Plant Pathol. J. 21(1) : 21-26 (2005)

The Plant Pathology Journal

Symposium

The 7th Molecular Plant-Microbe Interactions

December 3, 2004, Seoul, Korea

Intercellular Trafficking of Homeodomain Proteins

Seon Won Kim[1,2], Ju Yeon Moon[2], Jin Hee Jung[2], Xiongyan Chen[2], Chunlin Shi[2], Yeong Gil Rim[4], Hey-Jin Kwon[4], David Jackson[5], Raju Datla[6], Alain Joliot[7] and Jae-Yean Kim[2,3,4]*

[1]Department of Food Science & Nutrition, [2]Division of Applied Life Science (BK21), [3]Plant Molecular Biology & Biotechnology Research Center, and [4]Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea, [5]Cold Spring Habor Laboratory, 1 bungtown Rd, Cold Spring Harbor NY11724, USA

[6]Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada S7N 0W9

[7]Biologie cellulaire des homéoprotéines, CNRS UMR8542 Ecole Normale Supérieure 46 rue d'Ulm F-75005 Paris, France

(Received on January 17, 2005; Accepted on February 11, 2005)

Homeotic proteins have pivotal roles during the development of both plant and animals. Many homeotic proteins exert control over cell fate in cells where their genes are not expressed, i.e., in a non-cell autonomous manner. Cell-to-cell communication, which delivers critical information for position-dependent specification of cell fate, is an essential biological process in multicellular organisms. In plants, there are two pathways for intercellular communication that have been identified: the ligand/receptor-mediated apoplastic pathway and the plasmodesmata-mediated symplasmic pathway. Regulatory proteins and RNAs traffic symplasmically via plasmodesmata and play a critical role in intercellular communication. Thus, the non-cell autonomous function of homeotic proteins can be explained by the recent discovery of cell-to-cell trafficking of proteins or RNAs. This article specifically focuses on understanding the intercellular movement of homeodomain proteins, a family of homeotic proteins.

Keywords : cell-to-cell communication, homeodomain, intercellular trafficking, KNOTTED1, non-cell autonomous protein, plasmodesmata

Plant cell fate is generally determined late in plant development through positional information, rather than lineage (Dawe and Freeling, 1991; Huala and Sussex, 1993; Poethig, 1987). Cell-to-cell communication provides the means by which cells determine their position; e.g., by the use of both short- and long-range signals. A novel mechanism for cell-to-cell communication in plants involves the intercellular trafficking of regulatory proteins and mRNAs. Macromolecular signals may pass through the symplasm, via cytoplasmic channels called plasmodesmata (PD) (Jackson, 2000; Lucas et al., 1993, 1995; Zambryski and Crawford, 2000; Fig. 1A). However, an alternative pathway for macromolecular signaling exists in the apoplasm (Trotochaud et al., 1999).

PD are bounded by a sleeve of plasma membrane and traversed by a tube of appressed endoplasmic reticulum (ER). Passage of small molecules and regulated transport of macromolecules likely occurs through the cytoplasmic microchannels between the ER and plasma membrane (Jackson, 2000; Lucas et al., 1993, 1995; Zambryski and Crawford, 2000). PD are classified as primary, if formed during cytokinesis, or secondary, if synthesized through an existing cell wall. The latter class is important for connecting cells that do not share a recent division wall, for example those in adjacent layers of the shoot apical meristem (Satina et al., 1940). During later stages of leaf development, PD can become branched, and branching is correlated with changes in the PD size-exclusion limit (SEL) and ability to traffic specific proteins (Itaya et al., 1998; Oparka et al., 1999).

In animals, similar modes of transport to PD exist, e.g., gap junction- or membrane-lined tunneling nanotube-mediated communication pathways (Fig. 1B). Notably, recent observations of F-actin based intercellular nanotubular structures suggest functional and structural similarity between PD and nanotubes (Rustom et al., 2004).

