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CELL SCIENCE AT A GLANCE

Plasmodesmata at a glance

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ABSTRACT

Plasmodesmata are cytoplasmic communication channels that are vital for the physiology and development of all plants. They facilitate the intercellular movement of various cargos – ranging from small molecules, such as sugars, ions and other essential nutrients and chemicals, to large complex molecules, such as proteins and different types of RNA species – by bridging neighboring cells across their cell walls. Structurally, an individual channel consists of the cytoplasmic sleeve that is formed between the endoplasmic reticulum and the plasma membrane leaflets. Plasmodesmata are highly versatile channels; they vary in number and structure, and undergo constant adjustments to their permeability in response to many internal and external cues. In this Cell Science at a Glance article and accompanying poster, we provide an overview of plasmodesmata form and function, with highlights on their development and variation, associated components and mobile factors. In addition, we present methodologies that are currently used to study plasmodesmata-mediated intercellular communication.

KEY WORDS: Cell communication, Cell signaling, Fluorescent protein, Membrane nanotube, Plasmodesmata, Protein trafficking, Cell–cell channels, Cell-to-cell movement, Cytoplasmic bridges, Callose

Introduction

Plasmodesmata (singular plasmodesma) mediate direct cell-to-cell communication between neighboring cells; this facilitates the transport of cellular contents that vary in size and chemical form. They are one of the key cellular structures that distinguish plants from the animal system. Adjacent animal cells can form gap junction-mediated cytoplasmic pores to enable the exchange of small diffusible molecules across their plasma membranes (PMs). In plants, the cellulose walls of individual plant cells keep them from making direct contact with neighboring cells at their PM; instead, plasmodesmata serve as the PM-lined cytoplasmic bridges across the cell walls. Plasmodesmata are considered essential to support the diversification of cell and tissue

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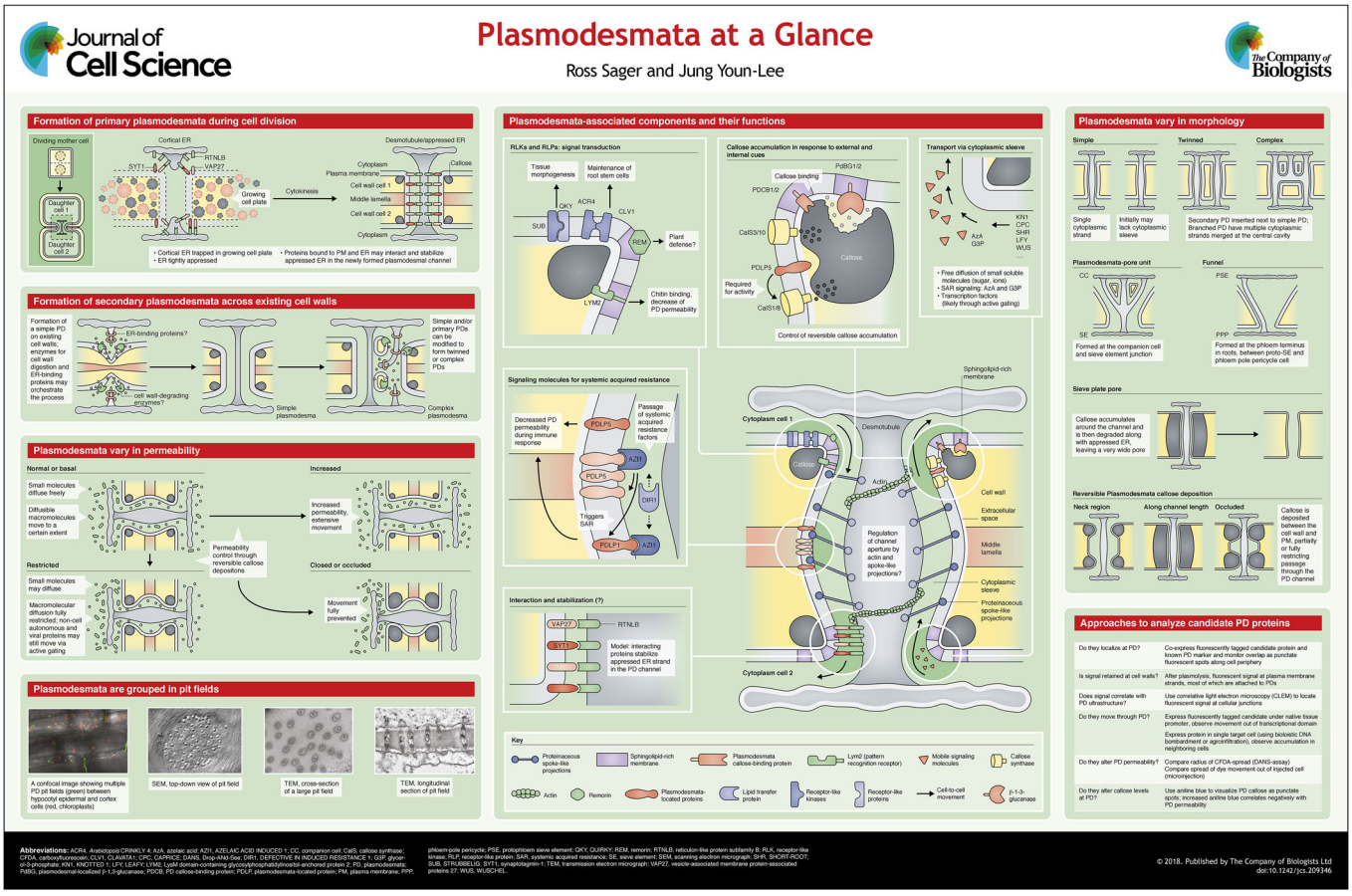

