RNA commutes to work: regulation of plant gene expression by systemically transported RNA molecules

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Summary
Although long-distance movement of endogenous mRNAs in plants is well established, the functional contributions of these transported RNA molecules has remained unclear. In a recent report, Kim et al.[1] showed that systemically transported mRNA is capable of causing phenotypic change in developing tissue. Here, this finding and its significance are reviewed and discussed in detail. In addition, in order to give proper perspective, long-distance transport of other types of RNAs, e.g., RNA elicitors of post-transcriptional gene silencing and RNA genomes of plant viruses, and its possible regulation are discussed. ***BioEssays*** **23:1087–1090, 2001.**

Background

Traditionally, RNA molecules are thought to function within the same cell in which they are generated. Recent evidence, however, indicates that, in addition to this conventional role, RNA also acts as a non-cell-autonomous signal molecule that travels intercellularly to regulate gene expression in remote target tissues. In plants, three general types of RNA molecules are known to travel long distances: cellular mRNA, RNA elicitors of post-transcriptional gene silencing (PTGS), and RNA genomes of invading viruses (reviewed in Refs. 2–7) (Fig. 1). Among these systemically transported RNAs, the biological relevance of intercellular transport of endogenous mRNA is the most controversial. A maize homeodomain protein Knotted1 (KN1), for example, has been shown to bind to its own mRNA, and transport it from cell to cell within the meristem.[8] While KN1-mediated transport of the *KN1* mRNA occurs only locally, between specific cell layers, other cellular mRNAs can be transported systemically through the plant vascular system. For example, mRNA for the leaf sucrose transporter SUT1 is transcribed in the companion cells and is then translocated into the adjacent sieve elements to be distributed systemically via the phloem.[9] In fact, RT-PCR analysis of pumpkin phloem sap showed that more than 100 different mRNA species exist in phloem.[10] Some phloem transcripts, such as potato *SUT1* mRNA[9] or rice *thioredoxin-h* mRNA,[11] are likely transported throughout the entire plant. Other mRNAs within the phloem, however, may be targeted, by as yet unknown mechanisms, to specific tissues. One such transcript, *CmNACP* mRNA, is transported to the shoot apex, suggesting a role of systemic mRNA transport in remote-controlled gene expression in meristem.[10] Identification of mRNA species that move between cells suggested the possibility that endogenous mRNAs serve as long distance, systemic signals for plant development and morphogenesis. However, none of these studies had clearly established that the transported mRNA actually functioned to control gene expression in the target tissues. Indeed, no experimental system had existed, in which obvious phenotypic changes occur in the target tissues in the presence of the known systemically translocated transcript. Such a system has now been reported by Kim et al.[1]

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Systemic transport of the PFP-LeT6 transcript induces changes in tomato leaf shape

Kim et al.[1] took advantage of two different tomato lines, *Xa* plants carrying the semidominant *Xanthophyllic* mutation that causes yellow leaves with wild-type morphology, and *Me* plants carrying a dominant *Mouse ears* mutation that causes leaves with extra orders of compounding. The *Me* mutation is caused by a gene fusion, termed *PFP-LeT6*, between *PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE (PFP)* and a tomato *KN1*-like homeobox gene *LeT6*.[12] The authors used these plants in grafting experiments similar to those previously employed to demonstrate systemic signaling in PTGS.[13] Specifically, it was shown that grafting *Xa* scions onto *Me* stocks caused distinct phenotypic changes in leaf morphology, resulting in extra orders of compounding. Examination of the heterografted shoot apex under a scanning electron microscope revealed that alterations in the leaf morphology occurred already early in the leaf development. These results indicated that the phenotypic effect in the *Xa* scion may be due to a signal substance that had originated

