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REVIEW

Mobile Macromolecules in Plant Development

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Abstract Plant cells transmit developmental signals and distribute nutrients via dynamic intercellular channels termed plasmodesmata (PD). Multidisciplinary inquiries have provided evidence that plasmodesmatal regulation is critical to fundamental plant functions, such as development, host–pathogen interactions, and systemic RNA silencing. This review focuses on macromolecules that transport cell-to-cell through PD and describes their implications on plant development.

Keywords Development · Intercellular transport · Macromolecules · Plasmodesmata · Protein · RNA

The development of a multicellular organism requires elaborate communication between cells to establish and maintain their fates. In *Arabidopsis*, clonal analyses and ablation experiments have demonstrated that cell fate is determined primarily by position (van den Berg et al. 1995; Hake and Char 1997; Berger et al. 1998; Kidner et al. 2000). Various modes of cell-to-cell communication exchange positional information. In animal systems, the secretion of ligands and their recognition by surface receptors on neighboring or distant cells is the foremost mechanism. In addition to secreted ligands and their corresponding receptors, plants have evolved unique intercellular connections called plasmodesmata (PD).

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PD were formerly considered to be merely a cytoplasmic continuity between plant cells, facilitating intercellular transport of low-molecular-weight growth regulators and nutrients. They were also seen as static 'holes' in the thick cell walls. However, this view has now drastically changed, and viral proteins and RNAs have been shown to traffic cell-to-cell through PD (Wolf et al. 1989, 1991; Ding et al. 1992; Waigmann et al. 1994). This finding has prompted suggestions that virus may usurp the plant's endogenous route; thereby endogenous plant proteins and nucleic acids may also use PD for short- and long-range signaling (Lucas et al. 1993).

PD are dynamic structures whose apertures fluctuate in different cell types during development and in response to the environment. Moreover, PD mediate the transport of endogenous macromolecules, e.g., some transcription factors (TFs), mRNAs, and gene-silencing RNA signals. Overall, they are critical gatekeepers for regulating cell fate. Our review briefly describes milestone studies of mobile proteins and RNAs and discusses their implications on plant development.

Intercellular Movement Through Plasmodesmata

Generic simple plasmodesmata (PD) consist of two major components—membranes and spaces (Fig. 1). Membranes form the boundaries of the PD channel, and the plasma membrane (PM) of two neighboring cells comprises the outer boundary. An appressed endoplasmic reticulum (ER), i.e., the desmotubule (D), runs through the axial core and constitutes the inner boundary. The space between PM and D is termed the cytoplasmic sleeve (CS); this primary passageway for molecular transport is continuous with the cytoplasm between adjacent cells. Instead of being empty,

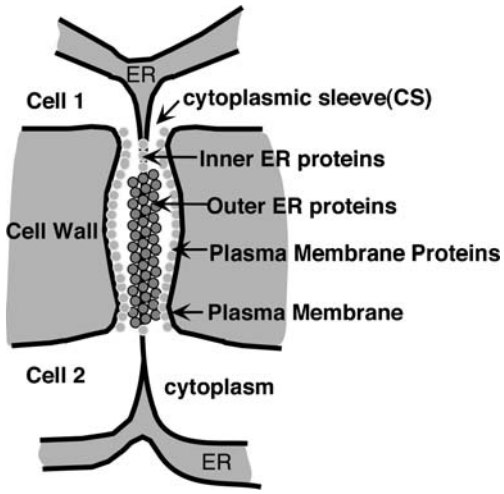

Fig. 1 Longitudinal view of simple plasmodesmata. Adapted with permission from Roberts (2005)

the CS is filled with proteinaceous molecules that likely regulate transport via PD. For example, actin and myosin along the length of the PD (see review by Baluska et al. 2001), and the centrin nanofilaments at the neck region (Blackman et al. 1999), may provide contractile elements to control PD apertures.

The functional measure of PD is their size exclusion limit (SEL), the maximum size of macromolecules that can freely diffuse between cells. PD SEL is regulated temporally, spatially, and physiologically throughout plant development. PD selectively allow for movement of proteins, e.g., some transcription factors, and RNAs, such as mRNAs and silencing RNAs, both of which are critical to cell-fate determination (see reviews by Ding et al. 2003; Kim 2005; and Kurata et al. 2005). PD in various tissues may be modulated differently, possibly by the involvement of developmentally regulated factors.

