

# Plasmodesmata: intercellular tunnels facilitating transport of macromolecules in plants

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Received: 28 June 2012 / Accepted: 18 December 2012 / Published online: 1 February 2013  
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**Abstract** In plants, intercellular structures named plasmodesmata (PD) form a continuous cytoplasmic network between neighboring cells. PD pores provide channels for intercellular symplasmic (cell-to-cell) transport throughout most tissues of the plant body. Cell-defining proteins, such as transcription factors, and regulatory non-coding sequences, such as short interfering RNA, micro RNA, protein-encoding messenger RNAs, viroids, and viral RNA/DNA genomes move via PD channels to adjacent cells. PD-mediated intercellular transport of macromolecules is a regulated process depending on the tissue, developmental stage, and nature of the transported macromolecule. In this review, PD channels and their similarity to tunneling nanotubes present in animals are highlighted. In addition, homeodomain protein movement and cellular components regulating transport are discussed.

**Keywords** Plasmodesmata · Nanotubes · Intercellular transport · Homeodomain protein · Cell differentiation · Plants

## Abbreviations

CPC	CAPRICE
ER	Endoplasmic reticulum
GFP	Green fluorescent protein
HD	Homeodomain protein
KN1	KNOTTED1
miRNA	Micro RNA
MP	Movement protein
MPB2C	MOVEMENT PROTEIN BINDING PROTEIN 2C
NCAP	Non-cell autonomous protein
PD	Plasmodesmata

siRNA	Small interfering RNA
TMV	Tobacco mosaic virus
TNTs	Tunneling nanotubes
TTG1	TRANSPARENT TESTA GLABRA 1

## Introduction

Multicellular organisms have evolved various strategies for efficient communication between cells and tissues. This is evident from organisms in which there is a division of metabolism and function between tissues, as observed in higher plants and animals. Growth and differentiation are consequences of genetic programs and coordinated responses to internal and external stimuli by individual cells. Obviously, for the coordination of tissue-wide responses, intercellular communication is essential to integrate such stimuli in order to build and maintain a proper body plan. In general terms, this coordination takes place by the exchange of signal molecules. In plants, in addition to canonical receptor-mediated signaling (cell-cell signaling) across membrane boundaries, intercellular signaling occurs through membrane-lined plasmodesmatal microchannels (cell-to-cell signaling). This review focuses on the symplasmic pathway provided by plasmodesmata (PD) paralleling the symplasmic tunneling nanotube (TNT) pathway found in animals.

## PD structure

Eduard Tangl discovered the presence of connections between plant cells more than a century ago; by conventional microscopy, he detected channels between plant cells and postulated that they serve as communication and transport pathways (Tangl 1880). Based on modern electron microscopy imaging techniques, the PD microchannels have been shown to form *de novo* during cell division in the newly formed cell plate