Many viruses encode movement proteins (MPs) that localize to PD and traffic themselves and MP–viral nucleic acid complexes between cells (Reichel et al., 1999). One

*Corresponding author
Phone) +82-55-751-6253, FAX) +82-55-759-9363
E-mail) kimjy@gsnu.ac.kr

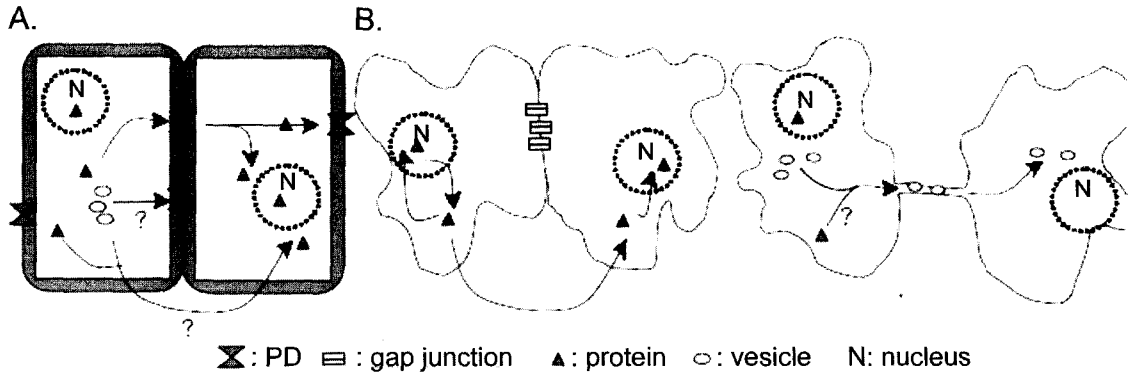

Fig. 1. Intercellular transport of macromolecular signals in plant and animal cells. **A.** In plant cells, trafficking of non-cell autonomous proteins most probably occurs symplasmically via PD. The trafficking of proteins bound to vesicles via secretion mechanism or via PD has not yet been proven and is shown with question marks. **B.** Symplasmic exchange of molecules in animal cells occurs via gap junctions (left panel) or nanotubes (right panel). Trafficking of animal homeodomain uses an unconventional secretion-internalization pathway. Recently, nanotubes similar to PD were found and thought to be a novel cell-to-cell communication pathway in animal. These nanotubes facilitate the transport of vesicles or membrane-linked proteins. Vesicle-bound or soluble HD proteins similar to those in the PD-based transport system of plants are hypothesized to use the nanotubular pathway (indicated with question mark).

hypothesis states that MPs hijack an endogenous intercellular trafficking pathway. This hypothesis is supported by the discovery of a plant MP-related phloem protein, PP16, which can traffic itself and RNA through PD (Xoconostle-Cazares et al., 1999).

Many developmental homeotic genes act non-cell autonomously, including members of the KNOTTED 1 (KN1)-related homeobox genes (KNOX) that were first characterized by dominant mutations affecting leaf cell fate (Hake and Freeling, 1986; Sinha, 1999). Qualitative differences in their non-autonomous action imply specific regulation of KNOX gene signaling (Jackson, 2000). In the case of KN1, cell-to-cell trafficking of the KN1 protein was suggested as a mechanism for its non-autonomy. Microinjection studies demonstrated its ability to traffic between cells, to gate PD, and to specifically traffic its mRNA (Lucas et al., 1995). Movement was a specific and regulated property of KN1, because a mutant, KN1 (M6), was unable to traffic.

Here, we discuss the non-cell autonomy, regulatory mechanisms, and biological significance of KN1 cell-to-cell trafficking. The focus of this article is to integrate the new data on KN1 movement, and homeodomain proteins in general.

Kn1 gene acts non-autonomously between cell layers to regulate cell proliferation

Homeodomain proteins play important roles during plant and animal development. In wild type plants, KN1 is expressed in the shoot apical and floral meristems but down-regulated at sites of leaf primordia initiation (Jackson, 2003; Jackson et al., 1994). The KN1 locus was first defined by dominant alleles that caused knots to form along the leaf lateral veins. The knotted phenotype, which results from excessive cell proliferation, is caused by ectopic KN1 expression in the leaf (Vollbrecht et al., 1991). Knock-out lines carrying loss-of-function *kn1* alleles show a defect in meristem maintenance (Vollbrecht et al., 2000). Therefore, KN1 may act to promote cell proliferation and to maintain cells in an undifferentiated state.

