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Plant Intercellular Communication via Plasmodesmata

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INTRODUCTION

The development and function of multicellular organisms rely on cell-to-cell communication. Detailed studies of animal cells show that this communication can occur by secretion of chemical signals, such as hormones and neurotransmitters, and by contact-dependent signals transmitted through extracellular matrix– and membrane-anchored molecules. Plants use similar modes of communication, although they are not as well characterized as those in animal systems. The transport of small signal molecules, such as hormones, regulates the proper growth of plant cells (see Creelman and Mullet, 1997; Kende and Zeevaart, 1997, in this issue), and cell-to-cell contact via an extracellular matrix–located glycoprotein and a receptor kinase plays a role in the self-incompatibility reaction between pollen and stigma (Stein et al., 1991). Plant cells, however, have an additional and unique mode of cell-to-cell communication derived from two of their characteristic features: the deposition of cell wall material, and the incomplete separation of the cytoplasm during cytokinesis.

Plasmodesmata (PD) are structurally complex channels that span the cell wall and connect the cytoplasm of one plant cell with that of its neighbors, consequently facilitating communication between cells. In higher plant embryos, initially all cells are interconnected by PD (Figure 1A; Schulz and Jensen, 1968; Mansfield and Briarty, 1991; see below) and integrated into a single symplast, the domain of common cytoplasm that is bounded by the plasma membranes of connected cells (Munch, 1930). As the plant differentiates, individual cells or groups of cells become isolated, possibly by loss of functional PD (Carr, 1976; Bergmans et al., 1993; Duckett et al., 1994). This symplastic isolation allows subsets of cells to function as distinct compartments within the plant. Indeed, mature flowering plants could best be described as mosaics of symplastic domains.

Because PD provide the symplastic connections between cells, communication and transport within and between these symplastic domains are intimately linked to the frequency, distribution, and function of PD. Plasmodesmal frequencies among cells have been used to characterize symplastic transport pathways, but this approach assumes that PD are both structurally and functionally uniform, which, as described below, does not appear to be the case (Van Bel and Oparka, 1995). Several techniques have been used to assay the function of PD in various cell types and in response to both environmental and developmental changes. Often PD function is characterized in regard to the plasmodesmal size exclusion limit (SEL) for passive, or diffusion-driven, movement of molecules. Plasmodesmal SEL typically is determined by monitoring the cell-to-cell movement of various sizes of microinjected fluorescently labeled dextrans.

More recently, global symplastic transport patterns have been examined using improved tools (Duckett et al., 1994; Oparka et al., 1994, 1995) such as new fluorescent tracer molecules and confocal laser scanning microscopy. In these experiments, loading of the tracer occurs far from the tissue of observation and is potentially less invasive than localized microinjection into cells of interest. Moreover, polar, relatively membrane-impermeant, low-molecular-weight fluorescent molecules, such as carboxyfluorescein diacetate and hydroxypyrene trisulphonic acid acetate, act as tracers for intercellular transport in various organs (Oparka et al., 1994; Wright and Oparka, 1996). Through the use of such techniques, plasmodesmal permeability has been shown to affect symplastic trafficking.

In response to physiological, developmental, or environmental changes, the permeability of PD between cells can be either downregulated to decrease or prevent symplastic trafficking between cells or upregulated to allow movement of large molecules (reviewed in Lucas et al., 1993; Ghoshroy et al., 1997). Thus, regulation of plasmodesmal transport may be the determining factor in cell-to-cell communication and the establishment of symplastic domains. Because PD establish the cytoplasmic continuity of the plant symplast, any factors that change plasmodesmal transport, by modifying either PD structure or function, can affect symplastic communication in the plant. Clearly, then, where and when PD form as well as how molecular transport through them is regulated may play important roles during plant development.

SYMPLASTIC DOMAINS

Characterization of Symplastic Domains

As mentioned above, the symplastic isolation of individual cells or groups of cells during the life of a plant allows them

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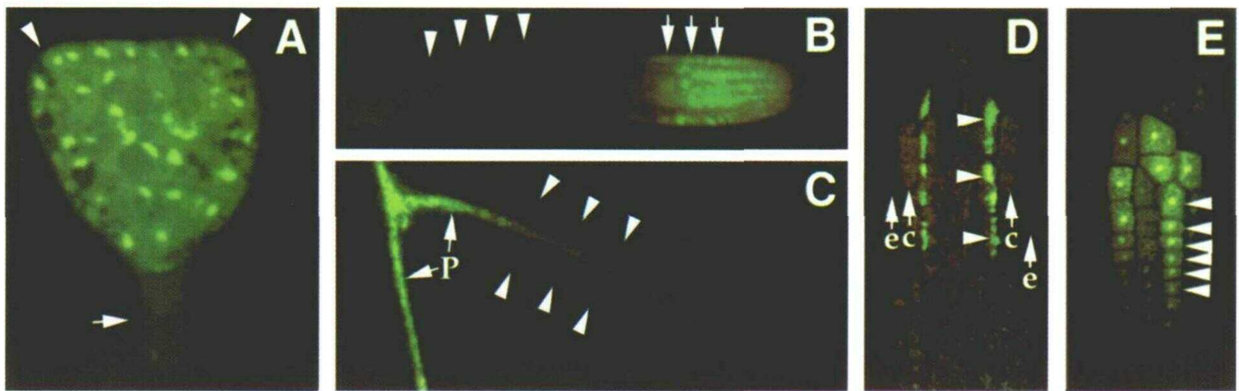

Figure 1. Dye Coupling in Arabidopsis.