The determination of which plant cells and tissues are in communication via PD is an area of active investigation. Such studies reveal where and when developmental signaling may occur. The plant is viewed as a mosaic of numerous symplastic domains, the common cytoplasm that is bounded by the plasma membranes of connected cells (Erwee and Goodwin 1985). In higher plants, the formation of symplastic domains often precedes apparent differentiation in morphogenetic development (McLean et al. 1997). For instance, *Arabidopsis* root epidermal cells are symplastically coupled in undifferentiated cells but become symplastically isolated as cells mature and differentiate to form root hairs (Duckett et al. 1994). In the embryo sac of *Torenia fournieri*, the permeability of PD down-regulates as the embryo sac matures. For instance, although 10-kDa dextrans can move between the central cell and the egg apparatus cells before anthesis, at 2 days after anthesis, the PD SEL decreases to 3 kDa (Han et al. 2000). This selective closure of PD at certain cell interfaces may lead to complex patterns of symplastic continuity and discontinuity during development. Examples of symplastic domains are summarized in Table 1.

In *Arabidopsis*, ultrastructural studies have revealed that all cells of the embryo are considered interconnected into a single symplastic unit via PD (Mansfield and Briarty 1991).

Table 1 Symplastic domains in plants

Cell types	Plant species	Functional Status	References
Embryos	*Arabidopsis thaliana*	Open for HPTS with formation of subdomains restricting different sized- GFPs	Kim et al. 2005
Seed Coats	*Arabidopsis thaliana*	Symplastic disconnection of the outer integuments from the inner integuments	Stadler et al. 2005
Cotton fibers (seed coat hairs)	*Gossypium hirsutum*	Temporary closure of PD accompanied by the elongation of single-celled seed coat hairs	Ruan et al. 2001
Shoot apex	*Arabidopsis thaliana*	Temporal isolation of meristem during floral transition	Gisel et al. 1999, 2002
Shoot apical meristem	*Betula pubescence*	Induction of symplastic isolation upon short photoperiod treatment	Rinne and van der Schoot 1998
CC-SE complex	*Nicotiana tabacum*	Symplastic isolation from non-phloem cells in source tissues	Imlau et al. 1999
	Arabidopsis thaliana		Oparka et al. 1999
Root and shoot epidermis	*Egeria densa*	Symplastic isolation of epidermis from underlying cortical cells	Erwee and Goodwin 1985
Root hair cells	*Arabidopsis thaliana*	Symplastic isolation from neighboring epidermal cells	Duckett et al. 1994
Stomatal guard cells	*Allium cepa*	Symplastic isolation upon stomata maturation	Palevitz and Hepler 1985

Functional assays with 0.5 kDa HPTS and 10 kDa dextrans have shown that embryo cells at the globular and heart stages are interconnected through open PD that permit the movement of symplastic tracers of up to 10-kDa dextrans (Kim et al. 2002a). As the embryo develops, PD SEL decreases so that torpedo embryos no longer allow those same-sized dextrans to move between cells.

Research using green fluorescent proteins (GFP) as symplastic tracers has further uncovered that *Arabidopsis* embryos form symplastic subdomains, where the cells within share a common PD SEL but distinctively restrict macromolecular traffic at the boundary between different subdomains (Kim et al. 2005). Intriguingly, those subdomains correspond to an axial–basal axis established during embryo development, which demonstrates the developmental significance of PD as a signaling channel.

Macromolecules Transported Through Plasmodesmata

Local and systemic spread of proteins and RNAs appears to follow the symplastic route. Therefore, mobile proteins and RNAs and their developmental functions in plants are research fields that have become more exciting because of recent advances (Table 2).

