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Seeing is better than believing: visualization of membrane transport in plants

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Recently, the plant transport field has shifted their research focus toward a more integrative investigation of transport networks thought to provide the basis for long-range transport routes. Substantial progress was provided by of a series of elegant techniques that allow for a visualization or prediction of substrate movements in plant tissues in contrast to established quantitative methods offering low spatial resolution. These methods are critically evaluated in respect to their spatio-temporal resolution, invasiveness, dynamics and overall quality. Current limitations of transport route predictions-based on transporter locations and transport modeling are addressed. Finally, the potential of new tools that have not yet been fully implemented into plant research is indicated.

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Introduction

> *Because you have seen me, you have believed;*
> *blessed are those who have not seen and yet have believed.*[1]
> John 20: 29

John 20: 29

The catalyzed movement of substrates across membranes, commonly referred to as transmembrane transport, has always been a hotspot in plant biology [1]. Throughout the 80s and 90s, the plant transport community concentrated mainly on biochemical characterization of single transporter isoforms with a focus on a kinetic level (the 'queen of transport experiments' [2]). In the last two decades, deep insights into transporter expression, trafficking, regulation and their role in plant physiology were provided. Recently, plant scientists have shifted their interest toward a more integrative investigation of long-range transport routes or transport networks [3–5] that is of water, nutrients or some hormones (for an overview, see Figure 1).

Transport was classically quantified on a whole organ or plant level using radiotracers, external microelectrodes or cell-type specific analyses of transport substrates (for reviews on these methods, see Ref. [6]). While these techniques allow for a high level of temporal resolution, they are limited in the spatial aspect. Therefore, direct and indirect techniques have been developed in order to image either local substrate concentrations or intra and intercellular transport *in planta*. Here, we present current methods that allow for a quantitative visualization of transmembrane transport and compare them with classical transport measurements and *in silico* techniques.

Inference of transport routes by transporter imaging

For several substrates, putative transport routes have been deduced from polar transporter localizations [3,5,7–10]. These cell-biological approaches have been partially backed up by analyses of reporter expression and transport modeling (see below).

The best-understood example is probably the polar transport of the auxin, indole-3-acetic acid (IAA), in the Arabidopsis root tip provided by a network of plasma membrane localized im- and exporters of the ABCB, AUX1/LAX and PIN-FORMED (PIN) families [3,11]. Different degrees of tissue-specific and polar locations of members of these families have been established by immunolocalization and fluorescent protein fusions (see Figure 3) that are in line with current models of a reversed fountain auxin flux pattern (see Figure 1; [8]). Similarly, the boric acid channel, NIP5;1, and the boron exporter, BOR1, have been localized in a polar fashion in the plasma membranes facing toward soil and stele, respectively, and are therefore suspected to function in the uptake and translocation of boron to support growth of various plant species (see Figure 1; [7]). Likewise, the ABCG-type SL transporter, PDR1, exhibits an asymmetrical localization in the plasma membrane of petunia root

[1] Please note that this bible citation is not at all meant as a religious statement but a stylistic element acknowledging the effort of the transport field to promote techniques allowing for a quantitative visualization of transport, which is the content of this review.