(A) Early heart-shaped embryo with complete symplastic coupling. Arrowheads indicate the cotyledons. The arrow indicates the embryonic suspensor.

(B) Dye coupling in the lateral root. Arrowheads indicate the region of cell maturation, and arrows designate the region of phloem unloading. The vascular system in the region of root cell maturation is not in the focal plane.

(C) Dye coupling between a primary root (at left) and a lateral root. Arrowheads indicate the region of cell maturation in the lateral root. P, phloem.

(D) Close-up of the region of symplastic phloem unloading in a lateral root apex. Arrowheads indicate endodermal cells in the region of phloem unloading. Unlike in **(B)**, movement of HPTS into the epidermis (e) is not yet evident. c, cortex cells.

(E) An optical section through the epidermis near the root tip. Arrowheads indicate a labeled longitudinal cell file containing more HPTS than does the cell file immediately to the left.

The fluorescent molecule 8-hydroxypyrene-1,3,6-trisulphonic acid (HPTS) acts as a tracer for symplastic transport. HPTS is primarily restricted to the cytoplasm, although it also can label nuclei (**[A]** and **[E]**). Optical sections were taken using confocal microscopy. HPTS was loaded overnight (**[B]**, **[C]**, and **[E]**) or for 1 hr **(D)**. For details on HPTS and dye coupling in the Arabidopsis root, see Oparka et al. (1994) and Wright and Oparka (1996). See Laux and Jürgens (1997, in this issue) for a discussion of the anatomy of the Arabidopsis embryo; see Schiefelbein et al. (1997, in this issue) for a description of the anatomy of Arabidopsis roots.

to function as distinct domains. This subdivision of the plant symplast into functional symplastic domains was clearly demonstrated by early studies using electrical pulses and radioactive or fluorescent tracer molecules (Spanswick and Costerton, 1967; Arisz, 1969; Tucker, 1982; Erwee and Goodwin, 1983; Palevitz and Hepler, 1985). These domains are obviously important for the basic functioning of the plant, for example, in the trafficking of photoassimilates (Fisher and Oparka, 1996; Sjölund, 1997, in this issue).

In Arabidopsis, symplastic transport pathways have been defined using real-time imaging. For example, the image shown in Figure 1A suggests that the Arabidopsis embryo functions as a single symplast because the fluorescent tracer molecule is seen in all cells of the heart-shaped embryo. Symplastic transport pathways have also been examined in detail in Arabidopsis roots (Duckett et al., 1994; Oparka et al., 1994, 1995; see Schiefelbein et al., 1997, in this issue, for a review of root development). In the lateral root, phloem unloading suggests that all cell layers are symplastically integrated within the elongation zone; however, cells in the maturation zone are evidently symplastically isolated because movement of the fluorescent tracer into these cells is restricted (Figure 1B). By contrast, phloem in the maturation zone of lateral roots comprises a symplastic domain that is isolated from the cells around it (Figure 1C).

These imaging techniques can also provide information concerning the rate and direction of symplastic transport. Indeed, precise patterns of phloem unloading are revealed by viewing roots soon after dye loading begins (Figure 1D); the endodermis appears symplastically coupled to the cortex cells, but dye movement to the epidermis is not yet evident (as in Figure 1B). Also, preferential dye movement within individual longitudinal cell files can occur even though all cells in the region of unloading are symplastically connected (Figure 1E).

Changes in Symplastic Transport and Communication during Development

Symplastic communication is implicated in the control of plant development because cell fate appears to depend strongly on position (Pennell et al., 1995; see Clark, 1997; Lax and Jürgens, 1997; Schiefelbein et al., 1997, in this issue). For example, disruption of the PD between cells in the prothallus of the fern gametophyte induces each cell to differentiate into a complete new prothallus. This suggests that a signal is transmitted symplastically to inhibit cell division and thus direct the prothallus to develop as a coordinated unit (Nagai, 1914; Nakazawa, 1963). Moreover, intercellular

signaling is essential for establishing global patterning in flowering plants. Recent cell ablation studies indicate that within the Arabidopsis root apex, older cells in a file are the source of developmental signals (i.e., positional information) that influence the identity of less differentiated cells within the same file (Van den Berg et al., 1995). Therefore, it is possible that positional information is communicated in roots by the symplastic movement of specific developmental signals within clonally related cell files (Figure 1E).

Which molecules serve to communicate this positional information? Because of their small size, a number of plant hormones and plant hormone conjugates can move readily through PD (Drake and Carr, 1979; Kwaitkowska, 1991; Epel et al., 1992). Even auxin, which can be transported by protein carriers, is also thought to move symplastically, particularly in its conjugated forms (Bandurski et al., 1995). Although little is known about symplastic hormone movement, such transport is likely to be important for plant development. In Arabidopsis, defects in auxin transport during the early stages of embryogenesis are thought to contribute to the phenotypes of a number of embryo-defective mutants, such as *pin-formed* (Liu et al., 1993).

