Transcription factor-mediated cell-to-cell signalling in plants

Xiao Han, Dhinesh Kumar, Huan Chen, Shuwei Wu and Jae-Yean Kim*

Division of Applied Life Science (BK21plus/WCU Program), Graduate School of Gyeongsang National University, Plant Molecular Biology & Biotechnology Research Center (PMBBRC), Jinju 660-701, Korea

* To whom correspondence should be addressed. E-mail: kimjy@gnu.ac.kr

Received 20 August 2013; Revised 22 October 2013; Accepted 11 November 2013

Abstract

Plant cells utilize mobile transcription factors to transmit intercellular signals when they perceive environmental stimuli or initiate developmental programmes. Studies on these novel cell-to-cell signals have accumulated multiple pieces of evidence showing that non-cell-autonomous transcription factors play pivotal roles in most processes related to the formation and development of plant organs. Recent studies have explored the evolution of mobile transcription factors and proposed mechanisms for their trafficking through plasmodesmata, where a selective system exists to facilitate this process. Mobile transcription factors contribute to the diversity of the intercellular signalling network, which is also established by peptides, hormones, and RNAs. Crosstalk between mobile transcription factors and other intercellular molecules leads to the development of complex biological signalling networks in plants. The regulation of plasmodesmata appears to have been another major step in controlling the intercellular trafficking of transcription factors based on studies of many plasmodesmal components. Furthermore, diverse omics approaches are being successfully applied to explore a large number of candidate transcription factors as mobile signals in plants. Here, we review these fascinating discoveries to integrate current knowledge of non-cell-autonomous transcription factors.

Key words: Cell-to-cell communication, intercellular signalling, non-cell-autonomous proteins, plasmodesmata, transcription factors.

Introduction

Land plants, which are sessile, have evolved developmental plasticity to cope up with environmental challenges. Cell-to-cell communication is one such regulatory event and represents a crucial step in the development of a complex signalling network at the multicellular level for establishing the spatiotemporal regulation that is essential for development. To transmit biological information, cells utilize various types of mobile molecules as a signalling code, including peptides, hormones, RNAs, and proteins. A large body of evidence has suggested that non-cell-autonomous proteins (NCAPs), especially non-cell-autonomous transcription factors (NCATFs), contribute to intercellular communication by acting as mobile signals capable of carrying information in plant cells. Plant organs such as the shoot meristem, flowers, and roots are established through cell fate determination processes under the control of NCAPs. This review describes some of the recent key findings related to NCATFs involved in plant development and attempts to integrate the current understanding of the evolution of non-cell-autonomous pathways. In addition, several representative types of crosstalk between NCAPs and other mobile molecules are reviewed to answer the question of how NCAPs can facilitate cell-to-cell communication in complicated signalling networks. This review also provides insights into how plasmodesmal symplasmic channels dexterously regulate NCAP movements. Finally, this review describes the fascinating exploitation of recent innovative omics approaches in the field of NCAP movement.
Mobile transcriptional regulators involved in developmental plasticity

Diverse plant cell patterning and organ formation processes are regulated by intercellular signalling to accomplish events ranging from reproduction to the responses to various environmental stimuli. It is now widely accepted that many developmental processes require mobile transcriptional regulators to transmit essential positional information in plants (Fig. 1).

Shoot development

The first endogenous NCAP to be discovered was the maize homeodomain (HD) transcription factor (TF) KNOTTED1 (KN1), which is essential for maintenance of the shoot apical meristem (SAM). KN1 is expressed in the L2 cells, and the KN1 protein has also been detected in the outer domain of expression, suggesting that this protein exhibits movement within the meristem (Lucas et al., 1995). In addition, KN1 was shown to move in a tissue-specific manner during Arabidopsis shoot development. This cell-to-cell trafficking ability of KN1 was found to be required for mutant rescue when expressed under layer-specific promoters (Kim et al., 2002). The Arabidopsis KN1 orthologues SHOOTMERISTEMLESS (STM) and KNAT1/BREVIPEDICELLUS (BP), and their highly conserved trafficking domains, including the HD, have been shown to confer a gain-of-trafficking function on cell-autonomous proteins within the SAM, leaves, and stem (Kim et al., 2003). Intercellular trafficking of KNAT1 from the cortex to epidermal cells in Arabidopsis stems is also essential for the determination of plant architecture and epidermal differentiation (Rim et al., 2009). Another essential NCAP involved in the maintenance of the Arabidopsis SAM is WUSCHEL (WUS) (Laux et al., 1996), a HD-containing TF that triggers downstream signals in the L1 and L2 layers following trafficking from the organizing centre of the SAM (Yadav et al., 2011).

Flowering induction and floral organ development

Various important aspects of flower development are controlled by mobile transcriptional regulators. During the process of transforming the SAM into an inflorescence meristem, the SAM transition is initiated by the mobile signals FLOWERING LOCUS T (FT) (Corbesier et al., 2007; Jaeger and Wigge, 2007) and TWIN SISTER OF FT (TSF) (Yamaguchi et al., 2005), members of the CETS (CENTRORADIALIS-TERMINAL FLOWER 1-SELFPRUNING) gene family in Arabidopsis, and orthologous proteins HEADING DATE 3A (HD3A) (Tamaki et al., 2007), SINGLE FLOWER TRUSS (SFT), and FT-Like 2 (FTL2) (Lin et al., 2007; Yoo et al., 2013) in rice, tomato, and squash, respectively. FT is loaded into the phloem sieve tube for long-distance transport from the leaf blade and cotyledons, unloaded at the proximal SAM unloading zone, and travels in a cell-to-cell manner to the SAM (Corbesier et al., 2007; Jaeger and Wigge, 2007). Arabidopsis TERMINAL FLOWER 1 (TFL1) and its tomato orthologue SELFPRUNING, which are also NCAPs, play a role in the maintenance of the indeterminate meristem. Thus, the ratio of FT to the antagonistic TFL1/SELFPRUNING protein specifies the transition of the SAM into an inflorescence meristem (Lifschitz et al., 2006; Conti and Bradley, 2007; Shalit et al., 2009). With a mature inflorescence meristem, the TFL1 protein travels to the epidermal cell layer and acts to inhibit the floral identity genes LEAFY (LFY) and APETALA1 (AP1). Interestingly, LFY, which is expressed in peripheral cells (anlagen) in the inflorescence and floral meristems, is indirectly required for TFL1 protein movement, thus establishing a TFL1–LFY feedback loop. LFY is also known as an NCAP; however, the biological significance of LFY movement is not clear because the patterns of LFY RNA and LFY protein expression in wild-type plants
are similar (Sessions et al., 2000). One possible explanation for this intricate phenomenon is that the non-cell-autonomic action of the LFY protein functions as a safe-guard mechanism to ensure complete conversion of a meristem into a flower. In addition, MADS domain transcription factors AGAMOUS (AG), AP3, PISTILLATA (PI), and SEPALLATA3 (SEP3), which serve as fundamental regulators of floral organ identity, are able to travel from cell to cell (Urbansus et al., 2010a), reinforcing the conclusion that cell-to-cell communication mediated by mobile transcription factors is an essential process for tissue patterning and organ formation.

**Organ size determination and epidermal patterning**

Coordinated proliferation between clonally distinct cell layers is essential for determining the size and shape of plant organs and has been proposed to be regulated in a non-cell-autonomous manner during leaf development (Savaldi-Goldstein and Chory, 2008; Bai et al., 2010; Kawade et al., 2010). Recently, the transcriptional coactivator ANGUSTIFOLIA3 (AN3), which is responsible for typical compensation for a decrease in cell proliferation via enhanced post-mitotic cell expansion in leaf primordia, was shown to travel from leaf mesophyll cells to epidermal cells in Arabidopsis. An interference with AN3 movement resulted in defective proliferation in leaf epidermal cells and abnormal leaf sizes and shapes, as shown in an an3 mutant, indicating that the intercellular movement of AN3 from subepidermal cells to epidermal cells is an essential process for AN3 function (Kawade et al., 2010; Kawade et al., 2013).

The most recent model for epidermal trichome and hair cell patterning in Arabidopsis relies on a bidirectional intercellular feedback circuit in which mobile TFs participate (Schellmann et al., 2002; Bernhardt et al., 2005; Kurata et al., 2005). This model has been well established through studies examining root hair patterning, with similarities being observed between shoot and root epidermal cell development. During root hair patterning, a WEREWOLF (WER)-GLABRA3 (GL3)—ENHANCER OF GLABRA3 (EGL3)—TRANSPARENT TESTA GLABRA1 (TTG1) complex forms in non-hair cells and induces the expression of GLABRA2 (GL2) and CARPRICE (CPC), which are a repressor and an activator of hair cell fate expression, respectively (Koshino-Kimura et al., 2005; Ryu et al., 2005). While GL2 specifies a non-hair cell fate, CPC travels to the neighbouring hair cells, and replaces WER in binding to the GL3–EGL3–TTG1 complex (Kurata et al., 2005). Then, the CPC-based complex inhibits GL2 expression, resulting in hair cell fate. GL3–EGL3 expression is activated by the CPC-based complex, and these proteins subsequently travel to non-hair cells, forming a lateral feedback loop (Bernhardt et al., 2003). In addition to the proposed simultaneous movement of CPC (or the CPC complex) and GL3–EGL3, it has been shown that CPC, GL3, and EGL3 exhibit autoinhibitory activity related to their own transcription, possibly enabling pattern determination to cease and tip-directed growth to commence. The CPC homologues ENHANCER OF TRY AND CPC 2/3 (ETC2/3) and TTG1 can also travel from cell to cell (Bouyer et al., 2008; Wester et al., 2009).

