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Minireview

# Intercellular Connections Are Developmentally Controlled to Help Move Molecules through the Plant

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In a recent issue of *Cell*, Karl Oparka and his several collaborators (Oparka et al., 1999) score a victory for noninvasive techniques of studying living cells by fluorescence microscopy as they revise our ideas about the role of plasmodesmata in controlling long-distance transfer of molecules through the plant.

### *What Is Phloem? A Quick Summary for Weekend Botanists*

For those unaccustomed to working with plants, a little introduction to the long-distance transfer system in question may be useful. A cellular conduit system called the phloem distributes large amounts of sugar and certain amino acids as well as lesser amounts of other small molecules from "source" regions where they are produced to "sink" regions such as flowers or developing leaves where they are utilized. Different kinds of plants have evolutionarily shuffled a variety of the cellular components of the phloem system while achieving the same basic function (Taiz and Zeiger, 1998; Oparka and Turgeon, 1999). It will here be useful to ignore the diversity in favor of a coherent description of how the phloem operates in a single kind of plant, tobacco.

Within a source tissue, companion cells use transmembrane porters to actively load their cytoplasm with the molecules destined for shipment through the plant. From the companion cells, intercellular connections called plasmodesmata form an open path for diffusion of the small molecules into sieve elements, elongate cells with a limited variety of organelles all packed around the periphery. Sieve elements are arranged end-to-end with conspicuous interconnecting pores, and the columns they form are called sieve tubes.

As sugar is loaded into the sieve tubes ramifying through the source tissue, water is drawn in osmotically across the cell membranes. Physical pressure builds, and the solution surges along the sieve tubes to less pressurized regions. Where they pass along major routes—as in leaf veins, stems, or roots—the sieve tubes gather into bundles and interconnect via specialized lateral pore systems. In the sink tissue, water and solute move out from the phloem to the surrounding cells, where solute will be utilized. In various proportions water may be retained for organ expansion, lost to the environment, and returned to sources via another system of pipelines called the xylem. In the sink tissues, the sieve elements, companion cells, and the surrounding cells that use the nutrient solutes are connected by plasmodesmata. Cells of source tissues also have plasmodesmata, although in comparison to sink cells they are sparser between the companion cells and the now mature photosynthetic cells and between the photosynthetic cells themselves.

### *What Are Plasmodesmata?*

Put simply, plasmodesmata are cytoplasmic channels between cells. In truth, however, plasmodesmal structure is far from simple. Within the limiting tubule formed by the cell membrane, the endoplasmic reticulum (ER) of the adjoining cells connects via a specialized domain called the desmotubule. Just as actin often coats the ER in the cytoplasm of the plant cell (e.g., Reuzeau et al., 1997; Boevink et al., 1998), it occurs at the ends of the desmotubule and perhaps accompanies it through the plasmodesma (e.g., Blackman and Overall, 1998).

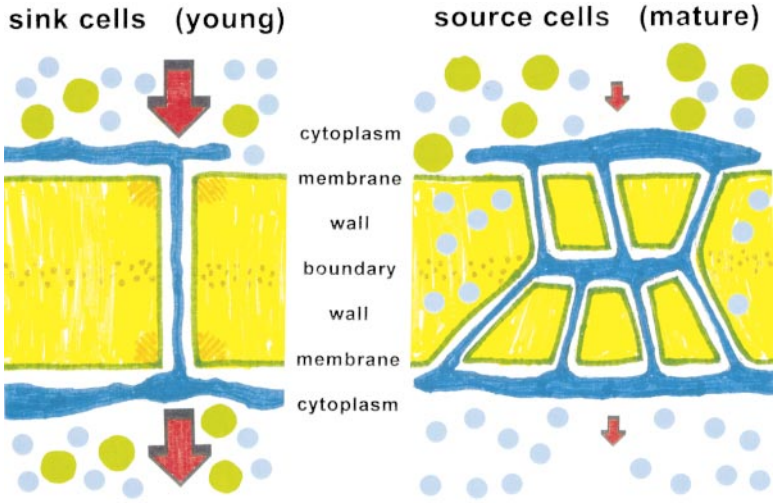


Figure 1. Representation of Molecular Permeation through Simple and Branched Plasmodesmata in Cells of a Tobacco Leaf