As a higher plant develops, its symplastic continuity changes. Detailed ultrastructural characterization of both *Capsella bursa-pastoris* (Schulz and Jensen, 1968) and Arabidopsis (Mansfield and Briarty, 1991) indicates that PD are present between all cells within young crucifer embryos. That these PD are functional is confirmed by the observation that fluorescent tracers move symplastically throughout the Arabidopsis embryo (Figure 1A). After germination, however, this single symplast is partitioned into symplastic domains (Duckett et al., 1994; Oparka et al., 1994). In the young Arabidopsis root, undifferentiated cells, such as in the meristem and elongation zone, are symplastically coupled, but as the cells differentiate, they become symplastically isolated. For example, the mature epidermis is symplastically isolated from underlying cells, and epidermal root hairs become symplastically isolated from other epidermal cells (Duckett et al., 1994). Interestingly, Arabidopsis mutants that are unable to establish symplastic domains have severe developmental defects. *knolle* embryos have many incomplete cell walls, and the resulting abnormal enhancement of cytoplasmic connections between normally distinct cells may cause *knolle* embryos to develop improperly (Lukowitz et al., 1996; reviewed in Laux and Jürgens, 1997, in this issue).

Perhaps the most dramatic example of developmentally regulated symplastic isolation is that of stomatal guard cells. Dye-coupling analyses in *Allium* and *Commelina* species reveal that as guard cells become fully differentiated, their symplastic communication with the rest of the leaf is lost (Palevitz and Hepler, 1985). Total disconnection is achieved through the degradation of PD, possibly via ubiquitin-mediated proteolysis of plasmodesmal components (Ehlers et al., 1996). Interestingly, symplastic isolation of the guard cells precedes obvious plasmodesmal degradation, which suggests that the presence of PD between cells does not necessarily indicate symplastic coupling (Palevitz and Hepler, 1985).

Developmentally associated changes in symplastic continuity are not restricted to the isolation of cells or tissues. Indeed, new symplastic connections can often establish or increase traffic between cells or tissues. For example, periwinkle carpels establish symplastic continuity via secondary PD after postgenital fusion (Van der Schoot et al., 1995), and dramatic increases in secondary PD correlate with the maturation of vascular tissue (Turgeon, 1996; Volk et al., 1996; see Fukuda, 1997; Sjölund, 1997, in this issue). In Arabidopsis, emerging lateral roots initially are symplastically isolated but become symplastically connected to the main root after differentiation of phloem connector elements (Figure 1C; Oparka et al., 1995). Developmental changes also can regulate plasmodesmal function. For example, the molecular permeability of PD from *Setcreasea purpurea* stamen hairs increases from a SEL of 1 kD in young buds and open flowers to 4.4 kD in senescent flowers (Yang et al., 1995).

Environmental Stimuli Affect Symplastic Communication

Because plants are sensitive to environmental changes, external stimuli may affect symplastic transport by influencing plasmodesmal function. In wheat roots subjected to anaerobic stress (and consequently to decreased ATP availability), plasmodesmal SEL increases from <1 kD to 5 to 10 kD (Cleland et al., 1994). Similarly, osmotic stress, which increases symplastic phloem unloading in pea root tips, is correlated with the dilation of the cytoplasmic sleeve and neck regions of PD (Schulz, 1995). In maize seedlings, changes in light quality alter symplastic trafficking rates (Epel and Erlanger, 1991). These data suggest that significant changes in light stimuli, such as those that promote flowering, may affect symplastic transport in leaves and meristems, although this has yet to be demonstrated directly. In *Nicotiana clevelandii* trichomes, differential turgor pressures between adjacent cells restrict symplastic coupling, suggesting that pressure gradients are involved in regulating plasmodesmal transport (Oparka and Prior, 1992). Such a pressure-generated mechanism for plasmodesmal closure would clearly be useful both for wound isolation and in response to a variety of environmental stresses. During wounding, PD can also be sealed off by callose deposition. Depending on the extent of deposition, which can be transient, the transport pathway is either constricted or completely blocked (Lucas et al., 1993).

The effects of the environment on plasmodesmal trafficking can also be demonstrated by the response of the plant to pathogens. Plant viruses can dramatically alter plasmodesmal function (Carrington et al., 1996; Ghoshroy et al., 1997), but viral transport through PD is apparently still subject to host-induced modification of plasmodesmal function. Indeed, factors that normally influence plasmodesmal transport, such as the developmental stage of the plant and

its physical environment, affect the movement of viruses (Matthews 1991). For example, under long-day photoperiods, Arabidopsis plants mature quickly and are relatively resistant to the systemic spread of the cauliflower mosaic virus. However, plants grown in short-day photoperiods are susceptible to the systemic spread of this virus (Leisner et al., 1993).

