Polar PIN Localization Directs Auxin Flow in Plants

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The phytohormone auxin plays a major role in the coordination of plant development (*1*). Classical models propose that the strict directionality of intercellular auxin transport depends on the polar subcellular localization of transport components (*2*). PIN auxin transport facilitators show such polar localization (*3*–*5*), but whether this is sufficient to determine the direction of auxin flow remains unclear. To investigate this question, we modified PIN polarity and examined directional auxin translocation during root gravitropism in *Arabidopsis thaliana*. This translocation occurs by upward PIN2-dependent auxin flow in epidermal cells along the lower side of the root from the place of gravity perception (root tip) to the place of growth response (elongation zone) (*6*, *7*).

We designed hemagglutinin (HA) epitope–tagged *PIN1* and *PIN2* genes under the transcriptional control of the *PIN2* promoter and introduced these into *pin2* mutants. PIN1 and PIN2 have distinct polar localization in their normal expression domains (*4*–*6*), and expressing them in the same cells could determine whether PIN polarity depends on cell type or PIN sequence–based signals. Consistent with normal PIN2 expression, we detected protein fusions in cortex and epidermal cells (fig. S1, A and B). In cortex cells, both proteins were localized at the lower (basal) side. However, in epidermal cells, endogenous PIN2 (in wild type) and PIN2:HA were always localized at the upper (apical) side, whereas PIN1:HA was observed at the lower side, creating a situation where PIN1 and PIN2 were localized to opposite sides of the same cell (Fig. 1A). These data demonstrate that the polarity of PIN localization is determined not only by cell type–specific signals, but also by sequence-specific signals within PIN proteins.

We also examined PIN1 constructs that may interfere with any sequence-based polarity signals, for example, the insertion of the green fluorescent protein (*GFP*) coding sequence at different positions within the *PIN1* coding sequence. These constructs were functional and showed normal localization patterns in the endogenous PIN1 domain (fig. S1H). When placed under the *PIN2* promoter, they typically (e.g., PIN1:GFP-2) showed localization, similar to PIN1:HA, at the lower side of epidermis cells, with the exception of PIN1:GFP-3, which showed localization at the upper side (Fig. 1A).

The availability of two versions of PIN1 with contrasting polarities allowed us to test the relationship between polarity and the direction of auxin flow by examining auxin translocation during gravity response. Auxin translocation to the lower side of the root (as visualized by the *DR5rev::GFP* auxin response reporter) after gravistimulation was not observed in *pin2* mutants but was completely restored in PIN2:HA lines (Fig. 1B). Whereas PIN1:HA protein at the lower side of epidermal cells failed, PIN1:GFP-3 at the upper side was able to mediate the gravitropic auxin translocation (Fig. 1B). Consequently, PIN2:HA and PIN1:GFP-3, but not PIN1:HA or PIN1:GFP-2, rescued the root gravitropic response of *pin2* mutants (Fig. 1, C and D). Thus, the localization of PIN proteins at the upper side of epidermal cells correlated with their ability to facilitate upward auxin movement for root gravitropic response.

The only variation between plants that express PIN1:HA or PIN1:GFP-3 is the engineered *PIN1* coding sequence. This difference alone is sufficient to change the polarity of PIN1 and, as a direct consequence, change its ability to mediate auxin flow in a given direction for the regulation of root gravitropism. This result shows that PIN polarity is a primary direction-determining factor in auxin transport in meristematic tissues and provides a crucial piece in the puzzle of how auxin flows can be redirected via rapid changes in PIN polarity in response to developmental and environmental signals.

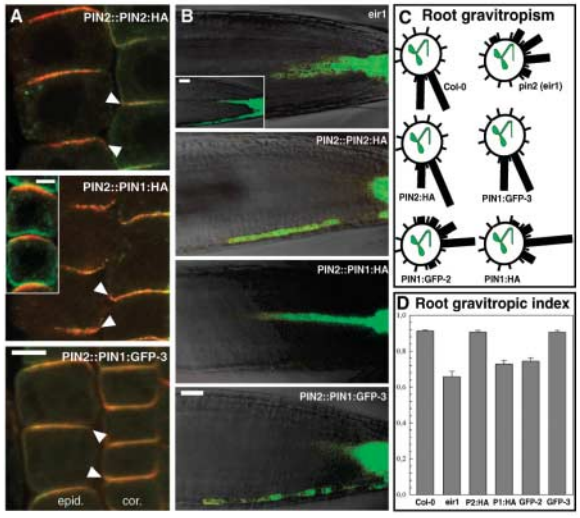

Fig. 1. (**A**) Polar localizations of PIN2:HA and PIN1:GFP-3 at the upper and PIN1:HA and PIN1:GFP-2 at the lower side of root epidermal cells as determined by coimmunolocalization with anti-PIN and anti-HA or anti-GFP antibodies. The inset shows localization of PIN1:GFP-2 (red) and PIN2 (green) on the opposite sides of the same wild-type cells. Arrowheads indicate the apical or basal polarity of PIN localization. (**B**) PIN2:HA and PIN1:GFP-3, but not PIN1:HA, when introduced in *pin2* mutants, mediate the unidirectional translocation of auxin to the lower side of the root after gravistimulation as monitored by DR5rev::GFP. The inset shows DR5rev::GFP pattern in the wild type. (**C**) In *pin2* mutants, PIN2:HA and PIN1:GFP-3, but not PIN1:HA and PIN1:GFP-2, mediate gravitropic root growth. (**D**) Quantitative evaluation of root gravitropism confirms that PIN2:HA and PIN1:GFP-3, but not PIN1:HA or PIN1:GFP-2, can functionally replace PIN2. Scale bars in (A), 5 μm; in (B), 25 μm.

References and Notes

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8. Supported by the VolkswagenStiftung, the EMBO Young Investigator Programme, the Foundation for Polish Science, the Margarete von Wrangell-Habilitationsprogramm, the Deutsche Forschungsgemeinschaft (grant SFB 446), and a PIONIER award from the Dutch Organization for Science.

Supporting Online Material

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Materials and Methods
Fig. S1
References and Notes

14 October 2005; accepted 23 January 2006
Published online 6 April 2006;
10.1126/science.1121356
Include this information when citing this paper.

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