On the left, signified by large red arrows, a simple plasmodesma permits extensive unloading of small molecules from the phloem because plasmodesmal permeability in sink tissue is high. The pale blue circles indicate small, normally phloem-translocated, nutrients such as sugar, and the green circles indicate permeant fluorescent test proteins of size up to 50 kDa introduced into the "upper" cytoplasm. The prominent central tubule of transcellular ER (dark blue) does not participate in diffusive passage of molecules from the cytoplasm of one cell to the next, but may be significant for facilitated movement of transcription factors and viruses through the sleeve of cytoplasm.

On the right, a branched plasmodesma in tissue producing sugar (blue circles) has a size exclusion limit of $<1$ kDa, as indicated by failure of diverse introduced fluorescent test molecules (green circles) to pass through. It may be $<0.4$ kDa. The very low permeability is symbolized by small red arrows. There are few plasmodesmata per unit area of wall. Movement of sugar from source cells to phloem occurs by release from source cells into and diffusion within the wall solution, from which it must be actively imported into the phloem.

Plasmodesmata may be constricted at the ends by a collar of specialized wall material.

Plasmodesmata of plants such as tobacco may be simple or branched (Figure 1, and see Itaya et al., 1998). When a cell divides, simple plasmodesmata form when new walls are being deposited between the daughters, although plasmodesmata can also form in established wall pairs. Branched plasmodesmata appear to differentiate from simple ones during cell expansion and maturation (Itaya et al., 1998). More or less commensurate with wall expansion, the number of plasmodesmata per unit surface area decreases.

### *Yesterday's Wisdom: How Are Plasmodesmata Controlled and How Do They Control Movement of Molecules?*

It is obvious that plasmodesmata form routes through which molecules pass from cell to cell. But what molecules? Under what control? Early evidence suggested that small solutes such as inorganic ions and sugars might diffuse readily through plasmodesmata. At the same time, cells must restrict movement of large proteins, messenger RNAs, and of course organelles. However, certain viruses traverse the plasmodesmata, apparently circumventing normal restraints. Following early studies of ion and fluorochrome passage, virologists intensified assessment of how molecules move through plasmodesmata because this figured in their quest to understand the infection process. Injecting cells with fluorescently tagged dextrans of various sizes, they concluded that, whereas foreign molecules above 1 kDa could not exit uninfected cells via plasmodesmata, foreign molecules up to about 20 kDa could exit cells infected by a virus such as the tobacco mosaic virus, or TMV (Oparka et al., 1997). The change in the permeability of the plasmodesmata is due to the TMV movement protein, a 30 kDa protein encoded by the virus. Since it is much larger than the plasmodesmal "size exclusion limit" or SEL, movement protein must have acquired specific functions that enable it to help the RNA genome of TMV move from cell to cell. Other viruses, including potato virus X, require coat proteins in addition to one or more movement proteins for cell-to-cell spread.

How viruses spread is a matter of critical interest for developmental biologists as well as for pathologists. A report by Lucas et al. (1995) suggested that mRNA of the maize *knotted1* gene, which conveys positional information at the plant apex, can be trafficked through plasmodesmata of photosynthetic cells of tobacco under the company and control of knotted1 protein. Paralleling the TMV story, maize knotted1 protein was reported to increase the SEL of tobacco plasmodesmata to 20 kDa or greater. It has been suggested (e.g., Kragler et al., 1998) that plant viruses may, in effect, write their own tickets to ride on a transport system evolved by the plant for controlling its own form and function. (Some appropriate readings are suggested by Oparka et al., 1999.)

Control of plasmodesmal permeability appears to be an active business: azide increases it, as does depolymerization of actin. Messengers such as $Ca^{2+}$ and IP3 participate in control of permeability. Yet, much remains unknown. How are plasmodesmata regulated during plant development? Is the "knotted1 system" a valid paradigm? If so, how many natural analogs does it have? Are virus movement proteins analogs of certain cellular proteins? What specific roles might plasmodesmata play in establishing the vector of phloem transport? Do plasmodesmata function differently in source and sink tissues?