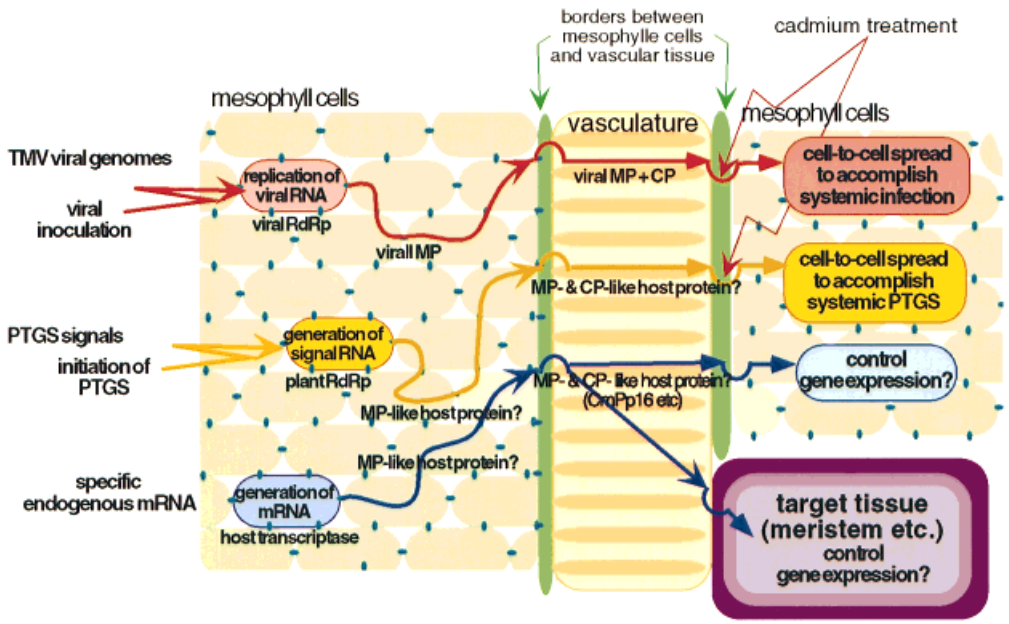

Figure 1. Three major types of systemic RNA transports in plants. RNA transport during viral infection and PTGS initiates with the insertion of foreign genetic material, i.e., virus inoculation or introduction of the transgene, respectively. RNA molecules, e.g., newly replicated viral genomes and PTGS elicitors, derived from this genetic material are then transported locally and/or systemically. Whereas viral RNAs must first travel cell-to-cell to reach vasculature, PTGS RNA signals and endogenous transported RNAs may originate directly in the phloem, circumventing the cell-to-cell movement step. For example, cell-to-cell movement of PTGS signals is not essential for development of PTGS.[20] All three types of RNA then enter the sieve elements of the vasculature and spread systemically, most likely in the source-to-sink direction. Finally, the signal molecules exit the vasculature in most tissues (viral RNAs and PTGS signals) or target to specific tissues by an as yet unknown mechanism (e.g., *PFP-LeT6* and *CmNACP* mRNAs).

from the *Me* stock. In reciprocal experiments, grafting *Me* scions onto *Xa* stocks did not alter the phenotype of these *Me* scions, suggesting that the change-inducing substance can travel only in the source-to-sink direction, i.e., from older to younger leaves, probably with the flow of photoassimilates. Similar unidirectional transport has been described for the systemic movement of PTGS elicitors and plant viruses (reviewed in Refs. 14,15).

The best candidates for this systemically transported signal are either *PFP-LeT6* mRNA itself, which is generated in *Me* tissues, or its protein product, which is directly responsible for the *Me* phenotype. Supporting the first possibility, the authors showed that, when *Xa* scions are grafted onto *Me* stocks, the *PFP-LeT6* chimeric transcripts are found in the *Xa* scions, indicating that this mRNA is graft-transmissible. Employing in situ RT-PCR, the authors also demonstrated that, in *Xa* scions grafted on *Me* stocks as well as in non-grafted *Me* plants, the *PFP-LeT6* fusion transcript was located in periphery of shoot apical meristem and tips of developing leaf and leaflet primordia. Importantly, in *Me* plants and in the heterografted *Xa* scions, the expression pattern of the *PFP* promoter-driven fusion transcripts was different from that of the *PFP* mRNA but resembled that of the *LeT6* mRNA in the wild-type (and *Xa*) plants, suggesting that the distribution of the *PFP-LeT6* transcripts is likely controlled by their long-distance transport rather than by the promoter activity. Finally, the *PFP-LeT6* transcripts were detected in the phloem but not xylem elements of the plant vasculature, directly confirming the role of the phloem as a conduit for the systemic movement of these chimeric RNA molecules. However, because both *PFP* and *LeT6* transcripts were found in *Xa* plants, albeit in small amounts, within the phloem, it was impossible to conclude which of these components of the fusion transcript is responsible for its systemic transport capacity.

