Meeting Report: Plant Vascular Biology 2013: Vascular trafficking

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Abstract

About 200 researchers from around the world attended the Third International Conference on Plant Vascular Biology (PVB 2013) held in July 2013 at the Rantapuisto Conference Center, in Helsinki, Finland (http://www.pvb2013.org). The plant vascular system, which connects every organ in the mature plant, continues to attract the interest of researchers representing a wide range of disciplines, including development, physiology, systems biology and computational biology. At the meeting, participants discussed the latest research advances in vascular development, long and short distance vascular transport and long-distance signaling in plant defense, in addition to providing a context for how these studies intersect with each other. The meeting provided an opportunity for researchers working across a broad range of fields to share ideas and to discuss future directions in the expanding field of vascular biology. In our report, we summarize the latest advances in understanding the mechanism of vascular trafficking presented at the meeting.

Key words: Plant Vascular Biology 2013, vascular development, vascular trafficking, phloem transport, plasmodesmata.

Introduction

The plant vascular system serves as the main route for long- and short distance transport of various compounds throughout the plant body as well as a means of long-distance communication (Fig. 1). It consists of two major tissues types – xylem, which

conducts water and nutrients, and phloem, which transports mainly organic compounds. Over the last decade, significant progress has been made in understanding how the vascular system forms and functions, and important new findings in this regard were presented at the PVB 2013 meeting. Despite remarkable progress in understanding the formation of vascular tissues, one of the central questions discussed during the conference was how various signals are transported via the vascular tissues to orchestrate various biological processes throughout the plant with such precision. In this brief report, we highlight some of the answers provided during the PVB 2013 meeting.

Long Distance Communication – Signaling Dynamics

In this session, molecular mechanisms which enable long-distance trafficking of macromolecules were presented, with a focus on the non-cell-autonomous function of those molecules.

Phloem is a major intermediary tissue through which many molecules move, including signaling molecules (De Lucas and Brady, 2013) (Fig. 1). Its structural property as a conduit was emphasized by Noll Gundula (University of Münster, Germany). He highlighted the functional differences between phloem proteins (p-proteins) and forisomes. Both are encoded by the same sieve occlusion gene family (SEO) which plays an important role in rapid wound sealing. (Ernst *et al.*, 2012; Ernst *et al.*, 2011; Froelich *et al.*, 2011; Jekat *et al.*, 2013). William Lucas and his colleague Ham Byung-kook (University of California, Davis, USA) shed light on the mechanism of phloem-mobile RNA translocation in cucurbits. Exploiting the histological advantages of phloem sap extraction, they demonstrated that the ribonucleoprotein (RNP) complex, an assembly between phloem-specific mobile RNAs and RNA-binding proteins (RBPs), mediates the cell-to-cell trafficking of RNA species (Li *et al.*, 2011). They have previously shown that the *Cucurbita maxima* RNA-binding protein 50 (CmRBP50) requires phosphorylation of its C-terminal serine residue for the establishment of a stable RNP complex (Li *et al.*, 2011). As an extension of this discovery, they presented an advanced regulatory mechanism of long-distance trafficking of phloem small-RNAs, additionally highlighting the biological meaning of this communication in terms of gene silencing. With regard to gene silencing, a presentation by Chiou Tzyy-Jen (Academia Sinica, Taiwan) gave us a new insight into how mobile microRNA is involved in the

maintenance of phosphate (Pi) homeostasis. Previous work has shown that miR399 plays an important role in the maintenance of Pi homeostasis by negatively regulating the expression of PHO2, a ubiquitin-conjugating E2 enzyme 24 (UBC24) (Aung *et al.*, 2006; Chiou *et al.*, 2006). In her talk, Tzyy-Jen proposed that initial upregulation of miRNA399 occurs in the shoot under Pi deficient conditions. Rootward movement of miR399 via phloem then results in the cleavage of PHO2 transcripts in the root, stimulating both Pi uptake from the roots and shootward translocation of Pi (Lin *et al.*, 2008; Pant *et al.*, 2008). Their recent studies identified PHO1 and several PHOSPHATE TRANSPORTER1 (PHT1) proteins as the downstream targets of PHO2 in this regulatory pathway (Huang *et al.*, 2013; Liu *et al.*, 2012).

