REGULATION OF PLANT INTERCELLULAR COMMUNICATION VIA PLASMODESMATA

Insoon Kim, Ken Kobayashi, Euna Cho and Patricia C. Zambryski

Department of Plant and Microbial Biology
111 Koshland Hall
University of California
Berkeley, CA 94720

INTRODUCTION

Plasmodesmata (PD) are unique to plants, and are utilized to establish dynamic intercellular continuity between groups of cells enabling the transport of nutrients, developmental cues and ribonucleoprotein complexes (reviewed in (1-4)). Multidisciplinary investigations over the last decade provide evidence that plasmodesmatal regulation is critical to various basic plant functions such as development, host–pathogen interactions and systemic RNA silencing. This chapter highlights various tools used to study PD, and elaborates on the regulation of PD during plant development.

PLASMODESMATA: STRUCTURES AND FUNCTIONS

Generic simple PD have two major components, membranes and spaces (5) (Figure 1). Membranes constitute boundaries of the PD channel. The plasma membrane (PM) of two neighboring cells form the outer boundary of PD.

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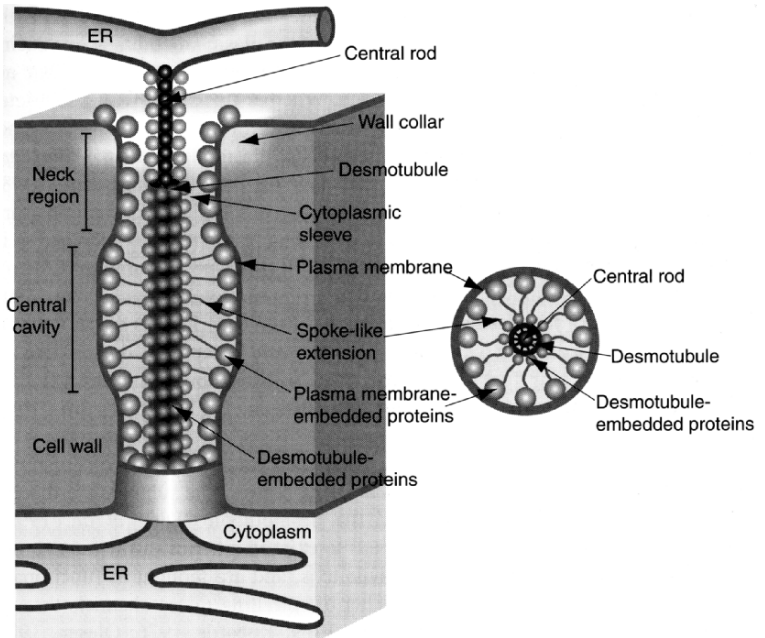

Figure 1. Diagram of simple PD. A longitudinal view in the left and a transverse view in the right, reprinted with permission from (5).

Appressed endoplasmic reticulum (ER), termed the desmotubule (D), runs through the axial core of PD and forms the inner boundary. The space between PM and D is the cytoplasmic sleeve (CS), the primary passageway for molecular transport, which is continuous with the cytoplasm between adjacent cells. The CS is not empty. Instead, the CS is filled with proteinaceous molecules that likely regulate transport *via* PD. For example, actin and myosin along the length of PD (reviewed in (6)), and centrin nanofilaments at the neck region (7), may provide contractile elements to control PD apertures.

The functional measure of PD is their size exclusion limit (SEL), the upper limit of the size of macromolecules that can freely diffuse from cell to cell. PD SEL is regulated temporally, spatially and physiologically throughout plant development. PD selectively allows movement of proteins, such as transcription factors, and RNAs, such as mRNAs and silencing RNAs, both critical in cell-fate determination (reviewed in (8, 9)). Therefore, PD in different tissues may be regulated differentially, possibly by the involvement of developmentally-regulated factors.

When cells and tissues exhibit cell-to-cell transport of micro- or macromolecular tracers they are said to form "symplastic domains" of shared cytoplasm. Cells within symplastic domains share a common PD aperture (SEL) compared to cells in surrounding regions. Because symplastic domains are thought to form during differentiation of tissues/organs, the determination of which cells and tissues in the plant are in communication *via* PD is an area of active investigation.

Such studies reveal communication domains for developmental/morphogenetic signaling. Below we review PD function during adult, seedling and embryonic plant development.