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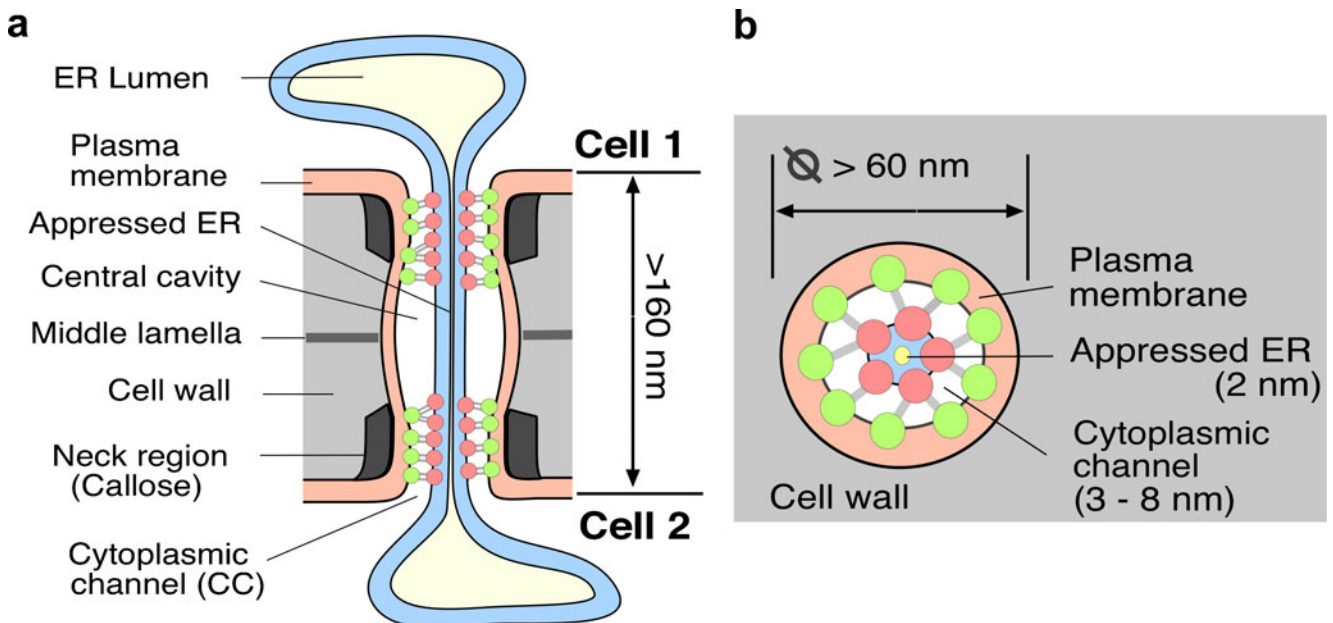
(primary PD; Hepler 1982). In addition, new PD appear later between non-clonal and clonal cells (secondary PD; Jones and Dropkin 1976). Secondary and primary PD appear morphologically similar in fully differentiated tissues: both classes of PD form higher order structures by building branches and forming a central expanded cavity at the junction of two neighboring cell walls, and both are outlined by the plasma membrane and contain an appressed membranous tube connected to the endoplasmic reticulum (ER; Fig. 1). Globular proteins seem to be present in the channel between the appressed ER and plasma membrane. These PD proteins have been suggested to form a helical structure with a width of approximately 5 nm forming a “nano” channel with a diameter of approximately 3 nm connecting the cytoplasm of the cells (Bayer et al. 2004; Waigmann et al. 1997; Overall and Blackman 1996; Ding et al. 1992; Burgess 1971). The appressed ER lumen appears to have a diameter of less than 2 nm (Robards and Lucas 1990). Between fully differentiated plant cells, several thousand PD channels are formed in clusters called pit fields (Faulkner et al. 2008). Thus, numerous PD build a symplasmic (continuous cytosol) and membranous network between neighboring cells forming a three-dimensional exchange system within and between tissues.

### Similarities and differences between TNTs and PD

The first evidence for tubular structures with a diameter of 50–200 nm connecting animal cells was found in cultured

rat and human kidney cells by the group of Gerdes (Rustom et al. 2004) who named these channels TNTs and who have shown the filamentous-actin-dependent transfer of organelles via TNTs (Bukoreshtliev et al. 2009). TNTs are symplasmic tunnels formed by highly dynamic membranous protrusions connecting cells in the vicinity. They display a continuous membrane to both connected cells as seen in transmission electron micrographs. These “novel” channels seem to have similar functions and dimensions as PDs (see Fig. 1; Baluska et al. 2004; Ruiz-Medrano et al. 2004). Indeed, if PD had not been uncovered a century ago, they would most likely have been named nano-tunnels today.

Several shared features exist between PD and TNTs. The PD diameter is in the same range as that observed in TNTs and can vary from approximately 60 nm to several 100 nm in the region of the central cavity (see Fig. 1). The length of both PD and TNTs can be highly variable and can extend over the cell diameter of a connected cell. Small fluorescent tracer molecules and green fluorescent protein (GFP) move via PD and TNTs to adjacent cells. PD and TNTs can connect non-clonal cells as they can be formed *de novo* between various cell types. In plants, PD are observed between all juvenile cell types. In mature tissues, connectivity can be lost by PD modification, such as the connection between guard cells forming stomata and epidermal cells. In the plant vasculature, PDs are highly modified or degraded to build the special transport vessels of the phloem or xylem tube system, respectively. In cell cultures, TNTs are reported to connect fibroblasts, epithelial cells, immune cells (Davis