**Root development**

NCAPs play a vital role in root radial cell patterning, vascular tissue patterning, and the transition from proliferation to differentiation. During root radial patterning, root endodermis cell fate is determined by SHORT-ROOT (SHR), a member of the GRAS gene family, whose transcripts are generally expressed only in the stele, although SHR protein is also detected in the endodermis (Nakajima et al., 2001). This movement is also required for asymmetric cell division during the formation of the adjacent ground tissue (endodermis, cortex, and middle cortex) (Helariutta et al., 2000; Nakajima et al., 2001). To understand the pattern of SHR movement, several studies have focused on the downstream targets of SHR and SHR-interacting proteins, such as SCARCROW (SCR) (Gallagher et al., 2004; Sena et al., 2004), JAKDOW (JKD), MAGPIE (MGP) (Welch et al., 2007), and SHR-INTERACTING EMBRYO LETHAL (SIEL) (Koizumi et al., 2011). Interestingly, SCR plays a special role in regulating SHR movement by forming SHR–SCR complex, sequestrating SHR into endodermal cell nuclei and thereby inhibiting further movement to the cortex cell layer (Cui et al., 2007).

The procambium/cambium tissue provides cells for establishing the xylem and phloem, and the organization of these tissue types is tightly controlled by the NCAP AT-HOOK MOTIF NUCLEAR-LOCALIZED PROTEIN 4 (AHL4). AHL4 travels from the procambium to the xylem in the root meristem, and its movement is required for vascular tissue patterning (Zhou et al., 2013). When the intercellular movement of AHL4 is impaired, AHL4 cannot complement the xylem phenotype observed in the ahl4 mutant. AHL4 movement is facilitated by its interaction with AHL3.

Growth in multicellular organisms requires maintaining the proper balance between cell proliferation and differentiation. A study was performed to identify TFs that regulate the first stages of the transition from cellular proliferation to differentiation by analysing root map gene expression data (Brady et al., 2007). Approximately 100 TFs show increased gene expression at the boundary between the meristematic and elongation zones. Among these TFs, a T-DNA insertion line harbouring an insertion in the UPBEAT1 (UPB1) gene, a member of the BHLH TF family, showed increased primary root growth. In addition, UPB1 has been revealed to display different distribution patterns in terms of mRNA and protein expression (Tsukagoshi et al., 2010). UPB1 travels from lateral root cap cells or vascular tissue to cells in the root elongation zone. As a consequence, different downstream targets of UPB1 operate a process that controls the balance of reactive oxygen species, which determine the cell proliferation and cell elongation zones where differentiation begins. Taken together, the findings described above indicate that NCAPs exhibit a prominent role in regulating the appropriate spatial and temporal occurrence of developmental programmes by controlling coordinated expression of a set of genes.
Evolutionary aspects of NCAPs and the plasmodesmal trafficking pathway

Evolutionary conservation of NCAPs in flowering and floral organ development

A good example of the evolutionary conservation of NCAP functioning is provided a group of genes involved in floral organ development. For example, Antirrhinum FLORICAULA (FLO) is required for the transition from vegetative to reproductive development through its intercellular movement in the floral meristem, which has also been observed for its Arabidopsis orthologue LFY (Carpenter and Coen, 1995). Analysis of periclinal chimeras demonstrated that when FLO was only expressed in a single cell layer, expression of the downstream target genes DEFICIENS (DEF) and PLENA (PLE) was also detected in all of the examined periclinal chimeras (Hantke et al., 1995). The MADS-domain Antirrhinum majus TFs DEF and GLOBOSA (GLO), which are required for establishing petal and stamen identity, have been known to travel towards the epidermis in the floral meristem, and it has been proposed that the control of the intradermal trafficking of these proteins could play a role in maintaining the boundaries of their expression domains (Perbal et al., 1996). In Arabidopsis, the orthologues of these two proteins, AP3 and PI, cannot travel through the secondary plasmodesmata (PD) between the L1 and L2 cell layers (Jenik and Irish, 2001; Urbanus et al., 2010b). However, photobleaching assays showed that both proteins can travel through the primary PD between epidermal cells, indicating evolutionary conservation in the maintenance of the boundaries of their expression domains (Urbanus et al., 2010a).