The importance of auxin transport as a major morphogenetic signal in plants was revisited by Ottoline Leyser (Sainsbury Laboratory University of Cambridge, UK). Continuous growth in plants is primarily dependent on the activity of meristems located at the tip of the root and shoot. Long distance source-to-sink transport of auxin has been proposed to play a critical role in coordinating the activity of meristems and systemic growth in general. This coordination process is exemplified by the formation of a vascular connection between axillary buds and the main stem (Domagalska and Leyser, 2011). In her keynote address, Leyser provided a comprehensive overview of bud activation, focusing especially on the role of the spatiotemporal regulation of auxin transport dynamics.

The research presented in this meeting reflected the increasing use of micrografting as a technique to dissect and understand long distance transport of signals between the shoot and root. An example of the successful application of this technique was provided during Leslie Sieburth's (University of Utah, USA) talk, where she presented a mechanism for the coordination of shoot growth and development by a signal derived from the root. In has been previously reported that the *Arabidopsis bypass* 1 (*bps1*) mutant root produces a mobile *bps1* signal (Adhikari *et al.*, 2013; Van Norman *et al.*, 2011) that functions in the shoot to arrest its growth. As an extension of this discovery, Sieburth provided evidence that the mobile *bps1* signal is also involved in maintenance of the shoot apical meristem (SAM).

Mechanisms and dynamics of vascular trafficking

Drought obviously has a major impact on water uptake by plants and the conductive properties of their vascular system. Despite significant progress in understanding how

plants tolerate drought, the mechanisms by which trees react to the drought remain elusive. Drought leads to impaired water transport from the soil to the leaves via the xylem and thus results in plant mortality via hydraulic failure (McDowell *et al.*, 2008; Sperry *et al.*, 1998). However, there have been very few reports about what happens in the phloem in response to drought. Sanna Sevanto (Los Alamos National Laboratory, USA) and colleagues showed that a loss of both conductivity and carbohydrate reserves can coincide in trees during drought. Furthermore, the loss of turgor in phloem restricts access to carbohydrate reserves while hydraulic control of respiration prolongs survival. These studies indicate that phloem plays a key role in drought-related mortality, although the exact cascade of events at the cellular level is a question which remains to be addressed.

Sucrose is the major transport form of carbohydrates in plants; it is transported from the sites of production in the leaves to sink tissues via the tube-like phloem network (Van Bel, 2003). This network is interconnected via plasmodesmata. As demonstrated previously, the flux and direction of sucrose is controlled by SUC/SUT type sucrose transporters (Aoki *et al.*, 2003; Riesmeier *et al.*, 1993; Sauer, 2007; Sauer and Stolz, 1994; Slewinski *et al.*, 2009), proton-driven sucrose transporters that play an essential role in cell-to-cell and long-distance distribution of sucrose within the plant body. Conformational changes in these transporters accompanying the transport of sucrose were previously observed (Carpaneto *et al.*, 2010). Furthermore, Dietmar Geiger (University Würzburg, Germany) and coworkers are investigating the mechanisms of reaction cycle of sucrose transporters – the binding of protons to the carrier and its effect on the protein trafficking.

Continuing on the theme of sucrose transport, David Braun (University of Missouri, USA) discussed how important it is to understand the molecular mechanisms of carbon partitioning, the process by which photoassimilates are transported from their site of synthesis in the leaves to the rest of the plant body. Although several studies have demonstrated that the control of carbon partitioning is essential for plant growth and development, an understanding of the genetic control of carbon partitioning remains elusive. The aforementioned sucrose transporters (SUTs) are the best known genes that directly load sucrose into the phloem (Lalonde *et al.*, 2004; Sauer, 2007). Characterization of all SUT family members will open up new directions of investigation into the control of carbon partitioning in plants. Furthermore, it will allow the development of new tools for biotechnological approaches to enhance crop yield and biofuel production (Slewinski *et al.*, 2009).