In the context of this review, it is notable that little attention has been paid to the role of intercellular transport in signal transduction pathways mediating plant defense responses; it is simply assumed that signaling molecules move. Hypothetically, genes induced in response to pathogen attack or elicitor treatment, such as those encoding the pathogenesis-related (PR) proteins, may be targeted to PD, where they may alter PD function or structure. In fact, a maize PR protein has recently been localized to PD (Murillo et al., 1997). Several PR protein genes also are induced during systemic acquired resistance, a suite of responses that confer resistance to a broad range of pathogens (Ryals et al., 1996). Potentially, then, systemic acquired resistance may also depend on intercellular transport of signaling molecules. Although most of these signaling molecules have yet to be identified, some may be transported symplastically through PD.

PLASMODESMAL STRUCTURE AND FUNCTION

General Structure of PD

Elegant electron microscopy studies of several plant species have revealed a general structure for PD as complex plasma membrane–lined pores containing appressed membranes of endoplasmic reticulum (ER), termed the desmotubule (DT), in their center (Figure 2A; reviewed in Lucas et al., 1993; Overall and Blackman, 1996). Diffusion of lipids along the ER of the DT demonstrates that it is contiguous with the ER in neighboring cells (Grabski et al., 1993). Additional unidentified plasmodesmal structural components are located along the membranous components and in the cytoplasmic sleeve between the DT and the plasma membrane (Figures 2B and 2C).

PD can be defined morphologically as simple (unbranched) and branched or developmentally as primary and secondary. Simple primary PD are single unbranched channels that form at the cell plate during cytokinesis (Strasburger 1901). Branched PD, which consist of multiple cytoplasmic strands ("channels") interconnected by a median cavity, form by the deposition of cell wall material over branched ER strands or by the coalescing of preexisting PD (Lucas et al., 1993; Ehlers and Kollmann, 1996). Secondary PD form completely de novo in preexisting cell walls. They occur in graft unions, chimeras, and during host–pathogen interactions (reviewed in Robards and Lucas, 1990; Lucas et al., 1993). The synthesis of secondary PD during plant development allows the symplastic integration of cells that are not immediately clonally related (i.e., that do not share a cell plate).

Symplastic Transport via Dynamic Plasmodesmal Channels

Passive, diffusion-driven symplastic transport of small molecules, such as dextrans, through the PD is limited simply by the hydrodynamic size of the transported molecule (Terry and Robards, 1987). This transport is proposed to occur within the cytoplasmic sleeve, the space between the plasma membrane and the DT (Figure 2; Overall et al., 1982; Ding et al., 1992). From electron microscopy studies, the cytoplasmic sleeve of a PD in mesophyll cells appears to be subdivided into microchannels 1.5 to 2.5 nm in diameter (Ding et al., 1992). Microinjection experiments reveal that the basal SEL of mesophyll PD is 1 kD; this SEL corresponds to a 0.75-nm Stokes radius (Tucker, 1982; Goodwin, 1983; Wolf et al., 1989) and predicts a basal diameter for a microchannel of 1.5 nm, consistent with the dimensions observed by using electron microscopy. Microinjection studies also show that PD in different cell types can have different basal SELs. For example, PD connecting phloem sieve elements (SEs) and companion cells (CCs) were found to have a basal SEL of at least 10 kD compared with the 1-kD SEL of mesophyll PD (Kempers and Van Bel, 1997).

The discovery that plant viruses pirate PD for cell-to-cell movement of their genomes during infection provided compelling evidence that PD are inherently dynamic and can be upregulated for transport of large molecules such as plant viral movement proteins (MPs). Consequently, MPs have been used extensively to probe the regulation and function of PD (Carrington et al., 1996; Ghoshroy et al., 1997). Microinjection experiments show that MPs, alone or complexed with nucleic acid, can be transported by *N. tabacum* mesophyll PD (Fujiwara et al., 1993; Noueiry et al., 1994; Ding et al., 1995), suggesting that movement is likely conferred by the supporting MP. The nucleic acid binding activity of the MP presumably shapes viral genomes into thin structures that are compatible with the narrow dimensions of plasmodesmal channels (Citovsky and Zambryski, 1991).

MPs from several different plant viruses increase the plasmodesmal SEL of *N. tabacum* mesophyll cells to promote movement of 20- to 40-kD fluorescent dextrans (Wolf et al., 1989; Fujiwara et al., 1993; Poirson et al., 1993; Noueiry et al., 1994; Vaquero et al., 1994; Waigmann et al., 1994). For dextrans of 10 and 20 kD, which have Stokes radii of 2.4 and 3.2 nm, respectively, to move in gated mesophyll PD would require plasmodesmal microchannels with diameters >6 nm, more than double the observed basal size (Wolf et al., 1989; Fujiwara et al., 1993; Poirson et al., 1993; Noueiry et al., 1994; Vaquero et al., 1994; Waigmann et al., 1994). Thus, plasmodesmal channels must gate, that is, open, to allow the active transport of molecules >1 kD.