The first clue that KN1 can traffic cell-to-cell came from an analysis of genetic mosaic maize. Hake and Freeling (1986) used X-rays to create genetic sectors in maize that lack a dominant KN1 allele (*Kn1*^{d}) in the *Kn1*^{d} genetic background The sectors were marked by discovering a tightly linked locus that confers a white albino phenotype, the dysfunctional allele of the *lw* gene. When seeds of the heterozygotic genotype *Kn1*^{d} *Lw/Kn1 lw* are irradiated by X-rays, sectors that lose the dominant alleles (*Kn1*^{d} *Lw*) because of chromosome-induced breakage give rise to white normal *Kn1 lw* sectors in green knotted leaf. The authors found that the dominant *Kn1*^{d} genotype of the internal cell layers (mesophyll cells) was sufficient to induce a KN1 overexpression phenotype in the outer cell layer (epidermis), i.e., the information that induces epidermal cells to divide into knots moved from internal *Kn1*^{d} leaf cells to the non- *Kn1*^{d} epidermal cells. Jackson et al. (1994) reported subsequently reported an intriguing difference between the localization of the KN1 protein and the expression of KN1 mRNA. Whereas the protein was detected in all cell layers of the meristem, KN1 mRNA was not detected in the L1 layer of vegetative, inflorescence or floral meristems (Fig. 2A and B). In addition, in plants carrying *Kn1*^{d} overexpression alleles, KN1 mRNA is ectopically expressed in leaf vascular strands, but not in epidermal cells, while KN1 protein is detected in epidermal

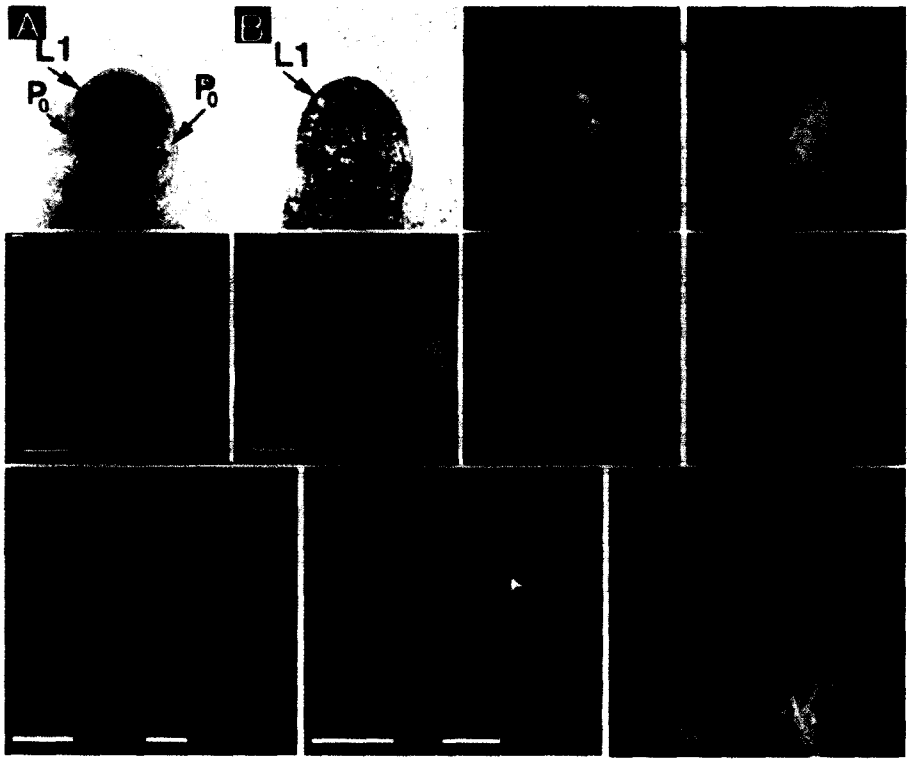

Fig. 2. Cell-to-cell trafficking of KN1 in plant cells. **A.** KN1 mRNA localization by *in situ* hybridization in maize shoot apical meristem. KN1 mRNA is predominantly localized in the L2 layer (subepidermal layer), but missing in the L1 (epidermal layer) and in the P0 region producing new leaf primordia. **B.** KN1 protein localization. KN1 protein is detected outside the domain of mRNA expression. **C.** Tobacco mesophyll cell microinjected with 10 kD-dextran-FITC. 10 kDa-dextran-FITC does not show intercellular trafficking. **D.** Tobacco mesophyll cell microinjected with KN1-FITC. The brightest cell was injected and fluorescence in surrounding cells was caused from the KN1-FITC protein trafficking. **E.** Confocal image (green and red channels) of a leaf cross-section of an *Arabidopsis* GAL4 enhancer trap (J2111 line) line showing ER-localized GFP (GFP-ER, cell autonomous reporter) expression in the vascular tissue. Red fluorescence comes from chlorophyll autofluorescence. **F.** Confocal image of J2111 transgenic plant expressing USA-GFP-KN1 showing GFP fluorescence in epidermal and mesophyll cells as well as vascular cells. This suggests that GFP-KN1 can traffic from vascular tissue to mesophyll and epidermal cells. **G.** Confocal image (green channel only) of a leaf cross-section of a transgenic plant carrying an epidermal specific LTP1 promoter driving GFP-ER expression. **H.** Confocal image of pLTP1-GFP-KN1 plant leaf. GFP-KN1 expressed in epidermal cells cannot move into mesophyll cells. **I.** Confocal image from plant meristem carrying L1 specific AtML1 promoter driven GFP-ER expression. Inset shows non-nuclear GFP signal (ER localization). **J.** Confocal image from plant expressing GFP-KN1 under the control of pAtML1. GFP-KN1 can move from the L1 to the L2/L3 layers showing developmental regulation of KN1 trafficking. Inset shows predominant nuclear localization of GFP-KN1. **K.** Functional complementation of *shoot meristemless* (*stm*) mutation by the L1 specific KN1 expression. *stm-11* strong allele (left), pAtML1-GFP-KN1/*stm-11* (middle) and pAtML1-KN/*stm-11* (right).

cells as well as subepidermal cells. One explanation for the discrepancy could be cell-specific differences in stability of KN1 mRNA. An alternative hypothesis is that KN1 protein could move between cells, since mosaic clonal analysis showed that a signal moved from internal cell layers to instruct the epidermis to divide. This movement was suggested to be short-ranged or selective, since protein does not move into the P0 or young leaf primordia, and in knotted leaves does not move to adjacent veins (Hake and Freeling, 1986).

To test if KN1 protein traffics cell-to-cell, microinjection was used (Lucas et al., 1995). *E. coli*- expressed KN1 was labeled with fluorescein isothiocyanate (FITC) and microinjected into the cytoplasm of tobacco mesophyll cells. The KN1-FITC conjugate injected into the cytoplasm moved rapidly into neighboring cells (Fig. 2D). An increase in the PD SEL was also associated with KN1 intercellular trafficking in maize and tobacco plants. Microinjected FITC-dextran of 9.4 or 20 kDa alone did not move out of the injected cell (Fig. 2C), but coinjection of these FITC-dextrans with unlabeled KN1 gave rise to their movement. The KN1-induced increase in PD SEL also permitted the cell-to-cell trafficking of a 20 kDa soybean cytosolic protein, soybean trypsin inhibitor, which does not normally traffic cell-to-cell. Microinjection studies determined that the upper PD SEL associated with KN1 gating is greater than 20 kDa and close to 39 kDa (Lucas et al., 1995). A series of experiments using KN1 alanine-scanning mutants

showed that only one mutation (M6) among nine mutations tested showed a significant reduction in its ability to move cell-to-cell (Lucas et al., 1995). The M6 mutation resides in a potential nuclear localization sequence that is present in the N-terminal region of the homeodomain. These studies suggest receptor-mediated and energy-dependant transport through PD.

KN1 intercellular trafficking requires unfolding and binding to a putative PD receptor