PD Function During Adult Plant Development

Research on PD has made exponential progress in the last several years due to technical innovations. The major targets of PD research in adult plants are leaves (see Figure 2 for plant diagram), due to their ready accessibility. The first approach used to examine PD function was microinjection of fluorescent probes. Historically, this approach revealed that PD SEL was less than 1 kDa (10), and only few specialized viral (11) or homeodomain proteins (12) could dilate PD beyond their innate small apertures.

The use of green fluorescent protein (GFP) and its introduction by biolistic bombardment dramatically altered this view, revealing an inherent complexity

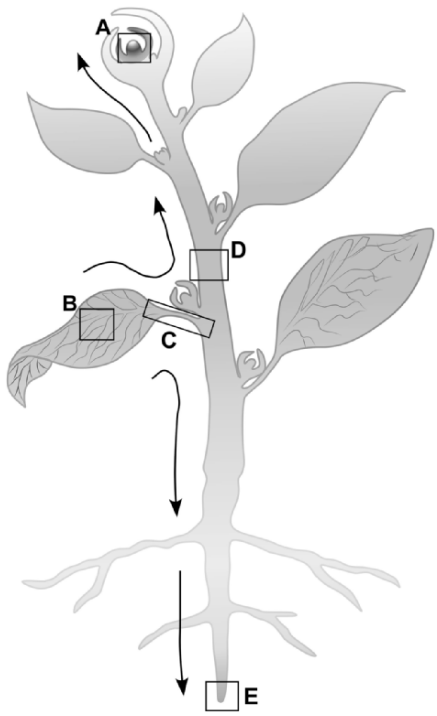

Figure 2. Plants use a combination of local and long-distance signaling to orchestrate proper function throughout the whole plant. Signals perceived/generated by leaves (B) are transmitted along the vascular systems of petioles (C) and stems (D), and then delivered to distant organs such as the shoot apical meristem (A) and the root tip (E). Signals generated in leaves and transported through the phloem reflect environmental changes (light, temperature, mineral nutrients, and water availability), physiological programs, developmental cues, and pathogenic attacks. Arrows indicate the transport by the phloem of the vascular system. Adapted from (3) with permission.

of PD function. Basically, plant leaves are bombarded with DNA constructs to express GFP (27 kDa) or its larger-sized protein fusions. Remarkably, such studies revealed that proteins at least 50 kDa were able to traffic cell-to-cell by passive diffusion (13, 14). PD aperture in leaves is developmentally regulated. Younger leaf cells contain PD with more dilated aperture than older leaves (as measured by different-sized GFP tracers) and this function is correlated with structural changes in PD that occur during leaf maturation. Quantitative studies, one of the benefits offered by biolistic bombardment over microinjection, reveal that even a single leaf is composed of PD with various apertures that likely respond dynamically to environmental and physiological changes (15).

PD aperture and protein size obviously govern passive macromolecular traffic. Given that size and aperture are synchronous with each other, can all macromolecules move cell-to-cell like GFP? Such rampant exchange would lead to loss of critical cell components. By fusing GFP to several localization sequences, such as ER retention or cytoskeleton anchoring, it was determined that cellular location dictates whether or not a protein can move cell-to-cell (14). Thus, exogenous tracers such as GFP can move by default as they do not contain cellular targeting signals. However, cells likely sequester or anchor their proteins according to their functions and thereby protect against non-specific intercellular transport.

Another method to measure PD conductivity, phloem loading, takes advantage of the plant vascular system. Fluorescent membrane impermeable tracers (once they are in the cytoplasm, they can move cell-to-cell only *via* PD) are loaded from the end of cut petioles, the little branch remaining after removal of leaves (see Figure 2 for plant parts). Tracers load into and move along the phloem. Tracers can then "unload" *via* PD connections between the phloem and surrounding cells in sink leaves or at the shoot apex. As tracer movement is imaged at a distance from the site of initial wounding and loading, this method is less invasive than microinjection or biolistic bombardment. Tracers can even move up to the top of the plant, to the shoot apical meristem (SAM), a group of stem cells that gives rise to all the above-ground plant organs following germination.