Overall, this paper confirmed that plant cellular mRNA can be transported systemically, and, for the first time, clearly showed that this transport can function to control morphological development. However, several aspects of the *PFP-LeT6* mRNA transport and its biological effects still elude our understanding. For example, is the movement of the *PFP-LeT6* mRNA solely responsible for the phenotypic changes observed in this study? Or does its protein product, similarly to several transcription factors known to move between plant cells and control tissue development,[8,16,17] also move into *Xa* scions out of *Me* stocks? In addition, in wild-type plants, do the sink leaves produce the *LeT6* and/or *PFP* mRNAs

themselves; if they do, what is the biological rationale for the systemic transport of these messages into these tissues?

Systemic RNA transport and its regulation during PTGS and viral infection

While the study by Kim et al.[1] illustrates the functional importance of long-distance mRNA movement, several fundamental questions about this process remain unanswered. Specifically, it is still unknown (i) whether the RNA molecules travel between cells alone or in complex with transport proteins, and (ii) how this RNA transport, with its far reaching biological effects, is regulated. Insights into these aspects of mRNA transport come from our knowledge of transport of two other types of RNA: PTGS elicitors and plant viral genomes (illustrated in Fig. 1).

PTGS is thought to occur following production of antisense RNA[18] which pairs with the mRNA of the transgene, causing degradation of the resulting double-stranded (ds) RNA by cellular dsRNA-specific Rnases.[19] Thus, small antisense and/or dsRNA molecules found in the phloem of gene-silenced plants have been proposed to serve as PTGS elicitors.[18] An important feature of PTGS is that it is not cell autonomous and, once initiated, spreads systemically throughout most parts of the plant.[13,20] Similar to the systemic transport of the *PFP-LeT6* mRNA, systemic transport of PTGS signals was inferred from the observations that silenced tobacco stocks induced PTGS in scions expressing the corresponding transgene.[13]

Another major type of RNA molecule systemically transported within plants is the genomes of the invading viruses. Systemic movement of the best-studied plant viruses, tobacco mosaic virus (TMV),[21] is aided by the viral movement protein (MP) and coat protein (CP) (reviewed in Refs. 2,7,22,23). The absolute requirement for these proteins, which associate with the TMV genome,[24,25] suggests that viral RNA travels between cells as a nucleoprotein complex. In fact, in most known cases, RNA molecules move between and within cells as nucleic acid–protein complexes.[26] It is, therefore, natural to assume that plants possess proteins, similar in function to the viral MP and/or CP, that bind to the endogenous RNA and mediate its transport. Indeed, a plant RNA-binding protein, CmPp16, has been identified in pumpkin sap and proposed to function as a paralog of viral MP, assisting RNA translocation between companion cells and sieve elements of the phloem.[27]

Systemic transport of biologically active RNA molecules is unlikely to occur indiscriminately; thus, a mechanism should exist to control this process. The studies showing that systemic but not local movement of tobamoviruses in tobacco plants is specifically inhibited by low, non-toxic concentrations of heavy metal cadmium[28,29] suggest that such control may be exerted by cellular factors induced by this treatment. Recently, one such factor, a cadmium-induced glycine-rich protein, designated cdiGRP (accession number AY034091), has been identified in tobacco and shown to inhibit tobamoviral systemic movement when expressed constitutively in transgenic plants (S.U. and V.C., unpublished). Furthermore, non-toxic levels of cadmium also inhibited the systemic onset of PTGS,[30] suggesting that PTGS elicitors and tobamoviruses share common regulatory steps in their systemic transport pathways.

Systemic transport of PTGS elicitors and several plant viruses, such as cucumber mosaic virus (CMV), differs from systemic transport of cellular transcripts, such as *PFP-LeT6*[1] and *CmNACP* mRNAs,[10] in that the former occurs throughout most of the plant, but often does not reach the very young meristematic tissues,[31] while the latter is specifically targeted to the meristems.[1,10] Thus, plants may have evolved a communication "fire wall" around their meristems, which protects these developmentally important tissues from viral infection and PTGS, yet remains permeable for specific endogenous mRNAs.

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References

1. Kim M, Canio W, Kessler S, Sinha N. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science 2001;293:287–289.
2. Ueki S, