

MEETING REPORT

Plasmodesma 2001: On Safari through the Symplast

Plant cells need to communicate with each other to orchestrate lifelong development, to integrate physiological processes, and to coordinate pathogen defense responses. The transmission of intercellular signals is an important means of regulating all plant life processes, from fertilization to senescence. In this framework, plasmodesmata (PD)—nanopores lined by plasma membrane that bridge the cytoplasm of most plant cells to their neighbors—play a pivotal role. PD function as relay stations in a unique cellular internet for the rapid exchange of water, metabolites, and even macromolecules. Exciting progress has been made in the field of plasmodesmal research, as reported at the 4th International Plasmodesma meeting in Cape Town, South Africa, in August 2001.

STRUCTURE AND REGULATION OF PD

In his plenary talk, Robert Turgeon (Cornell University, Ithaca, NY) challenged the conference with the fractured vision of PD currently shown to the world. Cartoons depicting PD as grossly oversimplified tubes of cytoplasm often are the only presentation to students in college textbooks. His concern was that such misrepresentation, quite unlike that of other aspects of plant cell biology, could lose the attention of ambitious young scientists in their future careers. At this meeting, the presentation of new data and novel techniques revealed the tremendous intricacy of PD and met Turgeon's call to acknowledge their true diversity and complexity.

Rosemary White (Commonwealth Scientific and Industrial Research Organization Plant Industry, Canberra, Australia) examined the complex interactions of

PD and the cytoskeleton. White's group showed that disrupting myosin function decreased the size exclusion limit (SEL) in Arabidopsis root epidermis but increased it in mature staminal hairs from *Tradescantia virginiana* flowers. Interestingly, myosin's role in regulating the SEL is contradictory in these systems. Actin inhibitors had no effect on the PD SEL in either system, suggesting that myosin may regulate the SEL without the engagement of actin. The evidence for the opposing role of myosin in these systems clearly shows the necessity of understanding the role of the cytoskeleton and molecular motors in PD regulation in different plants and tissues.

In sieve elements, PD are modified to form sieve pores, and their conductivity might be controlled by an entirely novel mechanism. Michael Knoblauch (Justus-Liebig-Universität, Giessen, Germany) proposed that the rapid decrease of sieve pore conductivity in response to injury results from the dispersal of crystalloid phloem-specific proteins. In an elegant video presentation, injury inflicted by micropipettes or the addition of the divalent cations Ca^{2+} , Sr^{2+} , and Ba^{2+} , but not Mg^{2+} , were shown to cause crystalloid dispersal that was rapidly reversible after the addition of a chelator. The crystalloid phloem-specific protein is located adjacent to the sieve plate, and its dispersal there might provide a rapid mechanism for blocking traffic through sieve pores in response to injury.

The traditional, oversimplified view of PD does not take into account the structural variations of these intercellular lifelines. Alison Roberts (Scottish Crop Research Institute, Dundee) convincingly demonstrated that in tobacco leaves, structural modifications from simple to branched PD correlated with a decrease in the SEL. Simple PD con-

sist of a single channel, whereas branched PD consist of numerous branches running almost parallel from a central cavity (Ehlers and Kollmann, 2001). As leaves progressed from sink (net importer of photosynthate) to source (net exporter of photosynthate), the frequency of simple PD decreased, whereas the frequency of branched PD increased. Most interestingly, Roberts used green fluorescent protein (GFP) fused with the 30-kD movement protein (MP30) of *Tobacco mosaic virus* (TMV), which targets branched PD exclusively, to show that PD branching is initiated in different cell layers at different times, beginning in the basal cells of trichomes (Figure 1). GFP fluorescence followed in the lower spongy mesophyll layers and finally in the anticlinal walls between adjoining palisade mesophyll cells. The sequence of PD branching closely correlated with the cessation of cell division, suggesting that dividing cells maintain simple PD longer than nondividing cells. It is not yet clear, however, whether the increase in the frequency of branched PD correlates to primary simple PD (formed during cytokinesis) becoming branched or if it results from the formation of new secondary branched PD (formed across mature cell walls). Likewise, the decrease of simple PD as leaves progress from sink to source can be attributable either to their modification to branched PD or to their loss (Roberts et al., 2001).

Robyn Overall (University of Sydney, Australia) has been using high-resolution scanning electron microscopy to provide a detailed picture of PD structure. By studying the PD in the young nodes of *Chara corallina*, Overall discovered that spokes spanned a gap between the cell membrane and the surrounding cell wall, perhaps serving

MEETING REPORT

as anchors or modifiers of PD structure. The spokes occasionally were sensitive to digestion by proteinase K, suggesting that the spokes are proteinaceous. Her work clearly showed that the structure of PD is more complex than was thought previously and that high-resolution scanning electron microscopy is a useful tool with which to study PD structural conformations.

Overall also reopened debate about transport pathways available through PD. Like most aspects of PD structure, the function of the continuous strand of endoplasmic reticulum (ER), which often is described as solid along the axis of the channel, is unclear. Evidence of a microinjectable network that is continuous from cell to cell and that resembles cortical ER suggests that the ER lumen might provide a route for intercellular trafficking (Cantrill et al., 1999). Recent work on trichomes of *Nicotiana benthamiana* by Overall and colleagues has localized microinjected lissamine rhodamine B to transcellular strands that colocalize with ER-targeted GFP, providing evidence of an ER luminal pathway.

VIRUS, RNA, AND PROTEIN MOVEMENT

Conflicting data on the role of microtubules in the movement of TMV infection ignited spirited debate. Manfred Heinlein (Friedrich-Miescher-Institute, Basel, Switzerland) identified a motif in MP30 with significant similarity to a sequence in tubulin that is known to make lateral contacts between microtubule protofilaments. The point mutation *Ls1*, long known to confer temperature sensitivity to viral movement, maps to this motif in MP30. Studies using virus derivatives expressing MP30 in fusion with GFP demonstrated that this mutation disrupts microtubule binding and viral

RNA transport in a temperature-sensitive manner. These data provide support for a role of microtubules in viral RNA movement and suggest the possibility that MP30 may mimic tubulin for microtubule associations.

Using a gene-shuffled mutant MP30, *shuffMP*, Karl Oparka (Scottish Crop Research Institute, Dundee) reported data that suggest that MP localization on microtubules was not essential for TMV movement. The *shuffMP* accumulates in clusters on the ER but does not transfer onto microtubules. In related studies, Petra Boevink (Scottish Crop Research Institute, Dundee) used colchicine to disrupt microtubules and examined TMV movement and MP localization to PD. Despite microtubule disruption, TMV was able to move intercellularly and targeted the MP to PD. Oparka and Boevink suggested that the role of microtubules in TMV infection is to recycle the MP via the proteasome degradation pathway. Furthermore, *shuffMP* might avoid the degradation pathway, thereby working more efficiently. Future research by both groups may clarify the role of microtubules in TMV infection.

David Jackson (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) presented a first look at the domains of transcription factor movement in Arabidopsis meristems. Defined domains of protein localization play a critical role in pattern formation and leaf initiation. KNOTTED1 (KN1), a maize homeodomain transcription factor expressed in the shoot meristem, is thought to traffic via PD. Using the meristem-specific *Wuschel* promoter driving a GFP-KN1 fusion, Jackson demonstrated that KN1 can move from the inner layers of the meristem to the outer L1 layer, presumably via PD. Differences between domains of GFP-KN1 and GFP-MP movement in the meristem led to a discussion of whether viral MPs and endogenous transcription factors use the same mechanism for gating and transport via PD.

MOLECULAR COMPONENTS OF PD

Plasmodesma 2001 included intriguing data that reflected the growing impact of proteomics and gene manipulation on our knowledge of PD structure and function. Elisabeth Waigmann (University of Vienna, Austria) linked viral movement to the newer area of endogenous plasmodesmal proteins. Her group identified a novel protein, MPB2C, whose sequence is specific to plants. The unique properties of MPB2C include colocalization with MP30 and the subsequent inhibition of intercellular movement. However, MPB2C is TMV specific in that it does not bind or prevent the movement of CMV3a, the MP from *Cucumber mosaic virus*. MPB2C colocalizes with MP30 on microtubules and is associated with the development of large MP30-GFP aggregates that are characteristic of progressing viral infections (Heinlein et al., 1998). MPB2C's role, although not yet defined, may be in transferring MP30 between its locations on microtubules and cytoplasmic bodies.

Until very recently, specific genes and molecular components of PD have been frustratingly inaccessible, particularly in that PD are comparatively rare structures among the complex cell wall matrix. To address this problem, Leila Blackman and Christine Faulkner (University of Sydney, Australia) compared protein extracts from PD-rich nodal walls to extracts from external walls of internodal cells, which do not have PD. Antibody labeling of a *Chara* cDNA expression library and parallel work using two-dimensional gel electrophoresis identified some proteins unique to the extracts from the nodal walls, including homologs of a tyrosine phosphatase and a plant lipoxygenase. Manue Bayer in Andy Maule's group (John Innes Centre, Norwich, UK) is taking a related proteomics approach with higher plant cells. Their work will strengthen this important broad effort to characterize

MEETING REPORT

plasmodesmal components. Michelle Cilia in David Jackson's group (Watson School of Biological Sciences, Cold Spring Harbor, NY) presented her rationale for a genetic screen using tissue-specific promoters driving GFP to search for protein-trafficking mutants. Her screen is intended to reveal the genes involved in regulating PD function and coding for functional components of PD in *Arabidopsis*.

David Ehrhardt (Carnegie Institution of Washington, Stanford University, Palo Alto, CA) presented some exciting fluorescence images resulting from an

Arabidopsis cDNA-GFP fusion library. D41, a GFP fusion protein containing the C-terminal domain of a syntaxin, displayed a range of features that closely associated it with PD. D41 accumulates in a punctate fashion on adjacent plasma membranes, it marks sites of plasma membrane contact during plasmolysis, and it is absent from guard cells, which lack PD. Ehrhardt's planned characterization of the protein should clarify its structural and functional role in PD and whether or not it shares the lipid bilayer fusion properties of other syntaxins.