For example, this approach reveals that PD in the SAM are dynamically regulated. During vegetative development, when the plant continuously produces new leaves, the cells at the SAM allow transport of small (~0.5 kDa, see below) symplastic tracers. However, during the transition from vegetative to reproductive development, when the plant starts producing flowers, PD at the apex are downregulated and no transport of tracers occurs (16, 17). Potentially, a signal molecule that regulates flowering is symplastically transported to the apex from leaves. The apex may then shut down further communication while it undergoes the profound morphological changes that accompany the switch to floral production. Interestingly, symplastic transport to the apex resumes once floral commitment is established. Such studies highlight the important role of PD during plant development.

The use of tissue-specific promoters to drive expression of fluorescent reporter proteins offered the next significant leap for PD research. In this approach, transgenic plants were constructed to express a soluble diffusible GFP (or GFP-fusion protein) in specific cells/tissues using a specific promoter. For example, soluble GFP expressed in the companion cells (CC) of source leaves (net

export of photosynthetic products) of tobacco and Arabidopsis (13, 18) moves toward regions of new growth, such as sink leaves (net import of photosynthetic products) and newly-emerging floral organs. Strikingly, GFP was observed to move throughout all plant tissues and organs, albeit to more or less extents depending on the tissue. Thus the PD SEL is at least 27 kDa in many regions of the plant. Such movement implies that endogenous macromolecular signals may traffic the phloem to facilitate new development.

PD Function During Seedling Development

Phloem-loading together with novel fluorescent probes made it possible to track the cell-to-cell movement of symplastic probes, both locally and long distance, in whole seedlings just after germination from the seed (19). This approach is especially suited to the model plant Arabidopsis, as seedlings are small (~1 cm for the shoot and ~3 cm for the root of one-week-old seedling) and thus the whole plantlet can be viewed easily under the fluorescent microscope. Early studies used small (~0.5 kDa) tracers such as carboxyfluorescein (CF) diacetate and 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS). The ester (uncharged) form of CF diacetate freely moves across plasma membrane. A cytosolic esterase then converts this probe to the anionic membrane impermeable form, trapping CF in the cytoplasm. CF diacetate applied to cut leaves of Arabidopsis seedlings translocates *via* the phloem and unloads into growing root tips (19) to reveal symplastic coupling between young root cells (see Figure 2). HPTS was found to be a more reliable tracer as it is highly anionic and localizes entirely to the cytoplasmic compartment for intercellular transport *via* PD. HPTS loading revealed that the epidermis of the root becomes symplastically isolated from inner cells as development proceeds (20). While such phloem loading offers an excellent non-invasive means to monitor PD function, especially over long distances, this method can only measure the transport of small probes.

An elegant series of experiments revealed macromolecular movement in Arabidopsis seedling roots, and the precision whereby PD can control such movement. In particular, the SHORTROOT (SHR) transcription factor (TF) was found to move cell-to-cell in developing roots. The SHR TF is required for the normal differentiation of cortex/endodermal initial cells that control the formation of the endodermis. Surprisingly, transcription of *SHR* (shown by *in situ* mRNA localization and a transcriptional fusion of GFP to the promoter of *SHR*) is absent from cortex/endodermal initial cells or its daughter cells. Instead, *SHR* mRNA is present in the internally adjacent cells of the stele (Figure 3, inset). However, SHR protein (shown by immunolocalization and a translational fusion of GFP to the coding sequence of SHR) localizes to the stele and the cells of the adjacent cell layer of the endodermis, which includes the cortex/endodermal initial cells and the quiescent center (Figure 3). These results imply SHR-GFP traffics from the stele to a single adjacent layer of cells, where it functions to promote asymmetric cell division and endodermal cell fate.

Over the years, additional studies using genetics and *in situ* gene expression have revealed that other plant TFs can move cell-to-cell *via* PD. The classical example is the maize KNOTTED1 (KN1) protein, discovered in 1994 to move

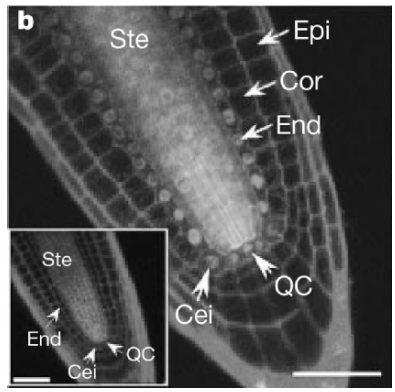

Figure 3. Intercellular movement of SHORTROOT (SHR) protein in Arabidopsis root. SHR proteins as GFP fusions (shown as grey regions) localize both in the stele (Ste) and endoderrmis (End), while *SHR* transcript locates only in the stele (inset). Reprinted from (59) with permission.