**Fig. 1** Representation of the complex membrane organization of a single plasmodesma (ER endoplasmic reticulum). Longitudinal (a) and cross section (b) of a plasmodesmal channel embedded in cell wall material

formed between two neighboring cells. Note that the end-to-end distance of a plasmodesmata (PD) channel can be long (up to several micrometers) depending on the cell wall width. **a, b** Modified after Lucas et al. (2001)

and Sowinski 2008; Gerdes and Carvalho 2008), primary neurons, and astrocytes (Wang and Gerdes 2012). Confocal laser scanning microscopy imaging of living tissues has revealed that TNTs connect putative dendritic cells in the cornea of mice (Chinnery et al. 2008) and non-neural ectoderm cells in mouse embryos (Pyrgaki et al. 2010). Another similarity to the plant PD transport mechanism is found in the capacity of TNTs to facilitate cell-to-cell transport of viruses (Sowinski et al. 2008). Other transport features also seem to be shared between PD and TNTs. Uni-directional transport occurs from epidermal cells towards trichome cells via PD (Christensen et al. 2009; Fig. 2), as has also been observed between cultured normal rat kidney cells (Gurke et al. 2008).

In a hypothetical line, if a conserved tubule-forming mechanism exists in plants and animals, one would predict that a plant cell devoid of cell wall material might also build structures similar to that found in animal tissues. Indeed, plant protoplasts (cells lacking cell wall material) when producing a viral movement protein (MP) associated with PD form tubular structures protruding from the cell surface (Huang et al. 2000; Canto and Palukaitis 1999; Fig. 2). Although no evidence exists that such protoplasmic tubules connect cells, they show striking parallels to TNTs. Both animal and plant cells might use a commonly conserved cellular mechanism to form connections in order to exchange macromolecules and organelles between cells.

### Mobility and motifs of macromolecules transported via PD

One of the best-known functions of PD is that they facilitate the intercellular transport of small nutritional molecules such as amino acids and sugars; however, more recently, it has become obvious that macromolecules such as RNA and proteins also move from cell to cell via PD (Haywood et al. 2002; Oparka 2004; Tilsner et al. 2011; Lucas et al. 2009; Maule 2008; Barton et al. 2011; Genoves et al. 2011; Kaido et al. 2011; Lucas et al. 2001; Ruiz-Medrano et al. 2012). Proteins moving via PD are generally termed non-cell autonomous proteins (NCAPs). NCAPs are suggested to interact with PD components to increase the basic size exclusion limit of the PD pore as measured by metabolically inert fluorescent dextrans (Haywood et al. 2002). This increase in PD pore size is thought to facilitate the passage of endogenous RNA, proteins, and viral genomic information (Lucas and Gilbertson 1994; Kragler et al. 1998, 2000; Wolf et al. 1989, 1991). A number of mobile endogenous macromolecules have been suggested to be transported via PD and include transcription factors, mRNAs, and small RNAs such as micro RNA (miRNA) and small interfering RNA (siRNA) molecules, which function as signals that co-

ordinate growth, regulate differentiation, and mediate systemic responses to environmental cues (Kim et al. 2001; Kim et al. 2003, 2005; Xu et al. 2011; Carlsbecker et al. 2010; Nakajima et al. 2001; Pant et al. 2008; Dunoyer et al. 2010a, 2010b). Interestingly, the intercellular transport of viral and endogenous macromolecules, such as viroid RNAs, viral MPs, and transcription factors, is selective and depends on unrelated motifs. For example, the intercellular transport capacity of mutant forms of viroid RNA lacking distinct specific hairpin structures changes in a tissue-specific manner (Qi et al. 2004). Moreover, structurally unrelated viral MPs and transcription factors, such as GRAS (named after GAI, RGA, and SCR) domain harboring SHORT ROOT or the homeodomain protein KNOTTED1 (KN1) move via PD, and their mobility varies depending on the nature of the tissue (Oparka et al. 1999; Kim et al. 2005b; Itaya et al. 1998; Zhong et al. 2008; Qi et al. 2004; Gallagher and Benfey 2009; Kim et al. 2002, 2003, 2005).