FT and its orthologues have been shown to function as florigens in diverse plant species including Arabidopsis (Navarro et al., 2011; Yamagishi and Yoshikawa, 2011; Yamagishi et al., 2011), rice (Kojima et al., 2002), tomato (Lifschitz et al., 2006), Cucurbita maxima (Lin et al., 2007), Pharbitis nil (Hayama et al., 2007), Populus deltoids (Böhlenius et al., 2006), and Vitis vinifera (Sreekantan et al., 2006; Carmona et al., 2007). These observations suggest an ancestral origin of NCAPs that function in the same developmental process.

Evolutionary aspects of proteins involved in plasmodesmal trafficking pathways

During the evolution of NCAPs, how many plasmodesmal trafficking pathways have occurred in plants? While LFY has been suggested to travel in a cell-to-cell manner via diffusion, several selective types of NCAP trafficking have also been reported. The selective non-cell-autonomous modes of trafficking involve a specific interaction between an NCAP and the components of a non-cell-autonomous protein pathway (Kim, 2005; Kim et al., 2005). FT movement, especially FT loading from companion cells to sieve elements, is at least partly dependent on an endoplasmic reticulum membrane protein, FT-INTERACTING PROTEIN 1 (FTIP1), in Arabidopsis (Fig. 2A). Accordingly, ftip1 null mutants exhibit late flowering under long days but still shows early flowering compared to ft mutants, suggesting the existence of an FTIP-independent FT movement pathway(s). A critical motif in Arabidopsis FT and its Cucurbita moschata homologue FTL2 for the execution of their non-cell-autonomous nature was
determined through analyses employing a zucchini yellow mosaic virus expression vector (Yoo et al., 2013). In this study, FT trafficking was distinguished between phloem loading and post-phloem unloading. A protein analysis performed on phloem sap collected from just beneath the vegetative apex of C. moschata plants indicated that all of the mutant proteins tested maintained their ability to travel to the phloem sieve tubes, possibly suggesting the existence of a non-selective mechanism. However, a number of FTL2/FT mutants could not be detected in the post-phloem zone in immunolocalization studies. The fact that FTIP is expressed in the leaf phloem, but not in the proximal SAM unloading zone, suggests that a putative FTIP-independent selective mechanism for FT trafficking exists during the post-phloem unloading step. These results, together with the confirmation of FT and FTL2 movement in microinjection and trichome rescue studies in Arabidopsis, indicates the existence of several pathways for FT/FTL2 movement: (i) FTIP-mediated selective movement; (ii) FTIP-independent non-selective movement in phloem loading; and (iii) FTIP-independent selective movement in the post-phloem unloading step (Fig. 2A and Fig. 3).

In another independent screen of plasmodesmal-enriched cell-wall proteins, it was shown that NON-CELL-AUTONOMOUS PROTEIN PATHWAY PROTEIN 1 (NCAPP1), an endoplasmic reticulum/PD-localized intercellular pathway component, is a component of the NCAP translocation machinery and facilitates the movement of CmPP16 (Lee et al., 2003) (Fig. 2B). A dominant-negative form of NCAPP1Δ1–22 blocks the capacity of CmPP16 and TMV movement proteins to modulate plasmodesmal channel size, but not that of KN1 and cucumber mosaic virus movement proteins, again suggesting diverse pathways of NCAP movement.

The long-standing notion that partial unfolding or refolding of KN1 (Kragler et al., 1998) could enhance its movement was experimentally verified in a recent genetic mutant screen that identified a class-II chaperonin subunit, CHARPERONIN-CONTAINING TCP1 8 (CCT8), which is responsible for the required refolding of KN1 and STM following their transport into destination cells (Xu et al., 2011) (Fig. 2C). In addition to KNOX HD proteins, CCT8 facilitates the movement of TRANSPARENT TESTA GLABROUS1 (TTG1) (Bouyer et al., 2008; Xu et al., 2011) and the spreading of the Oilseed rape mosaic virus (ORMV) in Arabidopsis (Fichtenbauer et al., 2012). However, the movement of SHR and AN3, two other NCAPs, is not influenced by CCT8, suggesting that at least two groups of NCAPs have evolved.

In the case of SHR, the intercellular trafficking of this protein occurs through a conserved mechanism in both Arabidopsis and rice. The movement of SHR from the stеле to the neighbouring cells requires SIEL and intact microtubules (Koizumi et al., 2011; Wu and Gallagher, 2013). SIEL null mutants are embryonic lethal, and hypomorphic alleles of this gene result in defects in root patterning and reduce SHR movement (Fig. 2D). In root cells, SIEL localizes to the nucleus and the cytoplasm, where it is associated with endosomes. Interestingly, microtubule malfunction results in mislocalization of the SIEL protein. SIEL also interacts with intercellular trafficking TFs including CPC, TARGET OF MP7 (TM07), and AGAMOUS-LIKE 21 (AGL21), but not with LFY and STM (Koizumi et al., 2011). However, whether SIEL is required for the intercellular trafficking of these TFs remains to be tested.