Although the PD SEL undergoes a significant increase from 10 to 40 kDa during KN1 trafficking, the extent of a protein-mediated increase in the PD SEL does not correlate well with the physical size of the translocated protein (up to 200 kDa). Thus, larger proteins must either undergo partial unfolding for translocation or PD must undergo a significant physical rearrangement to dilate the microchannels. Studies performed with cross-linked KN1 revealed that a conformational change is required for its cell-to-cell movement (Kragler et al., 1998). In addition, competition experiments between KN1 and cross-linked KN1 demonstrated that cross-linked KN1 functions as a strong inhibitor of KN1 gating activity (the ability of KN1 to increase the PD SEL) and subsequent KN1 trafficking. KN1 bound to 1.5 nm gold particles can normally traffic cell-to cell. However, KN1 bound to 6 or 15 nm gold behaves like cross-linked KN1 suggesting that the PD microchannels are incapable of dilation to the extent required to permit trafficking of KN1 bound to 6 or 15 nm gold particles. Co-injection of KN1-gold with unbound KN1 or Cucumber Mosaic Virus (CMV) MP established that the KN1-gold conjugate is highly effective at blocking PD transport of KN1 and MP. Thus, it is possible that KN1 and MP use a common receptor in their cell-to-cell trafficking pathways. An *in vitro* binding assay using KN1 and CMV MP revealed the presence of the putative common receptor in a PD-enriched cell wall protein fraction (W2). Subsequently Kragler et al. (2000) used a phage-display screening system to identify peptides capable of interacting with proteins present in W2 fraction. W2-fraction proteins were dotted onto a nitrocellulose membrane and incubated with a suspension of the phage library exposing 12mer amino acid sequences. After extensive washing, specific-bound sequences were eluted by exposure to excess levels of KN1 protein. After five rounds of amplification, incubation and elution steps, the authors obtained 100% enrichment of a specific epitope (KN1pep). KN1pep exhibited approximately 75% homology with the N-terminal motif of KN1. *In vitro* binding/competition studies and microinjection experiments established that KN1pep or its corresponding KN1 systhetic peptide interacted with proteins in W2 fractions. KN1pep prevented the KN1-mediated movement of an 11 kD F-dextran but not KN1-FITC intercellular trafficking. This result suggested that KN1 minimally requires two physically separated motifs for trafficking, i.e., one signal is required to dilate PD and a second signal is required for protein translocation through PD.

KN1 can also facilitate the specific trafficking of its sense mRNA (Lucas et al., 1995). KN1 protein trafficking is required since the non-trafficking mutant M6 did not facilitate trafficking of KN1 mRNA. In addition, KN1 gating activity appears to be essential for KN1 RNA trafficking, since KN1pep can block its trafficking. KN1 shares similar characteristics with viral MPs in its ability for cell-to-cell trafficking, PD gating activity and RNA trafficking, except that MPs can mediate the movement of nucleic acids in non-specific manner. The inhibitory effect of KN1pep on the increase of PD SEL by CMV MP confirmed that KN1 and CMV MP compete for common components in the PD transport pathway.

Developmental regulation and function of KN1 cell-to-cell trafficking

Although genetic mosaic analysis, *in situ* localization and microinjection assays using recombinant KN1 proteins as probes indicated intercellular KN1 movement, *in vivo* evidence of the regulated intercellular transport of KN1 trafficking was lacking. Our group provided the first evidence of *in vivo* cell-to-cell trafficking of KN1 (Kim et al., 2002). We used green fluorescent protein (GFP) fusion to KN1 to trace the trafficking of KN1 in the model plant *Arabidopsis thaliana*. KN1 fused to GFP is able to traffic between epidermal cells of *Arabidopsis* and onion. When expressed *in vivo*, the GFP-KN1 fusion trafficked from inner, mesophyll tissues of the leaf to the outer, epidermal tissue, thus providing the first direct evidence that KN1 can traffic across different tissue layers in the leaf (Kim et al., 2002, 2003; Fig. 2F). However, intercellular KN1 trafficking was position-dependent since KN1-GFP fusion proteins could not move from the epidermal cells into the mesophyll layers (Kim et al., 2003; Fig. 2H). Only the movement of KN1 was position-dependent because cytoplasmic GFP and the GFP~MP fusion moved readily out of the epidermis. Collectively, these data suggest that the KN1 trafficking pathway is distinct from that of free GFP diffusion and selective TVCV MP trafficking. In addition to positional constraints, KN trafficking is tissue-specific and depends on the developmental stage. In the shoot meristem, where cells are in a relatively undifferentiated state, the GFP-KN1 fusion was able to traffic out of the epidermal (L1) layer to subepidermal (L2/L3) layers (Kim et al., 2003; Fig. 2J). GFP fusions to KNAT1/

BREVIPEDICELLUS and SHOOT MERISTEMLESS (STM), KN1 homeobox-related proteins in *Arabidopsis thaliana*, also trafficked from the L1 to the L2/L3 layers of the meristem. The analysis of KNAT1 mutants suggested that KNAT1 may also regulate epidermal cell fate non-cell autonomously (Venglat et al., 2002). These observations suggest that intercellular trafficking is a general feature of class I KNOX homeodomain (HD) proteins.