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types in multicellular plant bodies; however, despite their importance, their molecular composition, as well as their underlying mechanisms of transport and biogenesis, remain largely enigmatic. In this Cell Science at a Glance article and the accompanying poster, we will highlight the formation and morphology of plasmodesmata, along with signaling molecules that are localized to or pass through them, to demonstrate their dynamic and adaptable nature. Furthermore, we will summarize methods to dissect whether a protein is associated with or is mobile across plasmodesmata, as well as techniques to monitor and assess the state of plasmodesmal permeability.

Plasmodesmata are membrane-lined cytoplasmic channels

Plasmodesmata bridge adjacent cells across cell wall matrices as membrane-lined cytoplasmic channels. In land plants, primary plasmodesmata form during cell division when the endoplasmic reticulum (ER) is incorporated into the developing cell plate, which creates the cytoplasmic pore – termed the cytoplasmic sleeve – between the PM and ER membrane linings (see poster). The ER within the plasmodesma (the desmotubule, or appressed ER) is continuous with the cortical ER, but appressed tightly, leaving no luminal space (Robards and Lucas, 1990). Interestingly, certain membrane microdomains within plasmodesmata have recently been found to be enriched in sterols and sphingolipids (Grison et al., 2015), which may attract proteins that carry the glycosylphosphatidylinositol (GPI) moiety, or other lipid-based post-translational modifications [as is the case for PLASMODESMATA CALLOSE BINDING PROTEIN (PDCB), β-1,3-glucanase (PdBG2), remorin, etc.]. Actin filaments and myosin-like proteins are localized within the cytoplasmic sleeve (Radford and White, 1998; White et al., 1994), and proteinaceous spoke-like projections that are regularly positioned within the cytoplasmic sleeve are thought to create nanochannels of varying size (Fisher, 1999; Terry and Robards, 1987). Callose, a β-1,3-glucan polymer, appears to serve as both a structural and a regulatory element of plasmodesmata. During cytokinesis, callose is deposited at the cell plate, but after cell division is completed, it is degraded from the new cell wall, except in the portions of cell walls that are adjacent to plasmodesmata (Northcote et al., 1989). Moreover, callose accumulates around plasmodesmata at various levels, which provides a mechanism to regulate their permeability (see poster).