### *New Insight: Plasmodesmal Function Changes Developmentally from Sink to Source*

Oparka and colleagues (1999) addressed critical questions about developmental control of long-range solute transport with stunning success.

Their approach was enabled by an expression system developed by Norbert Sauer, whose group (see Imlau et al., 1999) placed a gene encoding green fluorescent protein (GFP) under the control of a promoter from a gene encoding a sucrose transporter specific to companion cells. Imlau et al. showed that GFP synthesized in companion cells was delivered via the phloem to the major sink tissues of *Arabidopsis* and tobacco plants. Based on experiments at the organismic level, these authors surmised that plasmodesmal permeabilities must be important in determining how nutrients and other materials are distributed from source to sink.

The Oparka group extended the work of Imlau et al. (1999) by examining the behavior of tobacco plasmodesmata at cellular and molecular levels.

In mature source leaves, GFP fluorescence was seen only in phloem cells of the veins. In relatively young sink leaves, the fluorescence spread from the phloem into the surrounding tissues. In leaves of intermediate stage, which undergo a tip-to-base wave of cell expansion and maturation during which sink tissue becomes source tissue, there was a graded pattern of GFP distribution: fluorescence in the tip did not move out from the veins, whereas in the base it spread away from them.

To check the correlation between GFP distribution and sugar loading and unloading, Oparka et al. first imaged GFP patterns in the expanding leaves of intact plants, and then provided older source leaves with $^{14}CO_2$ to pulse label the sugars being produced. After a suitable interval, distribution of radioactivity in the previously imaged expanding leaves was determined by autoradiography. Unloading patterns of the $^{14}C$-labeled material from the phloem matched the unloading pattern of the GFP. In a complementary experiment, a small phloem-mobile fluorochrome was loaded in the old leaves. In expanding leaves this label, like the radiocarbon, was unloaded in the same pattern as GFP.

Previous measurements suggested that plasmodesmata normally have an SEL of less than 1 kDa. Based on the experiments with GFP expressed in tobacco leaves, such a limit appears valid only in tissue serving as a source for phloem redistribution—the plasmodesmal SEL for the sink region is considerably bigger. How much bigger? To find out, Oparka et al. (1999) used a bombardment method to transiently express GFP fusions to proteins of selected sizes. The spreading patterns of GFP fusions up to 50 kDa were similar to that of free GFP. These data establish that in sink but not source tissue the SEL of plasmodesmata is at least 50 times the previously accepted value.

***What Is the Structural Basis of the Functional Shift?***

Can structural differences between source and sink plasmodesmata be correlated with the functional SEL differences? As described above, tobacco leaf cell development was found by the group of Ding to include a shift in plasmodesmal architecture (Itaya et al., 1998). Examining electron micrographs of a series of cells from the immature base toward the maturing or mature tip of young leaves, they first observed simple plasmodesmata, then closely paired simple plasmodesmata and also what appear to be plasmodesmal pairs joined by a tubular cross-link to form an H shape. In larger cells closer to the mature tip, they found sparsely distributed "branched plasmodesmata," which look like clusters of plasmodesmata pinched together and joined not by a simple tubule but rather by an expanded chamber with a large central cavity. Branched plasmodesmata lack wall collars. The origin of H-type and branched plasmodesmata has not been investigated. Consistent with the idea that they nucleate in some way from the simple tubular plasmodesmata that originate in the new walls separating cells after mitosis, branched plasmodesmata are sparsely distributed.

Oparka and colleagues examined plasmodesmata in an expanding leaf for which sink, source, and transition regions had been mapped using GFP. Sink tissue had simple plasmodesmata, transition tissue had paired plasmodesmata joined by tubular cross-links, and source tissue had branched plasmodesmata. The branched form became predominant at the tip of the leaf, where the sink–source transition is initiated. Another feature of the developmental sink–source shift was a progressive decrease in the number of plasmodesmata per unit area of cell wall.