**Evolutionary scenario of intercellular trafficking domains**

Plants have evolved cell-autonomous and non-cell-autonomous TFs. Based on the concept that PD evolved from simple algal PD lacking a central desmotubule into highly complex PD, we previously proposed a hypothetical sequential evolutionary scenario: (i) sophistication of PD structure via the insertion of endoplasmic reticulum into the centre of the PD; (ii) evolution of cell-autonomous proteins (CAPs) from initial NCAPs in nature; and (iii) the emergence of a mechanism for selective NCAP trafficking (Lucas et al., 2009). To develop cell-autonomous TFs, several strategies—including (i) increases in protein size, (ii) the formation of complexes through protein-protein interaction, (iii) subcellular...
sequestration or anchoring, and (iv) control at the protein expression level—have been proposed (Rim et al., 2011). According to these hypotheses, selective trafficking of TFs might have evolved following the establishment of the land plant PD system. Indeed, KNOX family proteins, which share a HD that has been shown to be pivotal in cell-to-cell movements, seem to fall within this category. It is evident that the movement ability of closely related KNOX HD proteins is quite conserved in diverse plant species (Fig. 3). For example, HDs found in KNOX family transcription factors in Arabidopsis, Zea mays, and Physcomitrella show intercellular trafficking activity in Arabidopsis (Kim et al., 2003, 2005; Chen et al., 2013). However, the HD of the GSM1 KNOX protein of a unicellular alga, Chlamydomonas reinhardtii, exhibits little intercellular trafficking activity compared to the KNOX HD observed in other plant species, including Physcomitrella. One possible evolutionary scenario that might explain this finding is that land plants have developed a unique mechanism during evolution allowing them to efficiently transport KNOX HD proteins through endoplasmic reticulum-containing complex PD (Chen et al., 2013).

Recently, a plant-specific TF family member, Dof4-1, was shown to be a NCATF, and its Dof-domain-spanning region (the intercellular trafficking motif) is necessary and sufficient for cell-to-cell trafficking (Lee et al., 2006; Chen et al., 2013). An evolutionary analysis suggested that an ancestor of the intercellular trafficking motif served to foster diverse selectivity among Dof NCATFs (Fig. 3). When Dof of unicellular Chlamydomonas algae, which do not exhibit plasmodesmata, was tested, it was found to show a movement ability in higher plants similar to that observed in Physcomitrella patens, Nicotiana tabacum (tobacco), and Glycine max (soybean). This study therefore led a totally new concept, indicating that during evolution, higher plants might have developed a plasmodesmal pathway for the selective trafficking of non-cell-autonomous Dof TFs, where the Dof intercellular trafficking motif might have served as a molecular template.

The intercellular trafficking signal domains of many NCATFs, including Dof, KNOX, and GRAS (Gallagher and Benfey, 2009), include a DNA-binding domain. It is interesting to speculate that similar mechanisms or related interacting partners exist between the DNA binding motifs and intercellular trafficking motifs of these proteins. Given that knowledge of the molecular details of evolutionarily related NCATFs among different species is limited, further exploration of this field in the future is of interest.

NCAPs, peptides, RNAs, and hormones: the major mobile messengers involved in intercellular signalling networks

Cells respond not only to signals from NCATFs but also to the cumulative systemic information coming from other intercellular molecules, such as small peptides, RNAs, and hormones, at the same time. These molecules exploit different mechanisms to accomplish cell-to-cell trafficking, but the crosstalk among them incorporates the entire intercellular signalling network.

Signalling integration between NCATFs and mobile peptide ligands

In the shoot apical meristem, WUS-mediated shoot meristem homeostasis is essential (Mayer et al., 1998; Yadav and Reddy, 2012). In Arabidopsis, wus mutants failed to maintain the shoot meristem and plant growth, whereas when WUS was ectopically expressed, the meristem was expanded, which is a phenotype reminiscent of the fasciation observed in loss-of-function mutants for each of the three CLAVATA genes (CLV1, CLV2, CLV3) found in Arabidopsis. CLV3 belongs to a large gene family referred to as the CLE family, for CLAVATA/ESR-related, and encodes a protein processed into a post-translationally modified 13-amino acid arabinosylated glycopeptide (Ohyama et al., 2009). CLV3 peptides are secreted from the L1/L2 cells and travel to the L2/L3 cells, where they bind to the CLV1 receptor-like complex (Fletcher et al., 1999). This functional complex leads to signalling that eventually inhibits the expression of WUS in the SAM. Strikingly, CLV3 expression is positively promoted by WUS, which is indeed one of the NCATFs expressed in the organizing centre (Schoof et al., 2000; Yadav et al., 2011). This indicates that WUS builds an autoregulatory feedback loop through the CLV3 peptide signal via precise intercellular movements to maintain a constant cell number in the shoot meristem (Fig. 4A).

Signalling integration between NCATFs, hormones, and mobile miRNAs

During root development, NCATFs, hormones, and mobile miRNAs occur in the same places to establish a complicate intercellular signalling network. A genome-wide direct target analysis illustrated that a cytokinin catabolism enzyme, cytokinin oxidase 3, is directly upregulated by SHR (Cui et al., 2011). It is well established that cytokinins can repress PIN gene expression (Ruzicka et al., 2009; Zhang et al., 2011). By downregulating cytokinin synthesis, SHR might indirectly regulate PIN proteins, which are the major efflux transporters for auxin. In addition, auxin, which is a plant morphogen, has been shown to undergo cell-to-cell trafficking under various circumstances and to exert multiple functions in plant development. These findings may be included in a signalling pathway model in which NCATF and hormones would function together to fine-tune spatial and temporal signalling for programming cell patterns in plants. Yet another layer of interaction comes into play when the regulation of miRNA165/166 by SHR and SCR is considered (Carlsbecker et al., 2010) (Fig. 4B). In an elegant study, both shr and scr mutants were shown to reduce miR165/166 levels, without affecting the small RNA biogenesis pathway in the root tip. Surprisingly, these miRNAs have been shown to behave in a non-cell-autonomous manner: they are generated in the endodermis and spread to the stele to control PHABULOS (PHB) (Miyashima et al., 2011). In turn, PHB has been extensively studied to examine its definitive role in regulating the PIN-mediated auxin flow during embryogenesis and vascular patterning (Izhaki and Bowman, 2007; Zhou et al., 2007; Smith and Long, 2010).
Intercellular signalling by transcription factors

The cell types found in plant root are initially specified early during embryogenesis. Auxin plays a pivotal role in embryonic root initiation, including the specification of the hypophysis, which is the uppermost cell in the suspensor and the precursor of the root cap and the quiescent centre (Hamann et al., 1999). The auxin-dependent TF AUXIN RESPONSE FACTOR 5 (ARF5)/MONOPTEROS (MP) functions non-cell-autonomously in embryonic cells (Hardtke and Berleth, 1998) and drives hypophysis specification by activating polar auxin transport from the embryo to the hypophysis precursor. Auxin, itself, is one of the signals involved; however, its accumulation is neither restricted to only the uppermost suspensor cell nor sufficient to promote hypophysis specification (Weijers et al., 2006). Recently, additional MP-dependent mobile signals were identified through microarray analysis. TMO7, encoding a basic helix–loop–helix (bHLH) transcription factor, was shown to be required for MP-dependent embryonic root initiation (Schlereth et al., 2010). This gene is expressed in the hypophysis-adjacent embryo cells and travels to the hypophysis precursor, thus representing an MP-dependent intercellular signal involved in embryonic root specification (Fig. 4C). MP controls hypophysis specification by sending two mobile factors whose transport or expression is activated by MP. How these two signals converge during this process remains to be determined. Taking these findings together, it appears that plant organs have found ways to manipulate developmental aspects by integrating pathways that are individually employed by intercellular regulators in each plant.

**Signalling integration between NCATFs and mobile mRNAs**

As shown for RNA-binding NCAPs such as CmPP16 and viral movement proteins, KN1/KN1 mRNA travels from cell to cell and FT/FT mRNA/Arabidopsis CENTRORADIALIS homologue (ATC) mRNA moves systemically through the phloem. While KN1 mRNA is transported through the formation of KN1 RNA-KN1 protein complexes (Lucas et al., 1995), how FT mRNA/ATC mRNA enters the phloem stream is not clear. As KN1 mRNA is undetectable in L1 cells in the SAM, where KN1 protein is detected, the biological function of KN1 mRNA is not clear. In contrast, FT mRNA and ATC mRNA act non-cell-autonomously to stimulate or inhibit floral initiation, respectively (Huang et al., 2012; Lu et al., 2012) (Fig. 4D). Thus, the balance between FT (florigenic) and ATC (antiflorigenic) impacts flowering time. Interestingly, independent of FT protein trafficking, the trafficking of FT RNA alone is sufficient to promote flowering. This conclusion is supported by the three lines of evidence: (i) expression of the cell-autonomous protein marker red fluorescent protein (RFP)-FT in leaf companion cells stimulates flowering; (ii) removal of RFP-FT RNA from the meristem by introducing FDp::amiR-FT delays flowering; and (iii) surprisingly, translation-free FT mutants can still induce flowering in tobacco. These findings suggested that non-translatable FT mRNA is mobilized to the meristems, possibly facilitating the long-distance transport of the tobacco FT protein (Li et al., 2011).

