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Plasmodesmata: Channels Under Pressure

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Abstract

Multicellularity has emerged multiple times in evolution, enabling groups of cells to share a living space and reducing the burden of solitary tasks. While unicellular organisms exhibit individuality and independence, cooperation among cells in multicellular organisms brings specialization and flexibility. However, multicellularity also necessitates intercellular dependence and relies on intercellular communication. In plants, this communication is facilitated by plasmodesmata: intercellular bridges that allow the direct (cytoplasm-to-cytoplasm) transfer of information between cells. Plasmodesmata transport essential molecules that regulate plant growth, development, and stress responses. They are embedded in the extracellular matrix but exhibit flexibility, adapting intercellular flux to meet the plant's needs.

In this review, we delve into the formation and functionality of plasmodesmata and examine the capacity of the plant communication network to respond to developmental and environmental cues. We illustrate how environmental pressure shapes cellular interactions and aids the plant in adapting its growth.

Contents

1. INTRODUCTION

Achieving collective behavior and maintaining cohesion are essential for any group of individuals living together, requiring the exchange of information among individuals to coordinate their responses and work toward a common objective. Likewise, in multicellular organisms such as plants, cell-to-cell communication is crucial for enabling individual cells to coordinate their activities and operate as a cohesive unit (25). In plants, plasmodesmata bridges serve as direct physical connections that facilitate information exchange (15, 70, 117) (**Figure 1**).

Plasmodesmata are nanoscopic membrane-lined conduits (**Figure 1**) inserted within cell walls, establishing hundreds of cytosolic bridges between adjacent cells. These bridges collectively form the plant symplast, facilitating the direct exchange of signaling molecules (e.g., proteins, RNAs, and hormones) and nutrients. This connectivity within the symplast is often exploited by pathogens (5, 56, 57, 71, 137). Although multicellular organisms gain advantages from cell-to-cell communication, such as increased access to information, there is a trade-off in effectively managing and processing this information. Unrestrained and unfiltered molecular exchange can impede accurate decision-making by overflooding the system (79). Precise and balanced communication is therefore needed, which implies careful consideration of when to communicate broadly versus selectively and when not to communicate at all. Plasmodesmata act as gatekeepers capable of opening or closing communication as required.

Plant symplast: cytosolic continuum of cells interconnected by plasmodesmata bridges

Remarkably, plasmodesmata embody both rigidity (stemming from their location at the extracellular matrix) and flexibility as they undergo alterations in diameter and shape to regulate communication (105, 116). The flexibility of the intercellular communication network is crucial in plants, which are immobile and spatially constrained and thus must swiftly and precisely respond

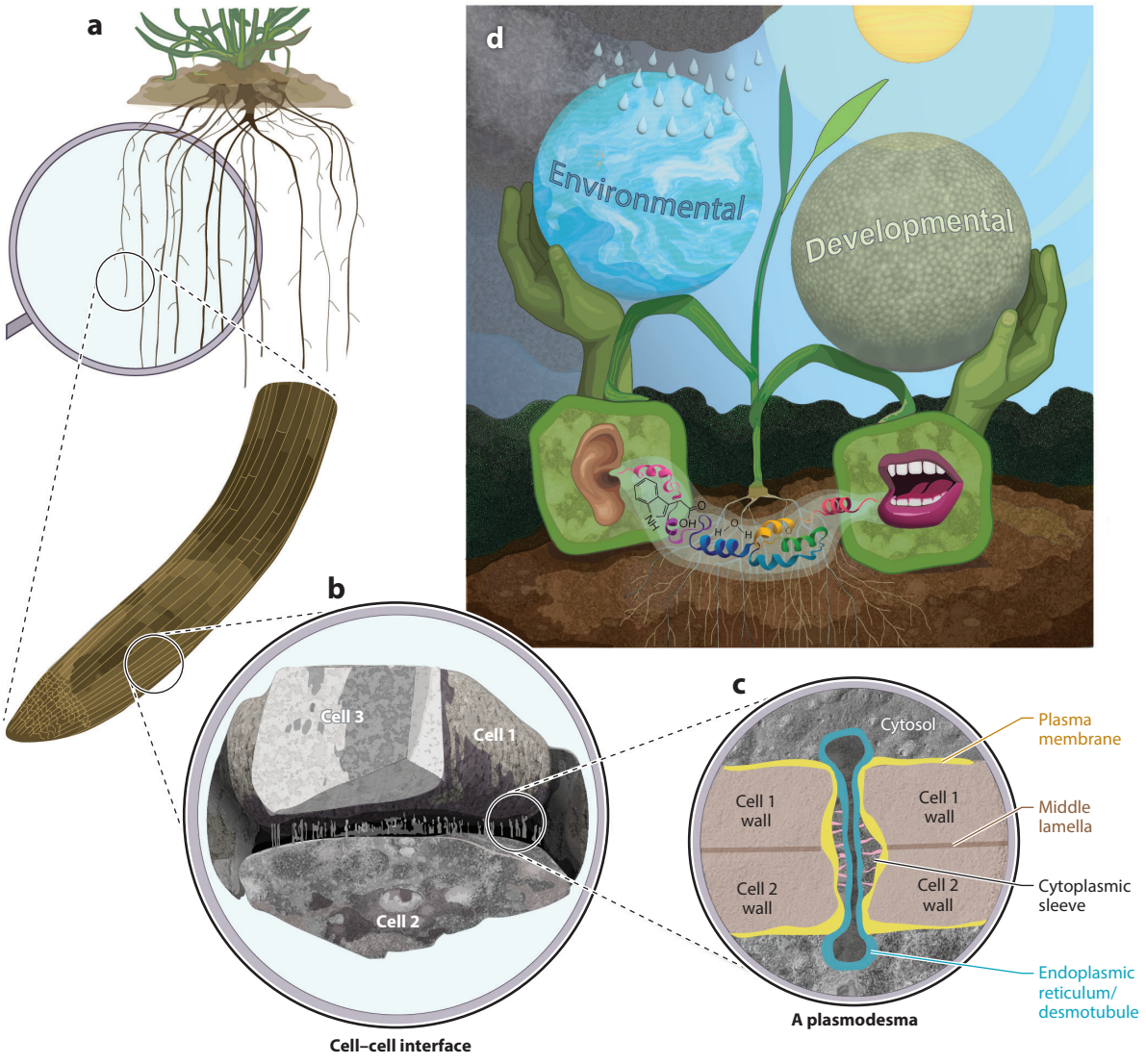

Figure 1

Plasmodesmata intercellular bridges connect plant cells, forming a communication network that is under developmental and environmental pressures. (*a–c*) Plasmodesmata interconnect virtually every cell throughout the plant body. (*b*) They create numerous communication channels at the cell–cell interface, which enable the transfer of information and nutritional molecules. (*c*) Plasmodesmata are embedded in the cell wall (*brown*) and present a concentric arrangement of an endoplasmic reticulum tube (*teal*), named the desmotubule, surrounded by and tethered (*pink spokes*) to the plasma membrane domain (*yellow*). The space between the two membranes is called the cytoplasmic sleeve and is thought to be the main path allowing molecules to flow from one cell to another. (*d*) Artistic representation showing that the communication network created by plasmodesmata facilitates the exchange of hormones, nucleic acids, proteins, and small molecules such as water between cells. By dynamically modulating the transfer of information, plants adapt and respond to different developmental and environmental cues and stressors—a concept we highlight in this review. Figure created by Tobin Florio (**https://flozbox-science.com/**).

to environmental changes. Plasmodesmata, with other cellular players such as phytohormones, enable plants to efficiently modulate their growth and physiological responses (**Figure 1**). There are many examples illustrating how plasmodesmata dynamically respond to external and internal clues to facilitate organ and plant adaptation (10, 16, 22, 30, 31, 102, 107, 123, 135, 139, 140, 143, 145). In all cases, the symplast functions as a highly intricate and adaptable network that regulates intercellular interactions in a dynamic and spatiotemporal manner.

Research is progressing toward understanding the mechanisms behind molecular exchange through plasmodesmata, including the physical parameters and molecular components regulating these processes. This review offers insights into (*a*) plasmodesmata structure, formation, and composition; (*b*) the regulation of biomechanical properties and modeling approaches; and (*c*) the influence of developmental and environmental cues on plasmodesmata regulation. Our goal is to highlight the influence of environmental and developmental pressures on the functionality of the plasmodesmata communication network and the demand for optimal performance to support plant growth and adaptation.

Desmotubule: an endoplasmic reticulum strand constricted to approximately 15 nm that forms a narrow membrane tubule within plasmodesmata

Size exclusion limit (SEL): refers to the maximum size of molecules or particles that can pass through the pores of plasmodesmata

Turgor pressure: the pressure exerted by the fluid inside the cells (such as cell sap) against the cell walls

Callose: β-1,3-glucan that accumulates at plasmodesmata cell wall microdomains and modulates cell-to-cell trafficking

2. PLASMODESMATA STRUCTURAL DOMAINS AND REGULATORY COMPONENTS

In most textbooks, plasmodesmata are depicted as single pores formed by a specialized ring of cell walls surrounding two interconnected membranes: the plasma membrane (PM) and an inner endoplasmic reticulum (ER)-derived membrane named the desmotubule (**Figure 1**). The space between the PM and the ER is named the cytoplasmic sleeve, and it is the region for symplasmic (cytoplasm-to-cytoplasm) transport. The size of the cytoplasmic sleeve is thought to determine the size exclusion limit (SEL). Our current understanding of plasmodesmata structural and molecular organization is based on electron microscopy and on proteomic profiling, combined with cell biology and genetic approaches. The emerging picture is that plasmodesmata consist of interacting layers of molecular constituents, including lipids, proteins, and wall polymers (**Figure 2**). These components work together in a coordinated manner to regulate the function of plasmodesmata (84).

In classical models, the surrounding cell wall is primarily responsible for constricting or relaxing the pore and determines the aperture of the cytoplasmic sleeve, which acts as a sieve for molecular transport. Intercellular transport also depends on the number and/or density of plasmodesmata; their spatial organization, such as clustering or random; the formation of complex structures in mature tissues; and concentration gradients between neighboring cells (**Figure 3**). Transport also depends on plasmodesmata architecture (branching), cell wall thickness, and the radius of the mobile molecule. A computational model of plasmodesmata (PDinsight) uses these parameters to predict plasmodesmata permeability from imaging (32). An alternative model considers the influence of cell mechanics/turgor pressure on the displacement of the ER/desmotubule and other geometric factors on permeability values (24, 111, 113, 116).

In this section, we discuss the knowledge cumulated in recent years regarding the functional molecular players of plasmodesmata.

2.1. The Dynamics of Plasmodesmata Cell Walls and Membranes

Plasmodesmata cell walls are regulated by the deposition and removal of callose. Callose is a β-1,3-glucan locally synthesized by callose synthases (CALSs) and degraded by β-1,3-glucanases [(BGs), members of glycosyl hydrolase family 17 (GH17)] (**Figures 2** and **3*d***). Excessive callose temporarily blocks or narrows the channels, reducing their permeability (2). At a more structural level, callose might help to maintain the shape and position of the plasmodesmata within the cell