Small (<1 kDa) soluble molecules move freely and extensively between cells through the cytoplasmic sleeve. The possibility exists that the appressed ER may also provide a membrane surface as a route for molecular transport within the plasmodesmata (Grabski et al., 1993; Guenoune-Gelbart et al., 2008), but this aspect remains to be further explored. Large diffusible molecules can also move between cells if their three-dimensional shape allows passage through the cytoplasmic nanochannels (e.g. free GFP, which has a molecular mass of ∼27 kDa, likely diffuses between cells because it forms a cylindrical shape that can fit in the 3–4 nm-wide microchannels; see poster). When plasmodesmal permeability is significantly increased though dilation or active gating, or through structural remodeling of widening the pore size (see poster), various macromolecules, including transcription factors, signaling molecules and various types of RNA–protein complexes, may traffic across specific cellular boundaries or domains (Lucas and Lee, 2004). Exploiting this endogenous trafficking mechanism, plant viruses can spread infectious materials between host cells (Heinlein, 2015; Schoelz et al., 2011) (see Box 1).

The number of plasmodesmata can increase or decrease in plant cell walls

In land plants, plasmodesmata are, by default, formed during cell division as strands of cortical ER membrane are caught within the developing cell plate (see poster). Although the process of appressed ER formation is not fully understood, it is likely to be mediated by the combined effects of pressure from the growing cell wall and the interaction between ER and PM proteins, such as *RETICULON-LIKE PROTEIN SUBFAMILY B 3* and *6* (*RTNLB3/6*), *SYNAPTOTAGMIN 1* (*SYT1*) and *VESICLE-ASSOCIATED MEMBRANE PROTEIN-ASSOCIATED PROTEIN 27* (*VAP27*), which stabilize this process (Knox et al., 2015; Kriechbaumer et al., 2015). Primary plasmodesmata are typically grouped in clusters, called pit fields, that exist in thinner areas of the cell walls (see poster).

As cells grow and expand in their cell wall surfaces, the density of primary plasmodesmata naturally decreases if no secondary plasmodesmata are produced across existing cell walls. This reduced plasmodesmal density is common within cells that have undergone substantial and rapid expansion, such as the cell walls that separate the epidermal layer from the underlying cortex in mature roots, or phloem sieve elements from parenchyma cells (Kollmann and Glockmann, 1999). Moreover, in some cells, plasmodesmata are actively disintegrated as part of a required cell differentiation process – for example, during guard cell maturation or germ cell development (Sager and Lee, 2014). By gradually reducing the density, or active degradation of plasmodesmata, individual cells, or groups of cells, can become symplasmically isolated or lose cytoplasmic connectivity from their neighbors.

Box 1. Plasmodesmata-like intercellular channels in animals and bacteria?

Plasmodesmata were first discovered in higher plants in 1885 by Edward Tangl, and later found to be present in all green plants. Similar, but simpler plasmodesmal structures consisting of only the PM-lined cytoplasmic pore can be seen in the closely-related charophycean algae (Cook et al., 1997; Franceschi et al., 1994), as well as outside of the plant lineage in fungi (Hawker et al., 1966) and brown algae (Terauchi et al., 2015). However, it was not until recently that the presence of plasmodesmata-like intercellular channels was also uncovered in animal cells, and subsequently even in bacteria; these intercellular channels are termed tunneling (or membrane) nanotubes (TNTs) (Dubey and Ben-Yehuda, 2011; Rustom et al., 2004). TNTs function as conduits for cell-to-cell trafficking of small or large molecules between animal or bacterial cells (Davis and Sowinski, 2008), and also have other analogous functions to plasmodesmata. For instance, the actin cytoskeletal core of animal TNTs can facilitate the intercellular transport of vesicular organelles. The plant appressed ER cannot do this, but in special circumstances, for instance between connected pollen mother cells, the callose walls and inner components of plasmodesmata can be degraded, leaving substantially larger pores called cytomictic channels (see poster) that can allow nuclei and other organelles to move intercellularly (Mursalimov et al., 2010). In bacteria, TNTs can function between cells of either the same or different species (Dubey and Ben-Yehuda, 2011); in plants, plasmodesmata can connect different species through graft unions (Kollmann and Glockmann, 1985) or parasitic interactions (Kim et al., 2014). Viruses have even evolved to exploit TNTs by spreading infectious viral materials through them; animal cells can even pass immune signals to uninfected neighboring cells through TNTs to defend themselves (Sowinski et al., 2008). Similarly, as viruses attempt to exploit plasmodesmata, plant cells can transmit symplasmic defense signals to protect themselves (Lim et al., 2016). Hence, although they may differ in compositional and mechanical details, TNTs may be considered, in essence to be membrane-lined, symplasmic communication channels, as are plasmodesmata (Lee, 2014). Given that these channels occur across almost all domains of life (the observation of plasmodesmata in a certain fungal species is noteworthy; Hawker et al., 1966), it seems that the strategy of using membrane-lined cytoplasmic pores is widespread in biology.