**Plasmodesmata: symplasmic channels for NCAPs**

Regulatory components of the PD

NCATFs travel through plasmodesmata, which are intercellular symplasmic channels that interconnect the cytoplasm of neighbouring cells in plants. Such intercellular trafficking can be achieved through either selective or diffusion-based non-selective movements through PD. The former involves an interaction between NCATFs and other non-cell-autonomous pathway proteins and/or protein unfolding/folding by chaperon proteins, as shown for the KN1 and STM proteins required for shoot meristem development (Lucas et al., 1995; Sessions et al., 2000; Kim et al., 2002; Xu et al., 2011). The latter is dependent on the molecule size/dimension of NCATFs, as proposed for the movement of LFY and GFP. The PD size exclusion limit is measured using symplasmic tracers, such as GFP fusion proteins and fluorescent dyes of...
different sizes (e.g. 8-hydroxyquinone 1,3,6-trisulphonic acid and carboxy fluorescein). Several regulators of the PD channels have been identified and localized in PD (e.g. callose synthases, beta-1,3-glucanases, PD CALLOSE-BINDING PROTEINS (PDCBs), PD-LOCALIZED RECEPTOR-LIKE PROTEINS (PDLPs), and PD-GERMIN-LIKE PROTEINS (PDGLPs); Fig. 5; Levy et al., 2007; Simpson et al., 2009; Amari et al., 2010; Guseman et al., 2010; Lee et al., 2011; Vaten et al., 2011; Ham et al., 2012). In addition, PD regulators have been observed at diverse subcellular locations, including within mitochondria (INCREASED SIZE EXCLUSION LIMIT OF PLASMODESMATA 1 (ISE1)), chloroplasts (ISE2, GFP ARRESTED TRAFFICKING 1 (GAT1), SUCROSE EXPORT DEFECTIVE 1 (SED1); Benitez-Alfonso et al., 2009), and nuclei (DECREASED SIZE EXCLUSION LIMIT OF PLASMODESMATA 1 (DSE1); Fig. 5; Burch-Smith and Zambryski, 2010). In the case of GAT1, ISE1, and ISE2, mutant forms of these proteins result in altered ROS levels, causing a decrease or increase in the PD size exclusion limit. Although the upstream signalling pathways involved in PD regulation include diverse components such as reactive oxygen species (ROS), salicylic acid and biotic/abiotic stimuli, the final phenotype associated with mutant forms of most regulators is correlated with PD callose level or PD structure. For example, ISE1, ISE2, GAT1, and DSE1 are likely involved in the modification of PD structure and function. In addition, the expression of GSL, PDBG, PDCB, PDLP, ISE1, ISE2, GAT1, and SED1 affects the deposition of PD callose.

Callose: an important component of PD regulation

The most compelling evidence of the role of endogenous callose levels in protein movement was provided by studies involving a mutation of *chorus* (chor)glucan synthase-like8 (gsl8) that results in excessive proliferation of stomatal lineage cells mediated by SPEECHLESS (SPCH) (Guseman et al., 2010). Intercellular diffusion of SPCH, which does not normally travel in a cell-to-cell manner, was observed. This suggests that restriction of symplastic TF movement is required for the proper segregation of cell fate determinants during stomatal development. More recently, a semi-dominant allele (*cals3-d*) of the gene *CALLOSE SYNTHASE-3* (CALS-3) was found to be defective in GFP unloading from the phloem and in the patterning and specification of the phloem and the xylem tissues via ectopically producing callose at PD, hinting at a link between a reduced PD aperture and NCATF signalling (Vaten et al., 2011). Consistent with these results, overexpression of PD-localized beta-1,3-glucanase was able to rescue this mutant phenotype. The most stunning aspect of this study was that the movement of SHR was reduced in a *cals3-d* mutant background. As described above, SHR can travel from its original location in stele tissue to the neighbouring endodermis, initial cells and QC. However, in *cals3-d* plants, the root endodermis showed lower levels of SHR, suggesting that movement of SHR from the stele was reduced. In accord with this finding, *cals3-d* plants exhibited disturbed patterning of the ground tissue and vasculature. Surprisingly, the intercellular movement of established mobile RNAs miRNA165/66 was abolished following ectopic callose synthesis in *cals3-d* mutants. The observation that this dysregulation of callose synthase affected the cell-to-cell trafficking of endogenous miRNAs suggests that the PD transport of RNAs might be controlled by a regulatory mechanism similar to that observed for NCAPs. Thus, it is tempting to speculate that a cellular level of callose that is capable of forming symplasmic domains may act as an endogenous regulator of macromolecular transport.

