IN THIS ISSUE

Penetrating Plasmodesmata

Living plant cells are connected to one another by plasmodesmata, thin cytoplasmic channels that bridge the cell wall. These structures are thought to play an essential role in cell–cell communication, providing routes by which small molecules, such as ions, can flow from cell to cell (for review, see Robards and Lucas, 1990). Plasmodesmata are important in virus infection as well, viruses having evolved ways to modify and move through plasmodesmata, often as unencapsidated nucleic acids, during the infection process. Despite the central role plasmodesmata are postulated to play in coordinating plant cell function, little is actually known about their structure and regulation, both during normal plant life and during viral infection.

What is known about plasmodesmal structure comes mainly from electron microscopy studies, which have revealed that plasmodesmata are membrane-lined pores that contain a strand of appressed endoplasmic reticulum (ER) that is continuous with the ER of the adjacent cells. From ultrastructural analyses and dye injection experiments, it appears that the presence and arrangement of numerous proteins in both the ER and plasma membranes create microchannels narrow enough to restrict diffusion to small (<1 kD) molecules (Ding et al., 1992). Plasmodesmata are just beginning to be isolated biochemically (e.g., Kotlizky et al., 1992), however, and nothing is yet known about what, and how many, proteins they contain.

No specific plant protein is known to move through plasmodesmata, although the proteins in phloem sieve elements, which are enucleate, are thought to be transported there via plasmodesmata from adjacent companion cells (Fisher et al., 1992). Most studies of plasmodesmal function have, therefore, focused on studying how viruses are able to move through these pores. Genetic studies of plant viruses have defined viral “movement” proteins that allow the virus to move out of infected cells and into neighboring healthy cells, and several lines of evidence indicate that these proteins function by modifying plasmodesmata. For example, the tobacco mosaic virus (TMV) movement protein is localized to the plasmodesmata of both infected plants (Tornemius et al., 1987) and movement protein–expressing transgenic plants (Atkins et al., 1991). Moreover, TMV movement protein expression in transgenic plants raises the size exclusion limit to ~10 kD (Wolf et al., 1989).

Movement proteins probably do more than just “open” plasmodesmata, however, because the increased pore size may still be too small to permit free diffusion of viral nucleic acids. A hint about another possible movement protein function came from the observation that in vitro, movement proteins from a number of viruses bind cooperatively and nonspecifically to single-stranded nucleic acids (e.g., Citovsky et al., 1990). Binding of the TMV movement protein to nonspecific RNAs appears to unfold them, resulting in a complex that should be thin enough to pass through plasmodesmata that have been diluted by movement protein activity (Citovsky et al., 1992). Interestingly, sequence analysis of a number of viral movement proteins has shown that they fall into two large families (Koonin et al., 1991); members of the larger family, which includes the TMV movement protein, contain a conserved motif shared by HSP90s, cellular heat shock proteins that may function as chaperones.

How movement proteins actually interact with plasmodesmata has remained an open question. One possibility has been that movement proteins escort bound nucleic acids through plasmodesmata, but there has been no experimental evidence for this. In this issue, Fujiwara and coworkers (pages 1783–1794) now provide a colorful demonstration that the 35-kD movement protein from red clover necrotic mosaic dianthovirus (RCNMV), an RNA virus, moves rapidly from cell to cell—both in the presence and absence of viral RNA. This observation provides definitive evidence that a protein can be transported through plasmodesmata. And the RCNMV movement protein is not alone in this capability: members of the same group have recently found that the movement protein of bean dwarf mosaic geminivirus, a DNA virus, spreads from cell to cell as well (A. Noueiry, W.J. Lucas, and R. Gilbertson, unpublished data).

To test the ability of the RCNMV movement protein to move through plasmodesmata, Fujiwara and coworkers labeled bacterially produced movement protein with a fluorescent dye and injected the protein into leaf mesophyll cells of cowpea, a host for the virus. Within seconds after injection, wild-type movement protein moved into adjacent cells, as visualized by fluorescence. By contrast, mutant 278, an alanine scanning mutant movement protein that is unable to promote virus spread in the context of an infection (Giesman-Cookmeyer and Lommel, 1993), did not spread out of the injected cell.

Like the movement protein from TMV, the movement protein from RCNMV increased plasmodesmal pore size: when a 9.4-kD fluorescein-conjugated dextran (F-dextran) was coinjected with wild-type movement protein, the F-dextran began to spread into adjacent cells less than a minute after injection. Only those alanine scanning mutants known to promote virus movement in infected plants increased the plasmodesmal size exclusion limit, with one exception: mutant 280, which does not promote virus movement in vivo (Giesman-Cookmeyer and Lommel, 1993), nevertheless allowed the 9.4-kD
F-dextran to spread beyond the injected cell. The properties of this mutant movement protein suggest that distinct domains of the movement protein are responsible for mediating cell-to-cell spread of the viral RNA and for opening plasmodesmata; furthermore, they indicate that an increase in plasmodesmal pore size is not sufficient for viral spread.