STM is maize KN1 ortholog in *Arabidopsis* (Long et al., 1996). A knock-out mutant of STM (*stm-11*) shows a defect in meristem initiation and maintenance. *stm-11* plants have a fused cotyledon without forming shoot and are seedling-lethal. In order that intercellular protein trafficking should act as a mechanism of cell-to-cell communication, non-cell autonomous proteins have to maintain its biological function after intercellular trafficking. In a test for the functional significance of KN1 trafficking, we showed that L1-specific expression of KN1, or of KNAT1, was able to partially complement the strong *stm-11* mutant (Kim et al., 2003; Fig. 2K). However, a cell-autonomous beta-glucuronidase (GUS) fusion to KN1 showed neither trafficking ability nor complementation of *stm-11* when expressed in the L1. These results suggest that the activity of KN1 and related homeobox proteins is maintained following intercellular trafficking, and that trafficking may be required for their normal developmental function.

Evolutionary analogy

Intriguingly, the animal HD transcription factors Engrailed (EN), Hox and Antennapedia move from cell-to-cell via an unconventional secretion–internalization mechanism (Maizel et al., 1999, 2002). Deletion assays showed that the homeodomain of these proteins was sufficient for movement. EN cell-to-cell trafficking was proposed to involve four events: nuclear import, nuclear export, secretion and internalization. The essential signal motif that mediates the intercellular transport activity of these transcription factors is located in a short motif of approximately 16 amino acids within the HD. Interestingly, Prochiantz and Joliot noted that the HD of KN1 also moves from cell to cell in tissue-cultured mammalian cells, although not full length protein (Prochiantz and Joliot, 2003). This surprising observation raises many questions regarding the mechanism that recognizes and mediates HD protein transport and the evolutionary relationship of the trafficking signal motifs between animal and plant proteins, and further suggests that some components of HD trafficking may also be conserved. It would be interesting to use the reverse approach to test whether animal HD proteins can move between plant cells. To date, however, no secretion mechanism similar to that used by EN has been found in plants. Considering that the intercellular transport of the transcription factors EN and Antennapedia in mammalian tissue cultures depends on the HD, it is plausible that a trafficking signal motif might reside in the HD of plant class I KNOX HD proteins. Consistent with such an assumption, a mutant KN1 protein (M6) that has an altered amino-acid composition in the HD motif remains cell-autonomous. However, further studies are needed to show that the KN1 HD motif is necessary and sufficient to act as a PD transport signal and whether specialized PD pathway protein(s) could recognize such a motif.

Conclusion and perspectives

Clearly, cell-to-cell communication by intercellular trafficking of homeotic transcription factors plays a pivotal role in plant cell biology and development. However, *in vivo* functions and mechanisms of protein trafficking are mostly uncharacterized. Although PD can provide a pathway for protein trafficking in plant cells, direct evidence is still missing in most cases. Furthermore, we cannot exclude the possibility that plant proteins might use a novel secondary pathway as shown animal homeodomain proteins. Thus, a future challenge is to understand the different modes in protein trafficking regulation. An additional challenge will be to illuminate the biological functions and mechanisms of intercellular trafficking of ribonucleoprotein complexes, e.g., the KN1 protein-RNA complex. Identification of a trafficking-signal motif or domain will be essential to unlock the mystery of a selective trafficking mechanism. The exploration of dark PD channels is exciting, and essential to bring light to plant cell biology.

Acknowledgement

This work was supported by a Korean Research Foundation Grant (KRF-2003-042-C00124). We thank Michelle Cilia for proofreading this article.

References

Dawe, R. K. and Freeling, M. 1991. Cell lineage and its consequences in higher plants. *Plant J.* 1:3-8.

Hake, S. and Freeling, M. 1986. Analysis of genetic mosaics shows that the extraepidermal cell divisions in *knotted1* mutant maize plants are induced by adjacent mesophyll cells. *Nature* 320:621-623.

Huala, E. and Sussex, I. M. 1993. Determination and Cell Interactions in Reproductive Meristems. *Plant Cell* 5:1157-1165.

Itaya, A., Woo, Y. M., Masuta, C., Bao, Y., Nelson, R. S. and Ding, B. 1998. Developmental regulation of intercellular protein trafficking through plasmodesmata in tobacco leaf epidermis. *Plant Physiol.* 118:373-385.

Jackson, D. 2000. Opening up the communication channels: recent insights into plasmodesmal function. *Curr. Opin. Plant Biol.* 3:394-399.

Jackson, D. and Kim, J. Y. 2003. Dispatch. Intercellular signaling: an elusive player steps forth. *Curr. Biol.* 13:R349-R350.

Jackson, D., Veit, B. and Hake, S. 1994. Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* 120:405-413.