The osmotically active transport of sugars via phloem is a key process in phloem function (Ayre, 2011) because it leads to the creation of the hydrostatic pressure gradient between source and sink, the mechanism postulated by (Münch, 1930) to drive the mass flow of phloem sap. Phloem transport is already known to be controlled by an active loading step between bundle sheath cells (BSCs) and the sieve element companion cell complex (SECCC). The abundance of cell connections between the BSC and SECCC determines whether phloem loading will be symplasmic or apoplasmic (Liesche and Schulz, 2012). Alexander Schulz (University of Copenhagen, Denmark) and colleagues are investigating the conversion of diffusional pre-phloem transport into bulk flow via symplastic loaders. Their findings suggest an important role for cell-specific water accessibility along the pre-phloem pathway that needs further investigation.

Nitrogen is one the most important nutrients for plants and serves as a major precursor of such critical compounds as chlorophyll, nucleic acids and amino acids. Lack of nitrogen severely affects plant metabolism and growth. Following uptake, nitrogen is immediately used in metabolism, transiently stored, or transported via the vasculature to sink tissues by a large number of well-described nitrogen transporters (Rentsch *et al.*, 2007). Several studies suggest that these transporters play an important role in organic nitrogen uptake, source-to-sink transport, and sink loading for growth and development. Mechthild Tegeder (Washington State University, USA) discussed the identification and characterization of transport processes controlling the partitioning of organic nitrogen between source and sink. She demonstrated that cellular import systems are essential for the partitioning of nitrogen at the scale of the whole plant body. In addition, she provided data demonstrating the importance of nitrogen transporters as key regulators of plant metabolism, growth and development (Tegeder, 2012; Tegeder and Rentsch, 2010).

Michael Knoblauch (Washington State University, USA) highlighted the importance of sieve tube structure and its impact on long distance transport. Sieve tube structure has a major impact on transport and unloading; however, questions concerning the basic structure and function of sieve tubes remain unanswered due to their inaccessibility and sensitivity to injury. The detailed structure of sieve plates has been previously investigated by using scanning electron microscopy (Mullendore *et al.*, 2010). These studies provided vital insight into the geometry of pores, plates and sieve elements. New methods to investigate sieve tubes based on *in vivo* high resolution microscopy

were discussed, together with the role of phloem proteins encoded by the sieve element occlusion gene family.

An earlier study performed by Carel Windt and colleagues (Forschungszentrum Jülich, Germany) in tomato truss demonstrated that most water transport occurs via the xylem (Windt *et al.*, 2009). This was determined with the help of Magnetic Resonance Imaging (MRI) flowmetry, which provides information not only about the volume of the flow but also flow conducting area and average linear velocity (Windt *et al.*, 2006). Carel Windt is using this technique in various plant species to investigate what fraction of the xylem cross-sectional area conducts water flow.

A novel technique to analyze the three-dimensional xylem network of *Arabidopsis* was presented by Ildoo Hwang (Pohang University of Science and Technology, Korea). Based on this technique, a flexible framework of the xylem network for water management with multiple layers of regulation was proposed. Such a framework may explain how hydraulic regulation is accomplished at the whole-plant level.

The role of plasmodesmata in vascular trafficking

Neighboring plant cells communicate with each other via symplastic transport through the plasmodesmata (PDs) or via the extracellular apoplastic space. PDs are microscopic plasma membrane-lined channels which traverse the cell wall of adjacent cells allowing the movement of various molecules through the symplastic space (Burch-Smith *et al.*, 2011; Cilia and Jackson, 2004; Lucas *et al.*, 2009; Maule, 2008; Oparka and Roberts, 2001; Sevilem *et al.*, 2013). Despite their role in plant development, physiology and defense, the mechanism and regulation of trafficking through plasmodesmata remains poorly understood. A new perspective on the role of PD in the transport of sugars and amino acids was presented by Sylvie Dinant (INRA, France) during the PVB 2013 meeting. Sylvie Dinant and her colleagues described the role of NHL26, a phloem protein targeted to the PD, in regulating PD permeability, which affects sugar signaling at the interface between companion cells and sieve elements. Overexpression of *NHL26* leads to slow growth, accumulation of carbohydrates in leaves, higher shoot biomass and defects in sugar export. These data suggest that *NHL26* plays an important role in the regulation of plasmodesmata permeability and sugar signaling in companion cells (Vilaine *et al.*, 2013).