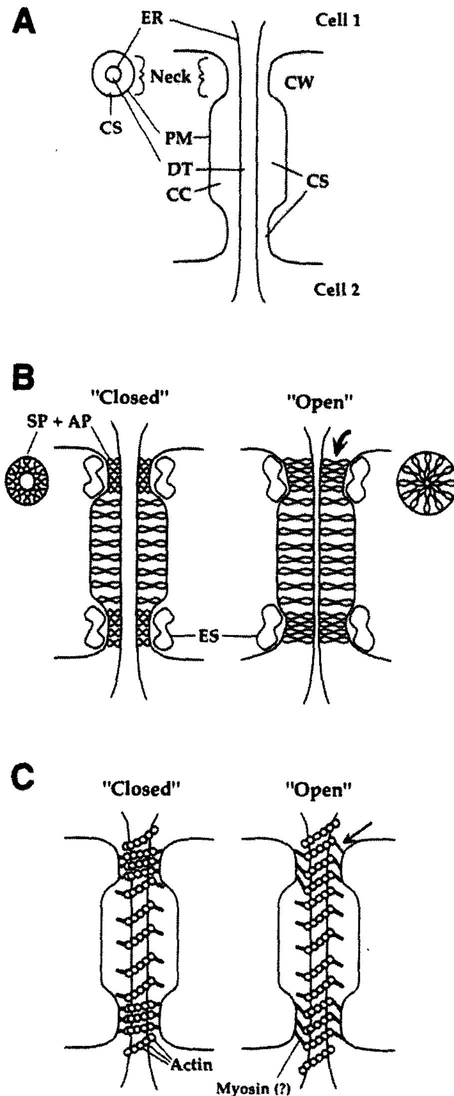

Figure 2. Structural Models of PD.

(A) "Skeleton" model emphasizing the membranes that delimit PD. The plasma membrane (PM), adjacent to the cell wall (CW, shaded), forms an outer boundary and is continuous between two cells. The DT, a tube of appressed ER, is located in the center of the PD. Between the DT and plasma membrane is the cytoplasmic sleeve (CS), which may include a central cavity (CC) region.

(B) Model for plasmodesmal function emphasizing structures seen using transmission electron microscopy. For simplicity, spokes (SP) and their associated proteins (AP) are shown here as a single (looped) structure. In this model, the DT provides a region for expansion of the neck and/or cytoplasmic sleeve. The arrow indicates the channel for movement through the cytoplasmic sleeve. An extracellular sphincter (ES) has also been proposed to modulate the structure of the PD (see Overall and Blackman, 1996).

(C) An actin-based model for plasmodesmal function. The packing of a polymer such as actin filaments (actin monomers are indicated by open circles) in the neck region may regulate molecular movement through PD. Changes in the polymer arrangement, possibly by a mechanism such as an actin–myosin interaction, could expand (indicated by an arrow) or contract a helical cytoplasmic pathway through the cytoplasmic sleeve.

Models for Plasmodesmal Architecture during Gating

Although limited data exist, many possible mechanisms and structural components are likely to be involved in gating PD. Potential mechanisms involving the modification of two plasmodesmal regions, the cytoplasmic sleeve and the neck, during active gating are illustrated in Figure 2. As shown in Figure 2B, appression of the ER in the DT results in increased space for macromolecular transport through the cytoplasmic sleeve. An open DT conformation where the ER is not appressed may reflect the physiological state of PD, that is, a closed state that only allows transport of molecules below the basal SEL. Gamalei et al. (1994) have argued that ER membranes compress under conditions of stress, such as cold temperatures or during fixation. Thus, the typical ultrastructural observation of desmotubular structures of PD, in which the membranes are either completely or partially appressed (Robinson-Beers and Evert, 1991; Ding et al., 1992; Botha et al., 1993; Evert et al., 1996), may be an artifact of sample preparation. Figure 2B also shows an extracellular sphincter, which has been proposed to modulate neck permeability and structure (Overall and Blackman, 1996).

Another potential mechanism involving rearrangement of components of the neck of the PD is shown in Figure 2C. Here, the neck opens for transport by shifting proteinaceous particles, perhaps actin (see below), that are associated with the plasma membrane and the DT. In some observations of plasmodesmal structure, "spokes" extend across the cytoplasmic sleeve. These spokes, which are postulated to link membrane-associated protein particles (reviewed in Overall and Blackman, 1996), could provide a contractile function to shift the proteins "blocking" the neck. For example, the interaction between contractile proteins, such as actin and myosin, may open the neck and increase the size of the cytoplasmic sleeve, thus expanding a cytoplasmic pathway through the PD (see below).

Expansion and contraction of plasmodesmal microchannels are easy to imagine but difficult to detect. Models mostly depict plasmodesmal channels as a linear tubular arrangement of microchannels traversing the cell wall. However, direct observation as well as computer-generated models suggest that microchannels are spirally arranged (Ding et al., 1992; Botha et al., 1993; Badelt et al., 1994; Overall and Blackman, 1996). Such a spiral arrangement may simplify the mechanics of moving macromolecules. In any case, studies of the nuclear pore, which reveal a highly elaborate double iris controlled by tens of different structural and regulatory

subunits (Davis, 1995), suggest that our current view of plasmodesmal architecture is undoubtedly an oversimplification.

