Plasmodesmata. A Not So Open-and-Shut Case

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In 1897, Eduard Tangl observed striations between cells in the cotyledons of *Strychnos nuxvomica* and hypothesized that the "the protoplasmic bodies. . . are united by thin strands passing through connecting ducts in the walls, which put the cells into connection with each other and so unite them to an entity of higher order" (for review, see 2). This was a prophetic view of the symplasm, a term coined much later by Münch to describe the cytoplasmic continuum that occurs between higher plant cells. Tangl's work challenged the then-held view that plant cells functioned as autonomous units and stimulated several studies on other species. However, it was Strasburger in 1901 who finally named these delicate structures plasmodesmata (2). Space constraints do not permit us to do justice to the several pioneers of plamodesmal research. However, we have attempted to select some notable milestones in the development of the field.

Although the concept of the symplasm became accepted by many plant physiologists, it was not until the advent of electron microscopy that the fine structure of plasmodesmata was resolved. Following the introduction of glutaraldehyde as a fixative, several studies showed plasmodesmata to be plasma membrane-lined channels containing a central endoplasmic reticulum (ER)-derived structure termed the desmotubule (16). The demonstration of ER continuity between adjacent cells clearly distinguished the plasmodesma from its functional counterpart in animal cells, the gap junction. Although some studies suggested that there may be homologies between plasmodesmata and gap junctions, the consensus today is that these structures differ considerably in both form and function. With the introduction of immunological techniques, plasmodesmata have been shown to be extremely complex structures containing several unique proteins, including cytoskeletal elements (13), and recent studies suggest that there may be important differences between simple and branched plasmodesmal architectures with respect to molecular trafficking (11).

PROBING PLASMODESMAL FUNCTION

Functional studies of plasmodesmata lagged behind ultrastructural studies, due mainly to a lack of suitable techniques for studying the movement of molecules through plasmodesmata. This was remedied in the 1980s when a number of groups began to microinject fluorescent probes into plant cells to study the functional size exclusion limit (SEL) of plasmodesmata. These studies gave rise to a general consensus that only relatively small molecules (<1 kD) pass freely between plant cells. The seminal work of Terry and Robards (20) suggested that the Stokes radius (R_s), rather than molecular mass, was the key determinant for passage of small molecules through plasmodesmata. The R_s is the molecular dimension of an equivalent sphere with the same hydrodynamic drag as the molecule in question. Small changes in R_s have significant consequences for the mobility of molecular probes, particularly if the functional plasmodesmal channel is close to the R_s of the diffusive molecule.

SYMPLASMIC DOMAINS

The initial studies of plasmodesmal SEL were technically limited to cells or tissues that could easily be microinjected. Further studies, however, revealed that not all cells in the plant body were connected with plasmodesmata of uniform SEL. The work of Erwee and Goodwin (6) was instrumental in introducing the concept that plasmodesmal conductance is reduced between some tissues as a natural consequence of differentiation, giving rise to the view that the symplasm may be segregated into domains, allowing cells within a domain to "talk" freely to each other while restricting communication between domains. Loss, or restriction, of plasmodesmatal conductance appears to be common during differentiation. An extreme case of plasmodesmal down-regulation occurs around stomatal complexes. Guard cells initially are coupled symplastically to adjoining epidermal cells. With time, however, their plasmodesmata become truncated and eventually nonfunctional, eliminating intercellular communication between mature guard cells and surrounding epidermal cells (14). In other cases, plasmodesmatal numbers become greatly reduced but not eliminated altogether. Such a situation occurs around the phloem of species with putative apoplastic loading mechanisms. Here, a small number of plasmodesmata are retained between the bundle sheath/phloem parenchyma cells and the sieve element-companion cell (SE-CC) complexes. For the plant, these plasmodesmata represent an "Achilles'

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heel" that can be exploited by phloem-mobile viruses (for review, see 12). Why are these plasmodesmata retained in apoplastically loading species? One possibility is that during differentiation the plant must retain some degree of intercellular communication to allow essential signals to pass between cells, and also to move long distances in the phloem. Such signals may be electrical, hormonal, or as discussed below, macromolecular.

MODULATION OF SEL

If one accepts the premise that the plant is forced to retain intercellular communication at critical cellular boundaries, perhaps it is not surprising that the SEL has been found to be plastic under certain circumstances. Plasmodesmata are sensitive to wounding, which causes rapid callose deposition around the neck of the plasmodesmal pore. Other factors shown to reduce the SEL include turgor pressure differentials and elevated cellular Ca^{2+} levels (for review, see 5). In contrast, some physiological or pharmacological treatments have been shown to increase the SEL of plasmodesmata (5). The picture that emerges is one of the plasmodesma as a dynamic structure that is capable of altering SEL depending on a range of intracellular cues.