Cell-to-cell signaling has been shown to play a critical role in the response to various environmental changes, including plant defense. Before an immune signal spreads

throughout the plant body, local cell-cell signaling is required to confine the infection. During this process, PD undergo various structural modifications. Several studies have demonstrated that the permeability, dilation, or structure of PD can be altered in response to infection by microbial pathogens (Benitez-Alfonso *et al.*, 2010; Carpaneto *et al.*, 2010; Schoelz *et al.*, 2011; Ueki and Citovsky, 2011). Jung-Youn Lee (University of Delaware, USA) presented evidence for the role of salicylic acid (SA) signaling components in regulating cell-to-cell connectivity. Application of SA leads to increased callose deposition at the PD and subsequent closure, a response which requires the presence of plasmodesmata-located protein, PDLP5. Mutations in SA signal transduction cause PD closure upon infection by bacterial pathogens. These data suggest that the crosstalk between PDLP5 and the SA signaling pathway plays a critical role in regulating PD permeability upon bacterial pathogen attack (Lee *et al.*, 2011; Wang *et al.*, 2013).

Kay Schneitz (Technische Universität München, Germany) presented an interesting talk about the role of the kinase STRUBBELIG in regulating inter-cell-layer communication and tissue morphogenesis. In *Arabidopsis*, the atypical transmembrane leucine-rich repeat receptor-like (LRR-RLK) *STRUBBELIG* (*SUB*) has been shown to be critical for a number of developmental processes (Chevalier *et al.*, 2005; Fulton *et al.*, 2009; Kwak *et al.*, 2005; Vaddepalli *et al.*, 2011; Yadav *et al.*, 2008). *SUB* functions in a non-cell-autonomous manner to mediate inter-cell-layer signaling across cell layers in the ovule, the floral meristem (Yadav *et al.*, 2008), and the root (Kwak and Schiefelbein, 2008). The three STRUBBELIG-LIKE MUTANT (SLM) genes *ANGUSTIFOLIA (AN)*, *QUIRKY (QKY)* and *ZERZAUST (ZET)* are considered central players in SUB-mediated signal transduction pathways, as the phenotype of these mutants is similar to *sub* mutants in certain respects.

Short range cell-to-cell communication in plants involves the selective trafficking of various transcription factors through PD. For example the *KNOTTED1* (*KN1*) homeobox (KNOX) family transcription factors and are essential for stem cell establishment and maintenance and use the PD pathway (Bolduc *et al.*, 2008; Kim *et al.*, 2002b; Lucas *et al.*, 1995; Xu *et al.*, 2011). David Jackson and coworkers showed that KN1 trafficking requires the chaperonins, a group of cytosolic chaperones which are necessary to fold specific substrate proteins. Chaperonins are known to be essential for the cell-to-cell trafficking of a subset of mobile transcription factors, and a critical role for chaperonin-dependent protein trafficking has also been demonstrated in plant stem cell function (Xu *et al.*, 2011). Currently, David Jackson and colleagues are

investigating the mechanisms by which chaperonins mediate plasmodesmal transport. These studies may lead to the discovery of new genes controlling trafficking through the PD.

The symplastic movement of various signaling molecules through the PD has been shown to play an important role in some developmental programs, including embryonic cell fate and postembryonic organ development (Chevalier *et al.*, 2005; Kim *et al.*, 2002a; Nakajima *et al.*, 2001; Xu and Jackson, 2010; Xu *et al.*, 2011). Beyond the well-studied role of hormones, the mechanisms that define lateral root architecture remain poorly understood. Yoselin Benitez-Alfonso (University of Leeds, United Kingdom) and colleagues demonstrated that symplastic connectivity plays a critical role in initiating lateral root meristems and determining their position. This connectivity is regulated by PD-localized glucanases, which control callose accumulation and molecular trafficking through PD. This discovery represents a great step forward in our understanding of lateral root formation (Benitez-Alfonso *et al.*, 2013).

