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Grand challenge: viewing transporter function in a pointillist landscape

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Membrane transport proteins play vital roles in plant development, signaling, environmental interactions, and biosynthesis. Transporter activity has been traditionally studied either at the level of whole plant physiology or in single cells and artificial systems. The tools available for these studies have expanded dramatically over the past decade. Genomic, transcriptomic, proteomic, metabolomic, and associomic/interactomic methodologies (Lalonde, et al., 2010) have produced large datasets that can be probed with bioinformatic tools to visualize transport networks within whole plants, organs, and tissues. Large collections of characterized mutants and mapped natural variation collections provide tools for rapid turnaround of forward genetic screens and reverse genetic assessment of gene function. Improved systems for heterologous expression of plant membrane proteins and an expanding set of defined pharmacological inhibitors provide the capability to rapidly evaluate the function of transporters in relative isolation and to identify proteins that regulate their activity. Live imaging of functional fluorescently tagged proteins has revolutionized plant cell biology and accelerated the understanding of membrane protein trafficking mechanisms (see Gilroy, 2011). Finally, improved crystallization and structural resolution technologies have increased the number of membrane protein structures available for threading of plant amino acid sequences to create testable models for experimental design. *Frontiers in Plant Traffic and Transport* is expected to serve as a forum for research utilizing all of these approaches and to function as a specialized journal for the presentation of functional evaluations of plant membrane transporters and the mechanisms by which they are trafficked and transported.

The grand challenge for work in the plant transport field is the integration of multiple information streams into models and understandings that fully realize the complexity of plant transport systems. There is hope that well-crafted transformations of datasets will reveal structured networks of processes in a manner similar to the visual assembly that occurs when viewing a pointillist painting. Using filters derived from evolutionary concepts or pattern-recognition algorithms, such bioinformatic exercises have produced important results. However, outputs are generally useful only when all of the available datasets were generated under identical conditions and validated to the same standards (Baxter et al., 2010). It is hoped that the creation of this specialty journal will aid the process of standardization of methodologies and provide a ready resource for investigators desiring to select the best systems for validation of datasets pertinent to their own analyses of plant transport function.

It is hoped that a number of realities and experimental limitations encountered in plant transport research will be openly presented and discussed in this journal. Apart from the difficulty inherent in exploration of complex natural systems, there are specific challenges and practices that the journal seeks to overcome:

1. Investigators tend to ignore the substrate promiscuity exhibited by a transporter of interest as assiduously as most would regard the sexual activity of their own teenage children. Multi-substrate transporter activity is quietly accepted but never publicly acknowledged. Transporter nomenclature established when transport activity is first discovered is subsequently promulgated in the literature and then propagated in successive (and often unwarranted) assignments of function via sequence homology comparisons. As one example of the phenomenon, consider the plight of some of the relatively chaste plant ABC transporters that must bear the stigma of the nomenclature "multidrug transporter" due to the licentious activities of other (non-plant) family members.
2. Changes in subcellular location or membrane stability are often accepted as the only indication of altered protein function. The use of live imaging of fluorescent protein fusions has revealed completely new mechanisms that localize clusters and complexes of proteins, detect direct protein interactions, and place transport proteins in polarized membrane domains (Moore and Murphy, 2010). Certainly, when the subcellular localization of a membrane transporter is altered, the aggregate activity of the population of those transporter molecules on that membrane surface is altered. On the other hand, altered localization or stability of a transporter does not establish its activity on that surface. The protein may be regulated by small molecules, protein modification, protein–protein interactions, or a combination of all of these. Too often, the lack of change in the visible position of a protein passes into the literature as a positive indication of activity.
3. Activity of plant transporters visualized in heterologous systems is always an approximation-even when a plant cell culture is used. Essential plant regulators will be missing in such systems, and interactions not found *in planta* may be present. Understanding of transporter activity often requires assimilation of results from multiple systems, analysis of the shortcomings of each, and synthesis of a consensus that can then be experimentally tested *in planta.* The subcellular localization of membrane proteins is often dependent on a tissue/cell type or developmental stage. Perhaps the best examples are the full-length PIN auxin transporters,

which exhibit polar and apolar distributions on the plasma membrane as well as endomembrane internalizations depending on cell type, developmental stage, membrane domain composition, cell wall interactions, transcriptional activity, and environmental inputs (reviewed in Grunewald and Friml, 2010; Feraru et al., 2011). With so many regulatory factors involved, assays of transport activity from heterologous single cell expression systems, especially those lacking a cell wall, are unlikely to reflect the full complexity of transport activity and regulation observed in an intact cell (Zazímalová et al., 2010).

4. Pharmacological agents that are used to inhibit transport activities are generally somewhat shabby toxins used by other plants, fungi, animals, or antisocial chemists to cause harm to competitors. However, qualified use of an inhibitor in a high profile biological assay often results its repackaging as a highly specific antagonist. These characterizations usually are as credible as the mythological "surgical bombing strikes" reported in military press briefings. Inevitably, the exciting discovery of a pharmacological transport inhibitor is followed by reports of activity with other targets. For example, wortmannin, a fungal toxin, inhibits phosphoinositide-3-kinase signaling at the plasma membrane (Templeton and Moorhead, 2005), clathrin-mediated endocytosis (Van Damme et al., 2011), and trafficking to the vacuole (daSilva et al., 2005), yet is often described as "specific."
5. There are still only a very small number of solved structures for plant transport proteins. This is a direct result of the difficulty encountered in producing high resolution X-ray crystal structures of membrane proteins. Linked to this difficulty is the financial reality that funding agencies have historically preferentially supported efforts to solve bacterial and mammalian protein structures. Although it is hoped that this bias will change as new technologies for solving structures with smaller crystals take hold, current efforts to thread plant amino acid sequences onto existing crystal structures is still more like ordering a mail order suit than visiting a custom tailor.

The necessity of improving our understanding of plant transport function in the face of these challenges is greater than ever. Growing demands on food supplies, water availability, and energy resources place a premium on technologies that will increase and improve the quality of plant food, fiber, and biomass production. Improvement efforts will only be successful if the tools and approaches required to map molecular data into whole plant physiology are developed and refined rapidly over the course of the next decade. Elucidation of plant transport processes must be a central component of these efforts, as membrane transport proteins are the workhorses that mobilize the nutrients, metabolites, signaling molecules, and structural components required for plant productivity. Arguably, the development of sensor technologies that allow monitoring of small molecule movements in intact tissues (Chaudhuri et al., 2011) is the highest priority for the field. The inherent limitations of non-invasive sensors that perturb the cellular environment must be overcome by technologies that maximize signal to noise ratios and minimize response times. We expect that *Frontiers in Plant Traffic and Transport* will be the premier specialty journal for publication of applications of this type of sensor research and modeling approaches that will reveal paradigms as elegant as Seurat's Sunday afternoon figures at la Grand Jatte.

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