Proteins

The first endogenous plant protein known to traffic cell-to-cell was KNOTTED1 (KN1). KN1 is a homeodomain transcription factor (TF) that regulates leaf and shoot meristem development (Vollbrecht et al. 1991; Reiser et al. 2000). Non-cell-autonomous activity of the dominant *Kn1* allele was first reported in the 1980s (Hake et al. 1989). Subsequent investigations with localization experiments have shown that KN1 protein is present outside the visible domain of KN1 mRNA expression in the leaf primordia of *Kn1* mutants (Smith et al. 1992; Jackson et al. 1994), suggesting that the protein itself could traffic cell-to-cell to promote cell fate in *Kn1* leaves. Microinjection experiments with maize and tobacco leaves (Lucas et al. 1995), micro-projectile bombardment, and tissue-specific expression (Kim et al. 2002b; Kim 2003) have demonstrated that KN1 protein is a developmentally functional signal that moves cell-to-cell through plasmodesmata.

In *Arabidopsis*, about 6% of its 1,500 genes encode TFs (Riechmann et al. 2000). By activating downstream target genes, these proteins are essential regulators of physiological, environmental, and developmental responses (Helariutta et al. 2000). Since the discovery of this cell-to-cell mobility for KN1, many TFs have been found to act non-cell-autonomously, by cell-to-cell movement, to exert developmental functioning in various plant species (Table 2).

One elegant example of cell-fate determination by a mobile protein is SHORT-ROOT (SHR) (Nakajima et al. 2001). *SHR* encodes a putative TF that is required for asymmetric divisions for the cortex/endodermal initial daughter cells and for the specification of endodermal fate (Helariutta et al. 2000). *SHR* mRNA is transcribed in the center of the root tip, the stele, but excluded from cortex/endodermal initial cells or in its daughter cells where it functions. These proteins move only to one adjacent cell

Table 2 Mobile macromolecules in plants

Proteins	RNA	Plant species	References
Local			
KNOTTED1(KN1)	*KN1* mRNA	*Zea mays*	Lucas et al. 1995
KNAT1/BREVIPED-ICILLUS		*Arabidopsis*	Kim et al. 2002b
SHOOTMERISTEM-LESS(STM)		*Arabidopsis*	Kim et al. 2002b
GLOBOSA(GLO)		*Antirrhinum*	Perbal et al. 1996
FLORICAULA(FLO)		*Antirrhinum*	Hantke et al. 1995
LEAFY(LFY)		*Arabidopsis*	Sessions et al. 2000
Shortroot(Shr)		*Arabidopsis*	Nakajima et al. 2001
CAPRICE(CPC)		*Arabidopsis*	Wada et al. 2002
Systemic			
CmPP16		*Cucurbita maxima*	Xoconostle-Cazares et al. 1999
	MOUSE EARS(Me) mRNA	tomato	Kim et al. 2001
	SUCROSE TRANSPORTER1 mRNA		Kuhn et al. 1997;
CmNACP	*CmNACP* mRNA		Ruiz-Medrano et al. 1999
CmPSRP1	siRNAs, miRNAs	*Cucurbita maxima*	Yoo et al. 2004
FLOWERING LOCUS T(FT)		*Arabidopsis*	Corbesier et al. 2007
Hd3		rice	Tamaki et al. 2007

file, e.g., from the stele cells to the outer cell layer, inducing the expression of *SCARECROW* (*SCR*) and, ultimately, the differentiation of endodermal cells.

Expression of *SHR* in the stele and subsequent movement to the single adjacent layer ensures correct division of the cortex/endodermal initial cell through the action of the *SCR* target gene. Therefore, SHR movement provides essential positional information that the endodermal cell layer is steadfastly positioned adjacent to the stele. This may be the concrete example of TF movement that is definitely necessary for its functioning.

The mobility of SHR, as SHR–GFP fusion proteins, has been examined in other root tissues, using cell-type specific promoters (Sena et al. 2004). Upon expression in companion cells or epidermal cells, a SHR–GFP protein does not move out of the cells where it is expressed. This suggests that tissue-specific factors may be required for SHR movement. When a SHR–GFP is expressed by its native promoter in plants with multiple endodermal layers, the protein moves only to the first endodermal layer and stops, indicating that SHR movement is not limited by a mechanism that recognizes boundaries between different cell types. Interestingly, a SHR–GFP protein expressed in the epidermis moves cell-to-cell in a *scarecrow* (*scr*) mutant background, implying that SCR limits the movement of SHR in the epidermis. Overall, the direction and extent of SHR intercellular movement are tightly regulated in a tissue-specific way. However, the mechanism by which it is controlled remains unknown.