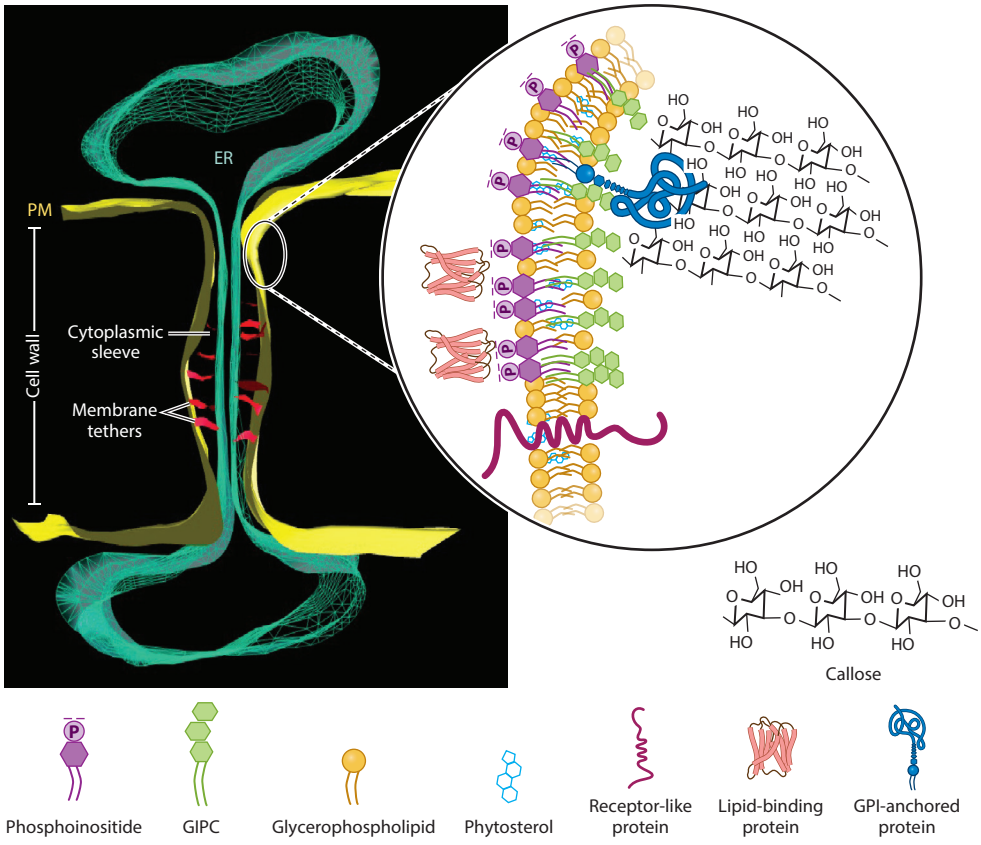

Figure 2

Plasmodesmata structure and function are governed by interactions between lipids, proteins, and wall polymers. The structure of plasmodesmata consists of a cytoplasmic sleeve delimited by a tubular structure made of ER membrane and the surrounding PM. Both the membrane and the surrounding cell wall exhibit a distinct molecular composition that ensures the proper functioning of plasmodesmata. Various protein families, including GPI-anchored callose–interacting enzymes/proteins, receptor-like proteins, and lipid-binding proteins, have been identified as components that either are embedded in the lipid bilayers (of the ER and/or PM) or interact with the PM through surface charges. PM-anchored proteins such as callose metabolic enzymes directly interact with wall polymers. Plasmodesmata display a layered arrangement of lipids, proteins, and wall polymers, which collectively contribute to their structure and function. Abbreviations: ER, endoplasmic reticulum; GIPC, glycosyl inositol phospho ceramides; GPI, glycosylphosphatidylinositol; PM, plasma membrane.

wall, preventing collapse or distortion of the channels. Regulation of callose is associated with responses to developmental or environmental cues and the spreading of pathogens (2, 45).

Besides callose, evidence indicates modifications of xyloglucans and pectins at plasmodesmata microdomains (2, 47, 115). Pectin modification is supported by pectin methyl esterase activity localized at plasmodesmata. Pectin subpopulations (rich in arabinan side-chain rhamnogalacturonan I) have been described around pit fields (clustered plasmodesmata), although their relevance remains elusive. A putative pectate lyase is required for the dedifferentiation of plasmodesmata into sieve pores (pores connecting the sieve elements to form the conductive tubes of the phloem). A callose synthetic enzyme (CALS7) also participates in this process, suggesting that there are interactions between callose and pectin regulatory activities during phloem development (66). Besides

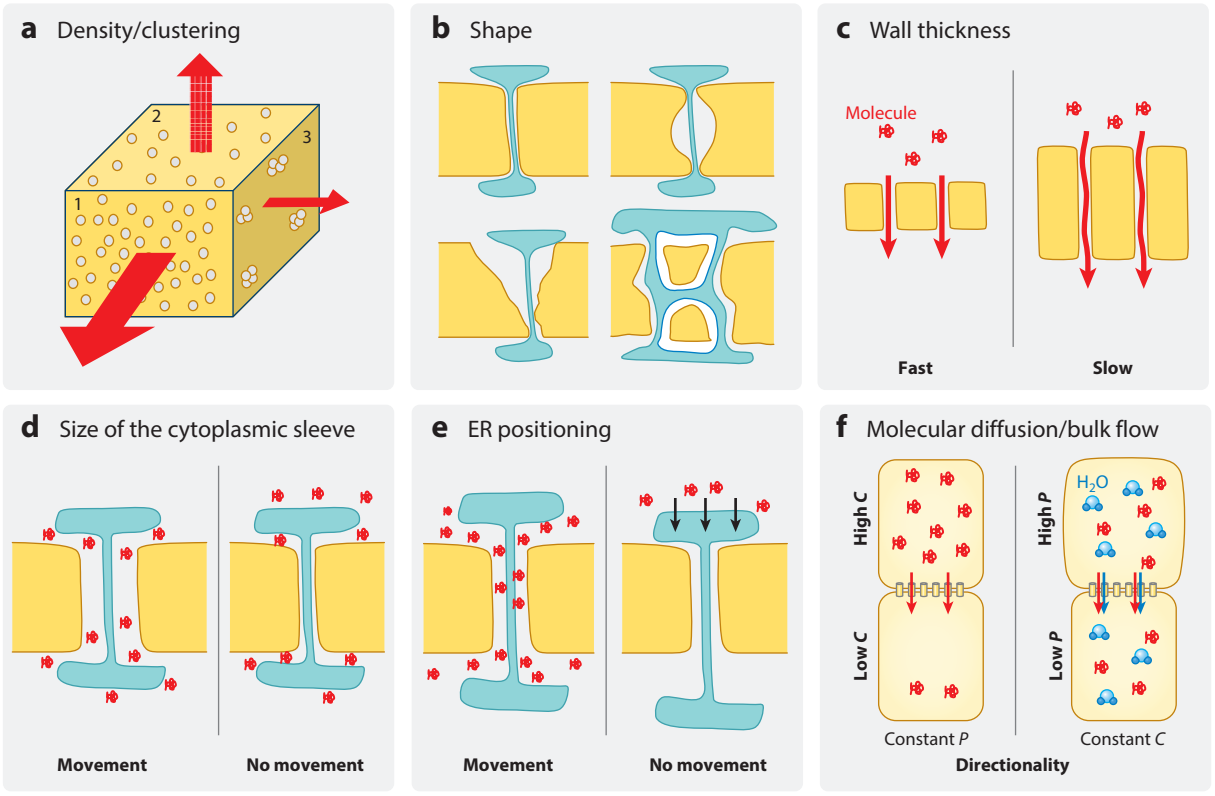

Figure 3

Multiple factors influence molecular trafficking through plasmodesmata. (*a*) Plasmodesmata density and clustering. Face 1 represents a cell wall with numerous randomly distributed plasmodesmata, leading to higher molecular flux compared to face 2, which has fewer pores. While faces 2 and 3 have the same plasmodesmata count, their distribution patterns, either random or grouped in pit fields, further impact the flux capacity at the cell–cell interface. (*b*) Plasmodesmata exhibit a range of geometries that vary depending on the tissue type and developmental stage. These shapes include straight, with or without a central cavity; branched; and funnel-like structures. Each of these architectures influences transport. (*c*) Wall thickness affects molecular transport between cells. As the cell wall thickens, the time taken for molecules to reach neighboring cells increases. (*d*) The radius of the cytoplasmic sleeve. The larger the distance between the desmotubule and the plasma membrane, the greater the available space for molecular transport. (*e*) Cortical ER positioning. The displacement of the dumbbell-shaped ER at the entrance leads to the closure of the plasmodesmata aperture. (*f*) Molecular diffusion and bulk flow. (*Left*) Concentration gradients can drive directional molecular diffusion between cells from a higher to a lower concentration. (*Right*) Bulk flow is the directional movement of fluid such as water carrying along molecules by a pressure gradient. Please note that bulk flow can also work against the molecular concentration gradient. Abbreviations: *C*, molecular concentration; ER, endoplasmic reticulum; *P*, hydrostatic pressure.

Glycosylphosphatidylinositol (GPI): a lipid anchor for membrane-bound proteins, consisting of a glycan (sugar) chain attached to a phosphatidylinositol lipid

pectins, analysis of plasmodesmata cell walls found the presence of fucosylated xyloglucans, although their role in plasmodesmata function is still under investigation (115). Arabinogalactan proteins are also found at plasmodesmata walls, and recent data suggest that they are required for plasmodesmata biogenesis (106).

Perhaps linked to cell wall modifications, the lipid composition of the plasmodesmata membrane also differs from other membrane domains (**Figure 2**). Lipidomic profiling showed an abundance of sphingolipids and sterols as well as saturated fatty acid phospholipids (48, 90, 154). These lipids are important for the localization of glycosylphosphatidylinositol (GPI)-anchored proteins, a feature found in several plasmodesmata proteins [such as BGs and plasmodesmata

callose-binding proteins (PDCBs)]. Anionic lipids, such as the low-abundant phosphatidylinositol-4-phosphate, are proposed as molecular determinants for the localization and activity of multiple C2 domains transmembrane region proteins (MCTPs), which tether plasmodesmata membranes (13, 117). However, experimental evidence supporting the presence and function of this lipid at plasmodesmata is missing.

Mutants with disrupted sphingolipid accumulation displayed defects in plasmodesmata ultrastructure and transport capacity (149). Lipids interact with plasmodesmata proteins, such as the plasmodesmata-located protein 5 (PDLP5), which binds t18:0-based sphingolipids and acts as an important regulator of callose (90) (**Figure 2**). Similarly, the hypothesis that lipids may interact with wall components is under investigation (142).

t18:0: refers to the long-chain base (LCB) of sphingolipid molecules containing a trihydroxylated C18 carbon chain with no double bonds

2.2. Progress Toward Defining the Plasmodesmata Proteome

The plasmodesmata proteome from *Arabidopsis* cell cultures (6, 13, 39) and leaves (65, 78); poplar cells (81); virus-infected tobacco cells (114); and, more recently, the moss *Physcomitrium patens* (47, 65) has been experimentally determined. The work involved cell wall fractionations, digestion, and mass spectrometry of the membrane fraction. These fractions remained contaminated; thus, comparative approaches that filter for predicted domains and validation using fluorescent fusions are required to confirm structural components. A resource was generated that compiles plasmodesmata proteomes of different plant species predicted and verified by independent studies (47; see also **https://pddb.uni-hohenheim.de/home.html**). Using experimental information, an in silico tool was also released [plasmodesmata in silico proteome 1 (PIP1) (69)] for the prediction of plasmodesmata proteins in species in which proteomic information is not yet available. Using comparative phyloproteomics, Johnston et al. (65) identified evolutionarily conserved proteins that associate with plasmodesmata (65) (**Table 1**). Together, in silico and experimental approaches have generated lists of plasmodesmata proteins including BGs, CALSs, PDCBs, PDLPs, MCTPs, tetraspanins, and leucine-rich repeat receptor-like kinases that are more abundant components (70) (**Figure 2**; **Table 1**).

In parallel to proteomic studies, several reports have identified plasmodesmata-localized proteins and contributed to our knowledge of their biological function (discussed in Section 5) (10, 13, 41, 85, 140, 153). Knowledge has been gathered about structural domains that determine protein targeting to plasmodesmata, but, so far, a unique signature is unknown (95).

2.3. Plasmodesmata Transient Components: Mobile Proteins and Transcripts

Data are still emerging on plasmodesmata transient components, including proteins and RNAs. Bioinformatic analysis by Kirk et al. (69) revealed significant overlap (more than 10× greater than chance) between proteins identified in the plasmodesmata proteomes and mobile factors exchanged between *Arabidopsis thaliana* and the parasitic plant *Cuscuta australis* (dodder) (89). One hypothesis proposes that, during isolation, mobile proteins are captured while transiently associated with plasmodesmata.

Early work identified mobile proteins serendipitously by characterizing their subcellular localization. For example, the transcription factors SHORTROOT (SHR) (103) and KNOTTED 1 (KN1) (94, 140) were identified by comparing their promoter and protein expression domains. Similarly, microRNAs (e.g., miRNA165/miRNA166) that move in a plasmodesmata-dependent manner were identified by comparing their expression (using in situ hybridization) and the degradation of their targets (18, 140).

The discovery that proteins such as KN1 traffic (likely as a ribonucleoprotein complex) with their messenger RNAs (mRNAs) made a fundamental conceptual contribution to the field (68, 94).