WHAT HAVE WE LEARNED FROM PLANT VIRUSES?

The movement potential of a given virus is an important factor determining its virulence and pathogenicity. In an early study of tobacco mosaic virus (TMV) movement, Samuel (18) suggested that plasmodesmata were the probable route of virus movement from one cell to the next. Sheffield (19) subsequently showed that virus did not enter mature guard cells due to the lack of "plasmodesms" between these and neighboring cells. Early electron micrographs often depicted intact virus particles in plasmodesmata, although it is now clear that viruses employ different strategies to move through plasmodesmata. In the comovirus group, typified by cowpea mosaic virus, the viral genome passes from one cell to the next in the form of intact particles through protein tubules that span the plasmodesmal pore. In some potexviruses, such as potato virus X, filamentous virus particles appear to represent the functional movement complex that traverses plasmodesmata, whereas in other viral groups (e.g. tobamoviruses) the viral genome appears to pass through plasmodesmata in the form of a linear ribonucleoprotein complex (for review, see 3, 12).

All plant viruses that move through plasmodesmata rely for movement on one or more gene products encoded by the viral genome. These were loosely termed "transport proteins" (1), a term later modified to movement proteins (MPs). The most studied of these is the 30-kD MP of TMV, which appears to perform several related functions including interacting with microtubules, binding single-stranded RNA, targeting the plasmodesmal pore, and "gating" the pore (increasing its SEL) to allow passage of the viral genome (3, 12; Figure 1). In other viruses, the different facets of cell-to-cell movement may be performed by separate, interacting gene products, and it appears that some viral MPs may play an indirect role in the movement process, perhaps delivering the viral ge-

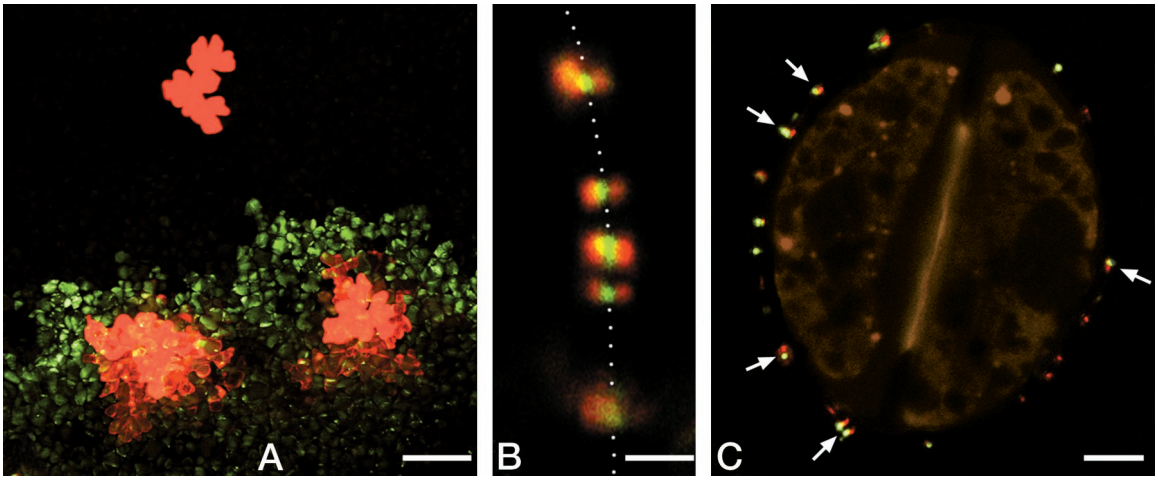

Figure 1. Different facets of the movement process of TMV. A, Plasmodesmal gating. A GFP-tagged TMV was used to delineate the viral infection front (green). Two injections of Texas Red dextran (10 kD) made immediately inside the infection front show cell-cell movement of the dextran (red). A third injection outside the infection front shows no cell-cell movement. Bar = 200 μm. B, Accumulation of TMV MP-GFP fusion (green) in the central cavity of epidermal cell plasmodesmata. The MP shows strong colocalization with callose (red). The white dotted line represents the position of the cell wall. Bar = 2 μm. C, Accumulation of TMV MP-GFP fusion (green) in the half plasmodesmata of mature guard cells and its colocalization (arrows) with callose (red). Although these plasmodesmata are nonfunctional, they are still targeted by the viral MP. Autofluorescence of the guard cell cytoplasm is shown in orange. Bar = 10 μm.

nome from the replication complex to the plasmodesmal pore (3). A major breakthrough in determining the "gating" role of MPs came from studies by Wolf et al. (23), who constructed transgenic plants expressing the 30-kD MP of TMV. The leaves of these plants were shown to traffic dextrans of 10 kD whereas control leaves showed the usual <1 kD exclusion limit.