one cell layer in the shoot apex. KN1 is a homeodomain containing protein that regulates leaf and shoot meristem development. KN1 and its mRNA traffic from cell-to-cell in the shoot apical meristems and leaves (21). LEAFY (22) that controls floral meristems identity, and CAPRICE (23), that is central to root hair cell formation, are other examples of TFs that move cell-to-cell. More information about the movement of TFs *via* PD is reviewed in (9, 24-26).

For readers interested in additional studies on the role of PD during postembryonic development, we mention a few articles as starting points. Two recent studies analyze the transport of GFP tracers in early seedling leaves (27, 28) and roots (28). See also the role of symplastic communication in morphogenesis of postembryonic tissues such as gametophytes, leaf, root, stem, flower and shoot apical meristem of land plants and algae (20, 29-34). Note that symplastic isolation occurs in different manners and to various degrees, permanent *versus* transient, and complete PD closure *versus* reduced PD aperture, and symplastic domains differentiate into tissues with distinct structures and functions.

PD Function During Embryonic Stages: A Transient Assay to Identify PD Genes

While PD function and ultrastructure have been extensively analyzed, until recently few studies have addressed what genes control PD. Genetics is a powerful tool to isolate potential PD genes, yet PD research using genetics is quite limited (35-37). One obstacle to such an approach is, given that PDs are essential to plant growth, most PD mutants are unlikely to grow to adult plants. However, while PD mutants cannot be easily identified at the adult plant level, PD mutants should manifest early in development during embryogenesis. Such lethal PD mutants can be propagated as heterozygous plants that then display their homozygous defective phenotype in embryos segregating in seedpods or fruit. One Arabidopsis fruit (called silique) contains 40-60 seeds in which embryos are enclosed (think of a pea

pod with 40 peas, but much smaller) (Figure 4A). Thus, 10-15 homozygous mutant embryos will be segregating in a single silique.

The next hurdle was to develop a strategy to test PD function during embryogenesis. First embryos need to be released from their seed coats. Seeds are extruded from siliques and collected in a glass slide. Application of a cover glass and slight pressure releases embryos. This extrusion process induces sublethal tears in plasma membranes and cell walls in the outermost cell layer of embryos. Such breaks provide initial entrance sites for symplastic tracers of various sizes (Figure 4D). Probes larger than PD SEL of the cells at the break site are trapped in the initial cells (Figure 4B,C) and cannot move, whereas tracers smaller than the PD SEL move cell-to-cell *via* PD (Figure 4D).