Table 1 Evolutionarily conserved plasmodesmata proteins[a]

Panther family	Family name	Example of verified protein (reference)	At-1	At-2	At-3	Pt	Nb
PTHR32227	GLUCAN ENDO-1,3-β-GLUCOSIDASE	AT3G13560/PdBG1 (10)	8	6	2	6	3
PTHR31279	PROTEIN EXORDIUM-LIKE 5	Pp3c19_8770/ EXO1 (47)	0	4	1	8	1
PTHR32191	TETRASPANIN-8-RELATED	At3g45600/TET3 (39)	3	4	0	4	1
PTHR31707	PECTINESTERASE	Pp3c18_22120/PME (47)	0	3	2	6	1
PTHR31682	UDP-ARABINOSE MUTASE	RGP2 (124)	1	2	0	5	1
PTHR45648	GDSL LIPASE/ACYLHYDROLASE	Pp3c12_25610 (47)	1	3	3	0	1
PTHR11119	XANTHINE-URACIL/VITAMIN C PERMEASE	Pp3c16_840 (47)	6	7	0	7	0
PTHR31235	PEROXIDASE 25–RELATED	Pp3c12_19290/PRX71 (47)	0	7	3	5	0
PTHR31867	EXPANSIN-A15	NbEXP1 (114)	0	1	0	7	2
PTHR31425	PHOSPHORIBOSYLANTHRANILATE TRANSFERASE ISOFORM 1	AT1G51570/MCTP4 (13)	4	3	0	2	0
PTHR31062	XYLOGLUCAN ENDOTRANSGLUCOSYLASE	Pp3c6_480/XTH_1 (47)	3	1	0	0	2
PTHR22298	ENDO-1,4-β-GLUCANASE	Pp3c3_7980 (47)	0	1	0	3	1
PTHR10774	EXTENDED SYNAPTOTAGMIN-RELATED	AT2G20990/SYTA (82)	0	3	0	3	0
PTHR31238	GERMIN-LIKE PROTEIN SUBFAMILY 3	Nt-PDGLP1 (52)	0	0	3	3	0
PTHR31044	CARBOHYDRATE-BINDING X8 DOMAIN SUPERFAMILY PROTEIN	AT5G61130/PDCB1 (131)	3	0	0	0	1
PTHR23050	CALCIUM-BINDING PROTEIN	CML41 (146)	0	1	0	2	0
PTHR33734	LYSM DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 2	AT2G17120/LYM2 (38)	1	1	0	0	0

[a]The table lists Panther protein families identified in the moss proteome (47, 65), containing at least one member with verified plasmodesmata localization. These protein families are also identified in plant proteomes obtained for *Arabidopsis thaliana* [At-1 (13); At-2 (39); At-3 (65)], *Populus trichocarpa* [Pt (81)], and *Nicotiana benthamiana* [Nb (114)]. An example of a family member with confirmed localization is mentioned (reference in parentheses) as well as the number of family members identified in each of the plant proteomes.

More recent evidence supports the importance of mobile transcripts and miRNAs in diverse processes (reviewed in 56, 72, 76), including leaf patterning (55), meristem development (e.g., 93), and sink–source relations (e.g., 23, 150). To identify transcripts that move for long distances from shoot to root and root to shoot, researchers have used grafting and/or profiling of phloem sap exudates (reviewed in 67). Grafting involves connecting the upper part of one plant with the lower section of another plant, leading to the formation of new plasmodesmata, reconnection of the vascular tissue, and restoration of molecular trafficking. RNA sequencing and mapping of single-nucleotide polymorphisms after grafting two different *Arabidopsis* ecotypes detected thousands of transcripts, ribosomal RNAs, transfer RNAs, and various classes of small RNAs that moved uni- or bidirectionally across the graft (junction), suggesting that they were transported via the newly formed plasmodesmata. These molecules are unlikely to move alone in the phloem sap, and it is hypothesized that they form complexes with ribosome and proteasome components present in the exudate (74, 110). The mechanism for transport and unloading of silencing RNAs from source-to-sink tissues was found coupled to ARGONAUTE (AGO) proteins and their consumption mechanism (34). Interestingly, up to a quarter of identified mobile transcripts move against the source-to-sink gradient, which indicates alternative mechanisms for transport.

How the plant controls the long-distance transport of RNAs and proteins in the phloem and their unloading is still under investigation. A screen based on trichome rescue of the *glabrous 1* (*gl1*) mutant, via mesophyll expression of the cell-autonomous protein GL1 fused with the mobile KN1 homeodomain, was used to identify factors affecting symplasmic transport. This screen identified chaperonins (147) and an RNA exosome subunit (73) presumably involved in binding and modifying the structure of protein and mRNA targets preceding their intercellular movement. A separate study in *Arabidopsis* proposes that plasmodesmata targeting of mobile RNAs is mediated by organelle-localized RNA-binding proteins [such as rotamase cyclophilins (ROCs)] and dependent on actin and microtubules (96). MS2-based mRNA live cell imaging (a tagging technique based on the affinity between the MS2 coat protein and the hairpin RNAs) showed that mobile mRNA colocalizes with a marker for multivesicular bodies and plasmodesmata targeting is impaired in the quadruple *roc* mutants. Despite these advances, many questions remain about the mechanisms underlying plasmodesmata targeting and transport of both RNA molecules and proteins.

Incomplete cytokinesis: partial division of the cytoplasm during cell division, resulting in the formation of cells with shared cytoplasmic content

3. CURRENT INSIGHT INTO THE FORMATION OF PLASMODESMATA

Plants continuously generate plasmodesmata, and their regulated insertion is important in shaping communication pathways between plant tissues. In this section, we delve into our current understanding of plasmodesmata formation and emphasize the plasticity and intricate interplay between the emergence of plasmodesmata, plant growth, and development.

3.1. Failing to Insert Plasmodesmata During Cytokinesis

Plant cells undergo simultaneous division and establishment of cell–cell connections, synergistically integrating these two cellular processes that are essential for multicellularity. When cells divide, plasmodesmata are inserted into the new cell wall, and, once cellular replication is complete, the twinned daughter cells are linked by hundreds of plasmodesmata, allowing immediate cell-to-cell communication (46, 58, 155).

Cell division involves two main processes: mitosis and cytokinesis. During mitosis, the nuclear components are divided, while cytokinesis involves the partitioning of cytoplasmic and membrane components, resulting in the physical separation of daughter cells and the loss of membrane and cytosolic continuity. In contrast to textbook models, cell division in plants does not terminate with the complete separation of daughter cells. Instead, twinned cells remain interconnected through plasmodesmata, ensuring cytoplasmic, PM, and ER continuity, through incomplete cytokinesis. Cytokinetic cytoplasmic bridges are not exclusive to plants but are also found in various eukaryotic lineages, including animals, fungi, and red and brown algae, presenting clonal multicellularity (19). Consequently, they have been suggested as a crucial structural basis for multicellularity, enabling cells to remain connected and exchange signals following division (19). Although incomplete cytokinesis occurs in specific cell types in animals (97), plants employ this mechanism systematically to establish their communication network.

The molecular mechanisms of plasmodesmata insertion during cytokinesis remain poorly understood. Most of the foundational work on this subject was performed in the 1970s and 1980s using electron microscopy and chemical fixation (54, 58, 125) and, more recently, by applying high-pressure freezing (130). Plant cytokinesis involves the formation of the cell plate, a primary structure built through the cooperation of the post-Golgi endomembrane system and the phragmoplast, an array of microtubules and actin filaments (134). The cell plate originates as a disk and gradually grows outward until it reaches and fuses with the side walls, creating a new wall that separates the parental cell into two daughter cells. Electron microscopy suggests entanglement of

the ER in the cell plate, contributing to the prevailing notion of accidental entrapment. However, given the highly dynamic nature of the ER (112), cellular coordination of both ER positioning and maintenance across the cell plate is likely necessary. Members of the reticulon family, with ER-shaping function, were reported to associate with cytokinetic plasmodesmata (75). To date, however, their implication in plasmodesmata biogenesis is unknown. Likewise, the molecular mechanisms responsible for incomplete cytokinesis in plants are currently unknown.

Auxin: a plant hormone that moves through plasmodesmata and auxin membrane transporters and regulates various aspects of growth and development

3.2. Shaping the Intercellular Landscape: The Emergence of Plasmodesmata During Development

In the mature plant body, cells are interconnected by plasmodesmata, but the density of these connections varies depending on cell type, tissue, and developmental stage. Not all cell–cell interfaces have the same capacity to transfer molecules, hormones, or nutrients to neighboring cells. This distribution reflects preferential symplasmic transport pathways that are important for maintaining auxin gradients (101) or facilitating communication within specific cell groups (44). The intercellular communication network requires modularity, where not all cells should be equally connected.

Communication capacity varies even at early stages of a cell's life. For example, in the root apical meristem, plasmodesmata density differs in division walls of adjacent cell types but also within a single tissue/cell lineage (155). Root epidermal cells have more plasmodesmata in their apical and basal walls than in their side walls, and macromolecule transport primarily occurs longitudinally (44).

Plasmodesmata distribution also changes during cell expansion and differentiation (40). As cells elongate, the walls undergo significant surface area increases, sometimes reaching magnitudes that are many hundredfold larger than the initial cell (27). Without additional communication channels, plasmodesmata formed during cytokinesis could become diluted, compromising cell-to-cell communication. During cell expansion and differentiation, the architecture and structure of plasmodesmata are also changing. Plasmodesmata go from type I single-stranded pores with a narrow diameter to a more complex structure with a modification of their trafficking properties, such as directionality for funnel-shaped plasmodesmata (105, 111).

The insertion of plasmodesmata in postmitotic cells involves wall perforation, ER strand insertion, and PM tube reformation. The molecular machinery behind this phenomenon is still unknown, but primary plasmodesmata were proposed to act as priming sites for secondary plasmodesmata insertion. Wall expansion triggers the insertion of new plasmodesmata along existing ones, and this iterative process leads to the formation of clustered plasmodesmata (pit fields) (37). It is important to note that, according to modeling, the clustering of plasmodesmata reduces the capacity for cell-to-cell transport in comparison to a random distribution (32) (**Figure 3**). In addition to wall expansion, hormones such as cytokinin influence plasmodesmata formation (109). In the vegetative shoot apical meristem, the application of cytokinin leads to an approximately threefold increase in plasmodesmata occurrence within just 20 h. A similar increase in density was observed during the transition from vegetative to floral stages, occurring 24 h after exposure to long-day conditions. Although we still do not know how this translates into the modification of transport capacity, these findings collectively suggest that plasmodesmata formation is regulated by multiple pathways.

Removal of plasmodesmata is equally important in shaping the intercellular landscape. For instance, during the maturation of stomata, the guard cells lose their plasmodesmata connections, leading to symplasmic isolation (30). This process allows the stomatal complex to attain autonomy and control over internal solute concentration, enabling the buildup of turgor pressure

essential for gas exchange and water balance. Removal of cytokinetic plasmodesmata also occurs during cell differentiation in the root meristem, most likely contributing to the experimentally observed decrease in cell-to-cell diffusion in the elongation zone (44). The mechanisms and speed of plasmodesmata removal are still poorly understood. These aspects deserve attention as they confer an additional layer of flexibility to the adaptive characteristics of the intercellular network.

Mechanical properties: physical characteristics and behaviors of a material or structure in response to mechanical forces, such as stress, strain, deformation, and elasticity

4. PLASMODESMATA MECHANOBIOLOGY AND BIOMECHANICS

Plasmodesmata exhibit structural plasticity, meaning that they can change their architecture and distribution in response to developmental or environmental signals. Fast opening/closing mechanisms and rapid changes in symplasmic transport rely on plasmodesmata mechanical properties (their deformability or the resistance to deformation when subjected to mechanical forces), which are, in turn, under the influence of structural components (protein/lipid/wall polymers) and changes in turgor pressure (59, 113). In this section, we review the ongoing research in plasmodesmata mechanobiology and biomechanics, establishing correlations and comparisons with other physical systems.

4.1. Plasmodesmata Under Cell Wall Pressure

Callose accumulation reduces plasmodesmata permeability as shown by genetic studies [e.g., *callose synthase 3 mutant* (*cals3m*), *plasmodesmatal beta 1,3 glucanase 1* (*pdbg1*), *pdgb2*, and *gfp-altered trafficking 1* (*gat1*)] (9, 10, 140). Both callose synthetic and degrading enzymes are localized at plasmodesmata; thus, callose are assumed to experience a rapid turnover underpinning a tight regulatory mechanism. Callose accumulation is thought to impose physical barriers that constrict the channel aperture (**Figure 3*d***), although callose may also play a role in channel shape and influence wall thickness.

Knowledge from other systems, such as pollen cell walls and fungal-induced papillae, has contributed to a widely accepted model suggesting that callose strengthens plant cell walls (45). Experimental evidence testing the properties of callose (using β-1,3-glucan analogs) in ionic liquid and hydrogel mixtures contradicts this model (1). Increasing the callose concentration in cellulose mixtures led to a decrease in the Young's modulus (i.e., elastic modulus) and a displacement in the fracture point, suggesting an increase in ductility and elasticity. This result was supported by a decrease in cellulose organization when comparing cell walls extracted from wild type and seedlings expressing the callose synthase–activated version *cals3m*. Another important conclusion from this study (45) indicates the capacity of cellulose to interact with callose, a concept further corroborated using molecular simulations (78a). The degree of callose deposition at plasmodesmata is thought to determine the level of restriction imposed on molecular transport. However, the proposed cellulose–callose interactions indicate that this effect is nonlinear (very small changes in callose can exponentially impact wall properties). Predictions from the molecular simulation also indicate that callose hydrophilicity might strongly impact cell wall mechanical properties. This aligns well with the increased degradability (enzymatic accessibility) of transgenic poplar woody biomass ectopically expressing a hyperactive callose synthase (12). Together, the data point to callose as a plasticizer, making cellulosic walls more flexible to accommodate the passage of macromolecules through plasmodesmata.

Cellulose, pectins, hemicelluloses, and other structural proteins might also contribute to plasmodesmata stability, positioning, and function. In contrast to callose, nothing is known about the properties of these components at plasmodesmata or how changes in the callose structure modify its biophysical properties.

4.2. Plasmodesmata and Changes in Cell Turgor Pressure

Bulk flow: the movement of fluids driven by pressure differences

Molecular diffusion: the spontaneous movement of molecules from an area of higher concentration to an area of lower concentration

In plants, turgor pressure is crucial for maintaining cell shape and rigidity and for supporting physiological processes such as nutrient transport and cell expansion (59). Turgor pressure is generated by the influx of water into the cells through osmosis, driven by the concentration of intracellular solutes (59). When water enters the cells, it fills the vacuole and exerts pressure on the walls. This pressure is transmitted to neighboring cells through plasmodesmata, creating a balance of turgor pressure across the plant tissue. Plasmodesmata play an essential role in maintaining turgor pressure equilibrium because they allow the free flow of water and solutes between cells. As they move, water and solutes help equalize the turgor pressure among neighboring cells, ensuring that it is evenly distributed throughout the tissue and protecting the mechanical integrity of tissues.

A recent study measured the translational water diffusion through plasmodesmata in maize roots, using the nuclear magnetic resonance spin echo method (3) to find that the rate of water flow via plasmodesmata along a linear cell chain was in the range of 4.5×10^{-7} to 8.8×10^{-7} m/s. Methods were also developed to quantify symplasmic (cell-to-cell) transport of water in 12-day-old lupin roots (152). Zarebanadkouki et al. (152) combined the quantification of deuterated water distribution imaged by rapid neutron tomography with an inverse simulation of water transport across root tissues to calculate the total flow of water across the apoplastic and the symplasmic pathways. They found a higher radial flux through the apoplastic pathway ($104 \pm 73\times$ higher at 0.06 cm from the root center), but, at the endodermis, the overall contribution was rather similar in both pathways at a flow rate of ~0.03 cm^3/s. Accessible models [e.g., MECHA (28)] were developed to compute the flow of water across the root. The model predicts that radial hydraulic conductivity values are particularly sensitive to changes in plasmodesmata conductance, especially after endodermal suberization (i.e., the reinforcement of apoplastic barriers by the addition of suberins).

The importance of turgor pressure on the dumbbell-shaped desmotubule and its capacity to obstruct molecular flow was considered by Park et al. (113). The authors developed a model of pressure-regulated plasmodesmata permeability, where the position of the dumbbell-shaped desmotubule changes to obstruct plasmodesmata apertures (113) (**Figure 3*e***). Using this model, the authors predict slow decreases in permeability at low pressure but dramatic modifications when changes in pressure are >150 kPa. These predictions align well with experimental observations and explain responses that are presumably faster (on the order of seconds) than changes in callose metabolism.

4.3. Symplasmic Molecular Flow Driven by Changes in Plasmodesmata Architecture

Changes in the architecture of plasmodesmata influence the rate at which molecules flow through plasmodesmata (i.e., the symplasmic molecular flow). Plasmodesmata can undergo remodeling, such as expansion or contraction, and branching in response to developmental and environmental cues. In rare cases, the desmotubule can be removed, eliminating obstacles for transport, such as in nitrogen-fixing actinorhizal root nodules of the Australian tree *Casuarina glauca* (33).

It is not difficult to conceptualize how changes in shape can influence flow mechanics across plasmodesmata. Plasmodesmata geometry determines their resistance to diffusion and convective flow as well as the SEL. For instance, funnel-shaped, instead of simple cylindrical, plasmodesmata connect the protophloem cells with surrounding cells in multiple species (111). These conical geometries reduce the physical resistance to symplasmic phloem unloading by bulk flow and molecular diffusion (**Figure 3*f***). Theoretical evaluations indicated that small opening angles of about 3° reduce the hydraulic resistance by over 90%, and the diffusive resistance by some 70% when compared to cylindrical straight geometries (111). The effect of structural shape on

plasmodesmata permeability is so relevant that it can override the effect of increasing plasmodesmata density or SEL.

Plasmodesmata shape changes have also been suggested to be linked to modifications in PM-ER membrane contact sites (MCSs) (105, 149). Looking for protein tethers bridging the two membranes, Brault et al. (13) identified MCTPs that specifically associated with plasmodesmata (13). Other proteins, such as SYNAPTOTAGMIN 1 (SYT1), SYT5, and SYT7, have also been described as general (cortical ER–bulk PM) and plasmodesmata-associated tethers and shown to positively regulate virus movement from cell to cell without affecting the symplasmic molecular flow between cells (20, 63, 82). A tight PM-ER tethering is correlated with type I plasmodesmata. Theoretical models suggest that tight PM-ER tethering together with type I plasmodesmata geometry affects symplasmic permeability differently depending on whether it is present in thin (approximately 100 nm in width) or thick (average 200 nm) cell walls (32). Defects in the transition from type I to type II (with a visible cytoplasmic sleeve) plasmodesmata (as found in mutants in the *PHLOEM UNLOADING MODULATOR* gene) increased symplasmic trafficking (149). Insights obtained from theoretical models suggest that transport time scales quadratically with the radius of the central region, whereas the relative transport volume depends on length. Thus, straight (type I–like) plasmodesmata perform better in thin cell walls (32). Other modifications, such as branching and clustering, create physical and mechanical constrictions to symplasmic transport (**Figure 3*a*,*b***). Some models have considered these factors, but experimental and ultrastructural evidence is missing to dissect quantitatively their impacts on cell-to-cell trafficking.

Membrane contact site (MCS): the physical apposition of two membranes driven by tether proteins without membrane fusion

Symplasmic domains: interconnected regions within a plant's tissues where movement of substances occurs through the cytoplasmic continuum created by plasmodesmata

Abscisic acid (ABA): a key signaling hormone that helps plants adapt and respond to changing environmental cues such as temperature changes and water stress

5. PLASMODESMATA UNDER DEVELOPMENTAL AND ENVIRONMENTAL PRESSURES

Plasmodesmata formation and function are under the continuous influence of developmental and environmental cues. In this section, we explore recent research linking growth, plant responses to stress, and cell-to-cell trafficking.

5.1. Plasmodesmata in the Development of Plant Organs

The density and permeability of plasmodesmata determine symplasmic domains within tissues, leading to the exchange and confinement of signals and cell fate transitions. Apical meristem development, the initiation of lateral organs, stomata and root hair formation, sink-to-source transitions, and distinction between abaxial and adaxial sides in leaves, among other processes, are all preceded by symplasmic domain formation (71, 126). The development of sink tissues relies on sugar unloading via plasmodesmata, which is regulated by the TARGET OF RAPAMYCIN (TOR) metabolic signaling network in leaves (14). The FLOWERING LOCUS T (FT) family members are loaded in the phloem of source leaves and unloaded via the symplast in the apex where they induce flowering (151). Recent work has focused on the role of FT and plasmodesmata in the break of bud dormancy in response to photoperiod (121, 133). Another developmental process controlled by FT-like proteins and the formation of symplasmic domains is tuberization (104). Recent research found that the FT-like SELF-PRUNING 6A (SP6A) protein interacts with the conserved transcription factor BRC1b, and this interaction blocks tuberization in aerial nodes of potato. BRC1b promotes bud dormancy, preventing the formation of sugar sinks in the bud that compete with the stolons (enlarged stems that thicken to develop into potato tubers). The mechanism involves the hormone abscisic acid (ABA), the expression of ABA-related genes, and a reduction in the number of plasmodesmata, which blocks SP6A movement into the buds. RNA interference lines reducing BRC1b expression led to the formation of more plasmodesmata, enhancing the expression of SP6A in the bud and the formation of aerial tubers (104).

Brassinosteroid (BR): a class of plant hormones that plays crucial roles in promoting plant growth and development, including cell elongation, cell division, and differentiation; BR precursors move through plasmodesmata

Plasmodesmata also serve as conduits for other mobile proteins and RNAs that regulate development. For example, mobility of the transcription factor WUSCHEL (WUS) within the shoot apical meristem and the WUS-RELATED HOMEOBOX 5 (WOX5) in the root apical meristem, together with miR394 targeting repression factors, generates a feedback loop that promotes meristem development while maintaining the stem cell pool (see 71 and references therein). However, plasmodesmata-mediated regulation of the mobile transcription factor SPEECHLESS (SPCH), which controls stomatal formation and patterning, is required to avoid the formation of clustered stomata and disorganized cell divisions in the stomatal lineage (50, 77).

The relationship of plasmodesmata with relevant phytohormones such as auxins, cytokinins and, more recently, brassinosteroids (BRs) has emerged (126). A new article highlights the importance of short-distance intercellular movement of BR precursors through plasmodesmata to establish and maintain BR signaling maxima in the root elongation zone (8, 144). In turn, the cellular BR content modifies plasmodesmata permeability to optimize its own transport.

Auxins control the expression of plasmodesmata proteins (including CALSs, PdBGs, and PDCBs) and the formation of symplasmic domains during lateral root development and in the tropic response (53, 98). Meanwhile, the ectopic expression of a hyperactive CALS3 (*cals3m*) in the quiescent center led to a failure in the formation of local auxin maxima and in the expression gradient of PLETHORA proteins (transcription factors essential for root meristem maintenance) (36, 91, 127). Computational modeling predicted that symplasmic transport is critical for the formation of informative auxin gradients across root tissues (101). Consideration of auxin transport via the symplasm improves the predictions of auxin cellular concentrations by computer models. This is also true when generating predictions for auxin transport from leaf tips to the petioles: a signal mediating the shade-avoidance response (41). A model based on photoactivation assays indicates that the rate of symplasmic transport is higher in the longitudinal versus the transversal direction in elongated petiole cells, and this directionality was essential for proper auxin distribution in response to shade. Supporting the model, *gsl8* (a mutant in a CALS gene) was impaired in auxin distribution and, concomitantly, in leaf movement upon application of radioactive auxin at the tip.

The links between auxin transport and plasmodesmata remain of interest as auxin is a key regulatory factor in the development of multiple organs. Blocking plasmodesmata in the root cap using the *icals3m* system (i.e., the induction of hyperactive *cals3m*) altered auxin distribution and root meristem development (83). Linh & Scarpella (86) indicate that regulated symplasmic movement of auxin or an auxin-dependent signal is required for proper leaf vein patterning (86). Lateral root emergence is also controlled by the transport of auxin which, in turn, triggers a feedback loop that regulates the expression of the PDLP5 in the tissue overlying the primordia (123). Besides auxins and ABA, cytokinins are also regulators of symplasmic transport, providing another hormone-mediated mechanism with implications for the development of multiple plant organs (61).

In summary, plasmodesmata control the transportation of developmental signals, including sugars and phytohormones, while being affected by the concentrations of these same phytohormones. This interaction influences RNA and protein expression domains crucial for cell fate transitions, cell specification, and organogenesis.

5.2. Plasmodesmata in Environmental Signaling

Plants need to adapt to fluctuations in their environment, including temperature shifts, water and nutrient availability, and light intensity. To thrive in their ever-changing environment, plants modify their growth pattern, and these adaptative measures involve changes in cell-to-cell communication (70, 129).

Due to the escalating impact of global warming, there is growing interest in studying how plants respond to temperature fluctuations. Each plant species has an optimal temperature range for growth and development, and deviations from this range can hinder or even halt growth, prioritizing survival over growth (35, 60). In addition to short-term temperature stress, plants such as perennial trees also protect themselves from the cold winter through dormancy, an adaptative process that involves the suspension of active growth, shedding of leaves, and slowdown of metabolic processes (99). Dormancy allows plants to conserve energy, protect themselves from harsh winter conditions, and resume growth when conditions improve in spring.

How does cell–cell communication contribute to plant responses to temperature stress? Recent research has revealed the influence of short-term cold or heat stress on intercellular transport and its effects on shoot and root meristems.

For instance, exposing *Arabidopsis* seedlings to high temperatures (around 30°C) for 3–4 days hinders phloem unloading, disrupting the supply of photosynthetic products to the root and inhibiting growth (87). This blockage of phloem unloading involves CALS8, which operates at the interface between the sieve element and the phloem pole pericycle, a key interface facilitating photosynthetic product unloading (122). The significance of CALS8 extends beyond temperature stress, as it has been previously identified as regulating plasmodesmata trafficking in response to pathogen infection and wounding (29). A similar reduction in the export of photosynthesis assimilates in maize leaves is linked to callose-induced plasmodesmata closure in response to moderate chilling [14°C; 28 h (11)]. In both scenarios, changes in cell-to-cell communication happen in specific cell types. In a similar line, cold stress leads to an increase in callose accumulation in both the shoot apical and floral meristems of tomatoes (145). This callose build up leads to symplastic isolation and altered intercellular movement of WUS, expressed in the organizing center that non-cell-autonomously activates the expression of CLAVATA 3 (CLV3) and AGAMOUS in the L1 and L2 layers (31, 92, 128, 148). Similarly, inhibition of cell-to-cell transport of *Solanum lycopersicum* WUS (SlWUS) prevents the activation of *SlCLV3* and *TAG1* (the homologous gene of AGAMOUS in tomato) genes, resulting in an expanded stem cell population and malformed fruits (145).

The modulation of callose dynamics in response to cold employs a mechanism akin to bud dormancy, involving changes in the hormonal balance of ABA and gibberellic acid (GA) (**Figure 4*a***). During winter, perennial plants enter dormancy, ceasing growth and intercellular communication. Growth resumes in spring after dormancy is released, triggered by prolonged exposure to cold and the restoration of intercellular communication (119, 133, 139). As winter progresses, the cell–cell network transitions from a lock-down state to an open state in preparation for spring growth. The survival of trees depends on precise timing and response to cold, which itself relies on the restoration of cell-to-cell communication. In perennial trees, as in tomato meristem, callose deposition/degradation is linked with ABA and GA signaling (139, 141).

Bud dormancy is activated through the ABA pathway, upregulating CALS1 and suppressing BG proteins to block plasmodesmata (139). GA signaling counteracts callose, promoting the dormancy break by restoring the symplastic route through BG upregulation (42, 120, 133). In seeds, however, ABA has an opposite effect by inducing BG14, which reduces callose at plasmodesmata, influencing seed longevity and dormancy in *Arabidopsis* (143).

Plasmodesmata also play a significant role in adaptive plant responses to water availability (100) (**Figure 4*b***). Treatment of *Arabidopsis* seedlings with osmolytes such as polyethylene glycol, NaCl, and mannitol, which reduces the water potential, triggers plasmodesmata closure via the deposition of callose (48, 69). This mechanism integrates with hormonal pathways to control root branching in response to changes in water availability. Hence, when roots lose contact with moist soil, lateral root formation is repressed through a process called xerobranching (108). The

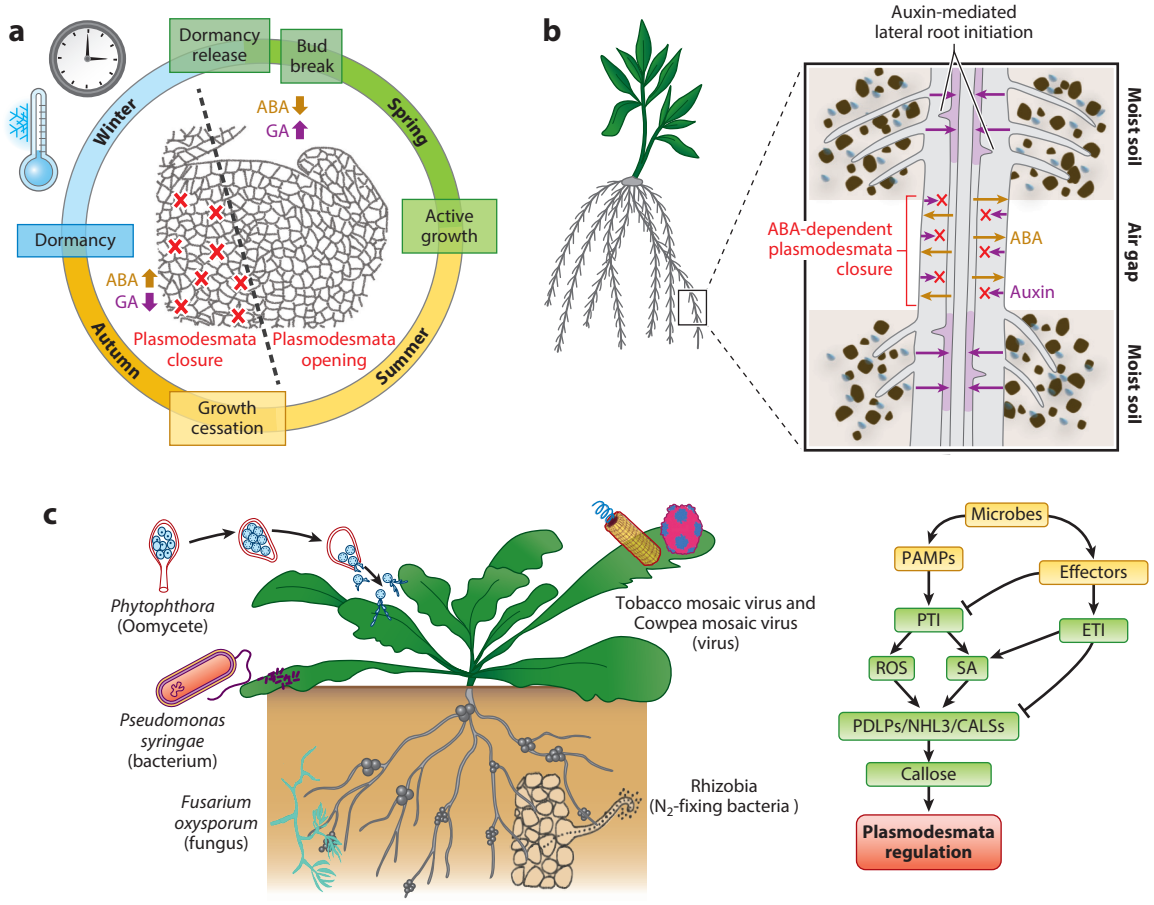

Figure 4

Dynamic response and adaptation of plasmodesmata cell-to-cell communication to environmental pressure. (*a*) Plasmodesmata undergo a lockdown during winter dormancy resulting from ABA-triggered callose deposition (119, 133, 139). Closed plasmodesmata are indicated by red × symbols. To break dormancy, buds require extended exposure to low-temperature conditions, simulating a winter cue. Prolonged cold induces the reopening of plasmodesmata and movement of growth factors into the bud. The regulation of the opening and closing of plasmodesmata in response to cold is modulated by the plant hormones ABA and GA. (*b*) Inhibition of lateral root growth during transient water deficit. The growth of lateral roots is hindered under water scarcity conditions, a phenomenon known as xerobranching (108). Lateral roots typically emerge from cells located in the pericycle layer of the root and are regulated by the hormone auxin and ABA. However, when roots no longer have access to moist soil, the direction of water transport changes, along with changes in the distribution of the hormone ABA. ABA-triggered callose deposition results in the closure of plasmodesmata, which prevents the inward movement of auxin. Consequently, the concentration of auxin in pericycle cells decreases, leading to the inhibition of lateral root formation in the air gap regions. (*c*) Plants engage in continuous interactions with a diverse array of microorganisms, including fungi, oomycetes, bacteria, and viruses (reviewed in 21, 45, 64, 88). These interactions can be either advantageous, as seen with the soil bacteria rhizobia, or hazardous. As part of the plant's defense response, plasmodesmata are initially closed upon pathogen detection through PAMP-triggered signaling involving the production of ROS and SA, along with the involvement of plasmodesmata-associated protein nodes such as PDLPs/NHL3 that regulate callose production via CALSs. However, microbial pathogens counteract this defense mechanism by producing virulent effector proteins that target the plasmodesmata defense layer. Abbreviations: ABA, abscisic acid; CALS, callose synthase; ETI, effector-triggered immunity; GA, gibberellic acid; NHL3, NON-RACE-SPECIFIC DISEASE RESISTANCE/HIN1 HAIRPIN-INDUCED-LIKE protein 3; PAMP, pathogen-associated molecular pattern; PDLP, plasmodesmata-located protein; PTI, PAMP-triggered immunity; ROS, reactive oxygen species; SA, salicylic acid.

regulation of root branching in response to soil water variations involves the redistribution of ABA and auxin hormones and adjustments in plasmodesmata permeability. In situations where roots lose contact with moist soil, the phloem-derived hormone ABA moves from inner to outer root tissues, causing plasmodesmata closure. This disrupts the inward movement of auxin with water flow, inhibiting lateral root formation. Once the root regains contact with moisture, the ABA response diminishes, plasmodesmata open, and auxin moves inward with the water flow, reactivating lateral root initiation. Mutants unable to close plasmodesmata (e.g., *cals7* and *pdlp2 pdlp3*) show defects in the xerobranching response. Plasmodesmata regulation enables plants to optimize water uptake during drought or limited moisture conditions by controlling the radial mobilization of hormones.

In all these instances, hormones, callose, and cell-to-cell transport form a cohesive network, with ABA emerging as a key regulator of plasmodesmata permeability in response to environmental pressure (7). Understanding this network will provide valuable insights into how plants adapt and cope with fluctuating temperatures and water stresses.

5.3. Plasmodesmata in Plant–Microbe Interactions

Throughout their life span, plants interact with a multitude of microorganisms (**Figure 4*c***). These interactions can be mutually beneficial or detrimental, leading to pathogenesis. Plants detect microorganisms through microbial signatures, activating extracellular pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and intracellular effector-triggered immunity (ETI) recognition and immune signaling (136). PTI acts as the primary line of defense, recognizing extracellular conserved molecular patterns (PAMPs) such as flagellin (flg22; from *Pseudomonas syringae* bacteria) or chitin (from fungi) through plant receptors on the cell surface. Host-adapted pathogens deploy virulent factors called effectors that are delivered inside the cells to impede host defense. In response, plants employ intracellular immune receptors to unleash a potent second line of defense known as ETI. In both PTI and ETI, a crucial element of the cellular defense response involves modifying plasmodesmata to counteract invading microbes. Viruses, bacteria, oomycetes, and fungi also possess the ability to manipulate plasmodesmata to invade plants (reviewed in 21, 45, 64, 88). A battle ensues between the plant and microorganisms as they strive to gain control over plasmodesmata.

Among these pathogens, viruses represent one of the earliest and possibly most thoroughly explored areas in the context of plasmodesmata–pathogen interactions. Viruses utilize plasmodesmata as conduits for cell-to-cell movement of their viral genome and encode movement proteins to force their way through the channels. This field of study holds historical significance in recognizing the transport capacity of plasmodesmata, together with the discovery that plasmodesmata can be dynamically regulated (recently reviewed in 62). In response to viral infection, plants produce callose to limit viral spreading, but viruses manipulate callose levels to counteract this defense. This strategy, relying on plasmodesmata and callose, is also applicable to bacterial, oomycete, and fungal infections as PAMPs and effectors induce modifications in callose at plasmodesmata (17, 21, 88).

In studies investigating plasmodesmata modifications during plant–microbe interactions, callose is consistently identified as the primary target, regardless of the specific signaling pathway activated by pathogens. However, a remaining question is how plants integrate multiple signaling pathways to achieve the common outcome of plasmodesmata closure. For example, fungal PAMP chitin and bacterial PAMP flg22 trigger distinct immune signaling pathways through different receptors, but both converge on callose-dependent plasmodesmata closure (22, 38, 80). At the top of the signaling cascade, flg22 and chitin perception by the plant triggers the production of reactive oxygen species (ROS) through NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), which, in turn, leads to callose deposition (22, 38, 146).

Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI): a plant defense response that is activated upon recognition of PAMPs by cell surface receptors, leading to a cascade of immune responses

Effector-triggered immunity (ETI): a plant defense response that is activated upon recognition of pathogen effectors inside the host cells

In parallel, the defense hormone salicylic acid (SA) also triggers callose deposition at plasmodesmata, likely serving to relay or amplify the PAMP-triggered response. The production of ROS by RBOHD and the SA-induced signal both converge at PDLP1 and PDLP5, which, in coordination with their interacting partner NON-RACE-SPECIFIC DISEASE RESISTANCE/HIN1 HAIRPIN-INDUCED-LIKE protein 3 (NHL3), integrate the information and activate CALS1 (135) (**Figure 4*c***). As pathogen infection progresses, effectors are produced and secreted into host cells to suppress plant defenses, including plasmodesmata responses. For instance, the *Pseudomonas syringae* effector protein HopO1-1 destabilizes PDLP5 and PDLP7, key components of the PAMP-triggered responses regulating callose levels upon flg22 perception (4, 135). The oomycete *Phytophthora brassicae* secretes the effector protein RxRL3, which targets callose synthases and suppresses callose accumulation, thereby promoting its intercellular movement (138). Similarly, the fungus *Fusarium oxysporum* utilizes the Avr2-Six5 effectors to modify the plasmodesmata SEL, facilitating the movement of Avr2 and enhancing virulence (17).