Figure 1

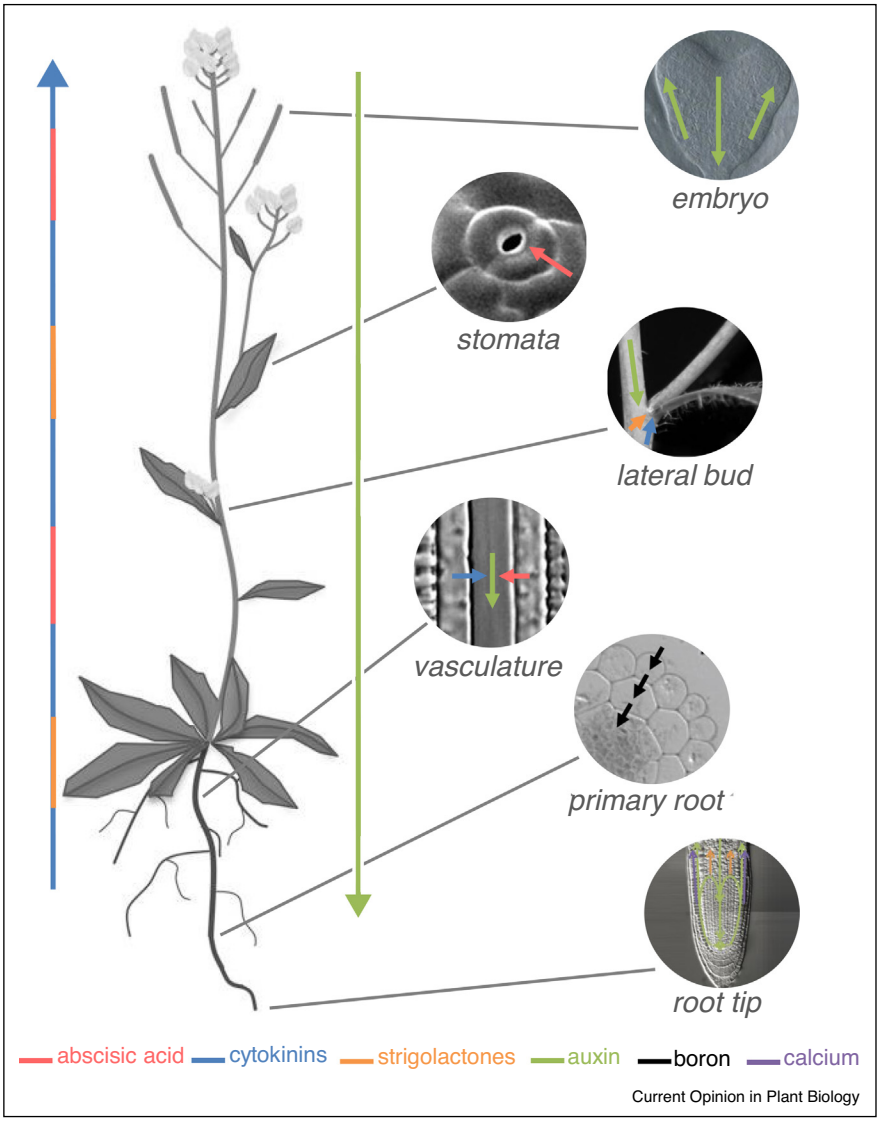

Illustration of trans-membrane, long-range transport routes in plants.
Exemplary long-range transport routes of indicated phytohormones, boron and calcium are indicated by arrows. Note that only long-distance signaling of Ca^{2+} where it acts as a second messenger but not as a nutrient is indicated. In contrast, boron paths are indicating nutrition routes. Overall figure outline is based on [5]; usage of Arabidopsis model was granted by Mary Lou Guerinot.

tips indicating a directional cell-to-cell transport process at least in this region of the root (see Figure 1; [5]). However, presence of a transporter on a cellular subdomain does not automatically reflect local activity levels. Moreover, this approach failed so far in the prediction of transport routes for non-polar transporters known to be involved in long-distance transport [12].

Quantification of transporter activity or regulation

A better proxy might be the recently developed *transport activity sensors*, such as AmTrac and MepTrac [13,14], NiTrac1 and PepTrac [15]. Although these transport activity sensors do not strictly monitor transport, and have not yet been tested *in planta*, they will most likely become valuable, especially in the context of analytes for which no tracers exist.

An alternative approach might be imaging transporter regulation, such as by protein phosphorylation [16]. AGC kinases were shown to phosphorylate and thus alter transport activities of auxin transporters of the PIN and ABCB families [16–20], but have not yet been imaged *in planta*. A future alternative might be offered by novel GFP-based kinase reporters, called SPARK (Separation of Phases-based Activity Reporter of Kinase). SPARK