MOVEMENT OF HOST PROTEINS THROUGH PLASMODESMATA

It appears that many plant viruses, at some stage in the coevolution with their hosts, exploited the use of the endogenous host pathway, including the capacity to traffic RNA between cells. However, whether all of the movement mechanisms displayed by plant viruses were usurped from their hosts remains questionable. It seems reasonable that under certain conditions plant cells require the exchange of informational macromolecules that dictate, for example, cell position, cell fate, or the onset of attack by pathogens. The first evidence for plasmodesmal trafficking of a plant protein came from studies of the maize transcription factor KNOTTED1 (KN1; 41 kD), a protein involved in meristem identity. Microinjection studies showed that fluorescently labeled KN1 moved between mesophyll cells of tobacco and maize. Furthermore, microinjected KN1 increased the plasmodesmal SEL of mesophyll cells and mediated the selective trafficking of its own mRNA (10). Since these studies, the number of host proteins reported to increase plasmodesmal SEL (and move between cells) has expanded to include pathogenesis-related proteins, and phloem proteins found in sieve tube exudate (for review, see 12). To date, it remains unclear why meristem transcription factors and phloem-derived proteins should traffic through mesophyll plasmodesmata.

THE SEL GETS BIGGER. . . AND BIGGER

Following initial observations that plasmodesmata allowed only the movement of small molecules, a growing number of cases became apparent where this general "rule" did not apply. The most studied have been those plasmodesmata connecting SEs and CCs. Here, nonspecific trafficking of dextrans of at least 10 kD has been reported (9). It appears that exceptionally high SELs are not restricted to the SE-CC complex, and a recent study has found SELs as high as 50 kD in sink leaves of tobacco (11). The apparent increase in SEL may be due to advances in alternative approaches to microinjection for studying macromolecular trafficking. For example, Imlau et al. (8) used the promoter of the CC-specific Suc transporter (*SUC2*) to drive the production of green fluorescent protein (GFP) within CCs. The free protein (27 kD) entered SEs and was unloaded in a range of sink tissues throughout the plant. At present it appears that both nonspecific (diffusional) and specific (facilitated) protein trafficking may occur through plasmodesmata. A clear challenge for the future will be to determine the precise mechanism(s) for trafficking macromolecules in different cells and tissues, and unraveling the factors that influence such movement. In this context, the Stokes radius becomes increasingly important. A 25-kD dextran has the same Stokes radius as a 51-kD globular protein, emphasizing the point that at large SELs the molecular mass may be a poor indicator of the potential for transport through plasmodemata (12).

SYSTEMIC GENE SILENCING: THE ROLE OF PLASMODESMATA

Recent evidence suggests that the signals involved in systemic gene silencing may move through plasmodesmata (22). As these signaling molecules are likely to be small (approximately 25 nucleotides) RNA species (7), it is conceivable that these small RNAs could move freely through plasmodesmata. Such silencing signals have been shown to enter the phloem and subsequently unload in sink tissues in a pattern that resembles the pathway of assimilate and virus unloading (12, 22). In young sink leaves the silencing signal moves into immature guard-cell complexes but is excluded from the guard cells of mature leaves, consistent with the passage of the signal through plasmodesmata (22). Given the high SEL of sink tissues it is possible that such small RNA signaling species may pass through plasmodesmata by simple diffusion. Further work is required to demonstrate the mechanism of movement of these RNA molecules in source tissues where the plasmodesmal SEL is particularly low.

THE FUTURE: BRIDGING THE GAPS

Although much has been learned in the last 10 years concerning the structure/function relations of plasmodesmata, a number of questions remain outstanding. A clear challenge will be to understand how plasmodesmata at different cellular interfaces function within the plant, and to more closely link plasmodesmal architecture with function. Progress is being made in understanding the unique role of plasmodesmata between SEs and CCs. In this respect, the ability to express specific proteins within CCs (8) is likely to provide valuable insights into the trafficking mechanisms that operate at this interface. At present, plant apical meristems represent a "black box" concerning plasmodesmal structure and function. It has been shown that plasmodesmal architecture differs in adjoining layers of the shoot apical meristem and these variations may reflect important differences in the ability of plasmodesmata to traffic informational macromolecules during early tissue differentiation (21). Detailed studies in this area are long overdue.