Plants face a delicate balance between closing plasmodesmata to restrict pathogen spread and maintaining intercellular communication to prime defense responses to neighboring cells (132). Distantly located, uninfected cells will then activate systemic acquired resistance (SAR), serving as an immune memory to enhance the speed and effectiveness of defense responses upon pathogen encounter (49, 51, 118). Mobile signaling molecules, including azelaic acid and glycerol-3-phosphate, play a crucial role in SAR establishment through plasmodesmata transport (132). How plants balance the need to close plasmodesmata to limit pathogen propagation while maintaining communication for non-cell-autonomous defense response is still not well understood. Precise spatiotemporal regulation of plasmodesmata closure may be critical. In addition, the apoplastic trafficking of immune molecules could serve as an alternative transport pathway, with SA being preferentially transported via the apoplast, whereas phloem loading of azelaic acid and glycerol-3-phosphate occurs via the symplast (85, 132).

Plant–microbe interactions are not always detrimental and can also be a bonus for the plants when a symbiotic mutualistic interaction is engaged. So far, limited research has been conducted on plasmodesmata in mutualistic symbioses. However, the available data suggest that when plants are colonized by beneficial microorganisms such as nitrogen-fixing bacteria, they promote symplastic communication within the colonized tissue (26, 43, 69). For example, within 24 h of *Medicago truncatula* infection by the soil-borne bacteria rhizobia, the expression of the endogenous plasmodesmata-located BG protein MtBG2 increases, leading to reduced callose levels (43). Nodulation is stimulated by ectopic expression of MtBG2, whereas silencing MtBG2 or overexpressing *cals3m* at the infection site reduces infection events and the number of nodules. PDLPs also play a role in this mechanism as the ectopic expression of a PDLP-like protein identified in *M. truncatula* modifies callose levels and improves infection and nodulation, particularly in the presence of nitrate (69).

Overall, it is evident that the regulation of plasmodesmata is vital in plant–microbe interactions, with plants and microbes having evolved intricate strategies to control plasmodesmata permeability for their benefit. Understanding the specific responses of plasmodesmata to different stress-related stimuli is crucial for comprehending how organism-level responses are regulated in a multicellular context. Future research will be instrumental in advancing our knowledge in this field.

6. SUMMARY AND FUTURE PERSPECTIVES

Ongoing research is investigating how plants control the transport of RNAs and proteins between cells over short and long distances. Plasmodesmata play a key role in regulating intercellular communication through their formation, regulation, and removal. However, there is still a lack of detailed understanding regarding the molecular factors and developmental/environmental cues

that finely tune these processes. Cell wall composition, properties such as elasticity and ductility, and the presence of proteins that interact with lipids and bridge the ER and the PM, as well as the desmotubule, are recognized as significant factors. Recent advances in electron tomography (for determination of plasmodesmata structure and geometric features), photoactivatable/photoswitchable probes (applied to calculate plasmodesmata permeability or symplasmic molecular flow rate), super-resolution approaches (for characterizing plasmodesmata components and protein–protein interactions), and modeling (to predict, for example, changes in permeability driven by changes in plasmodesmata geometries, density, and distribution) have improved our understanding of these enigmatic structures. Single-cell transcriptomics and new grafting methodologies are rapidly discovering a compendium of mobile proteins and RNA molecules. New technologies to study structural mechanics and the development of physical models and computer simulations are applied to unlock plasmodesmata regulation. Using these technological advances, future research will reveal how plasmodesmata components (protein, lipid, and wall polymers) integrate to regulate mechanical properties and transport capabilities across different tissues and in varying physiological and environmental conditions.

SUMMARY POINTS

1. Plasmodesmata are communication bridges essential for plant development and responses to the environment.
2. Plasmodesmata structure and molecular composition are dynamic and required for fine tuning cell–cell molecular trafficking.
3. Cell wall microdomains surrounding plasmodesmata are enriched in callose and experience structural modifications that control symplasmic permeability.
4. The expression, activity, and localization of plasmodesmata regulatory proteins are modified in response to changes in the developmental, physiological and environmental conditions.
5. Computational modeling and bioinformatic approaches aid in understanding intercellular transport at the micro and macro levels and how plasmodesmata geometric features and tissue distribution contribute to effective transport.
6. Cell turgor pressure and flow mechanics influence symplasmic connectivity.
7. The density and permeability of plasmodesmata determine symplasmic domains within tissues, leading to the exchange and confinement of signals and cell fate transitions.
8. Plants face a delicate balance between closing plasmodesmata to restrict pathogen spread and maintaining intercellular communication to prime defense responses in neighboring cells.

FUTURE ISSUES

1. More research should focus on the function of ER-PM contacts in plasmodesmata structure and function and interplay with the extracellular matrix.
2. What are the mechanisms underpinning callose action as part of different environmental and developmental responses?

3. Researchers should dig further into the interplay between plasmodesmata and hormonal signaling.
4. Research is required to unlock the physicomechanical factors influencing plasmodesmata communication.
5. Further research should be done on the interactions between the symplasmic and the apoplastic pathway and how they influence the establishment of information and water gradients.
6. How does the plant control the long-distance transport of RNAs and proteins in the phloem and integrate multiple biotic and abiotic signaling pathways to achieve plasmodesmata regulation?
7. Solving the challenge of in vivo visualization and coordination of plasmodesmata biogenesis, structural modifications, and function should be a priority.
8. Researchers should develop new system biology approaches that integrate knowledge on plasmodesmata.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Investigates for the first time the physicomechanical properties of callose analogs in cellulosic mixture.

1. **Abou-Saleh RH, Hernandez-Gomez MC, Amsbury S, Paniagua C, Bourdon M, et al. 2018. Interactions between callose and cellulose revealed through the analysis of biopolymer mixtures. *Nat. Commun.* 9:4538**
2. Amsbury S, Kirk P, Benitez-Alfonso Y. 2017. Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. *J. Exp. Bot.* 69:105–15
3. Anisimov AV, Suslov MA. 2023. Measuring of water transport selectively along the plant root plasmodesmata using gradient nuclear magnetic resonance with paramagnetic doping. *Plant Physiol. Biochem.* 194:263–70

4. Aung K, Kim P, Li Z, Joe A, Kvitko B, et al. 2020. Pathogenic bacteria target plant plasmodesmata to colonize and invade surrounding tissues. *Plant Cell* 32:595–611
5. Band LR. 2021. Auxin fluxes through plasmodesmata. *New Phytol*. 231:1686–92
6. Bayer EM, Bottrill AR, Walshaw J, Vigouroux M, Naldrett MJ, et al. 2006. *Arabidopsis* cell wall proteome defined using multidimensional protein identification technology. *Proteomics* 6:301–11
7. Benitez-Alfonso Y. 2019. The role of abscisic acid in the regulation of plasmodesmata and symplastic intercellular transport. *Plant Cell Physiol*. 60:713–14
8. Benitez-Alfonso Y, Cano-Delgado AI. 2023. Brassinosteroids en route. *Nat. Chem. Biol.* 19:1294–95
9. Benitez-Alfonso Y, Cilia M, San Roman A, Thomas C, Maule A, et al. 2009. Control of *Arabidopsis* meristem development by thioredoxin-dependent regulation of intercellular transport. *PNAS* 106:3615–20
10. Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y, Maule A. 2013. Symplastic intercellular connectivity regulates lateral root patterning. *Dev. Cell* 26:136–47
11. Bilska A, Sowinski P. 2010. Closure of plasmodesmata in maize (*Zea mays*) at low temperature: a new mechanism for inhibition of photosynthesis. *Ann. Bot.* 106:675–86
12. Bourdon M, Lyczakowski JJ, Cresswell R, Amsbury S, Vilaplana F, et al. 2023. Ectopic callose deposition into woody biomass modulates the nano-architecture of macrofibrils. *Nat. Plants* 9:1530–46
13. **Brault ML, Petit JD, Immel F, Nicolas WJ, Glavier M, et al. 2019. Multiple C2 domains and transmembrane region proteins (MCTPs) tether membranes at plasmodesmata. *EMBO Rep*. 20:e47182**
14. Brunkard JO, Xu M, Scarpin MR, Chatterjee S, Shemyakina EA, et al. 2020. TOR dynamically regulates plant cell–cell transport. *PNAS* 117:5049–58
15. Brunkard JO, Zambryski PC. 2017. Plasmodesmata enable multicellularity: new insights into their evolution, biogenesis, and functions in development and immunity. *Curr. Opin. Plant Biol.* 35:76–83
16. Caillaud MC, Wirthmueller L, Sklenar J, Findlay K, Piquerez SJ, et al. 2014. The plasmodesmal protein PDLP1 localises to haustoria-associated membranes during downy mildew infection and regulates callose deposition. *PLOS Pathog.* 10:e1004496
17. Cao L, Blekemolen MC, Tintor N, Cornelissen BJC, Takken FLW. 2018. The *Fusarium oxysporum* Avr2-Six5 effector pair alters plasmodesmatal exclusion selectivity to facilitate cell-to-cell movement of Avr2. *Mol. Plant* 11:691–705
18. Carlsbecker A, Lee JY, Roberts CJ, Dettmer J, Lehesranta S, et al. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465:316–21
19. Chaigne A, Brunet T. 2022. Incomplete abscission and cytoplasmic bridges in the evolution of eukaryotic multicellularity. *Curr. Biol.* 32:R385–97
20. Chen C, Vanneste S, Chen X. 2021. Review: Membrane tethers control plasmodesmal function and formation. *Plant Sci.* 304:110800
21. Cheval C, Faulkner C. 2018. Plasmodesmal regulation during plant–pathogen interactions. *New Phytol*. 217:62–67
22. Cheval C, Samwald S, Johnston MG, de Keijzer J, Breakspear A, et al. 2020. Chitin perception in plasmodesmata characterizes submembrane immune-signaling specificity in plants. *PNAS* 117:9621–29
23. Cho SK, Kang IH, Carr T, Hannapel DJ. 2012. Using the yeast three-hybrid system to identify proteins that interact with a phloem-mobile mRNA. *Front. Plant Sci.* 3:189
24. Christensen AH, Stone HA, Jensen KH. 2021. Diffusion and flow across shape-perturbed plasmodesmata nanopores in plants. *Eur. Phys. J. Plus* 136:872
25. Combarnous Y, Nguyen TMD. 2020. Cell communications among microorganisms, plants, and animals: origin, evolution, and interplays. *Int. J. Mol. Sci.* 21:8052
26. Complainville A, Brocard L, Roberts I, Dax E, Sever N, et al. 2003. Nodule initiation involves the creation of a new symplasmic field in specific root cells of *Medicago* species. *Plant Cell* 15:2778–91
27. Cosgrove DJ. 2022. Building an extensible cell wall. *Plant Physiol*. 189:1246–77
28. Couvreur V, Faget M, Lobet G, Javaux M, Chaumont F, Draye X. 2018. Going with the flow: multiscale insights into the composite nature of water transport in roots. *Plant Physiol*. 178:1689–703
29. Cui W, Lee JY. 2016. *Arabidopsis* callose synthases CalS1/8 regulate plasmodesmal permeability during stress. *Nat. Plants* 2:16034

13. Identified plasmodesmata-specific ER-PM tether candidates, suggesting a function of membrane contacts in regulating molecular flow.