A Potential Role for Cytoskeletal Elements in Plasmodesmal Structure and Function

Given the requisite dynamic nature of plasmodesmal channels, what provides the contractile function? Identification of cytoskeletal components in PD may have implications for how gating of PD occurs. Actin localizes to the plasmodesmal neck and along plasmodesmal channels, and treatment of cells with cytochalasin, an agent that depolymerizes actin filaments, causes the plasmodesmal neck region to appear greatly enlarged (White et al., 1994). Moreover, microinjection of substances that perturb actin filaments, such as cytochalasin or the actin binding protein profilin, dramatically increases mesophyll plasmodesmal SEL (Ding et al., 1996). Additional data suggest that myosin also localizes to PD (J. Radford and R. White, personal communication). Thus, actomyosin complexes may perform a contractile function at the neck region and may also determine the dimensions of the microchannels along the length of the cytoplasmic sleeve of the PD. It is intriguing to speculate that the spokes of electron-dense material between the DT and the plasma membrane may correspond to an actin–myosin contractile structure (Figure 2C; Overall and Blackman, 1996).

In plant and animal cells, reorganization of the actin cytoskeleton is linked via profilin to the production of the second messenger molecules inositol triphosphate (IP_3) and diacylglycerol (Goldschmidt-Clermont and Janmey, 1991; Aderem, 1992). IP_3 acts as a messenger to stimulate the release of calcium from intracellular stores (see Trewavas and Malhó, 1997, in this issue). In plants, IP_3 itself apparently moves through PD, and IP_3 and elevated free calcium levels decrease plasmodesmal SEL (Erwee and Goodwin, 1983; Tucker, 1988, 1990; Tucker and Boss, 1996). Potentially, the interplay between actin cytoskeletal rearrangement and physiological signals such as calcium and those in the inositol signaling pathway may direct the fine control of plasmodesmal permeability (Tucker and Boss, 1996).

The effect of calcium on cell-to-cell communication suggests that calcium binding proteins such as centrin could regulate the dimensions of the transport channel (Overall and Blackman, 1996). Centrin functions in a number of different filamentous structures that contract in response to increased intracellular calcium concentration and extend via ATP-mediated phosphorylation (Salisbury, 1995). Because reversible phosphorylation is involved in the regulation of many basic cellular processes, proof of the involvement of protein kinases in PD function is anticipated. In fact, a cell wall–associated protein kinase in tobacco leaves specifically phosphorylates the tobacco mosaic virus (TMV) at its C terminus (Citovsky et al., 1993). Because TMV MP traffics through PD, the cell wall–associated protein kinase may actually be an endogenous plasmodesmal component.

The study of viral MPs recently has highlighted another possible role for the cytoskeleton in the transport of molecules to and through PD. In tobacco protoplasts, which lack PD and thus restrict TMV MP movement to the cytoplasm, the TMV MP appears as filaments that colocalize primarily with microtubules (Heinlein et al., 1995; McLean et al., 1995). McLean et al. (1995) found that to a lesser extent, TMV MP colocalizes with actin filaments and that TMV MP binds to both actin and tubulin in vitro. The potential interaction between TMV MP and actin suggests that gating of PD by the MP involves an actin-based mechanism. Whether this presumed cytoskeletal association plays a primary role in driving intracellular transport of the MP (or MP–viral genome complexes) or in anchoring the MP in the cytoplasm for nucleoprotein complex formation remains to be determined. Cytoskeleton-mediated active intracellular transport of viral nucleoprotein complexes to PD is hypothetically advantageous to the virus because the viscosity of the cytoplasm would otherwise hinder the rapid diffusion of such elongated complexes.

Models for PD Transport

In addition to demonstrating that PD can gate to allow transport of molecules above the basal SEL, the use of viruses and their MPs also reveals that PD in various cell types can differ in response to viral MPs and/or in transport of dextrans. For example, potato virus X can promote the movement of large dextrans through *N. clevelandii* leaf trichome PD (Angell et al., 1996). TMV MP, however, cannot promote large dextran movement in these trichome PD, although it promotes such movement in *N. tabacum* mesophyll PD (Wolf et al., 1989; Waigmann et al., 1994; Waigmann and Zambryski, 1995). Whereas TMV MP cannot gate *N. clevelandii* leaf trichome PD to allow the diffusion of dextrans, TMV MP can mediate the movement of the 68-kD reporter protein β-glucuronidase (GUS) between these cells as a MP–GUS fusion protein (Waigmann and Zambryski, 1995). Thus, an essential plasmodesmal transport signal residing in the MP facilitates the transport of proteins between trichome cells. These data further imply that proteins and dextrans move through PD by different mechanisms; for example, size alone mediates diffusion of the dextran, whereas a signal sequence may help to shuttle the transported protein.

In an attempt to visualize macromolecular transport through PD, different strategies for mesophyll and trichome PD are presented in Figure 3 (Waigmann et al., 1997a). Two scenarios, both based on studies with TMV MP, are proposed for protein versus dextran transport in mesophyll (Figure 3A) and trichome (Figures 3B and 3C) PD. In Figure 3A, a protein containing a plasmodesmal transport signal is routed to the mesophyll PD along the cytoskeletal highway, first along the microtubules in the cytoplasm and then along the actin filaments associated with the PD. Upon arrival at the plas-