Even within a cell type, differences are observed; tobacco mosaic virus (TMV) uses subtypes of PD present within a cell and preferentially moves via branched PD (Oparka et al. 1999). In addition, unspecific transport of heterologous proteins via PD is regulated differently between cells of apical tissues. For example, the homeodomain transcription factor KN1 moves from the outer L1 layer of the apical meristem (stem cells) into the inner L2 and L3 cells but not vice versa (Kim et al. 2003, 2005). An example for developmental changes in PD connectivity is found during developmental transitions in embryos. Here, in early stages, PD allow the diffusion of GFP throughout the embryo, and in later stages, sub-domains corresponding to morphological regions of the plant are established with limited diffusion between cells (Kim and Zambryski 2005). In addition, PD permeability has recently been shown to be regulated depending on the redox status of the cells (Burch-Smith and Zambryski 2012; Barton et al. 2011).

Microinjection studies (Oparka 2004; Haywood et al. 2002; Ruiz-Medrano et al. 2004) have suggested that the transport of actively transported proteins via PD involves at least two consecutive processes. First, NCAPs are recognized by specific PD pathway receptors. Second, in the vicinity of the PD orifices, a predicted docking complex recognizes the presence of cargos and governs the passage of the cargo through PD. The predicted receptor(s) mediate structural modifications of the cargo and the diameter of PD pores (Haywood et al. 2002). In microinjection assays, a mobile protein such as the homeodomain protein KN1 seems to be unfolded, and the pore size is increased to allow passage (Kragler 2005; Kragler et al. 1998, 2000). In support of the results obtained by microinjection assays, a type II chaperonin complex containing the KN1 interacting protein CCT8 facilitates the proper refolding of KN1 after

transport via PD in vivo (Xu et al. 2011). Most likely, the same chaperonin complex also facilitates viral transport in the recipient cells (Fichtenbauer et al. 2012).

Highly divergent motifs seem to mediate intercellular protein transport. Domain shuffling or mutant studies of mobile proteins, such as the homeodomain transcription factor KN1 (Kim et al. 2005; Lucas et al. 1995), the GRAS family protein SHR (Gallagher and Benfey 2009; Vaten et al. 2011), a subclass of phloem-specific HSC70 chaperones (Aoki et al. 2002), or membrane-associated PD-LOCATED PROTEIN 1 (Thomas et al. 2008), or of viral RNAs (Lough et al. 2006) have revealed highly variant transport motifs that are necessary and sufficient for entry into the intercellular PD transport pathway. For example, the homeodomain motif of KN1 and other closely related homeodomain transcription factors have been shown to mediate intercellular transport via PD. For SHR, domain shuffling experiments indicate that the presence of a GRAS domain and a functional nuclear localization signal seem to play a crucial role (Gallagher and Benfey 2009). For mobile HSC70 variants, deletion and shuffling experiments have shown that a small non-conserved C-terminal part (short variable region) of the chaperone is sufficient and necessary to mediate transport via PD. On the other hand, the unspecific PD-mediated allocation of foreign proteins such as free GFP and ER-localized GFP has been reported suggesting that open (gated) PD pores allow the diffusion of small proteins (Oparka and Roberts 2001; Oparka et al. 1999; Kim and Zambryski 2005; Kim et al. 2005a). Such diffusion of freely available macromolecules and specific motif-driven transport can be regulated by cell-wall-modifying enzymes found in close association with PD (Boyer and South 1989; Zavaliev et al. 2011; Rinne et al. 2011; Levy et al. 2007a, 2007b). Specific subsets of membrane-associated cell-wall-modifying enzymes such as 1,3-beta-glucanases or callose synthases regulate the accumulation of callose present at PD (Fig. 1). For example, stress-induced callose deposition reversibly plugs PD structures within minutes. Glucanase or callose synthase activity can cause an increase or decrease of pore size, enabling or restricting the intercellular transport of macromolecules, respectively (Levy et al. 2007a, 2007b; Rinne et al. 2001; Bucher et al. 2001; Benitez-Alfonso and Jackson 2009).

