The Plant Cell, Vol. 13, 2569, December 2001, www.plantcell.org

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The Long and the Short of It: Signaling Development through Plasmodesmata

Developmental biologists seek to explain the generation of complex three-dimensional organisms from the starting point of a single cell and its genetic complement. During this incredible transformation, different cell fates rarely are specified intrinsically; rather, the fates of individual cells or groups of cells usually are under the control of external signals that are conserved and recycled throughout the development of the organism. Therefore, developmental mechanisms commonly involve cell-to-cell communication, using signals from neighboring cells or from distant tissues. Elegant mosaic analysis studies show us that many developmental genes act nonautonomously, indicating that they feed into pathways for intercellular signaling. For example, genes involved in leaf patterning (Harper and Freeling, 1996), in the floral transition (Colasanti et al., 1998), and in the homeotic control of flower development (Perbal et al., 1996; Jenik and Irish, 2001) act nonautonomously.

Intercellular signaling may occur via a traditional route of secreted ligands for transmembrane receptors; in fact, the presence of hundreds of orphan receptors encoded by the Arabidopsis genome implies that this means of communication is very important (Arabidopsis Genome Initiative, 2000). An alternative hypothesis that has gained increasing support in recent years is that plant cells communicate by the regulated transport of specific regulatory proteins and mRNAs through plasmodesmata and that these signals can operate over short distances as well as systemically throughout the plant. The evidence for cell-to-cell trafficking of plant gene products follows the paradigm of viral movement protein and nucleoprotein trafficking (Gilbertson and Lucas, 1996; Ghoshroy and Citovsky, 1997; Ding et al., 1999; Zambryski and Crawford, 2000) and comes from localization studies, observations of movement after transient expression by microinjection, and grafting experiments and analyses of genetic mosaics (Jackson et al., 1994; Lucas et al., 1995; Perbal et al., 1996; Balachandran et al., 1997; Ruiz-Medrano et al., 1999; Sessions et al., 2000). A role for systemic signaling in transgene and viral responses through a phloem- and plasmodesmata-transmitted signal is clear (Palauqui et al., 1997; Voinnet et al., 1998), although the exact nature of that signal remains elusive (Mallory et al., 2001). In developmental studies, the contrary is true; although there are clear demonstrations of cell-to-cell trafficking of specific regulatory mRNAs and proteins (Lucas et al., 1995; Perbal et al., 1996; Ruiz-Medrano et al., 1999; Sessions et al., 2000), a direct demonstration that plasmodesmal trafficking performs an essential developmental function has been lacking. Now, two new reports suggest that short- and long-range trafficking of specific mRNA and protein signals is important for developmental regulation, showing that this is in fact an important and novel plant-specific regulatory mechanism.

In the first report, Kim et al. (2001) at the University of California, Davis, used grafting experiments to investigate the autonomy of a dominant leaf mutant of tomato called *Mouse ears* (*Me*). Previously, grafting experiments had shown that sequence-specific transgene silencing signals were systemically transmitted in the phloem and that endogenous regulatory mRNAs were phloem transmissible (Palauqui et al., 1997; Jorgensen et al., 1998; Voinnet et al., 1998; Ruiz-Medrano et al., 1999). However, in this case, the striking finding was that long-distance movement of the mutant *Me* transcripts was correlated with a change in leaf morphology.

The *Me* mutation is caused by a chromosomal rearrangement that results in a fusion of *LeT6* (Chen et al., 1997) to the 5′ coding and promoter region of *PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE* (*PFP*), which encodes a metabolic enzyme. *LeT6* is a member of the class I *KNOX* gene family, which is important for shoot meristem function (Tsiantis, 2001). The *PFP-LeT6* fusion transcript is overexpressed in leaves and encodes a protein composed of the N-terminal region of PFP followed by most of the LeT6 polypeptide sequence. Accumulation of the overexpressed fusion protein leads to changes in tomato leaf morphology (hence the name *Mouse ears*). Surprisingly, when normal shoots (scions) are grafted onto *Me* mutant stocks, new leaves initiated by the scion develop mouse ears morphology (Figure 1). Therefore, the *Me* phenotype was graft transmissible. So the obvious question was What is the signal? To answer this, a sensitive in situ polymerase chain reaction technique was used to determine if *Me* transcripts could be detected in the scion apex. As it turns out, they could. Furthermore, the localization of the *Me* transcripts in the scion apex resembled that in nongrafted *Me* plants, implying that this specific pattern of transcript accumulation arises from the spatial control of trafficking of the *Me* transcripts rather than from the activity of the promoter, because the *Me* gene fusion is not present in the scion.

These remarkable findings suggest

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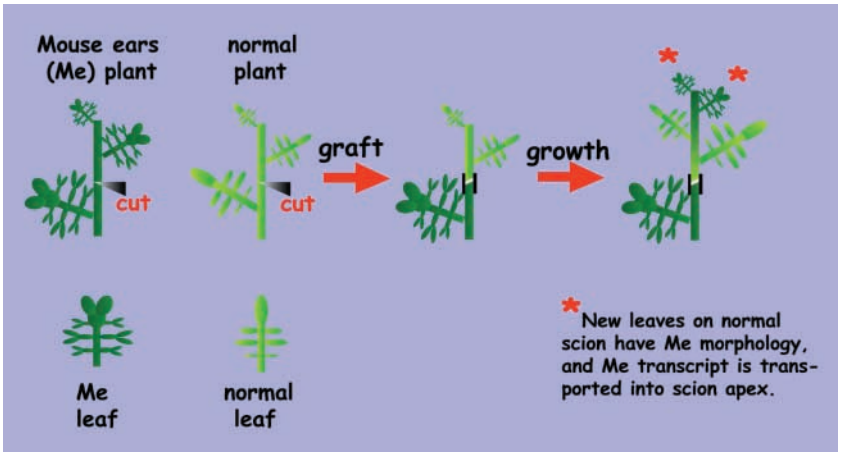

Figure 1. Tomato Grafting Studies Show Transmission of a Leaf Shape Signal and KNOX Fusion mRNA into the Graft Scion.

The *Me* mutant has leaves that are more dissected than normal. When a normal scion is grafted onto a *Me* stock, new leaves that initiate on the scion have the *Me* morphology, indicating that the leaf shape signal is graft transmissible. The mutant *Me* transcript also is detected in the normal scion apex, indicating that this transcript likely is the transmitted signal.

that the long-distance transport of regulatory mRNAs controls plant morphology. However, although the *Me* transcripts in this example clearly are transported into the graft scion, the results are correlative and do not prove that the *Me* transcripts are the signal responsible for the altered morphology. For example, it is possible that the graft-transmitted signal is a plant growth regulator, such as one of the cytokinins or gibberellins that are known to be misregulated in *KNOX*-overexpressing plants (Ori et al., 1999; Sakamoto et al., 2001; Tsiantis, 2001). If we assume that the mRNA is the signal, another question is How do these studies using an overexpressed mutant *KNOX* fusion transcript relate to the situation in normal plants? Are transcripts of *KNOX* genes or other regulatory genes normally transported from basal locations to the apical meristem, and if so, do they regulate development? Support for this idea comes from the observation that *KNOX* transcripts are detected in the provascular tissues that lie below the shoot apical meristem (Jackson et al., 1994). The KNOTTED1 protein also is detected in these provascular tissues (Smith et al., 1992), suggesting that it may be transported along with the mRNA and perhaps provides the sequence specificity for transport of a KNOTTED1–ribonucleoprotein complex, as suggested by Lucas et al. (1995). Furthermore, previous studies using interspecific grafts indicate that many regulatory mRNAs are in fact phloem mobile (Ruiz-Medrano et al., 1999). But does movement of these RNAs have a developmental consequence? If so, one would expect that in these grafts the morphology of the scion should begin to resemble that of the stock. Although such effects have not been reported, this possibility deserves careful attention. Of course, some classic plant signals are transmitted from the leaves to the apex, including the elusive flower-inducing signal florigen (Zeevaart, 1962), and it has been suggested that this signal could be a regulatory RNA molecule (Ruiz-Medrano et al., 1999). The study by Kim et al. is an important step in proving that mRNAs act as developmentally relevant long-range signals, and it opens up new opportunities and ideas to test whether this system is used for normal developmental regulation.

In the second report, Nakajima et al. (2001) at New York University studied the role of the *SHORT-ROOT* (*SHR*) gene in root development. The Arabidopsis root has a simple radial pattern that is created through the predictable division of initial cells and subsequent cell fate acquisition. In *shr* mutants, the cortex/endodermis initial daughter cells fail to divide, resulting in a single layer of cells that resembles cortex. Therefore, *SHR* is required not only for the asymmetric division of the initial daughter cells but also for setting endodermal fate. The *SHR* gene encodes a putative transcription factor of the GRAS family, implying that it functions through the transcriptional activation of downstream effector genes, which may include the related *SCARECROW* (*SCR*) gene. Surprisingly, *SHR* mRNA is detected neither in the cortex/endodermal initial cell nor in its daughter cells, where it functions; rather, it is present in the internally adjacent cells of the stele (Helariutta et al., 2000). To determine the mechanism by which *SHR* is able to signal adjacent cells nonautonomously, a green fluorescent protein (GFP) fusion of SHR was created and cloned downstream of the *SHR* promoter (*pSHR*), and the resulting construct was transformed into *shr* null plants. This construct fully rescued the *shr* phenotype, indicating that the fusion of GFP did not interfere with SHR function.

Imaging of *pSHR*::*SHR-GFP* roots showed that GFP fluorescence was detected not only in stele cells, where *pSHR* is active, but also in a single layer of cells outside of the stele that includes the quiescent center, the cortex/endodermal initial cells, and the endodermis. Therefore, the SHR-GFP fusion protein appeared to traffic from stele cells to the adjacent layer of cells. SHR-GFP

was present in the cytoplasm and nucleoplasm of stele cells, whereas it accumulated specifically in nuclei in the adjacent layer of cells into which it trafficked (Figure 2). In this case, trafficking appeared to be a specific property of the SHR (fusion) protein, and the authors presented convincing arguments against the likelihood of significant *SHR* mRNA transport.

To learn more about the mechanism of SHR movement, the *SHR* gene was also expressed using the *SCR* promoter (*pSCR*), which drives expression in the cell layer adjacent to the stele (that is, in the cells into which SHR protein normally moves). The *pSCR-SHR* transgenic plants had a very interesting phenotype: their roots developed several additional layers of cells that all expressed endodermal fate markers (Figure 2). This phenotype is explained by an autocatalytic relay mechanism; because *SCR* is a downstream target of SHR, the outward movement of the SHR protein from the cell layer where it is expressed by *pSCR* results in the activation of *pSCR-SHR* in this adjacent cell layer, and the cycle reiterates to generate multiple additional cell layers. The additional cells have endodermal fate as a result of the action of SHR.

These studies illustrate an ingenious mechanism for radial patterning of the Arabidopsis root. *SHR* is required for division of the cortex/endodermal initial daughter cells (working through *SCR*) and for specification of endodermal cell fate, and its expression in the stele and the subsequent movement of the SHR protein to the adjacent cell layer provides the necessary positional information to ensure that the endodermal layer is faithfully positioned adjacent to the stele. These findings also highlight possible future questions about the mechanism and specificity of movement. For example, what regulates the specific movement of the SHR protein and not of the similar SCR protein? Is the movement of SHR truly directional (outward)? This question has not been addressed by the present study because SHR normally is expressed in the innermost cells of the root. Also, what mechanism limits the range of movement to a single cell layer outside of the stele? Is it the translocation of SHR protein into the nucleus in the adjacent cell layer? Previous studies of the nontargeted movement of GFP suggest that nuclear targeting does not limit cell-to-cell movement (Crawford and Zambryski, 2000), although the mechanism may be different for targeted movement.

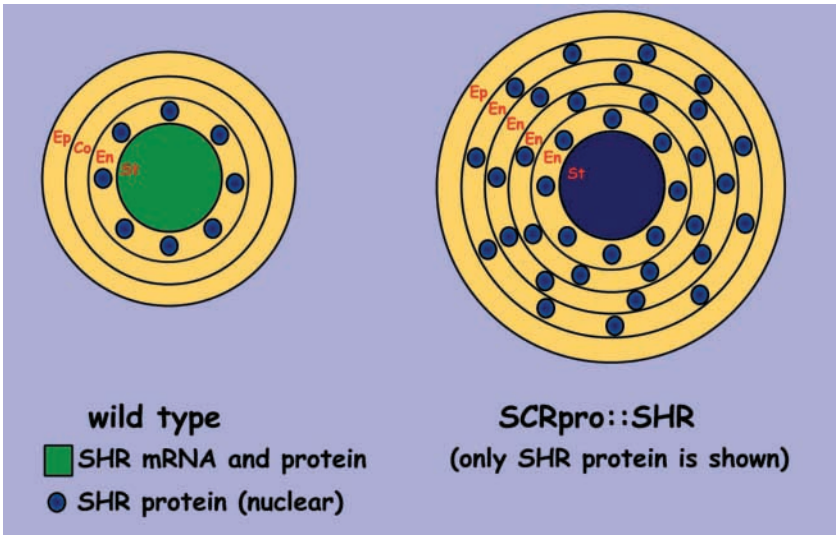

Figure 2. Scheme of the Arabidopsis Root, Highlighting Cell Fate Changes Caused by the Movement of the SHR Protein from the Stele to Endodermal Cells.

SHR mRNA is detected only in stele cells, where the SHR protein is present in the cytoplasm and nuclei. In the surrounding endodermal cells, the SHR protein is nuclear, presumably trafficking through plasmodesmata into these cells. When SHR is expressed ectopically in the endodermis, using the *SCARECROW* promoter, the SHR protein moves outward and generates an additional cell layer with endodermal identity. This cell layer also expresses SHR (controlled by the SCR promoter). A relay mechanism ensues to generate several concentric layers of endodermal-like cells. Ep, epidermis; Co, cortex; En, endodermis; St, stele.

CONCLUDING REMARKS

The two studies discussed here highlight examples of developmentally significant mRNA and protein translocation. They raise questions about how widespread this mechanism is in plants, and also whether the movement is through plasmodesmata, as suspected. Recent reports of transcription factor movement between animal cells (Maizel et al., 1999) should caution us that other translocation routes are possible. Nonetheless, these exciting studies remind us that development invents many novel and imaginative mechanisms for cell-to-cell communication and cell fate determination, and future studies should seek to determine how these intriguing mechanisms, and others yet to be discovered, are integrated to generate complex morphologies in biology.

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