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In contrast, the number of plasmodesmata can also increase in existing walls independently of cell division (see poster). These secondary plasmodesmata often form during cell expansion to maintain or increase plasmodesmal density in the growing walls (Burch-Smith et al., 2011). The mechanisms that underlie secondary plasmodesmata formation, including the cell wall-degrading enzymes and the proteins that are involved in ER membrane insertion, remain to be uncovered. However, various cellular signals have been implicated as inducers of this process: plants that are treated with phytohormones, such as salicylic acid (Fitzgibbon et al., 2013), cytokinin (Ormenese et al., 2006) or flower-inducing day length (Ormenese et al., 2000), have been shown to increase secondary plasmodesmal formation. New plasmodesmal connections are created between the fusion walls of graft partners during wound healing (Kollmann and Glockmann, 1991). Furthermore, parasitic plants appear to exploit the mechanism of secondary plasmodesmata formation to develop new plasmodesmata between themselves and their hosts, which allows them to siphon off host nutrients (Kim et al., 2014). These examples demonstrate a remarkable flexibility of plant cells to ensure the presence of the right number of plasmodesmata for their developmental or cellular context.

Plasmodesmata vary in morphology

Plasmodesmal morphology varies greatly during the growth and differentiation of a cell, or, depending on the intercellular transport requirements, between different cell–cell junctions (see poster). A simple plasmodesma consists of a single channel between cells, but this can become modified into a branched or complex plasmodesma, with multiple channels that merge, split and may share the same opening (Faulkner et al., 2008). Simple and complex plasmodesmata regulate intercellular transport differently, in that complex plasmodesmata seem to act somewhat like a filter, increasing the specificity of which molecules can cross between cells. For example, as tobacco leaf plasmodesmata undergo changes from simple to branched forms during the sink-source transition (the time when the increase in daily carbohydrate exchange rate exceeds the daily increase in leaf carbohydrate content), cell-to-cell diffusion of GFP that is expressed under the control of a phloem-specific promoter is greatly reduced (Oparka et al., 1999; Roberts et al., 2001).

Plasmodesmata can take on a highly specialized morphology to control movement between certain tissues. For instance, in phloem, asymmetric plasmodesmata develop between companion cells and sieve elements, which give rise to a unique complex called the plasmodesmata-pore unit (see poster). Also in phloem, plasmodesmata at the sieve plate (where the ends of sieve elements are joined) become substantially enlarged through callose-mediated cell wall restructuring, which creates expanded cytoplasmic pores through which the mass flow of phloem sap can occur (Esau and Thorsch, 1985). A recent study on the unloading of root phloem revealed unique ‘funnel’-shaped plasmodesmata in the cell wall junctions between phloem sieve elements and the phloem pole pericycle cells, with a much wider opening on the phloem sieve element side compared to the phloem pole pericycle side (Ross-Elliott et al., 2017). The funnel shape thereby helps to direct the majority of molecules into the phloem pole pericycle during unloading, prior to transport into the outer root. Another study has visualized the development of plasmodesmata in root tips, and revealed that the PM and ER membranes in newly formed plasmodesmata are in such close contact that they completely lack the cytoplasmic sleeve (Nicolas et al., 2017). Intriguingly, even without a clearly discernable cytoplasmic sleeve, these new plasmodesmata can still facilitate cell-to-cell movement of GFP. It is likely that, as imaging methodologies for studying plasmodesmal ultrastructure advance, using tools such as electron tomography (Nicolas et al., 2017) and 3D scanning electron microscopy (Kremer et al., 2015), we will learn more about the unique morphologies that plasmodesmata may have.