Studies of endogenous factors interacting with viral proteins, setting the stage for the transport of viral genomic information, have revealed a specific role of cytoskeleton-associated proteins and vesicular structures in intercellular transport. For example, viral produced MP has been shown to mediate the intercellular spread of the RNA genome of TMV. The TMV viral MP binds cooperatively to the viral RNA (Citovsky et al. 1990), associates to filamentous-actin, microtubule, ER, and PD pores. Association to filamentous-actin seems to serve as a pathway of the viral MP towards PD channels, whereas the localization to microtubules hinders the transport in the initial state of infection (Niehl and

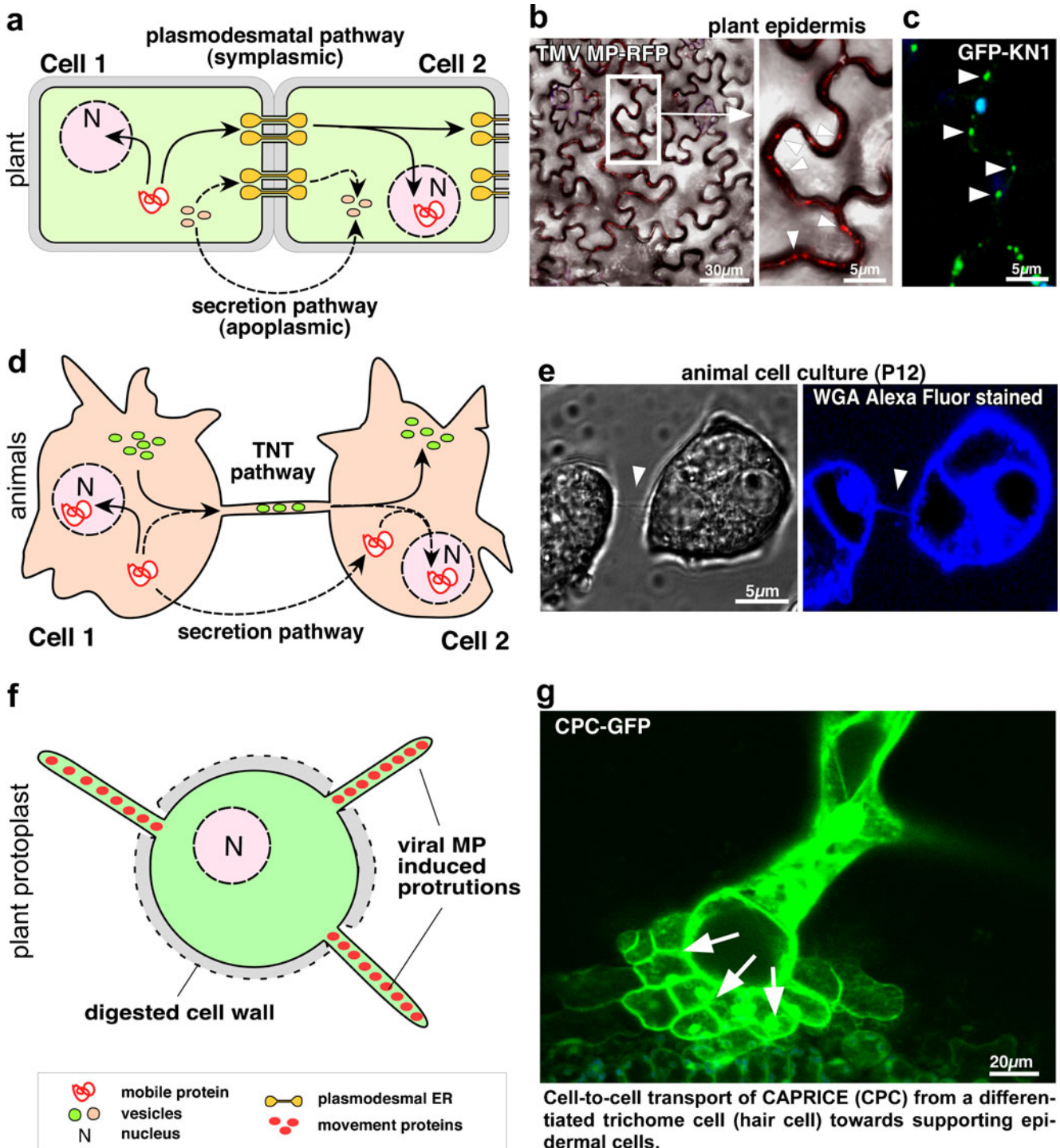
**Fig. 2** Similarities of membranous connections and transport pathways between cells found in plants and animals. **a** Plants cells are symplasmically connected by multiple PD organized in pitfields. Experimental evidence supports a model whereby symplasmic transport occurs from one cell (*Cell 1*) to a connected neighboring cell (*Cell 2*) via PD microchannels. Although indicated in the drawing, delivery of homeodomain transcription factors bound to vesicles via a secretion mechanism independent of PD has not yet been observed. **b, c** Confocal laser scanning images of epidermal cells transiently expressing the viral movement protein (*TMV-MP Tobacco mosaic virus movement protein*) fused to red fluorescent protein (*RFP, red*; boxed area is shown at higher magnification *right*) or the homeodomain transcription factor (*KN1*) fused to green fluorescent protein (*GFP, green*). Both proteins move from cell to cell via PD and are found in association with PD structures (*arrowheads*) connecting epidermal cells. **d** Animal cells exchange macromolecules via the tunneling nanotube (*TNT*) pathway or via secretion/internalization. Evidence exists for homeodomain transcription factor movement via an unconventional secretion/internalization mechanism (Prochiantz and Joliot 2003). However, tubular connections build a symplasmic continuum between animal cells and provide a means of filamentous-actin-dependent transport of organelles. In a hypothetical line, vesicles (*green*) and TNTs might also facilitate transcription factor transport in animals. **e** Confocal laser scanning image of a TNT (*arrowhead*) formed between two animal cells (rat pheochromocytoma cells; *PC12*) stained with a fluorescent membrane marker. *Right* Wheat-germ agglutinin (WGA)-bound Alexa fluorescence (*blue*). **f** Representation of a cultured plant cell (protoplast) with a disrupted cell wall. Because of the presence of a viral movement protein (*MP*), the cells form membranous protrusions similar to TNTs found in the animal cells. The protrusions contain viral MP and fibers resembling a cytoskeleton. However, whether the structures solely contain viral MP or also cytoskeletal components is currently not known (Heinlein et al. 1998). **g** Example of protein movement via PD between two different cell types. The myb transcription factor CAPRICE fused to GFP (*CPC-GFP, green*) moves from a trichome cell into the underlying basement cells and suppresses trichome formation in neighboring cells. **a, d** Modified after Ruiz-Medrano et al. (2004)

Heinlein 2011). Association to the ER establishes an assembly point to form a viral protein-RNA complex, which is guided most likely via the filamentous-actin to the PD pores (Heinlein 2002; Oparka 2004; Gillespie et al. 2002; Kawakami et al. 2004).

However, despite a number of cellular components having been identified that are involved in intercellular transport via PD, we know surprisingly little about the motifs and receptors facilitating the transfer of proteins or RNA across the PD “nano”-channel.

### Cell-to-cell transport of homeodomain transcription factors

The first plant endogenous protein to move via PD between cells was the homeodomain transcription factor KN1. Members of this protein family are essential for stem cell tissue maintenance in plants and have provided the initial evidence



for an ever-increasing number of mobile transcription factors in plants (Lucas et al. 1995). Kim et al. (2003, 2002) have described the position-dependent intercellular movement of KN1 in transgenic *Arabidopsis* plants; KN1 fused to GFP moves from the inner layers of a leaf into epidermal cells, but not in the opposite direction. In support of a general mobility of a subclass of homeodomain proteins, the KN1 homologous *Arabidopsis* proteins STM and KNAT1 move out of the

outermost L1 cell layer of the shoot apical meristem, and both trafficking proteins are able to complement a mutant *stm* allele. Consistent with the notion that KN1 intercellular transport is required for plant function, a cell-autonomous KN1 fusion protein expressed in the outermost L1 layer of the meristem cannot rescue an *stm* mutant. These results support the notion that the movement of homeodomain proteins is necessary to ensure the proper differentiation of plant stem cells.