RCNMV movement protein also promoted the rapid cell-to-cell spread of fluorescently labeled RCNMV RNAs, showing conclusively that movement protein is the only viral protein required for cell-to-cell movement of viral RNAs. Again, the potentiation of RNA movement was correlated with the ability of the movement protein to cause systemic infection; not surprisingly, mutant 280 did not promote RNA trafficking. In vitro, RCNMV movement protein binds cooperatively and with no apparent specificity to both RNA and single-stranded DNA (Osman et al., 1992). However, although the authors cite preliminary results suggesting that the RCNMV movement protein promotes cell-to-cell movement of a plasmid transcript, it did not potentiate the trafficking of single-stranded DNA. One possible explanation for this difference is that the structure of the movement protein–DNA complex somehow prevents the movement protein from binding to putative plasmodesmal receptors.

Binding of TMV movement protein has been proposed to extend nucleic acids so that they are able to fit through enlarged plasmodesmata (Citovsky et al., 1990, 1992). However, Fujiwara and coworkers found that RCNMV movement protein binding does not lengthen RCNMV RNAs in vitro. The RCNMV movement protein belongs to the movement protein family that lacks similarity to HSP90 (Koonin et al., 1991), and the different effects of the TMV and RCNMV movement proteins on RNA structure may indicate that these proteins interact differently with their own RNAs. In any case, assuming that the upper limit for free diffusion through movement protein–modified plasmodesmata is 10 kD, the RCNMV movement protein–RNA complex may be too large to diffuse freely. Thus, in addition to binding to viral transcripts and opening plasmodesmata, the RCNMV movement protein may undergo a further interaction with the plasmodesma that enables the protein–RNA complex to be actively transported across it. The latter function must be defective in mutant 280, which binds to RNA and increases plasmodesmal pore size but does not promote plasmodesmal transport of RCNMV RNA.

If enlarged plasmodesmata are not sufficient for viral RNA trafficking, at least in the case of RCNMV (and even, it has been suggested, in the case of TMV; Citovsky, 1993), then what is the function of the increase in pore size? Perhaps plasmodesmal opening is a two-step process; it could be that the initial enlargement of the pore is necessary for the movement protein–nucleic acid complex to contact plasmodesmal receptors that actively transport the complex across the plasmodesma. The fact that no movement protein mutant has been found that crosses plasmodesmata without dilating them is consistent with this idea.

What do Fujiwara and coworkers’ results imply about plasmodesmal transport in uninfected plants? The very fact that plasmodesmata become modified within seconds after the introduction of viral movement protein hints that plasmodesmal opening and macromolecular transport are normal events in the life of a plant and that viruses have evolved ways to piggyback on this plant capability. On the other hand, although it has been hypothesized that during plant development, the plasmodesmata of cells in a particular developmental domain might open to allow them to exchange informational macromolecules, plasmodesmata have never been observed to open except under viral influence. The plasmodesmata linking sieve elements to their companion cells allow the free diffusion of dextrans of at least 3 kD (Kemppers et al., 1993), but it is likely that some active process is necessary for the transport of large proteins.

Assuming that endogenous plant proteins do traffic through plasmodesmata, at least in certain cells and at certain times in development, it seems likely that plant movement proteins facilitate their transport (Lucas and Wolf, 1993). Just as proteins destined for the nucleus have sequences that mediate their binding to cytoplasmic receptors that shuttle them to the nuclear pore (Goldfarb and Michaud, 1991), proteins destined to cross plasmodesmata might have recognition sequences for endogenous plant proteins that then carry them to, or across, the plasmodesma. An alternative possibility is that there may be little specificity in plant movement protein binding; if so, the presence of movement proteins might allow many different proteins to cross the plasmodesma. In this case, endogenous movement protein activity would have to be tightly regulated.

Continued studies of viral movement proteins—such as mutant 280, which is defective in some but not all aspects of the interaction with plasmodesmata—will undoubtedly shed light on how plasmodesmata function. However, to understand whether, and how, plasmodesmata regulate intercellular transport of endogenous macromolecules, it will be necessary to identify endogenous plant proteins that are transported across plasmodesmata. One way to do this might be to fluorescently label a number of different candidate proteins, including those present in sieve elements, and assay them for their ability to move out of an injected cell. Another approach might be to use the polymerase chain reaction to isolate plant genes that share sequence similarity with viral movement protein genes; endogenous plant movement proteins might also be identified by screening plant sequences for their ability to complement cell-to-cell spread of viruses lacking functional movement proteins. The isolation of plasmodesmata and the purification of their protein components should also yield...
much-needed insights into the role of these organelles in coordinating plant development and physiology.

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REFERENCES


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Plant Cell 1993;5:1693-1695
DOI 10.1105/tpc.5.12.1693

This information is current as of October 20, 2017