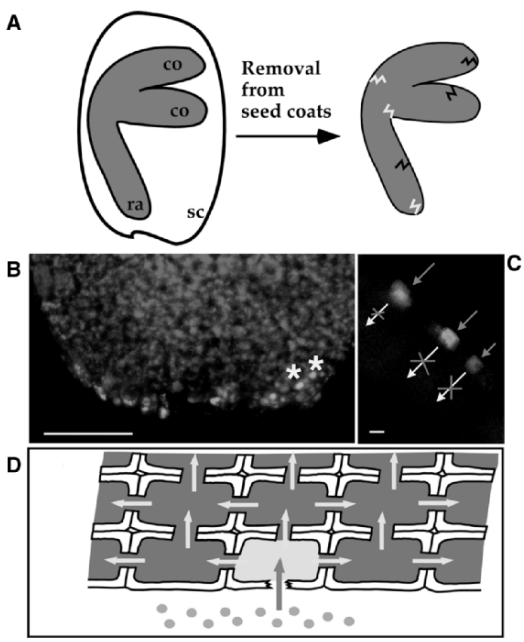

Figure 4. Uptake of symplastic probes in cells of Arabidopsis midtorpedo embryos. (A) When embryos are released from their seed coats, physical damage occurs in a subset of cells. As a result, small regions of cell walls and plasma membranes are broken to a sublethal level to provide an initial entrance site for uptake of symplastic tracers such as HPTS and F-dextran, which do not cross plasma membranes. Jagged lines indicate the most common site of damage. co, cotyledon; ra, radicle; sc, seed coat. (B) A small number of cells at the base of the detached cotyledons from midtorpedo embryos are cytoplasmically loaded with 10 kDa F-dextran (asterisks), yet further movement to neighboring cells does not occur. Scale bar, 50 µm. (C) A typical example of loaded cells in a region containing abrasion at the edge of the protodermal layer, marked as jagged lines in (A). Individual cells in the protodermal layer take up 10 kDa F-dextrans (arrows) and show cytoplasmic localization of the probe. However, subsequent movement of the probe is inhibited (arrows with X). Scale bar, 5 µm. (D) A diagram shows a partially broken cell wall and plasma membrane (jagged edge) may provide the initial entrance site for uptake of symplastic tracers, F-dextran or HPTS (circles). Further symplastic transport is then determined by the PD SEL and the size of symplastic tracers introduced. Reprinted with permission from (37).

In this transient assay, HPTS (0.5 kDa) or fluorescently (F)-labeled 10 kDa dextrans were exogenously introduced into developing embryos. HPTS moves through all cells of embryos throughout all stages of embryonic development examined (early heart to midtorpedo), demonstrating that the embryo is single symplast. However, the use of higher molecular weight tracers reveals that PD aperture is downregulated as development proceeds. 10 kDa F-dextrans are transported cell-to-cell in 50% of heart, 20% of early torpedo, and 0% of midtorpedo embryos. Thus, while symplastic connectivity remains (as measured by small tracers such as HPTS), PD SELs are altered during development.

Over 5,000 lines of Arabidopsis with an embryo defective phenotype were screened by the above assay to detect mutants that continued to traffic 10 kDa F-dextran at the midtorpedo stage. Fifteen lines, called *increased size exclusion limit of plasmodesmata* (*ise*), were identified (37). Two lines, *ise1* and *ise2*, are currently under investigation to identify their defective genes and characterize their role in PD function and/ or structure.

PD Function During Embryonic Stages: Analysis of Symplastic Domain Formation during Embryogenesis

Besides providing a genetic tool, embryos are innately interesting subjects for investigation of intercellular transport patterning. Embryogenesis is a critical stage of plant development that sets up basic body axes enabling the development of different tissues and organs. Arabidopsis embryos have regular pattern of cell divisions that allow the tracking of the origin of seedling structures back to specific groups of cells in the early embryo (38, 39). The seedling shows an apical–basal pattern along the main axis composed of structures such as shoot apical meristem (SAM), cotyledons, hypocotyl and root (Figure 5I). Clonal analyses and histological techniques predict the contribution of each embryonic cell to this body plan (40) (Figure 5I, compare heart and seedling). Generally, positional information determines the overall body pattern, and lineage-dependent cell fate specifies local patterning (40-42). Auxin signaling as well as differential gene expressions then facilitate specific morphogenesis (reviewed in (43, 44)).

Cell-to-cell signaling *via* PD is an important factor to coordinate embryonic development. However, until recently no studies have directly addressed PD function during embryogenesis. Now evidence suggests that PD also conveys positional information during axial patterning in late embryogenesis (see below). For these studies, stable (*versus* transient introduction of tracers) expression of GFP in specific regions of the embryo was investigated.

Subdomains Corresponding to Axial Body Pattern

Two different promoters were used to drive GFP expression in meristematic regions of Arabidopsis embryos. The *SHOOT MERISTEMLESS* (*STM*) promoter was used to express 1X, 2X and 3XsGFP (single 27 kDa, double 54 kDa and triple 81 kDa forms of sGFP) in the shoot apical meristem (SAM) and a subset of cells in the hypocotyl (45). In addition, the cell-type-specific enhancer of the J2341 line-induced expression of 2XsGFP in the SAM and the root apical