Although the role of MPs in assisting viral movement has been partially characterized, almost nothing is known of the plasmodesmal proteins that interact with viral MPs (or putative host MPs) to permit plasmodesmal gating and nucleic acid trafficking. What exactly are the plasmodesmal "receptors" alluded to in several studies? Recent promise comes from studies of the TMV MP where it was shown that a region of the MP interacts specifically with pectin methyl esterase, an enzyme involved in pectin modification of the cell wall (4). Might MPs interfere with pectin methyl esterase function, leading to localized wall loosening (with concurrent increase in SEL) around plasmodesmata? Also, how are nucleic acids chaperoned through plasmodemasta? Important clues might again come from plant viruses. Beet yellows closterovirus has been shown to encode an HPS70 homolog that is essential for cell-to-cell movement (15). Mutations of the ATPase domain of this protein eliminated cell-to-cell virus movement, indicating an energy requirement of the viral HSP70 protein to move through plasmodesmata. A recent study of the umbravirus, groundnut rosette virus, showed that the ORF3 of this virus had the capacity to translocate heterologous RNA over long distances in the phloem (17), suggesting that this protein might be a paralog of an elusive host long-distance RNA chaperone. It is clear that the isolation and characterization of host plasmodesmal chaperones is an important goal for the future.

The identification of plasmodesmal receptors/chaperones will almost certainly lead to a molecular dissection of cell-to-cell RNA trafficking mechanisms. However, several questions are outstanding. How are chaperone/RNA complexes moved through the plasmodesmal pore? What are the roles of the several cytoskeletal elements located in plasmodemata? In the final analysis, the answers are likely to come from closely aligned synergies in the fields of cell biology, molecular biology, and virology.

LITERATURE CITED

1. **Atabekov JG, Morozov YS** (1979) Adv Virus Res **25:** 1–91
2. **Carr DJ** (1976) *In* BES Gunning, AW Robards, eds, Intercellular Communication in Plants: Studies on Plasmodemata. Springer-Verlag, Berlin, pp 291–295
3. **Carrington JC, Kasschau KD, Mahajan SK, Schaad MC** (1996) Plant Cell **8:** 1669–1681
4. **Chen MH, Sheng JS, Hind G, Handa AK, Citovsky V** (2000) EMBO J **19:** 913–920
5. **Ding B, Itaya A, Woo Y** (1999) Annu Rev Cell Dev Biol **190:** 251–316
6. **Erwee MG, Goodwin PB** (1985) Symplast domains in extrastelar tissues of *Egeria densa* Planch. Planta **163:** 9–19
7. **Hamilton AJ, Baulcombe DC** (1999) Science **286:** 950–952
8. **Imlau A, Truernit E, Sauer N** (1999) Plant Cell **11:** 309–322
9. **Kempers R, van Bel AJE** (1997) Planta **201:** 195–201
10. **Lucas WJ, Bouche-Pillon S, Jackson DP, Nguyen L, Baker L, Ding B, Hake S** (1995) Science **270:** 1980–1983
11. **Oparka KJ, Roberts AG, Boevink P, Santa Cruz S, Roberts IM, Kotlszky G, Sauer N, Epel B** (1999) Cell **97:** 743–754
12. **Oparka KJ, Santa Cruz S** (2000) Annu Rev Plant Physiol Plant Mol Biol **51:** 323–347
13. **Overall R, Blackman LM** (1996) Trends Plant Sci **1:** 307–311
14. **Palevitz BA, Hepler P** (1985) Planta **164:** 473–479
15. **Peremyslov VV, Hagiwara Y, Dolja VV** (1999) Proc Natl Acad Sci USA **96:** 14771–14776
16. **Robards AW** (1971) Protoplasma **72:** 315–323
17. **Ryabov EV, Robinson DJ, Taliansky M** (1999) Proc Natl Acad Sci USA **96:** 1212–1217
18. **Samuel G** (1934) Ann Appl Biol **21:** 90–111
19. **Sheffield FML** (1936) Ann Appl Biol **23:** 506–508
20. **Terry BR, Robards AW** (1987) Planta **171:** 145–157
21. **van der Schoot C, Rinne P** (1999) Trends Plant Sci **4:** 31–37
22. **Voinnet O, Vain P, Angell S, Baulcombe DC** (1998) Cell **95:** 177–187
23. **Wolf S, Deom CM, Beachy RN, Lucas WJ** (1989) Science **246:** 377–379