for xylem loading of CKs for root-to-shoot CKs translocation in *Arabidopsis*. However, the molecular mechanism underlying ABCG14-mediated xylem loading of CKs remains unknown. Here, we studied transport properties of ABCG14 and spatial requirement of its activity. Characterization of CK transport activity in transgenic Arabidopsis T87 cultured cells expressing *ABCG14* indicated that ABCG14 mediates export of CK precursors. To test in which cell type ABCG14 activity is required for xylem loading of CKs, we generated a series of transgenic lines expressing *ABCG14* under cell type-specific promoters in *abcg14* background, and phenotypic recovery and cytokinin levels in xylem sap were evaluated. Though exo-vascular expression of *ABCG14* was not effective, endo-vascular expression with some cell type-specific promoter partially recovered the phenotype and xylem sap cytokinin. Based on these data, the mechanism for xylem loading of CKs will be discussed.

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IDENTIFICATION OF AN ARABIDOPSIS NRT1/PTR FAMILY PROTEIN INVOLVED IN ROOT GRAVITROPISM

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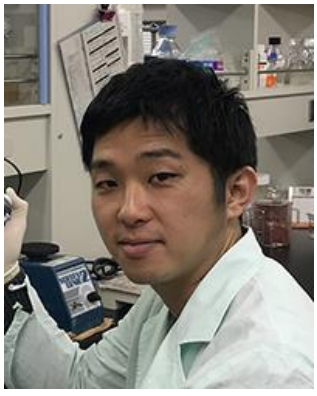
ABSTRACT

NITRATE TRANSPORTER 1/ PEPTIDE TRANSPORTER FAMILY (NPF) proteins have been shown to transport not only nitrate and small peptides (di- and tri-peptides) but also phytohormones (auxin, abscisic acid, jasmonates, and gibberellin) and secondary metabolites (glucosinolates and alkaloids). These suggest that NPFs play important roles in many aspects of plant development and environmental responses. Among 53 NPFs in Arabidopsis, we recently identified a protein involved in root gravitropism. Loss of function of the NPF disturbed the root responses to gravity. This phenotype was rescued by exogenous application of indole-3-acetic acid (IAA), the major endogenous auxin, indicating that the NPF is required for auxin-dependent root gravitropism. Interestingly, a decreased level of indole-3-butyric acid (IBA), which is considered as a precursor of IAA, was detected in the NPF mutant roots while the levels of IAA and IAA conjugates (IAA-aspartate, -glutamate, and -glucose) were comparable to those in wild-type. Furthermore, hormone transport assays in yeast showed that the NPF mediated the uptake of IBA into the cells whereas IAA was not efficiently transported by the same protein. Nevertheless, IBA supplementation did not fully recover the altered gravitropism in the NPF mutant roots. Based on these results, we speculate that the NPF is necessary for IBA uptake into the cells, where IBA is then converted to IAA to be distributed within root tissues to exhibit proper gravitropism.



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KINETICS OF ARABIDOPSIS PIN-FORMED PROTEINS- THEIR KINASE DEPENDENCY AND BIOLOGICAL CONSEQUENCES

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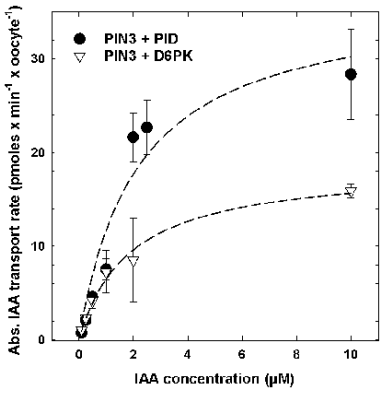
ABSTRACT

PIN-FORMED PROTEINs are the main facilitators of auxin efflux and play an important role in maintaining polar auxin transport, leading to the formation of auxin maxima and minima. The unique expression pattern of the PIN-FORMED PROTEINs and their phosphorylation-dependent activation by AGCVIII kinases are sufficient to explain this. The eight members of PINs from Arabidopsis thalianashare a highly conserved protein structure with 10 membrane-spanning α-helices and a hydrophilic loop domain. Based on the length of the loop domain, the PIN-FORMED PROTEINs are divided long PINs localize to the plasma membrane- often in a polar fashion and short PINs, which localize to the endoplasmic reticulum. The loop domain was identified as target for phosphorylation-dependent activation by the D6 PROTEIN KINASE (D6PK) and PINOID (PID) kinase. Kinetic studies performed in *Xenopus laevis* oocytes revealed that the activating kinase has a major influence on the affinity (Michaelis constant, K_m) and the maximum transport rate (V_{max}) of PIN1 and PIN3. This extends models of auxin distribution. The kinase dependence of the kinetic parameters may lead to varying auxin accumulation in plant cells depending on the combination of PIN-FORMED PROTEINs and kinase. Chimeras between PIN1, PIN2 and PIN3 were generated by domain swapping. The functionality of PIN chimeras in plantawas demonstrated by their ability to rescue the agravitropic phenotype of the pin2mutant. We suggest that the interaction of PINs and kinases goes beyond phosphorylation and the proteins interact in a stable complex and form a novel transport unit.



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