The concomitance of the plasmodesmal shifts from simple to branched forms and from large to small SEL is clear, at least for leaves of the species selected for study. So, also, is concomitance with the shift of the tissue from sugar import to export. A specific change in plasmodesmal form and function can thus be pinpointed as a critical regulatory event in the development and physiology of the leaf. Identification of these changes should greatly facilitate study of the mechanisms underlying plasmodesmal formation and differentiation.

***Spread of Virus Infection and Endogenous Regulators***

Virus genomes are believed to pass through plasmodesmata only in the presence of movement proteins. However, on discovering that the SEL of plasmodesmata in sink tissue is much higher than formerly appreciated, Oparka and colleagues used potato virus X to check whether in a sink leaf (as contrasted to a source leaf) a virus might pass through plasmodesmata unaided by movement proteins. The answer is no: it remains generally true that cell-to-cell transmission of the virus requires facilitation by movement proteins, even when the basal SEL is high.

Attention must now be focused on two central questions of viral infection. How do movement proteins enlarge the SEL of a source leaf plasmodesma? How does a virus genome make its way through a plasmodesma of already high or enlarged SEL? Further studies of how shape and charge distribution of molecules influence their intercellular movement should better delimit these questions. Breakthroughs in the realm of molecular interaction will perhaps be the next step toward solving the complex interactions between plant viruses and plasmodesmata.

Do viruses indeed steal a ride on a train for endogenous regulators? As pointed out above it was proposed that plant viruses evolved mechanisms to pass through plasmodesmata by taking advantage of a system for the cell-to-cell passage of proteins and mRNAs that the plant itself uses to coordinate its own development. The data underlying this proposal were collected before the Oparka group showed that the SEL of tobacco leaf plasmodesmata changes during tissue maturation, and were to a considerable extent based on observations of maize knotted1 mRNA and protein that were injected into tobacco leaf cells. More extensive controls should perhaps be carried out to assure that a normal gradient of developmental change in plasmodesmal function did not influence the outcome of the original studies. Then, assuming reassuring results, the behavior of endogenous regulators can be rethought in terms of different developmental forms of plasmodesmata. In any event, work in this area should accelerate with the combined power of genetics and genomic databases. And, in addition to the obvious reasons for interest in plant viruses, there is hope that they may provide an easier route to understanding plant development than the isolated study of plant genes and gene products per se. Much is already known about the proteins encoded by many plant viruses and about the cell and tissue biology of infection (Lazarowitz and Beachy, 1999). Virus or virus components are readily introduced to cells by a variety of methods, with or without alterations such as mutations and provision of GFP. The convenience of virus–host experimental systems should facilitate attempts to understand in detail the structures of movement and coat proteins, how they interact with the molecules of the cytoskeleton-clad ER on which they are intracellularly distributed, and how they interact with the molecular features of the plasmodesma including its central desmotubule of modified ER.

***Long-Term Benefits: Are Plasmodesmata a Key to Higher Crop Yields?***

The work of Oparka et al. (1999) will no doubt assume a prominent place in the textbooks, as opening up our understanding of plasmodesmata means opening up so many important areas in basic plant biology. This basic knowledge might be of practical importance as well. Could plasmodesmata be a target for the future crop engineer? There are at least two reasons this might be so. First, of course, understanding plasmodesmata may give new insights on how to prevent the spread of viral infections. Second, many biologists have speculated that understanding how the plant controls its flow of carbon- and nitrogen-rich compounds could be important for improving crop yields and quality. Often, only a particular component of the plant, such as seed, is harvested. Diversion of even a little more of the plant's total photosynthetic products into the desired organ might result in significant increases in yield. Plasmodesmata clearly play a central role in the delivery of such

products into harvestable sink tissues, and as such remain a prime target for genetic modification. Of course, plasmodesmata are only one feature of the plant's resource distribution network. Nevertheless, new understanding of developmental regulation of plasmodesmata is certain to have a large impact on how we look at practical agricultural problems as well as how we think about basic plant cell biology.

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