Arabidopsis mutants with altered plasmodesmal structure or formation

Genetic mutant screening using *Arabidopsis* has identified a number of genes that impact cell-to-cell movement of molecules and/or plasmodesmal structure. These genes include *INCREASED SIZE EXCLUSION LIMIT (ISE) 1* and *ISE2*, which encode a mitochondrial DEAD-box RNA helicase and chloroplast DEVH-type RNA helicase, respectively (Kobayashi et al., 2007; Stonebloom et al., 2009); *DECREASED SIZE EXCLUSION LIMIT 1* (*DSE1*), encoding a putative WD-repeat protein (Xu et al., 2012); *GFP ARRESTED TRAFFICKING 1 (GAT1*), encoding a chloroplast thioredoxin (Benitez-Alfonso et al., 2009); and the *CHOLINE TRANSPORTER-LIKE 1 (CHER1)* (Dettmer et al., 2014; Kraner et al., 2017). Mutations in these genes affect plasmodesmata in different ways. In *ise1/2* loss-of-function mutant embryos, branched plasmodesmata increase in frequency (Burch-Smith and Zambryski, 2010), whereas their frequency decreases in *dse1* embryos (Xu et al., 2012). In *gat1* loss-of-function mutants, GFP unloading from the phloem is restricted in the root, and plasmodesmata are often occluded with heavily deposited callose (Benitez-Alfonso et al., 2009). *ISE1* likely regulates the redox states of mitochondria and plastids, whereas *ISE2* and *GAT1* regulate those of plastids, altering organellar redox states to bring about changes in plasmodesmal permeability (Benitez-Alfonso et al., 2009; Stonebloom et al., 2012). Loss-of-function mutations in *CHER1* lead to alterations in choline metabolites and defects in phloem transport, which correlates with malformation of sieve pores and sieve plates (Dettmer et al., 2014). Additionally, *cher1* mutants develop fewer plasmodesmata per unit cell wall length, and the plasmodesmata that do form remain abnormal and consist of fewer branches (Kraner et al., 2017). The effects of these plasmodesmal mutants on its morphology are likely indirect, since the proteins encoded by their wild-type alleles associate with subcellular compartments other than plasmodesmata. However, these mutants illustrate the complexities that are associated with plasmodesmal form and regulation well.

What is the role of callose and how does it regulate plasmodesmal permeability?

Currently, there are only two different structural elements of plasmodesmata that are known to regulate cell-to-cell movement: the actin cytoskeleton and callose (see poster). It is speculated that F-actin and unconventional myosin motors gate and regulate the plasmodesmal size exclusion limit (SEL), but this is based solely on their localization to plasmodesmata, and the detrimental effects on cell-to-cell movement when actin filaments are disrupted (Tilsner et al., 2016). For callose, however, a considerable amount of physiological, cellular and genetic data is available, and this data supports the idea that an increase in plasmodesmal callose levels reduces molecular movement between cells. Whereas callose is considered a normal structural constituent of plasmodesmata, it usually accumulates only transiently, for example, at the cell plate during cell division or at wound sites (Jacobs et al., 2003; Nishimura et al., 2003; Northcote et al., 1989). At plasmodesmata, callose specifically enriches in the extracellular space between the cell wall and the PM lining, and it may span the entire length of the

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plasmodesmal channel, or be more focused around the neck regions at each orifice (Radford et al., 1998; Vatén et al., 2011) (see poster). Plasmodesmal morphology may even be altered by callose occlusion, for example as observed in mutants for *SUCROSE-EXPORT-DEFECTIVE* (*SXD-1*) (Botha et al., 2000).

Functionally, callose accumulation effectively reduces plasmodesmal permeability, which can eventually lead to plasmodesmal closure, by restricting passage through the cytoplasmic sleeve. As callose accumulation increases on the inflexible cell wall outside of the PM lining, it pushes the PM inward, and is therefore assumed to physically squeeze the cytoplasmic sleeve to narrow the plasmodesmal aperture (Radford et al., 1998). In contrast, callose degradation would reverse this spatial constraint, increasing plasmodesmal permeability. The levels of callose accumulation at plasmodesmata are determined by the balance between the activities of two types of enzymes, callose (or β-1,3-glucan) synthases and glucanases (Zavaliev et al., 2011).