Toolboxes for examining intercellular networks

Based on the tremendous advances in the understanding of the importance of NCAP movement during plant development in the last decade, novel technologies have been rapidly conceived and are being explored to study such scenarios at the genomics/proteomics level. One of the potentially most promising approaches for addressing intercellular regulation in individual cell types is based on macromolecular expression profiling. In *Arabidopsis*, a gene expression map of specific root cells has been developed using fluorescence-activated cell sorting, which involves cell-specific GFP marker-based cell sorting and transcriptomics/proteomics analyses (Birnbaum et al., 2003; Brady et al., 2007). Using this approach, it was shown that among 2000 proteins identified in *Arabidopsis* roots, only 35% were found in only a single root cell population, while the remained 65% were detected in more than two cell types (Petricka et al., 2012). Strikingly, the Pearson correlation coefficient between the root cellular proteome and transcriptome is significantly low for a given cell type (0.19–0.36).
Considering such inconsistencies between RNA and protein expression profiles, it seems possible that there might be several NCAPs, especially among the 65% of proteins that were detected in multiple cell types. Indeed, it was previously reported that 25% of the TFs tested undergo post-transcriptional regulation via microRNA-mediated mRNA degradation or intercellular protein movement (Lee et al., 2006).

Another approach that has advanced the comprehension of NCAPs is high-throughput omics analyses. Using this approach, studies in a number of plant species, including Populus (Dafoe et al., 2009; Abraham et al., 2012), pumpkin (Lin et al., 2009; Cho et al., 2010), Agrilus planipennis (Whitehill et al., 2011), white lupin (Rodriguez-Medina et al., 2011), rice (Aki et al., 2008), Chelidonium majus (Nawrot et al., 2007), and cucumber (Walz et al., 2004), have shown that transcripts, proteins, and small RNAs are present in the phloem sap. In pumpkin phloem sap, a minimum of 1209 non-redundant proteins were identified. These proteins are distinct from the observed total protein profiles and travel in a source-to-sink direction, exhibiting a wide range of functions, as observed in the case of proteins with functions in RNA binding, signal transduction/plant defence, protein synthesis, the cell cycle, cell fate, and metabolism (Lin et al., 2009). Likewise, a phloem proteomics approach resulted in the identification of a group of proteins involved in the control of embryonic development and flowering. In addition, through phloem sap proteomics analyses, TFs such as BTF3b, HAP5A, HAP5B, MFB1B, and ZFP30 have been identified in pumpkin and rice (Aki et al., 2008; Lin et al., 2009). The future issue facing this field is elucidation of the biological significance of these phloem macromolecules and the roles they play in the integration of whole-plant signalling.

Other technologies, including those deployed in reporter gene expression and tagged protein localization studies, have been applied for the large-scale screening of NCATFs, regarding their potential to coordinate specific intercellular regulatory networks (Rim et al., 2011). These researchers quantitatively investigated almost 70 Arabidopsis TFs, among which 22, including proteins belonging to the homeobox, GRAS, and MYB protein families, were shown to exhibit an extensive capacity for cell-to-cell movement as well as discrete trafficking patterns. Information gleaned from the application of these technologies can be directly applied to novel screens for regulators that exhibit movement and fluxes in various tissues related to different cellular processes at a cellular resolution.

**Concluding remarks**

In plants, direct cell-to-cell communication through the movement of TFs, together with diverse mobile signals including peptide ligands, RNAs, and hormones, regulates cell specification and patterning, associated with great developmental plasticity. The big picture concerning the evolution of non-cell-autonomous pathways together with components that regulate the transport of NCATFs has begun to emerge. Although there have been significant findings concerning the probable convergence of diverse intercellular regulators in signalling networks across cell types, the mechanistic details of how such adaptations are implemented on a molecular scale to control a specific developmental output remains to be determined. As NCATFs are divided among multiple protein families, it will be interesting to determine how many distinct non-cell-autonomous pathways have evolved and what components are shared among them. Adapting new emerging technologies, including genomics, proteomics, and bioinformatics methods, to be used alongside conventional approaches will be required to develop a complete picture of the non-cell-autonomous communication network through the PD. Conducting studies to obtain a comprehensive understanding of intercellular signalling via mobile TFs represents a large challenge to shed light on the mechanisms underlying the organization and evolution of multicellular plants.

**Acknowledgements**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2007230) and by the Next-Generation BioGreen 21 Program (SSAC, grant PJ009495), Rural Development Administration, Republic of Korea. The authors thank their colleagues for technical assistance, comments, and advice.

**References**


Bernhardt C, Zhao M, Gonzalez A, Lloyd A, Schiefelbein J. 2005. The bHLH genes GL3 and EGL3 participate in an intercellular


Gallagher KL, Benfey PN. 2009. Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement. The Plant Journal 57, 785–797.