Kim, J. Y., Yuan, Z., Cilia, M., Khalfan-Jagani, Z. and Jackson, D. 2002. Intercellular trafficking of a KNOTTED1 green fluorescent protein fusion in the leaf and shoot meristem of Arabidopsis. *Proc. Natl. Acad. Sci. USA* 99:4103-4108.

Kim, J. Y., Yuan, Z. and Jackson, D. 2003. Developmental regulation and significance of KNOX protein trafficking in Arabidopsis. *Development* 130:4351-4362.

Kragler, F., Monzer, J., Shash, K., Xoconostle-Cazares, B. and Lucas, W. J. 1998. cell-to-cell transport of proteins: requirement for unfolding and characterization of binding to a putative plasmodesmal receptor. *Plant J.* 15:367-381.

Kragler, F., Monzer, J., Xoconostle-Cazares, B. and Lucas, W. J. 2000. Peptide antagonists of the plasmodesmal macromolecular trafficking pathway. *EMBO J.* 19:2856-2868.

Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K. 1996. A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature* 379:66-69.

Lucas, W. J., Bouche-Pillon, S., Jackson, D. P., Nguyen, L., Baker, L., Ding, B. and Hake, S. 1995. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* 270:1980-1983.

Lucas, W. J., Ding, B. and C. Van der Schoot. 1993. Plasmodesmata and the supracellular nature of plants. *New Phytol.* 125:435-476.

Maizel, A., Bensaude, O., Prochiantz, A. and Joliot, A. 1999. A short region of its homeodomain is necessary for engrailed nuclear export and secretion. *Development* 126:3183-3190.

Maizel, A., Tassetto, M., Filhol, O., Cochet, C., Prochiantz, A. and Joliot, A. 2002. Engrailed homeoprotein secretion is a regulated process. *Development* 129:3545-3553.

Oparka, K. J., Roberts, A. G., Boevink, P., Santa Cruz, S., Roberts, I., Pradel, K. S., Imlau, A., Kotlizky, G., Sauer, N. and Epel, B. 1999. Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell* 97:743-754.

Poethig, R. S. 1987. Clonal analysis of cell lineage patterns in plant development. *Am. J. Bot.* 74:581-594.

Prochiantz, A. and Joliot, A. 2003. Can transcription factors function as cell-cell signalling molecules? *Nat. Rev. Mol. Cell Biol.* 4: 814-819.

Reichel, C., Mas, P. and Beachy, R. N. 1999. The role of the ER and cytoskeleton in plant viral trafficking. *Trends Plant Sci.* 4: 458-462.

Rustom, A., Saffrich, R., Markovic, I., Walther, P. and Gerdes, H. 2004. Nanotubular highways for intercellular organelle transport. *Science* 303:1007-1010.

Satina, S., Blakeslee, A. F. and Avery, A. G. 1940. Demonstration of the three germ layers in the shoot apex of Datura by means of induced polyploidy in periclinal chimeras. *Am. J. Bot.* 27: 895-905.

Sinha, N. 1999. Leaf Development in Angiosperms. *Annu Rev Plant Physiol. Plant Mol. Biol.* 50:419-446.

Trotochaud, A. E., Hao, T., Wu, G., Yang, Z. and Clark, S. E. 1999. The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. *Plant Cell* 11:393-406.

Venglat, S. P., Dumonceaux, T., Rozwadowski, K., Parnell, L., Babic, V., Keller, W., Martienssen, R., Selvaraj, G. and Datla, R. 2002. The homeobox gene BREVIPEDICELLUS is a key regulator of inflorescence architecture in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 99:4730-4735.

Vollbrecht, E., Reiser, L. and Hake, S. 2000. Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, knotted1. *Development* 127:3161-3172.

Vollbrecht, E., Veit, B., Sinha, N. and Hake, S. 1991. The developmental gene Knotted-1 is a member of a maize homeobox gene family. *Nature* 350:241-243.

Xoconostle-Cazares, B., Xiang, Y., Ruiz-Medrano, R., Wang, H. L., Monzer, J., Yoo, B. C., McFarland, K. C., Franceschi, V. R. and Lucas, W. J. 1999. Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* 283:94-98.

Zambryski, P. and Crawford, K. 2000. Plasmodesmata: gatekeepers for cell-to-cell transport of developmental signals in plants. *Annu. Rev. Cell Dev. Biol.* 16:393-421.