Specific callose enzymes modulate plasmodesmal callose levels

Key genes that regulate callose levels specifically at plasmodesmata include members of the *A. thaliana* β-1,3-glucanase, *AtBG_ppap* (Levy et al., 2007) and *PdBGs*, as well as the *A. thaliana* callose synthase (CalS) family, encoded by *CalS10/GSL8/CHORUS*, *CalS3/GSL12*, *CalS1/GSL6* or *CalS8/GSL4*. Mutations in the *PdBG1* or *PdBG2* genes increase callose accumulation and perturb normal lateral root formation (Benitez-Alfonso et al., 2013). In *cals10* mutants, callose deposition at the cell plate is abolished, causing seedling lethality and pleiotropic phenotypes that range from defects in cell division and guard cell patterning, to alterations in phototropic response and plasmodesmal permeability (Guseman et al., 2010; Han et al., 2014). Gain-of-function mutations in *CalS3* increase callose levels and alter root development (Vatén et al., 2011). Mutations in *CalS1* abolish the hyperaccumulation of callose induced by salicylic acid or pathogenic bacterial infection, while those in *CalS8* eliminate the callose deposition induced by mechanical wounding or reactive oxygen species. Furthermore, *cals8* mutants show an increase in basal plasmodesmal permeability compared to that found in wild-type plants (Cui and Lee, 2016). These data, together with the observations on additional proteins that are found at plasmodesmata (see below, Box 2 and poster), suggest that a network of callose-regulating factors control the permeability of plasmodesmal channels.

Plasmodesmata-located regulators of plasmodesmal permeability

A number of plasmodesmata-localized proteins, unrelated to any callose synthase or hydrolase family, have been found to regulate plasmodesmal permeability through callose-dependent or independent modes (see poster). *Arabidopsis PDCB1* encodes an extracellular protein that contains a GPI-anchor within its X8 domain (Simpson et al., 2009). PDCB1 binds to callose *in vitro*, and its ectopic overexpression increases callose deposition at plasmodesmata. A chitin-binding receptor-like protein, LYSIN MOTIF DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 2 (LYM2), is a PM protein that also accumulates at plasmodesmata (Faulkner et al., 2013). In *lym2* mutants, the normal chitin-induced reduction in cell-to-cell movement is abolished, and chitin-triggered defenses in *Arabidopsis* are also compromised. Whether LYM2 regulates plasmodesmal permeability through a callose-dependent mechanism is unknown.

The *PLASMODESMATA-LOCATED PROTEIN* (*PDLP*) family encodes eight members of the receptor-like integral membrane proteins in *Arabidopsis*; they consist of a functionally unknown extracellular domain and a short cytoplasmic tail across a transmembrane helix (Thomas et al., 2008) (see poster). The *pdlp1 pdlp3* and *pdlp2 pdlp3* double mutants exhibit increased GFP movement between cells, whereas overexpression of PDLP1 decreases GFP trafficking (Thomas et al., 2008). The *pdlp5* mutant leads to an increased basal plasmodesmal permeability, whereas overexpression of PDLP5 induces the opposite phenotype (Lee et al., 2011). PDLP5 regulates cell-to-cell movement by stimulating callose deposition at plasmodesmata through CalS1 and CalS8 (Cui and Lee, 2016) (see poster). PDLP5 is also required for a proper innate immune response to bacterial pathogens, as well as the normal salicylic acid-induced reduction in plasmodesmal permeability (Wang et al., 2013). Finally, both PDLP1 and PDLP5 are necessary for systemic acquired resistance in *Arabidopsis* (Lim et al., 2016) (see Box 2). It remains unknown whether PDLP1 and other PDLP members regulate plasmodesmal permeability in a callose-dependent manner, similar to PDLP5.

In the following sections, we summarize select methodologies for analyzing protein association with and impact on the permeability of plasmodesmata at the molecular and cellular level.

Box 2. Signaling proteins and transcription factors that localize to or move through plasmodesmata

Receptor-like protein kinases that are important for controlling growth and developmental processes are partially associated with plasmodesmata (see poster). For example, the receptor-like kinase STRUBBELIG (SUB) and C2-domain-containing receptor-like protein QUIRKY (QKY) interact at plasmodesmata, which is thought to promote movement of unidentified intercellular factors needed for tissue morphogenesis (Vaddepalli et al., 2014). Similarly, the receptor kinases ARABIDOPSIS CRINKLY 4 (ACR4) and CLAVATA 1 (CLV1) interact at plasmodesmata in the root meristem to restrict root meristematic activity to only a few designated cells (Stahl et al., 2013). A number of transcription factors move from cell to cell through plasmodesmata, including those that play important roles in cell type specification (see poster). KNOTTED 1 (KN1) moves from inner shoot apical meristem (SAM) tissue layers into overlying layers to establish meristematic cell identity and maintain the stemness of this tissue (Kim et al., 2002). LEAFY moves from the outermost SAM layer into inner layers to control the differentiation of vegetative cells into floral meristem cells (Sessions et al., 2000). WUSCHEL (WUS) moves from the SAM-organizing center into the central zone, where it maintains stem cell fate and number (Yadav et al., 2011). CAPRICE (CPC) is expressed in all non-hair root epidermal cells, but moves into the cells that will become root hairs to promote their differentiation (Kurata et al., 2005). SHORT ROOT (SHR) moves from root stele into neighboring endodermal cells to promote differentiation of the ground tissues (Nakajima et al., 2001). Signaling molecules that move through plasmodesmata also include those involved in defense signal propagation (see poster). For example, the lipid transfer protein AZELAIC ACID INDUCED 1 (AZI1) not only interacts with the plasmodesmal regulators PDLP5 and PDLP1, but also triggers systemic acquired resistance (SAR) in response to pathogen exposure. This in turn leads to symplasmic transport of the SAR signaling molecules azelaic acid (AzA) and glycerol-3-phosphate (G3P) (Lim et al., 2016). Another lipid transfer protein, DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1), has been found to move symplasmically into systemic tissue (Champigny et al., 2013). Taken together, these factors exemplify the variety of cellular signaling events that employ plasmodesmal communication.

Methods to analyze candidate plasmodesmata proteins

Protein association or movement through plasmodesmata

If a newly identified protein localizes to plasmodesmata, it should be present at the cell periphery as discrete puncta when tagged with

a fluorescent protein (FP) and viewed with confocal microscopy. Certain proteins may move out of the cells in which they are originally produced and into other cells, either locally or systemically (Gallagher et al., 2014). The mobility of these non-cell autonomous proteins can be examined after biolistic DNA delivery or by comparing the overall accumulation pattern of the candidate protein in tissues to its native transcriptional expression domain (see poster). It is important to validate that the punctate fluorescent signals only associate with junctional walls by performing a series of optical *z*-sections partially or completely through the depth of the cell that expresses the fluorescently tagged candidate protein (Lee et al., 2011). This method can be combined with techniques that cause plasmolysis in the cell; if the punctate fluorescent signals remain at the walls when the protoplast is pulled away, it is more likely that they are associated with plasmodesmata (see poster). In addition, plasmodesmal association can be confirmed by testing for colocalization of the candidate protein with cell wall callose by using a fluorescent stain such as Aniline Blue (Zavaliev and Epel, 2015). Alternatively, a known plasmodesmata-associated protein can be used as a marker for colocalization (see poster). Popular plasmodesmal markers include viral movement proteins, such as MP30, encoded by the *Tobacco mosaic virus*, or MP17, which is encoded by the *Potato leaf roll virus* (Hofius et al., 2001). Endogenous plasmodesmal proteins can also be used as markers, including all eight members of the PDLP family (Lee et al., 2011; Thomas et al., 2008), PDCB1/2 (Simpson et al., 2009) and PdBG1/2 (Benitez-Alfonso et al., 2013). Correlative light and electron microscopy (CLEM) (Lee et al., 2011; Modla et al., 2015) (see poster), and super resolution three-dimensional structured illumination microscopy (Fitzgibbon et al., 2010), are also suitable to address whether a protein locates within or around the plasmodesmata, as an alternative to immunogold labeling.