INDUSTRY AND NEW TECHNOLOGY

Moving away from purely academic studies, Gregory Pogue (Large Scale Biology, Inc., Vacaville, CA) introduced a striking spin-off of more than a decade of investigations into viral movement through PD. Using a mutagenesis technique known as "shuffling," Pogue, in association with the Scottish Crop Research Institute, developed TMV vectors with improved intercellular movement properties. These vectors have an improved ability to transport large foreign gene sequences and possess an extended host range. "Biomanufacturing" with these modified viral vectors is being used to transform fields of plants into protein factories. Target proteins for mass production include antibodies for cancer treatment and human enzymes for replacement therapies. Such developments demonstrate the significance of past and continued research into cell-to-cell communication in plants and also emphasize the contributions of basic research in plants to practical and revolutionary developments in the pharmaceutical industry.

In another area of new technology, Helle Martens (Royal Veterinary and Agricultural University, Copenhagen, Denmark) presented a talk entitled "Microinjection without Injection," introducing the method of photoactivation of caged probes to study symplasmic transport. Using multiphoton microscopy, the probe is uncaged in a single cell and observed in its fluorescent form. This technique could revolutionize studies of the intercellular movement of small molecules and could have an effect on these studies similar to that of GFP tagging on our knowledge of macromolecular trafficking. Photoactivation of caged probes is precise and noninvasive and could be of great advantage in the study of symplasmic communication.

These developments also illustrate the strengths of bringing together

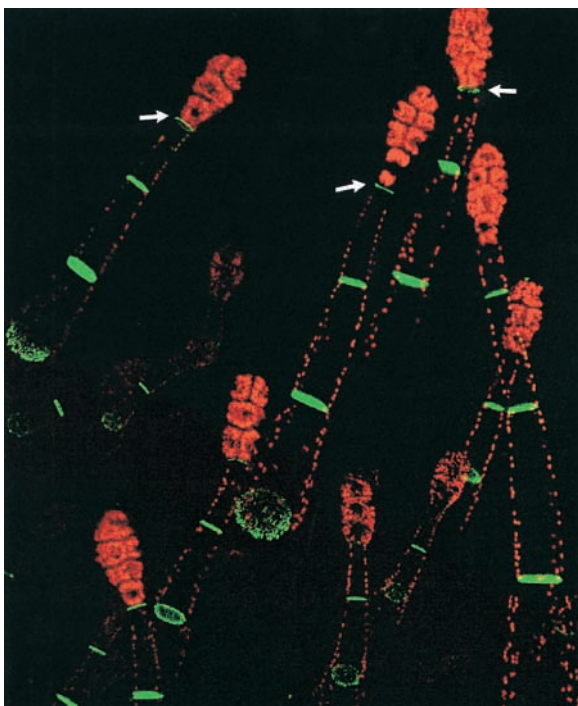


Figure 1. Trichomes of tobacco overexpressing MP-GFP.

The fusion protein is targeted to branched plasmodesmata (shown in green). Trichomes are one of the first cell types to show plasmodesmal branching in a leaf, and show a graded progression of plasmodesmal targeting by MP-GFP from tip to base. Plasmodesmal branching has commenced in some neck cells (arrows) and is more prevalent in basal cells. The immature, dividing tip cells show no fluorescence. Chlorophyll autofluorescence is shown in red. (Figure courtesy of Alison Roberts).

MEETING REPORT

researchers from different but related fields, a sentiment expressed by Ted Botha (Rhodes University, Grahamstown, South Africa) and Bob Turgeon, who called for an expansion in the fields participating at the next plasmodesma conference, including plant developmental biology. It is hoped that consideration of the potential environmental and social impacts of the genetic manipulation of intercellular communication in plants will be discussed in depth at the next meeting.

It was clear by the conclusion of the meeting that research into PD is continuing to flourish at many levels. Physiological, molecular, microscopic, and genetic techniques continue to add to our knowledge of this elusive structure. However, many important questions still remain, many of which were raised at the meeting and which are discussed in a recent book edited by Aart van Bel and Pim van Kesteren (van Bel et al., 1999). For example, what is the MP30's true function? How large is the diversity among PD? What are the long-distance signals controlling systemic gene silencing? What are the genes encoding the functional components of PD? How is the SEL controlled developmentally? Do viruses usurp an endogenous pathway for movement via PD? What is the role of

the cytoskeleton in protein trafficking? As these questions are addressed, researchers in diverse fields of plant biology will be able to determine that PD are important to their biological questions.

Local organizers Ted Botha and Ed Rybicki can be proud of putting together such an excellent meeting. Living in South Africa, they shared with meeting participants tremendous personal insight into the theme of the conference, which was diversity—diversity in experimental approaches to a complex problem, a call to consider PD a group of functionally diversified channels specialized for different tasks in different cells, and a reborn embrace of cultural diversity in the host nation, South Africa.

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