Another process where cell-to-cell communication through plasmodesmata plays a critical role is dormancy in trees. Earlier studies in trees showed that PD undergo structural modifications in response to a short photoperiod, resulting in a lower size exclusion limit of PD in the SAM (Rinne *et al.*, 2001; Ruonala *et al.*, 2008). Consequently, after the buds are formed, the SAM shifts into a dormant state by closing the PD in all its cells via callose deposition (Rinne *et al.*, 2001; Rinne and van der Schoot, 1998; Ruonala *et al.*, 2008). When the early winter chill comes, it results in the SAM shifting from its disconnected dormant state to a reconnected quiescent state, at the same time as it increases its freezing-tolerance. Christiaan van der Schoot and colleagues (Norwegian University of Life Sciences, Norway) identified a group of genes that are critical for the transition between the two phases. These genes are members of the GH17 family of carbohydrate-active 1,3-betaglucanases, which are enzymes that degrade PD-callose. Their expression is controlled by several factors, such as day length, temperature and genes involved in gibberellic acid biosynthesis and signaling (Rinne *et al.*, 2011).

Jae-Yean Kim (Gyeongsang National University, Korea) showed that callose deposition plays an important role in establishing PIN-driven auxin gradients.

Gene regulatory networks in vascular development

The establishment of proper tissue layers is a prerequisite for intercellular trafficking. With regard to this issue, an entire session was allocated to vascular development. Siobhan Brady (UC Davis, USA) presented a global-scale view of genetic regulatory networks in root vascular development, focusing especially on cell type specification (De Lucas and Brady, 2013). Ji-Young Lee (Seoul National University, Korea) proposed a genetic regulatory mechanism by which xylem cells are distinguished from the procambium domain. She presented two mobile transcription factors from an AT-hook family transcription factor, *AHL3* (*AT-HOOK MOTIF NUCLEAR LOCALIZED PROTEIN 3*) and *AHL4*. According to the proposed model, the movement of AHL4 from the procambium to the xylem plays a key role in establishing tissue boundaries in the root meristem. In particular, the protein complex of AHL4 and AHL3 was shown to propel AHL4 movement (Zhou *et al.*, 2013), implying an intriguing regulatory mechanism between transcription factors.

Plant hormones and their signaling pathways have been considered as a major controller of vascular cell division and specification (De Lucas and Brady, 2013), an issue that was addressed by several talks in the session. For example, Hiroo Fukuda (University of Tokyo, Japan) proposed a model integrating brassinosteroid (BR) signaling into vascular stem cell fate determination. It has been previously shown that the TDIF-TDR (Tracheary Element Differentiation Inhibitory Factor-TDIF Receptor) pathway plays a role in vascular stem cell maintenance. *WOX4*, in particular, is known to be a key gene involved in promoting cell proliferation in the procambium and cambium as a positive regulator of the TDIF-TDR pathway (Hirakawa *et al.*, 2010). Fukuda showed that BR signaling functions in conjunction with the TDIF-TDR-WOX4 signal in determining vascular stem cell fates, with the two pathways sharing a common target.

Biotic plant interactions

The importance of plants' response to biotic stimuli was evinced by several talks in this session. Speakers presented the result of research into the process of infection by root-knot nematodes and plant responses to this invasion. Upon invasion by root-knot nematodes, giant cells are formed in the stele of host roots. Since these cells later serve as feeding sites, it has been proposed that the formation of vascular tissues around them and sustained vascular continuity are critical for the survival of the nematodes (Absmanner *et al.*, 2013). Ulrich Hammes (Regensburg University,

Germany) provided evidence that the vascular tissues encircling the giant cells are actively dividing protophloem. In combination with his results, Derek Goto (Hokkaido University, Japan) showed various mutants that repress the nematode infection and proposed a genetic regulatory mechanism involved in nematode infection, focusing on the initial events in particular.