Evaluating the permeability of plasmodesmata

In response to varying exogenous and endogenous factors, plants constantly modify the extent of molecular trafficking between cells by fine-tuning plasmodesmal permeability (Sager and Lee, 2014). One method that is used to detect such changes in plasmodesmal permeability quantifies the extent to which free GFP (or other FPs in monomeric forms) diffuse from cell to cell (Stadler et al., 2005). The plasmodesmal SEL may differ in particular cell types, or change under specific conditions, and these changes can be monitored by using concatenated GFP (2× or 3× GFP) (Kim et al., 2005). Similar to the protein movement assays described earlier, free GFP as a symplasmic reporter is expressed either through DNA bombardment in individual epidermal cells or by transgenic expression in conjunction with a cell- and/or tissue-specific promoter. These FP-based cell-to-cell movement assays have been the most broadly used approaches in the field. Microinjection techniques using various fluorescent tracers of different molecular mass have been extensively employed to evaluate the SEL, as well as the permeability of plasmodesmata in different cell types of varying plant species (Kragler, 2015). Alternatively, membrane-permeable dyes could be applied to intact plants in a non-invasive manner to measure plasmodesmal permeability at the tissue level. For example, the Drop-ANd-See (DANS) dye-loading assay utilizes the membrane-permeable non-fluorescent dye carboxyfluorescein diacetate, which enters epidermal cells once loaded onto an intact plant surface and is immediately converted into the membrane-impermeable fluorescent tracer carboxyfluorescein (Cui and Lee, 2016; Cui et al., 2015; Gui et al., 2014; Lee et al., 2011; Lim et al., 2016; Wang et al., 2013) (see poster). This method is an ideal choice when addressing questions that require real-time, non-invasive analyses. It represents a good approach to determine whether or not plasmodesmal permeability may have been affected owing to genetic mutations or specific exogenous treatments (see poster). In addition, quantitative analysis of plasmodesmal callose levels using Aniline Blue stain can serve as an indirect measure for fluctuations in plasmodesmal gating (Zavaliev and Epel, 2015), based on the negative correlation between plasmodesmal permeability and callose accumulation. However, since plasmodesmal permeability might also be altered in a manner independent of callose, it is advisable to perform the callose measurement in conjunction with additional assays that directly report changes in cell-to-cell movement. Measuring changes in plasmodesmal permeability can be quite challenging, because plasmodesmal gating is a highly dynamic cellular event and the impact of a particular gene and/or protein may be specific to certain cell or tissue types, developmental stages or environmental conditions. Hence, multiple approaches may be necessary to address whether a novel gene and/or protein is related to plasmodesmata-mediated cell-to-cell communication.

Perspectives

Plasmodesmata are essential for the life of multicellular plants, yet we still lack a great deal of knowledge regarding their molecular anatomy, composition and regulatory mechanisms. For example, many of the molecules that constitute the plasmodesmal channel itself have yet to be discovered. Furthermore, the genetic factors that control primary and secondary plasmodesmal formation, as well as the proteins required for structural modification, remain to be identified. Finally, how specific proteins associate with plasmodesmata and transport through plasmodesmata, and how cells integrate specific intracellular signals with plasmodesmal components to execute intercellular responses, and vice versa, remain to be elucidated. However, those gaps in our understanding of plasmodesmata will be narrowed through genomic and proteomic data mining, and the development of new methodologies and technologies enabling real-time monitoring of cell-to-cell movement and structural analysis of developing plasmodesmata. In particular, current advances in imaging acquisition, resolution and analysis tools seem to hold much promise to unfold morphological details and variations in plasmodesmal ultrastructure. Since accumulating evidence now supports that animal cells and even bacteria form plasmodesmata-like intercellular channels (see Box 1), understanding the molecular details of plasmodesmata could provide significant insights into universal mechanisms that orchestrate communication between cells.

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Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.209346.supplemental

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