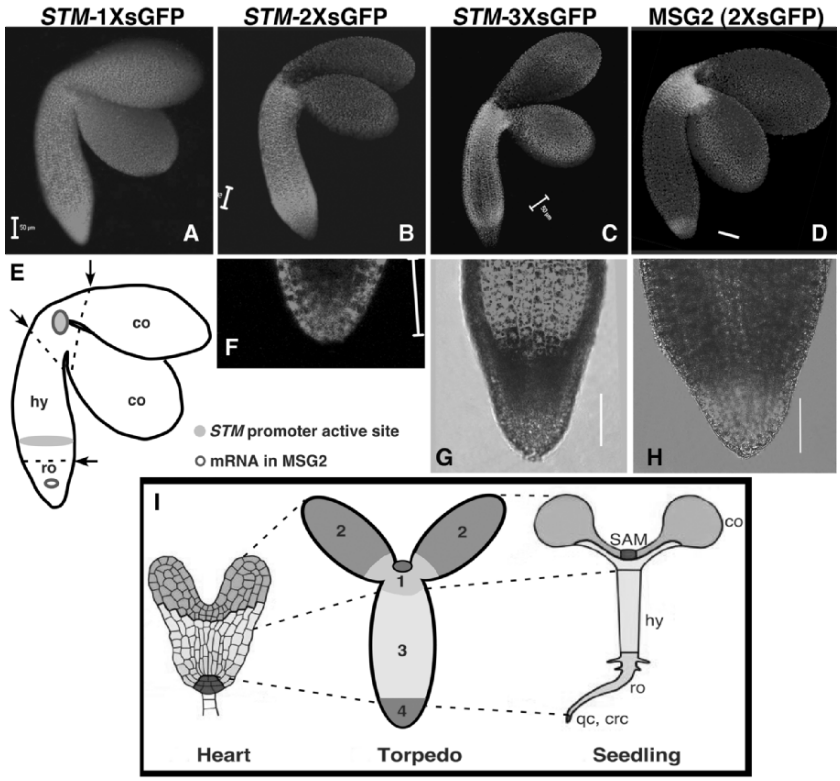

Figure 5. sGFP movement in Arabidopsis midtorpedo embryos. 1XsGFP expressed by the *STM* promoter in the SAM and the base of hypocotyls (hy) (E) freely moves throughout the whole embryo (A). 2XsGFP fails to move into cotyledons (co) (B) but moves to the root tip (F). 3XsGFP fails to move to the root (ro) as well as cotyledons (C,G). These results indicate the formation of at least two symplastic subdomains, e.g. the cotyledon and root. 2XsGFP expressed in the SAM and RAM in MSG2 line (E) stays within subdomains of the shoot apex and the root, respectively (D,H). These results, together with (B), reveal the boundary between the shoot apex and hypocotyl subdomains. Root subdomains from embryos in (C) and (D) are shown in larger magnification views under each whole midtorpedo image, and include quiescent center (qc), part of the RAM, and central root caps (crc). (E) Origin of MSG2-mediated expression is indicated by empty circles at SAM and RAM, and origin of *STM*-mediated expression is indicated by shaded circles at the SAM (same as MSG2) and the lower part of hypocotyl. (I) Four symplastic subdomains in torpedo embryos, shoot apex (1) including SAM (a dark circle), cotyledons (2), hypocotyl (3), and root (4) are extrapolated to the body parts in heart embryos and seedlings. Same shading in heart embryo and seedling represent regions of development with common clonal origins. Subdomains of the torpedo embryo, determined by their cell-to-cell transport *via* PD, also correspond to the apical-basal body pattern of the heart embryo (and seedling) by their positions; these regions are diagrammed with different shadings to indicate they were defined by a different assay. Scale bars, 50 μm. Reprinted with permission from (27).

meristem (RAM) in the MSG2 line (Figure 5E) (27). The subsequent movement of these various-sized tracers from their site of synthesis was monitored at three stages of embryogenesis to reveal two major findings. First, 2XsGFP (54 kDa) moves throughout the entire early heart embryo demonstrating that PD apertures

(interconnecting cells to form a single symplast) in early embryos are quite dilated. Secondly, different regions of the embryo have distinct PD SELs defining symplastic subdomains by the midtorpedo stage. These subdomains correspond to the major regions of the apical-basal body axis, the shoot apex, cotyledons, hypocotyls and root. (See Figure 5 and legend) (27). These subdomains can be extrapolated to regions of the early embryo (and seedling) defined by gene expression profiles and clonal analyses (Figure 5I).