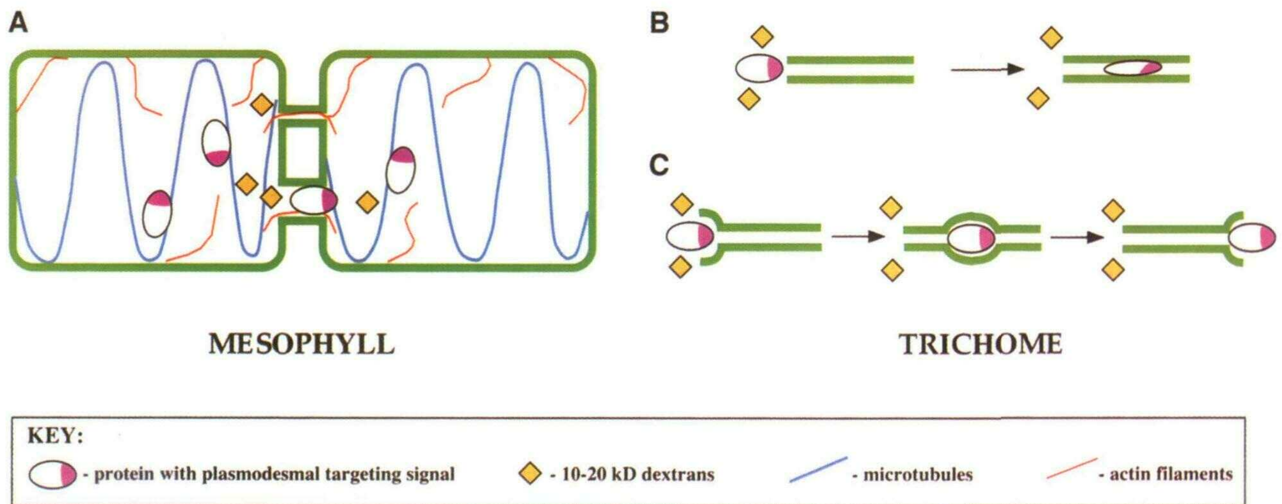

Figure 3. Mechanisms for Transport through PD.

(A) Mesophyll PD. A plasmodesmal-targeted protein (the plasmodesmal targeting signal is represented in pink) gates the whole length of the PD simultaneously (compare upper "closed" PD to lower "gated" PD). Interaction with cytoskeletal elements (see key) may transport the protein to and through the PD and/or may gate the plamodesmal neck. The gating of the PD along its entire length would provide easy access to the transport channel, facilitating diffusion of large dextrans (see key) from cell to cell.

(B) Trichome PD, model 1. In both this and Figure 3C, only the PD are depicted. The protein (containing the plasmodesmal targeting signal) is unfolded (indicated by shape change) to allow transport through the PD. Because dextrans are not covalently linked to a plasmodesmal targeting signal, dextrans larger than the basal SEL cannot move through the PD.

(C) Trichome PD, model 2. In this model, gating of trichome PD occurs, but unlike mesophyll PD, the entire length of the PD is not gated at the same time. Once the protein passes by, that region of the PD rapidly returns to the basal SEL, impeding the relatively slow diffusion of large dextrans into and through the long trichome PD.

modesmal neck, the protein gates the mesophyll PD (represented by the upper open PD), potentially by modifying actin at the neck; the protein then is transported actively through the channel by interacting with actin filaments traversing the PD. In this scenario, the whole length of the PD is gated simultaneously, which would provide easy access to the transport channel and thus facilitate the diffusion of large dextrans from cell to cell. Such gating may be a necessary prerequisite for proteins to gain access to the transport channel because mesophyll PD have a low basal SEL of 1 kD.

Two different models are presented for trichome PD (Figures 3B and 3C). Microinjection of TMV MP with fluorescent dextrans suggests that *N. clevelandii* trichome PD are not gated to allow diffusion of dextrans >7 kD, the basal SEL for these PD (Waigmann and Zambryski, 1995). Previous studies suggested that the basal SEL for *N. clevelandii* trichome PD is 1 kD (Derrick et al., 1992); the different SELs may be a consequence of variability in plant growth or experimental conditions. Unlike mesophyll PD, then, transport through trichome PD may require that (1) the molecule is linked directly to a plasmodesmal targeting signal and/or (2) the molecule is modified, that is, unfolded, to fit the basal SEL. As shown in Figure 3B, arrival of a protein with a plasmodesmal targeting signal at a trichome PD does not result in gating. Instead, the protein is unfolded, possibly by interacting with a chaperone, to allow transport through the PD. Here, the higher basal SEL of 7 kD is sufficient to allow protein transport. Because the PD is not gated, dextrans larger than the basal SEL could not diffuse through the PD. In this scenario, the native "size" of the transported molecule would not appear to be a major selection criterion for its movement through trichome PD (Waigmann and Zambryski, 1995). Instead, the molecule must fold into a transferable conformation; potentially, the plasmodesmal targeting signal may also include a "conformation change" signal.