The intercellular transport of homeodomain transcription factors also takes place between animal cells (Prochiantz and Joliot 2003). For example, the homeodomain transcription factor Engrailed acts as a non-cell autonomous signaling molecule that helps to specify crossvein formation on the wings of *Drosophila melanogaster* (Layalle et al. 2011). Another mobile Engrailed-like protein (En-2) present in *Xenopus* acts as a mobile signal involved in the proper formation of axons (Brunet et al. 2005). A further example of homeodomain protein transport in animals is found in the neuronal cells forming Antennapedia (Joliot et al. 1991). With respect to both Engrailed and Antennapedia, the mobility of cell-fate-deciding factors seems to depend on a so-called penetratin motif present in the homeodomain. These mobile transcription factors are thought not to utilize TNTs, as they seem to associate with vesicles that are secreted (Layalle et al. 2011; Maizel et al. 1999).

Interestingly, another similarity between animal and plant homeodomain transport has been found. The transport motif of animal homeodomain proteins is harbored in a similar structural context as plant homeodomain protein KN1. A mutation introduced in the proximity of the KN1 homeodomain motif (M6) or a deletion of the homeodomain disrupts its mobility in vivo (Lucas et al. 1995; Kim et al. 2005). Intriguingly, KN1 has also been shown to move between animal tissue-cultured cells (Prochiantz and Joliot 2003). So far, we do not know whether the transport mechanism used by Engrailed and Antennapedia or the TNTs are related to the PD-based transport system (Layalle et al. 2011). Nevertheless, this observation raises many questions regarding the mechanism recognizing and mediating homeodomain protein transport and the evolutionary relationship of the signal motifs.

More recent studies on two KN1-interacting proteins, namely MOVEMENT PROTEIN BINDING 2C (MPB2C) and CCT8 shed light on the mechanism of KN1 intercellular transport via PD. MPB2C is a plant-specific microtubule-associated factor initially identified as a viral MP interacting protein. *MPB2C* overexpression decreases the rate of viral infection spread, and *MPB2C* silencing increases the rate of viral infection spread. Interestingly, MPB2C also binds to the KN1 protein homeodomain region essential for mediating intercellular transport. As observed with the viral MP, the intercellular transport of KN1 and its relatives found in other plant species, such as the stem cell factors STM and KNAT1, can be negatively regulated by MPB2C (Winter et al. 2007). Because MPB2C is mainly expressed in the tissue neighboring the stem cell tissue present at the shoot apex, its function seems to restrict, with the support of other proteins, the presence of the intercellular transport activity of homeodomain proteins (F. Kragler, unpublished).

However, more experiments have to be performed to reveal the function of homeodomain protein transport via

PD in plants. Moreover, we need to show whether a specific PD-associated receptor protein can recognize a potential signal motif mediating the transport of a subclass of homeodomain proteins.

### Intercellular transport of cell-fate-deciding proteins and miRNA

The best current examples for specific movement of cell-fate-deciding proteins are found in the pattern-forming WD40 protein called TRANSPARENT TESTA GLABRA 1 (TTG1), the myb-related genes *TRYPTICHON* (*TRY*) and *CAPRICE* (*CPC*; Fig. 2g; Wada et al. 1997, 2002), and the GRAS family gene *SHORT ROOT* (*SHR*; Nakajima et al. 2001; Sena et al. 2004; Helariutta et al. 2000). Both TTG1 and CPC/TRY decide epidermal hair patterns in leaves (Balkunde et al. 2010; Pesch and Huelskamp 2011), whereas SHR is essential for the formation of proper radial patterns in roots. Trichomes (leaf hair cells) form on *Arabidopsis* rosette leaves in a highly ordered fashion; *CPC* and *TRY* are expressed in trichome initial cells and, with other factors, determine the distance to the next trichome by suppressing trichome formation in neighboring cells. To illustrate further the complexity of the system, TTG1 protein is produced in all epidermal cells and acts earlier to define trichome cells. TTG1 moves via PD from surrounding epidermal cells into trichome initials (Bouyer et al. 2008). By this means, a gradient forms in cells around the trichome initials. Because the trichome-defining TTG1 factor is depleted around the trichome initials, no new trichomes can form in the neighboring cells. This ensures that no trichomes appear in close proximity of each other, and that a regular pattern is formed over the planar leaf surface.