Boundaries Between Symplastic Subdomains of Cell-to-Cell Transport

The above data imply that there are boundaries between each of four symplastic subdomains where the embryo controls intercellular transport (45). Each boundary has a distinct PD SEL. For example, the boundary between the shoot apex and the cotyledons has a SEL between 27 and 54 kDa, as 1XsGFP but not 2XsGFP moves from the SAM to the cotyledons (Figure 5A, B, and E). The boundary between the hypocotyl and the root has a SEL between 54 and 81 kDa, as 2XsGFP but not 3XsGFP moves from the hypocotyl to the root (Figure 5F and G). The hypocotyl and shoot apex subdomains are indicated by the movement of 2XsGFP from its site of synthesis at the SAM and surrounding cells in MSG2, and its failure to move to the hypocotyl (Figure 5D and E). Movement of 2X and 3XsGFP in the hypocotyl subdomain results from upward movement from its site of synthesis (under the *STM* promoter) near the hypocotyl-root junction (Figure 5B, C, and E). The existence of the root and cotyledon subdomains was further investigated in transgenic plants expressing 1X or 2XsGFP fused to the P30 movement protein (MP) of *Tobacco mosaic virus* (TMV), also under the control of the same *STM* promoter (45).

TMV P30 localizes to PD in virus-infected cells (46) and in uninfected transgenic plants expressing P30 (47). TMV P30 acts as a molecular chaperone to bind the single-stranded viral RNA genome and targets this ribonucleoprotein complex to PD, where it triggers an increase in PD SEL (called gating) to facilitate movement of the TMV genome into adjacent uninfected cells (reviewed in (48)). In embryos, GFP-P30 targets to PD as in adult plants, and moves more extensively than similarly sized GFP tracers, confirming the functionality of P30. However, 1XGFP-P30 (57 kDa) and 2XGFP-P30 (84 kDa) behaved as the similarly-sized 2XsGFP (54 kDa) and 3XsGFP (81 kDa) in their inability to be transported into cotyledons and roots, respectively (45). These data reinforce the existence of boundaries between symplastic subdomains in embryos.

Further Refinement of Local Symplastic Subdomains

To date additional symplastic subdomains, corresponding to the protodermis and stele, have been observed. When 1XsGFP was expressed in the outermost protodermal layer of the hypocotyl, under the control of the Arabidopsis *GLABRA2 (AtGL2)* promoter, it moves uniformly inward to internal ground tissues and to neighboring protodermal cells in cotyledons at the heart stage (see Figure 3F of (49)). However, in the early torpedo stage, centripetal movement of 1XsGFP from the protodermis is reduced such that GFP signal intensity is now

much weaker in ground tissues, while movement among cells in the protodermis continues (Figure 6A). Similarly, 1XsGFP expressed in the root tip, by the Arabidopsis *SUCROSE TRANSPORTER3 (AtSUC3)* promoter, freely moves to the hypocotyl in earlier stages (49), but becomes restricted to the stele in the midtorpedo stage (Figure 6B).

Note that the extent of symplastic movement is significantly affected by the location of the initial site of sGFP synthesis. 1XsGFP freely moves to every cell in embryos following expression in the SAM (27, 45), but its movement is limited to within the stele upon expression from a subset of cells in the root tip (49) (compare Figures 5A and 6B). It makes sense that PD in and around the SAM are more active than those in the root tip, as meristems are likely the source of morphological signals to enable patterning during embryogenesis. Future studies need to address how the SAM (and RAM) contribute to the formation of symplastic subdomains to determine the apical-basal body pattern, and how symplastic sudomains corresponding to various tissue types are controlled locally.

Symplastic Domains in Developing Seed Coats

The Arabidopsis seed coat consists of five cell layers, the innermost endothelial layer, followed by two cell layers each of inner and outer integuments. Two symplastic domains, corresponding to the outer and the inner integuments, were identified in developing seed coats (49). GFP expressed in the outer integument cannot move to the inner integument layers (Figure 6D). Similarly, GFP