The model shown in Figure 3C suggests that gating of trichome PD is rapid and transient and takes into account that, because of the thick cell walls, trichome PD are longer than are mesophyll PD (Waigmann et al., 1997b). In this model, gating by proteins with plasmodesmal targeting signals occurs in trichome PD as well as in mesophyll PD, with the signal responsible for gating being rapidly transmitted along the PD. As shown in Figure 3C, upon arrival at the trichome PD, the protein gates the neck of the PD to enter the transport channel. The gating signal then moves along the PD coincident with the protein. Once the protein moves through, that region of the PD rapidly returns to the basal SEL. In the long PD of trichome cells, propagation of the gating signal results in the opening of small regions of the PD; the rapid closing of the entrance and transport channel of the PD

would impede the relatively slow diffusion of large dextrans into and through the long trichome PD. In the shorter PD of mesophyll cells, however, propagation of the gating signal allows the entire length of the PD to be open; consequently, dextrans can easily diffuse through during active protein transport. Thus, models presented in Figures 2 and 3 illustrate what is known and not known about the mechanisms that regulate molecular transport through PD in various cell types.

Plasmodesmal Transport of Endogenous Plant Proteins

Although the study of viral MPs demonstrated that PD could transport large proteins, evidence for the involvement of PD in the transfer of endogenous proteins between plant cells is only recent. More than 100 proteins, ranging in molecular weight from 10 to 90 kD, apparently move from CCs into the enucleate SE of the phloem, the long-distance photoassimilate transport system (Fisher et al., 1992; Nakamura et al., 1993; Sakuth et al., 1993). Because the mature SEs of the phloem lack nuclei and ribosomes and thus are incapable of protein synthesis, all macromolecules must be transported into SEs from neighboring CCs, presumably through PD (see Sjölund, 1997, in this issue). The presence of ubiquitin and chaperonins in castor bean phloem sap suggests that unfolding and other modifications are involved in protein translocation through PD connecting CCs and SEs (Schobert et al., 1995). Plasmodesmal transport from the CC into the SE also appears to be selective because not all proteins detected in CCs are found in SEs (Schobert et al., 1995).

The concept that plasmodesmal transport plays a role in development is strengthened by studies suggesting that plant developmental regulators can move between cells. For example, molecular, genetic, and microinjection studies suggest that a plant transcription factor, the maize homeodomain protein KNOTTED1 (KN1), moves from cell to cell. During maize development, RNA encoding KN1 is found in all cell layers of the meristem except the outermost layer; KN1 protein, however, is found in both the inner and outer layers (Smith et al., 1992; Jackson et al., 1994; see Clark, 1997; Kerstetter and Hake, 1997, in this issue), and clonal analyses show that *kn1* action in the inner layer influences adjacent outer-layer cells (Hake and Freeling, 1986; Sinha and Hake, 1990). Microinjection experiments show that the KN1 protein moves between tobacco mesophyll cells, facilitates the movement of dextrans and proteins <40 kD, and selectively transports *kn1* sense but not antisense RNA (Lucas et al., 1995). Two Antirrhinum MADS box family members, DEFICIENS and GLOBOSA, also may be transported from cell to cell. Similar to KN1, genetic, morphological, and molecular analyses of somatically stable chimeras suggest that these two floral regulators move from the inner cell layer to the epidermis (Perbal et al., 1996). A third Antirrhinum floral regulator, FLORICAULA, may also be transported from cell to cell; periclinal chimeras indicate that *FLORICAULA* expression in one cell layer results in the expression of other floral regulators in all layers (Carpenter and Coen, 1995).

That important regulators of plant development can move between cells has significant implications for the mechanisms plants use to program differentiation. For example, distribution as well as permeability of PD may regulate development by providing channels for the exchange of regulatory signals (Lucas, 1995; Zambryski, 1995). However, these intriguing studies provoke many questions. How does the cell regulate nuclear versus plasmodesmal localization of transported transcription factors? How common is macromolecular transport via PD during development? Do KN1, DEFICIENS, and GLOBOSA move selectively to the outermost cell layer, or do they move everywhere? What is the benefit of such transport? If *kn1* RNA is already actively expressed throughout the meristem, what does the plant gain by facilitating the movement of *kn1* RNA? These questions illustrate the complexity that plasmodesmal transport of developmental factors adds to the characterization and understanding of plant developmental processes.

FUTURE PROSPECTS

Although plasmodesmal research is progressing rapidly, our understanding of plasmodesmal structure and function is still naive. Crucial questions regarding the ultrastructure of PD may be resolved by new microscopy techniques, such as high resolution scanning electron microscopy, as well as by improvements in fixation methods. Molecular characterization of plasmodesmal structure and function will be greatly facilitated by selective extraction procedures for plasmodesmal components (Kotlizky et al., 1992; Turner et al., 1994). These methods have already led to the isolation of a 41-kD plasmodesmal protein from maize mesocotyl cell walls (Epel et al., 1996). Although well-known systems such as maize and tobacco continue to be indispensable for studying symplastic transport and signaling, several laboratories are now making use of Arabidopsis. That Arabidopsis is also the subject of intense genetic dissection should allow the molecular basis of homeostatic and regulated cell-to-cell communication to be defined. Indeed, Arabidopsis and maize mutants that are defective in cell-to-cell transport are currently being characterized as a way to identify plasmodesmal components genetically (Russin et al., 1996; V. Citovsky, unpublished results). This combination of ultrastructural, molecular, and genetic approaches should reveal the mechanisms and regulation of plasmodesmal transport as well as the roles of symplastic communication in the life of the plant.

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