30. Cui Y, He M, Liu D, Liu J, Liu J, Yan D. 2023. Intercellular communication during stomatal development with a focus on the role of symplastic connection. *Int. J. Mol. Sci.* 24:2593
31. Daum G, Medzihradszky A, Suzaki T, Lohmann JU. 2014. A mechanistic framework for noncell autonomous stem cell induction in *Arabidopsis*. *PNAS* 111:14619–24
32. Deinum EE, Mulder BM, Benitez-Alfonso Y. 2019. From plasmodesma geometry to effective symplasmic permeability through biophysical modelling. *eLife* 8:e4900
33. Demchenko KN, Voitsekhovskaja OV, Pawlowski K. 2014. Plasmodesmata without callose and calreticulin in higher plants—open channels for fast symplastic transport? *Front. Plant Sci.* 5:74
34. Devers EA, Brosnan CA, Sarazin A, Schott G, Lim P, et al. 2023. *In planta* dynamics, transport biases, and endogenous functions of mobile siRNAs in *Arabidopsis*. *Plant J.* 115:1377–93
35. Ding Y, Yang S. 2022. Surviving and thriving: how plants perceive and respond to temperature stress. *Dev. Cell* 57:947–58
36. Du Y, Scheres B. 2017. PLETHORA transcription factors orchestrate de novo organ patterning during *Arabidopsis* lateral root outgrowth. *PNAS* 114:11709–14
37. Faulkner C, Akman OE, Bell K, Jeffree C, Oparka K. 2008. Peeking into pit fields: a multiple twinning model of secondary plasmodesmata formation in tobacco. *Plant Cell* 20:1504–18
38. Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, et al. 2013. LYM2-dependent chitin perception limits molecular flux via plasmodesmata. *PNAS* 110:9166–70
39. Fernandez-Calvino L, Faulkner C, Walshaw J, Saalbach G, Bayer E, et al. 2011. Arabidopsis plasmodesmal proteome. *PLOS ONE* 6:e18880
40. Fitzgibbon J, Beck M, Zhou J, Faulkner C, Robatzek S, Oparka K. 2013. A developmental framework for complex plasmodesmata formation revealed by large-scale imaging of the *Arabidopsis* leaf epidermis. *Plant Cell* 25:57–70
41. Gao C, Liu X, De Storme N, Jensen KH, Xu Q, et al. 2020. Directionality of plasmodesmata-mediated transport in *Arabidopsis* leaves supports auxin channeling. *Curr. Biol.* 30:1970–77.e4
42. Gao X, Yuan Y, Liu Z, Liu C, Xin H, et al. 2021. Chilling and gibberellin acids hyperinduce β-1,3-glucanases to reopen transport corridor and break endodormancy in tree peony (*Paeonia suffruticosa*). *Plant Physiol. Biochem.* 167:771–84
43. Gaudioso-Pedraza R, Beck M, Frances L, Kirk P, Ripodas C, et al. 2018. Callose-regulated symplastic communication coordinates symbiotic root nodule development. *Curr. Biol.* 28:3562–77.e6
44. Gerlitz N, Gerum R, Sauer N, Stadler R. 2018. Photoinducible DRONPA-s: a new tool for investigating cell–cell connectivity. *Plant J.* 94:751–66
45. German L, Yeshvekar R, Benitez-Alfonso Y. 2023. Callose metabolism and the regulation of cell walls and plasmodesmata during plant mutualistic and pathogenic interactions. *Plant Cell Environ.* 46:391–404
46. Godel-Jedrychowska K, Kulinska-Lukaszek K, Kurczynska E. 2021. Similarities and differences in the GFP movement in the zygotic and somatic embryos of Arabidopsis. *Front. Plant Sci.* 12:649806
47. Gombos S, Miras M, Howe V, Xi L, Pottier M, et al. 2023. A high-confidence *Physcomitrium patens* plasmodesmata proteome by iterative scoring and validation reveals diversification of cell wall proteins during evolution. *New Phytol.* 238:637–53
48. Grison MS, Kirk P, Brault ML, Wu XN, Schulze WX, et al. 2019. Plasma membrane-associated receptor-like kinases relocalize to plasmodesmata in response to osmotic stress. *Plant Physiol.* 181:142–60
49. Guerra T, Schilling S, Hake K, Gorzolka K, Sylvester FP, et al. 2020. Calcium-dependent protein kinase 5 links calcium signaling with N-hydroxy-L-pipecolic acid- and *SARD1*-dependent immune memory in systemic acquired resistance. *New Phytol.* 225:310–25
50. Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, et al. 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis CHORUS* (*GLUCAN SYNTHASE-LIKE 8*). *Development* 137:1731–41
51. Hake K, Romeis T. 2019. Protein kinase-mediated signalling in priming: immune signal initiation, propagation, and establishment of long-term pathogen resistance in plants. *Plant Cell Environ.* 42:904–17
52. Ham BK, Li G, Kang BH, Zeng F, Lucas WJ. 2012. Overexpression of *Arabidopsis* plasmodesmata germin-like proteins disrupts root growth and development. *Plant Cell* 24:3630–48
53. Han X, Hyun TK, Zhang M, Kumar R, Koh EJ, et al. 2014. Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. *Dev. Cell* 28:132–46

32. Built a theoretical framework for modeling cell–cell movement from experimentally extracted geometrical properties of plasmodesmata.

47. Experimentally dissected the plasmodesmata proteome in moss, validated new PD proteins, and established a scoring algorithm.

54. Hawes CR, Juniper BE, Horne JC. 1981. Low and high voltage electron microscopy of mitosis and cytokinesis in maize roots. *Planta* 152:397–407
55. Haywood V, Yu TS, Huang NC, Lucas WJ. 2005. Phloem long-distance trafficking of *GIBBERELLIC ACID-INSENSITIVE* RNA regulates leaf development. *Plant J.* 42:49–68
56. Heeney M, Frank MH. 2023. The mRNA mobileome: challenges and opportunities for deciphering signals from the noise. *Plant Cell* 35:1817–33
57. Heinlein M. 2015. Plasmodesmata: channels for viruses on the move. *Methods Mol. Biol.* 1217:25–52
58. Hepler PK. 1982. Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. *Protoplasma* 111:121–33
59. Hernandez-Hernandez V, Benitez M, Boudaoud A. 2020. Interplay between turgor pressure and plasmodesmata during plant development. *J. Exp. Bot.* 71:768–77
60. Hong JH, Savina M, Du J, Devendran A, Kannivadi Ramakanth K, et al. 2017. A sacrifice-for-survival mechanism protects root stem cell niche from chilling stress. *Cell* 170:102–13.e14
61. Horner W, Brunkard JO. 2021. Cytokinins stimulate plasmodesmatal transport in leaves. *Front. Plant Sci.* 12:674128
62. Huang C, Heinlein M. 2022. Function of plasmodesmata in the interaction of plants with microbes and viruses. *Methods Mol. Biol.* 2457:23–54
63. Ishikawa K, Tamura K, Fukao Y, Shimada T. 2020. Structural and functional relationships between plasmodesmata and plant endoplasmic reticulum–plasma membrane contact sites consisting of three synaptotagmins. *New Phytol.* 226:798–808
64. Iswanto ABB, Vu MH, Pike S, Lee J, Kang H, et al. 2022. Pathogen effectors: What do they do at plasmodesmata? *Mol. Plant Pathol.* 23:795–804
65. Johnston MG, Breakspear A, Samwald S, Zhang D, Papp D, et al. 2023. Comparative phyloproteomics identifies conserved plasmodesmal proteins. *J. Exp. Bot.* 74:1821–35
66. Kalmbach L, Bourdon M, Belevich I, Safran J, Lemaire A, et al. 2023. Putative pectate lyase PLL12 and callose deposition through polar CALS7 are necessary for long-distance phloem transport in *Arabidopsis*. *Curr. Biol.* 33:926–39.e9
67. Kehr J, Morris RJ, Kragler F. 2022. Long-distance transported RNAs: from identity to function. *Annu. Rev. Plant Biol.* 73:457–74
68. Kim JY, Rim Y, Wang J, Jackson D. 2005. A novel cell-to-cell trafficking assay indicates that the KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. *Genes Dev.* 19:788–93
69. Kirk P, Amsbury S, German L, Gaudioso-Pedraza R, Benitez-Alfonso Y. 2022. A comparative meta-proteomic pipeline for the identification of plasmodesmata proteins and regulatory conditions in diverse plant species. *BMC Biol.* 20:128
70. Kirk P, Benitez-Alfonso Y. 2022. Plasmodesmata structural components and their role in signaling and plant development. *Methods Mol. Biol.* 2457:3–22
71. Kitagawa M, Jackson D. 2017. Plasmodesmata-mediated cell-to-cell communication in the shoot apical meristem: how stem cells talk. *Plants* 6:12
72. Kitagawa M, Tran TM, Jackson D. 2024. Traveling with purpose: cell-to-cell transport of plant mRNAs. *Trends Cell Biol.* 34:48–57
73. Kitagawa M, Wu P, Balkunde R, Cunniff P, Jackson D. 2022. An RNA exosome subunit mediates cell-to-cell trafficking of a homeobox mRNA via plasmodesmata. *Science* 375:177–82
74. Knoblauch M, Peters WS, Bell K, Ross-Elliott TJ, Oparka KJ. 2018. Sieve-element differentiation and phloem sap contamination. *Curr. Opin. Plant Biol.* 43:43–49
75. Knox K, Wang P, Kriechbaumer V, Tilsner J, Frigerio L, et al. 2015. Putting the squeeze on plasmodesmata: a role for reticulons in primary plasmodesmata formation. *Plant Physiol.* 168:1563–72
76. Kondhare KR, Patil NS, Banerjee AK. 2021. A historical overview of long-distance signalling in plants. *J. Exp. Bot.* 72:4218–36
77. Kong D, Karve R, Willet A, Chen MK, Oden J, Shpak ED. 2012. Regulation of plasmodesmatal permeability and stomatal patterning by the glycosyltransferase-like protein KOBITO1. *Plant Physiol.* 159:156–68

78. Kraner ME, Muller C, Sonnewald U. 2017. Comparative proteomic profiling of the choline transporter-like1 (CHER1) mutant provides insights into plasmodesmata composition of fully developed *Arabidopsis thaliana* leaves. *Plant J.* 92:696–709

78a. Kumari P, Ballone P, Paniagua C, Abou-Saleh RH, Benitez-Alfonso Y. 2024. Cellulose–callose hydrogels: computational exploration of their nanostructure and mechanical properties. *Biomacromolecules* 25:1989–2006

79. Laub MT. 2016. Keeping signals straight: how cells process information and make decisions. *PLOS Biol.* 14:e1002519

80. Lee JY, Wang X, Cui W, Sager R, Modla S, et al. 2011. A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in *Arabidopsis*. *Plant Cell* 23:3353–73

81. Leijon F, Melzer M, Zhou Q, Srivastava V, Bulone V. 2018. Proteomic analysis of plasmodesmata from populus cell suspension cultures in relation with callose biosynthesis. *Front. Plant Sci.* 9:1681

82. Levy A, Zheng JY, Lazarowitz SG. 2015. Synaptotagmin SYTA forms ER-plasma membrane junctions that are recruited to plasmodesmata for plant virus movement. *Curr. Biol.* 25:2018–25

83. Li M, Wang MX, Lin QY, Wang MY, Niu XF, et al. 2022. Symplastic communication in the root cap directs auxin distribution to modulate root development. *J. Integr. Plant Biol.* 64:859–70

84. Li ZP, Paterlini A, Glavier M, Bayer EM. 2021. Intercellular trafficking via plasmodesmata: molecular layers of complexity. *Cell Mol. Life Sci.* 78:799–816

85. Lim GH, Shine MB, de Lorenzo L, Yu K, Cui W, et al. 2016. Plasmodesmata localizing proteins regulate transport and signaling during systemic acquired immunity in plants. *Cell Host Microbe* 19:541–49

86. Showed that vein patterning relies on auxin movement through highly permeable plasmodesmata in forming veins.

86. Linh NM, Scarpella E. 2022. Leaf vein patterning is regulated by the aperture of plasmodesmata intercellular channels. *PLOS Biol*. 20:e3001781

87. Liu J, Liu Y, Wang S, Cui Y, Yan D. 2022. Heat stress reduces root meristem size via induction of plasmodesmal callose accumulation inhibiting phloem unloading in *Arabidopsis*. *Int. J. Mol. Sci.* 23:2063

88. Liu J, Zhang L, Yan D. 2021. Plasmodesmata-involved battle against pathogens and potential strategies for strengthening hosts. *Front. Plant Sci.* 12:644870

89. Liu N, Shen G, Xu Y, Liu H, Zhang J, et al. 2020. Extensive inter-plant protein transfer between *Cuscuta* parasites and their host plants. *Mol. Plant* 13:573–85

90. Liu N-J, Zhang T, Liu Z-H, Chen X, Guo H-S, et al. 2020. Phytosphinganine affects plasmodesmata permeability via facilitating PDLP5-stimulated callose accumulation in *Arabidopsis*. *Mol. Plant* 13:128–43

91. Liu Y, Xu M, Liang N, Zheng Y, Yu Q, Wu S. 2017. Symplastic communication spatially directs local auxin biosynthesis to maintain root stem cell niche in *Arabidopsis*. *PNAS* 114:4005–10

92. Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, et al. 2001. A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* 105:793–803

93. Lu K-J, Huang N-C, Liu Y-S, Lu C-A, Yu T-S. 2012. Long-distance movement of Arabidopsis *FLOWERING LOCUS T* RNA participates in systemic floral regulation. *RNA Biol*. 9:653–62

94. Lucas WJ, Bouche-Pillon S, Jackson DP, Nguyen L, Baker L, et al. 1995. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* 270:1980–83

95. Luna GR, Li J, Wang X, Liao L, Lee JY. 2023. Targeting of plasmodesmal proteins requires unconventional signals. *Plant Cell* 35:3035–52

96. Luo K-R, Huang N-C, Chang Y-H, Jan Y-W, Yu T-S. 2024. *Arabidopsis* cyclophilins direct intracellular transport of mobile mRNA via organelle hitchhiking. *Nat. Plants* 10:161–71

97. Mathieu J, Michel-Hissier P, Boucherit V, Huynh JR. 2022. The deubiquitinase USP8 targets ESCRT-III to promote incomplete cell division. *Science* 376:818–23

98. Maule AJ, Gaudioso-Pedraza R, Benitez-Alfonso Y. 2013. Callose deposition and symplastic connectivity are regulated prior to lateral root emergence. *Commun. Integr. Biol.* 6:e26531

99. Maurya JP, Bhalerao RP. 2017. Photoperiod- and temperature-mediated control of growth cessation and dormancy in trees: a molecular perspective. *Ann. Bot.* 120:351–60

100. Showed that lateral root development is controlled by auxin movement via plasmodesmata in response to soil–water conditions.

100. Mehra P, Pandey BK, Melebari D, Banda J, Leftley N, et al. 2022. Hydraulic flux-responsive hormone redistribution determines root branching. *Science* 378:762–68

101. Mellor NL, Voß U, Janes G, Bennett MJ, Wells DM, Band LR. 2020. Auxin fluxes through plasmodesmata modify root-tip auxin distribution. *Development* 147:dev181669

102. Miyashima S, Roszak P, Sevilem I, Toyokura K, Blob B, et al. 2019. Mobile PEAR transcription factors integrate positional cues to prime cambial growth. *Nature* 565:490–94
103. Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* 413:307–11
104. Nicolas M, Torres-Perez R, Wahl V, Cruz-Oro E, Rodriguez-Buey ML, et al. 2022. Spatial control of potato tuberization by the TCP transcription factor BRANCHED1b. *Nat. Plants* 8:281–94
105. Nicolas WJ, Grison MS, Trepout S, Gaston A, Fouche M, et al. 2017. Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nat. Plants* 3:17082
106. Okawa R, Hayashi Y, Yamashita Y, Matsubayashi Y, Ogawa-Ohnishi M. 2023. Arabinogalactan protein polysaccharide chains are required for normal biogenesis of plasmodesmata. *Plant J.* 113:493–503
107. O'Lexy R, Kasai K, Clark N, Fujiwara T, Sozzani R, Gallagher KL. 2018. Exposure to heavy metal stress triggers changes in plasmodesmatal permeability via deposition and breakdown of callose. *J. Exp. Bot.* 69:3715–28
108. Orman-Ligeza B, Morris EC, Parizot B, Lavigne T, Babe A, et al. 2018. The xerobranching response represses lateral root formation when roots are not in contact with water. *Curr. Biol.* 28:3165–73.e5
109. Ormenese S, Bernier G, Périlleux C. 2006. Cytokinin application to the shoot apical meristem of *Sinapis alba* enhances secondary plasmodesmata formation. *Planta* 224:1481–84
110. Ostendorp A, Pahlow S, Krussel L, Hanhart P, Garbe MY, et al. 2017. Functional analysis of *Brassica napus* phloem protein and ribonucleoprotein complexes. *New Phytol.* 214:1188–97
111. Ostermeyer GP, Jensen KH, Franzen AR, Peters WS, Knoblauch M. 2022. Diversity of funnel plasmodesmata in angiosperms: the impact of geometry on plasmodesmal resistance. *Plant J.* 110:707–19
112. Pain C, Kriechbaumer V, Kittelmann M, Hawes C, Fricker M. 2019. Quantitative analysis of plant ER architecture and dynamics. *Nat. Commun.* 10:984
113. Park K, Knoblauch J, Oparka K, Jensen KH. 2019. Controlling intercellular flow through mechanosensitive plasmodesmata nanopores. *Nat. Commun.* 10:3564
114. Park S-H, Li F, Renaud J, Shen W, Li Y, et al. 2017. NbEXPA1, an α-expansin, is plasmodesmata-specific and a novel host factor for potyviral infection. *Plant J.* 92:846–61
115. Paterlini A, Sechet J, Immel F, Grison MS, Pilard S, et al. 2022. Enzymatic fingerprinting reveals specific xyloglucan and pectin signatures in the cell wall purified with primary plasmodesmata. *Front. Plant Sci.* 13:1020506
116. Peters WS, Jensen KH, Stone HA, Knoblauch M. 2021. Plasmodesmata and the problems with size: interpreting the confusion. *J. Plant Physiol.* 257:153341
117. Petit JD, Li ZP, Nicolas WJ, Grison MS, Bayer EM. 2020. Dare to change, the dynamics behind plasmodesmata-mediated cell-to-cell communication. *Curr. Opin. Plant Biol.* 53:80–89
118. Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H. 2018. Plant immunity: from signaling to epigenetic control of defense. *Trends Plant Sci.* 23:833–44
119. Rinne PL, Kaikuranta PM, van der Schoot C. 2001. The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *Plant J.* 26:249–64
120. Rinne PL, Paul LK, Vahala J, Kangasjarvi J, van der Schoot C. 2016. Axillary buds are dwarfed shoots that tightly regulate GA pathway and GA-inducible 1,3-β-glucanase genes during branching in hybrid aspen. *J. Exp. Bot.* 67:5975–91
121. Rinne PLH, Paul LK, van der Schoot C. 2018. Decoupling photo- and thermoperiod by projected climate change perturbs bud development, dormancy establishment and vernalization in the model tree *Populus*. *BMC Plant Biol.* 18:220
122. Ross-Elliott TJ, Jensen KH, Haaning KS, Wager BM, Knoblauch J, et al. 2017. Phloem unloading in Arabidopsis roots is convective and regulated by the phloem-pole pericycle. *eLife* 6:e24125
123. Sager R, Wang X, Hill K, Yoo BC, Caplan J, et al. 2020. Auxin-dependent control of a plasmodesmal regulator creates a negative feedback loop modulating lateral root emergence. *Nat. Commun.* 11:364
124. Sagi G, Katz A, Guenoune-Gelbart D, Epel BL. 2005. Class 1 reversibly glycosylated polypeptides are plasmodesmal-associated proteins delivered to plasmodesmata via the Golgi apparatus. *Plant Cell* 17:1788–800
125. Samuels AL, Giddings TH Jr., Staehelin LA. 1995. Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants. *J. Cell Biol.* 130:1345–57

113. Proposed a new model for the regulation of intercellular flow by changes in desmotubule positioning.

126. Sankoh AF, Burch-Smith TM. 2021. Plasmodesmata and hormones: pathways for plant development. *Am. J. Bot.* 108:1580–83
127. Santuari L, Sanchez-Perez GF, Luijten M, Rutjens B, Terpstra I, et al. 2016. The PLETHORA gene regulatory network guides growth and cell differentiation in Arabidopsis roots. *Plant Cell* 28:2937–51
128. Schoof H, Lenhard M, Haecker A, Mayer KF, Jurgens G, Laux T. 2000. The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell* 100:635–44

129. Determined that plasmodesmata frequency in the mesophyll–bundle sheath interface is induced by light and C_4 plants.

129. Schreier TB, Müller KH, Eicke S, Faulkner C, Zeeman SC, Hibberd JM. 2024. Plasmodesmal connectivity in C_4 *Gynandropsis gynandra* is induced by light and dependent on photosynthesis. *New Phytol.* 241:298–313
130. Segui-Simarro JM, Austin JR 2nd, White EA, Staehelin LA. 2004. Electron tomographic analysis of somatic cell plate formation in meristematic cells of Arabidopsis preserved by high-pressure freezing. *Plant Cell* 16:836–56
131. Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ. 2009. An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. *Plant Cell* 21:581–94
132. Singh A, Lim GH, Kachroo P. 2017. Transport of chemical signals in systemic acquired resistance. *J. Integr. Plant Biol.* 59:336–44
133. Singh RK, Miskolczi P, Maurya JP, Bhalerao RP. 2019. A tree ortholog of *SHORT VEGETATIVE PHASE* floral repressor mediates photoperiodic control of bud dormancy. *Curr. Biol.* 29:128–33.e2
134. Smertenko A, Assaad F, Baluska F, Bezanilla M, Buschmann H, et al. 2017. Plant cytokinesis: terminology for structures and processes. *Trends Cell Biol.* 27:885–94

135. Discovered that the PDLP-NHL3 complex is a key node for integrating multiple immunity signals and regulating plasmodesmata.

135. Tee EE, Johnston MG, Papp D, Faulkner C. 2023. A PDLP-NHL3 complex integrates plasmodesmal immune signaling cascades. *PNAS* 120:e2216397120
136. Tena G. 2021. PTI and ETI are one. *Nat. Plants* 7:1527
137. Tilsner J, Nicolas W, Rosado A, Bayer EM. 2016. Staying tight: plasmodesmal membrane contact sites and the control of cell-to-cell connectivity in plants. *Annu. Rev. Plant Biol.* 67:337–64
138. Tomczynska I, Stumpe M, Doan TG, Mauch F. 2020. A *Phytophthora* effector protein promotes symplastic cell-to-cell trafficking by physical interaction with plasmodesmata-localised callose synthases. *New Phytol.* 227:1467–78
139. Tylewicz S, Petterle A, Marttila S, Miskolczi P, Azeez A, et al. 2018. Photoperiodic control of seasonal growth is mediated by ABA acting on cell-cell communication. *Science* 360:212–15
140. Vaten A, Dettmer J, Wu S, Stierhof YD, Miyashima S, et al. 2011. Callose biosynthesis regulates symplastic trafficking during root development. *Dev. Cell* 21:1144–55
141. Veerabagu M, van der Schoot C, Turečková V, Tarkowská D, Strnad M, Rinne PLH. 2023. Light on perenniality: Para-dormancy is based on ABA-GA antagonism and endo-dormancy on the shutdown of GA biosynthesis. *Plant Cell Environ.* 46:1785–804
142. Voxeur A, Fry SC. 2014. Glycosylinositol phosphorylceramides from *Rosa* cell cultures are boron-bridged in the plasma membrane and form complexes with rhamnogalacturonan II. *Plant J.* 79:139–49
143. Wang C, Lyu Y, Zhang Q, Guo H, Chen D, Chen X. 2023. Disruption of BG14 results in enhanced callose deposition in developing seeds and decreases seed longevity and seed dormancy in *Arabidopsis*. *Plant J.* 113:1080–94

144. Showed that brassinosteroid precursors move short distances cell to cell through plasmodesmata and fine-tune plasmodesmata permeability.

144. Wang Y, Perez-Sancho J, Platre MP, Callebaut B, Smokvarska M, et al. 2023. Plasmodesmata mediate cell-to-cell transport of brassinosteroid hormones. *Nat. Chem. Biol.* 19:1331–41
145. Wu J, Sun W, Sun C, Xu C, Li S, et al. 2023. Cold stress induces malformed tomato fruits by breaking the feedback loops of stem cell regulation in floral meristem. *New Phytol.* 237:2268–83
146. Xu B, Cheval C, Laohavisit A, Hocking B, Chiasson D, et al. 2017. A calmodulin-like protein regulates plasmodesmal closure during bacterial immune responses. *New Phytol.* 215:77–84
147. Xu XM, Wang J, Xuan Z, Goldshmidt A, Borrill PG, et al. 2011. Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function. *Science* 333:1141–44
148. Yadav RK, Perales M, Gruel J, Girke T, Jönsson H, Reddy GV. 2011. WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. *Genes Dev.* 25:2025–30

149. Yan D, Yadav SR, Paterlini A, Nicolas WJ, Petit JD, et al. 2019. Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading. *Nat. Plants* 5:604–15
150. Yang L, Perrera V, Saplaoura E, Apelt F, Bahin M, et al. 2019. m^5C Methylation guides systemic transport of messenger RNA over graft junctions in plants. *Curr. Biol.* 29:2465–76.e5
151. Yoo S-C, Chen C, Rojas M, Daimon Y, Ham B-K, et al. 2013. Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. *Plant J.* 75:456–68
152. Zarebanadkouki M, Trtik P, Hayat F, Carminati A, Kaestner A. 2019. Root water uptake and its pathways across the root: quantification at the cellular scale. *Sci. Rep.* 9:12979
153. Zavaliev R, Levy A, Gera A, Epel BL. 2013. Subcellular dynamics and role of *Arabidopsis* β-1,3-glucanases in cell-to-cell movement of tobamoviruses. *Mol. Plant Microbe Interact.* 26:1016–30
154. Zhang Y, Wang S, Wang L, Chang X, Fan Y, et al. 2022. Sphingolipids at plasmodesmata: structural components and functional modulators. *Int. J. Mol. Sci.* 23:5677
155. Zhu T, O'Quinn RL, Lucas WJ, Rost TL. 1998. Directional cell-to-cell communication in the *Arabidopsis* root apical meristem II. Dynamics of plasmodesmatal formation. *Protoplasma* 204:84–93