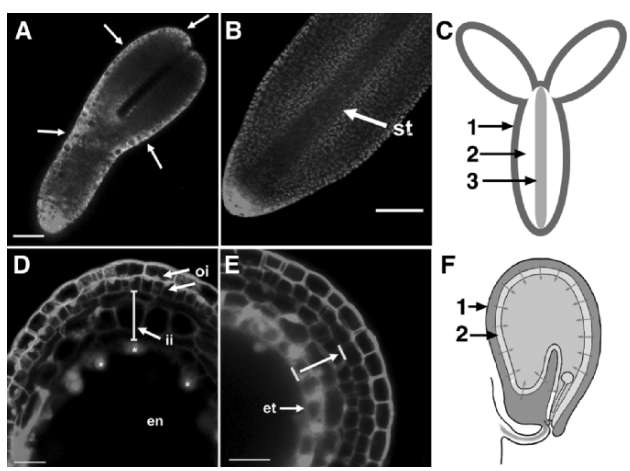

Figure 6. More subdomains in embryos and seed coats. The protodermis (A, arrows, and C1) and the stele (st) (B, C3) form subdomains where the movement of 1XsGFP, expressed by *AtGL2* and *AtSUC3* promoter, respectively, is allowed within domains but is reduced (A) or blocked (B) to cells beyond each domain. Outer integuments (oi) (D, F1) and inner integuments (ii) (E, F2) form separate symplastic domains where 1XsGFP movement is blocked across a boundary between the two domains. C2, ground tissues; en, endosperm; et, endothelium. Scale bars, 40 μm (A), 50 μm (B), 25 μm (D) and 20 μm (E). Reprinted with permission from (49).

expressed in the innermost endothelial layer moves to the inner integument layers, but cannot move to the outer integument layers (Figure 6E). Even small tracers such as HPTS (0.5 kDa) are not transported across the boundary between the outer and the inner integuments. Stadler *et al*. suggested that the outer integuments may provide a symplastic route for nutrient transport from maternal tissues to developing seeds, but that transfer between the outer integument and the inner integument, and the inner integument to the embryo may be apoplastic (49).

MORE APPROACHES TO IDENTIFY PD COMPONENTS

Although it is now established that PD have dynamic and critical roles in various aspects of plant life, no components specific to PD are known. In addition to the genetic approach mentioned above, several different approaches have been conducted in an effort to uncover structural or functional components of PD. A biochemical approach uncovered one *Nicotiana tabacum* NON-CELL AUTONOMOUS PATHWAY PROTEIN 1 (NtNCAPP1), from PD-enriched cell wall extracts as an interacting partner to a PD-trafficking protein (CmPP16) by affinity purification (50). NtNCAPP1 locates in the cell periphery and contains ER transmembrane domain which deletion blocks the movement of specific PD-trafficking proteins, suggesting that protein movement *via* PD is both selective and regulated. A plasmodesmal-associated protein kinase (PAPK) specifically interacts with plant viral proteins, such as TMV P30, and localizes to PD. Since P30 is known to manipulate PD, PAPK may act to regulate PD function (51).

A collection of random plant cDNA-GFP fusions and their localization in cells generated a library composed of GFP tags to specific plant organelles including PD (52). Another high-throughput screening where plant cDNA-GFP fusions were expressed by a viral expression system identified twelve proteins specifically localized to PD (53). A punctate pattern in cell walls is diagnostic for labeling and localization to PD. Half of the twelve-encoded proteins share no similarity with known proteins and may represent novel components of PD.

Proteomic technology is another approach to identify PD-specific proteins from purified PD or cell wall fractions enriched for PD (54). One protein found by several research groups is a class 1 reversibly glycosylated polypeptide (RGP). RGPs normally associate with the Golgi, but one RGP targeted to PD (55). The giant-celled green alga *Chara corallina* provides an advantageous system to apply proteomics (56) as cells are arranged in a single linear file and PD are localized to the cross walls between adjacent cells. Peptides isolated from PD-enriched cell wall fractions include previously known PD-associated proteins, validating the experiments, as well as novel proteins, providing new candidates for PD components.

PERSPECTIVES

The critical role of PD in plant development is supported by accumulating data of cell-to-cell movement of TFs critical in cell-fate determination. Recent data also suggest that RNAs, mRNAs and gene silencing RNAs (reviewed in (3, 57, 58)) also traffic *via* the vascular system and its connected PD. Besides

identifying the cargo of PD, little is known about potential regulatory molecules that signal PD to allow selective movement of macromolecules. Furthermore, what are the exact mechanics of transport *via* PD? Diverse approaches including cellular, genetic and genomic tools will need to be synergistically applied to answer these questions.

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