Long-distance signal trafficking serves as a pivotal mechanism for the communication between plant and bacteria. For instance, legume species that host rhizobacteria adopt an auto-regulatory mechanism controlling nodulation as a part of stable symbiotic relationship (Reid *et al.*, 2013). In this meeting, Peter Gresshoff (University of Queensland, Australia) introduced the CLAVATA1-like receptor kinase in soybean, which is responsible for the systemic regulation of nodulation. Dubbed GmNARK (Nodulation Auto-regulation Receptor Kinase), this kinase was previously found to interact with the CLE peptides (CLV3-related peptides) (Lim *et al.*, 2011; Reid *et al.*, 2011). Of the three CLE peptides in soybean – GmRIC1, GmRIC2, and GmNIC1 – GmRIC1 and GmRIC2 were shown to interact with GmNARK in the shoot, inducing the shoot-derived inhibitor (SDI) signal which subsequently regulates nodulation in the root. It was also proposed that the CLE domain has nodulation suppression residues that are crucial for long-distance signal transmission (Reid *et al.*, 2013). However, the underlying mechanism behind this is still unclear.

Pradeep Kachroo (University of Kentucky, USA) shared his research on unraveling the signaling pathways that control defense responses in plants. He highlighted the role of several components of the systemic acquired resistance (SAR) pathway, including the nine carbon dicarboxylic acid azelaic acid (AA), the phosphorylated sugar glycerol-3-phosphate (G3P), and two lipid transfer proteins DIR1 (Defective in Induced Resistance) and AZI1 (AA insensitive). SAR has been observed in a variety of flowering plants and can be induced by a broad spectrum of pathogens. Experimental evidence was presented demonstrating that a feedback regulatory loop involving G3P, DIR1, and AZI1 regulates the precise induction of the SAR (Glover and Kachroo, 2013; Kachroo and Robin, 2013; Yu *et al.*, 2013). Cyril Zipfel (The Sainsbury Laboratory, UK) presented evidence of an antagonistic interaction between BR signaling and the innate immune response in plants. Plant immunity is primarily triggered via the recognition of a pathogen-associated molecular pattern (PAMP) by surface-localized pattern-recognition receptors (PRRs). The interplay between BR signaling and the immune response was demonstrated on the grounds that BR signaling can inhibit PRRs-mediated immune signaling (Albrecht *et al.*, 2012).

Aart van Bel (University of Giessen, Germany) highlighted the effect of early biotic and abiotic stimuli on the gating of Ca^{2+}-permeable channels. As described earlier by Aart van Bel and colleagues, infection of plants by phytoplasma, bacteria that are obligate parasites of phloem, results in Ca^{2+} influx into phloem sieve tubes, leading to callose deposition and sieve plate occlusion. In addition, phytoplasma infection may cause gating of sieve-element Ca^{2+} channels, leading to sieve-tube occlusion with consequent dramatic effects on both the spread of the infection and photoassimilate distribution (Musetti *et al.*, 2013). Moreover, various injuries initiate longitudinal electrical waves which also enhance Ca^{2+} concentration in sieve elements by altering the gating of Ca^{2+} channels in the plasma membranes. The passage of an electrical wave associated with calcium influx leads to a temporary reorganization of the phloem symplasm. Lateral waves of calcium influx and the symplasmic reorganization may trigger the production of an arsenal of long-distance messages ranging from phytohormones to macromolecules in vascular cells along the sieve tubes (van Bel *et al.*, 2011a; van Bel *et al.*, 2011b).

Concluding remarks

Significant progress has been made in the past several years in the area of plant vascular biology. A number of recent insights into the molecular mechanisms controlling vascular patterning, trafficking and biotic interactions were presented during the meeting. At the same time, it illustrated our need to understand the integration of these processes to control plant growth and development. We noticed a growing interest in understanding long and short distance communication and its impact on vascular tissue formation. These studies will greatly contribute to biotechnologies that can improve agricultural performance. Overall, it was a truly productive meeting, and we look forward to the next Plant Vascular Biology meeting which will be held in China in 2016.

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Fig. 1. Representative image of vascular trafficking. The whole plant image in the middle demonstrates long-distance signal trafficking. Arrowheads indicate source-to-sink translocation of signaling molecules either through phloem (blue arrows) or xylem (pink arrows). Symplastic communication between sieve elements and companion cells is marked with green arrows in the magnified phloem image.

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