Another example of developmental controls by mobile proteins is FLOWERING LOCUS T (FT) (Corbesier et al. 2007). In photoperiodically sensitive plants, day length is perceived by the leaves, whereas flower formation occurs in the shoot apex. Extensive studies have been conducted to identify the long-distance mobile signals, e.g., florigen, from induced leaves to the shoot apex. FT protein has emerged as a very strong candidate for at least a part of that florigen.

In *Arabidopsis*, expression of *CONSTANS* (*CO*) is not regulated by long-day conditions, but it transcriptionally activates *FT* in the vascular tissue of leaves. Ectopic expression of CO induces early flowering, but not when expressed from a shoot meristem-specific promoter. In contrast, ectopic expression of FT in the shoot apical meristem causes early flowering, as does expression of *CO* from phloem companion cell-specific promoters. Together, these suggest that CO expressed in the leaf phloem regulates synthesis of a phloem-mobile signal that induces flowering in the shoot apex (An et al. 2004; Wigge et al. 2005). FT in the apex forms a complex with FLOWERING LOCUS D (LD) and promotes the developmental transition to flowering by activating downstream genes (Abe et al. 2005).

FT–GFP expressed by phloem-specific promoters is found in both the leaf phloem and the shoot apex. *FT:GFP*-transgenic *Arabidopsis* flowers earlier than *ft* plants, thereby showing that FT protein is expressed in the leaf phloem traffic to the shoot apex to induce flowering (Corbesier et al. 2007). To rule out the possibility of a secondary product of FT being involved, *FT:GFP* has been expressed by the *GALACTINOL SYNTHASE* (*GAS1*) promoter, which is active specifically in the companion cells of the minor veins of leaves but not in the companion cells of the shoots or major veins. In transgenic plants expressing *GAS1:FT:GFP*, GFP is detected only in the minor veins, and those transgenic flower as late as the *ft* plants. It has been proposed that the fusion protein is not mobile in the minor veins and, as a result, does not move to the shoot apex and induce flowering (Corbesier et al. 2007).

The protein encoded by *Hd3a*, a rice ortholog of *FT*, also moves from the leaf to the shoot apex and induces flowering (Tamaki et al. 2007). Under short days, expression is highest in leaf blades. *Hd3a:GFP*-transgenic rice, under the control of phloem-specific promoters, flowers early, and Hd3a:GFP proteins are detected in the vascular tissues of the leaf blade, stem, and shoot apex. Therefore, the Hd3a protein in rice moves from the leaf through the phloem to the shoot apex and induces flowering.

RNA

The critical role of intercellular transport for specific RNAs in regulating plant development has been examined. Systemic movement of RNA was first reported in the infectious spread of plant virus. Viral movement proteins (MP) facilitate the intercellular movement of viral RNA through PD by forming MP–RNA complexes (Lucas and Gilbertson 1994). A paralog to viral MP, CmPP16 from *Cucurbita maxima*, also carries various mRNA molecules cell-to-cell (Xoconostle-Cazares et al. 1999). More mRNA of endogenous proteins, such as *SUCROSE TRANSPORTER1* and *CmNACP*, also move cell-to-cell (Table 2). Along with mobile proteins, the functional implication of mRNA traffic in plant development is an active field of investigation. In fact, a homeobox fusion transcript in tomato offers an elegant example of developmental changes caused by its systemic movement.

Grafting experiments in tomato have shown that mRNA moves systemically and controls leaf morphology (Kim et al. 2001). The dominant mutant *Mouse ears* (*Me*) has up to octapinnate compound leaves, unlike wild-type tomato that bears unipinnate compound leaves. This phenotype of the *Me* mutant is caused by a gene fusion between *PYROPHOPHATE-DEPENDENT PHOSPHOFRUCTOKINASE* (*PFP*), an enzyme in the glycolytic pathway, and *LeT6*, a tomato *KNOTTED-1*-like homeobox (*KNOX*) gene.

Wild-type tomato plants have been grafted as scion onto *Me* plants as stock. Newly formed leaves from those scions

resemble those produced on plants carrying the *Me* mutations. *PFP–LET6* fusion DNA is detected only in the *Me* stocks but not in DNA from the wild-type scions. In contrast, both *PFP* and *LET6* mRNAs are detected in the scion. Therefore, the phenotypic alteration observed in scion leaves after grafting is caused by movement of the chimeric *PFP–LeT6* fusion RNA from *Me* stock into the wild-type scion.

The presence of short interfering RNAs (siRNAs) and micro-RNAs (miRNAs) in the phloem sap supports the prospect that RNA signals traffic through PD and likely functions as a developmental cue, thereby constructing RNA-based signaling networks (Yoo et al. 2004). RNA silencing is part of a wide range of phenomena that commonly involve small RNA species and play basic roles in virus-defense responses, transposon mobility, and development (Carrington and Ambros 2003; Finnegan and Matzke 2003; Lai 2003). Although this silencing was originally described differently as co-suppression and post-transcriptional gene silencing (PTGS) in plants (Marathe et al. 2000; Meins 2000), quelling in fungi (Pickford et al. 2002), or RNA interference (RNAi) in animals (Plasterk and Ketting 2000; Sharp 2001), it is generally induced by double-stranded (ds)-RNA and can act beyond the cells in which it is initiated both in plants and animals.

RNA silencing breaks down dsRNA and forms small RNA, which can specifically suppress the expression of homologous genes in single cells as well as in the whole organism (systemic silencing). Local silencing curbs the expression of homologous genes in cells where dsRNAs build, and produces silencing signals that are mobile and able to confer the degradation of homologous RNA molecules in neighboring cells in a sequence-specific manner. During this process, new signal molecules are generated to propagate RNA silencing from cell-to-cell (short range) and systemically (long distance) (Mlotshwa et al. 2002). This nature of the silencing signal to spread and replicate is similar to that of the infectious behavior of plant viruses. In fact, accumulating data suggest that, like plant viruses, signals for RNA silencing move through PD.

PTGS is an innate plant defense mechanism against the activity of transposons and viruses (Waterhouse et al. 2001). Grafting experiments on transgenic plants have shown that a signal from silenced rootstocks is transmitted to non-silenced scions expressing the respective transgenes, leading to PTGS in the scions (Palauqui et al. 1997). Small RNAs have been proposed as candidates for mobile signals (Mlotshwa et al. 2002). siRNA originating from a transgene or a virus infection has been detected in the phloem sap of silenced plants, but not in those that are not silenced, suggesting that siRNAs are the mobile signals. siRNAs are 21~26 nt, with 2-nt 3′ overhangs (Himber et al. 2003). Both sense and antisense strands of siRNAs are present at similar levels in the phloem sap and no double-stranded siRNAs are detected, implying that siRNAs transport through phloem connected via PD as single strands (Yoo et al. 2004). Another type of small non-coding RNAs is miRNAs, which are 21~24-nt single-stranded RNA molecules that are processed from endogenous hairpin RNA precursor transcripts derived from miRNA genes (Pasquinelli and Ruvkun 2002). They are present in the phloem sap of pumpkin (Yoo et al. 2004) and lupin (Atkins and Smith 2007), suggesting a role as mobile signal molecules. The phloem is a main highway for transport in plants. Analyses of macromolecules present in the sap have been conducted with various species (Vilaine et al. 2003; Doering-Saad et al. 2006; Pommerrenig et al. 2006; Omid et al. 2007). Uncovering the mechanism that confers specificity to mobility should be an exciting field of research.

Concluding Remarks

A pivotal role for plasmodesmata (PD) in plant development is supported by accumulating evidence for cell-to-cell movement of transcription factors critical to cell-fate determination. Data also suggest that RNAs, mRNAs, and gene-silencing RNAs traffic via the vascular system and the connected PD. In fact, it took about 70 years to learn the molecular nature of florigen, the least expected, i.e., of an approximately 20-kDa mobile FT protein, transported by a pathway involving PD. Other than the identities of PD cargo, potential regulatory molecules that signal PD to allow for selective movement of macromolecules are still elusive. Further questions concern the exact mechanics of transport through PD. These might be answered by applying multidisciplinary tools that bring a synergistic effect.

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