Another example of a pattern-forming mechanism occurring via intercellular signaling is found in the roots of plants. Correct radial patterning in *Arabidopsis* roots depends on the positional signal transmitted by SHR moving from the vascular cylinder to neighboring endodermal cells. In the endodermis, SHR by interacting with *SCARECROW* specifies endodermis identity (Sena et al. 2004; Nakajima et al. 2001). When expressed in other cell types, such as in phloem companion cells or in epidermal non-root hair cells, SHR does not move to neighboring cells. The radial pattern formed by SHR also underscores the complexity and specificity of this system, as it requires miRNA transport via PD. SHR protein moving into the endodermis activates *SCARECROW* expression. Together, these transcription factors specify the cortex-endodermal tissue and induce the expression of two miRNAs (miR165a and miR166b). These miRNAs are mobile and move most likely via PD into the vascular cylinder in which they inhibit the activity of specific homeodomain mRNAs and, by this means,

promote the differentiation of a subset of vascular cells (Carlsbecker et al. 2010; Furuta et al. 2012; Vaten et al. 2011; Miyashima et al. 2011).

### Are organelles transported via PD?

The function and mode of organelle transport between cells and the possible involvement of PD remain under discussion. Recent studies with grafting techniques have provided the first evidence for a transfer of whole organelles between plant cells. In grafting assays, plant tissues of two individual plants are fused to form a single chimeric plant body. This approach allows the screening of cellular components exchanged between genetically distinct cell types and has revealed that, after grafting, the chloroplasts of the tissue of one plant are transferred into the cells of the tissue of the other plant (Stegemann et al. 2012; Stegemann and Bock 2009). Despite the intercellular transfer of chloroplasts being limited to cells in close proximity to the graft junction, this finding establishes that whole organelles, including their genetic information, can be transferred between cells and can form new chimeric cell types.

Other cytoplasmic channels, which are in some aspects distinct from PD, have been observed between the meiotic cells present in anthers (plant male tissue containing cells undergoing meiosis). In transmission electron micrographs, wide open pores containing chromatin and organelles such as chloroplasts have been detected between meiotic cells (Mursalimov and Deineko 2011; Bellucci et al. 2003). Whether these structures devoid of ER components are related to or derived from PD is unknown. They have a large diameter of approximately 800 nm and are observed in extremely low frequencies between cells in somatic tissues. Some concerns exist as to whether these cytoplasmic channels are artifacts of fixation. However, in light of recent studies describing the exchange of chloroplasts between distinct cell types, we can reasonably assume that such structures are formed between cells and provide a pathway for the exchange of organelles and even chromosomes.

### Concluding remarks

In a hypothetical cell line, one would predict that, if a pore-forming mechanism exists in plants, plant cells devoid of cell wall material might also build tubular structures similar to that in animal tissues. Indeed, plant protoplasts when producing viral MPs have the capacity to form tubular structures protruding from the cell surface (Fig. 2; Huang et al. 2000; Canto and Palukaitis 1999). Although no evidence exists that such protoplasmic tubules connect cells, they show striking parallels to animal nanotubes and might

represent a conserved mechanism establishing connections between cells and might fulfill demands to exchange signals in the form of specific macromolecules.

Despite striking parallels, more evidence is needed showing that the plant PD transport pathway is related to the TNTs used by animal cells. Obviously, the movement of transcription factors would provide a simple answer for a tissue to establish differentiation patterns. On the other hand, why are some homeodomain transcription factors mobile, whereas others that are closely related are not? How is their transfer regulated? These are intriguing questions that remain to be answered. A potential default intercellular transport pathway via diffusion for all cytosolic soluble proteins would impose a challenge for tissue differentiation and pattern formation. Thus, a delicate mechanism seems to have evolved in higher organisms with regard to the mobility and/or the presence of cell-fate-deciding factors.

Interestingly, despite several attempts to identify protein structures acting as PD targeting signals, a simple common sequence motif necessary and sufficient to guide heterologous proteins and RNA molecules through PD has not been determined. Based on knowledge gained by high-throughput screening combined with advanced *in silico* algorithms, the identification of PD targeting motifs and receptors might be feasible and will allow us to assign non-cell-autonomous functions to gene products.

**Acknowledgments** I apologize to all of my colleagues whose work was not mentioned or addressed in depth because of space limitations. I thank the anonymous reviewers for their helpful comments for improving the manuscript.

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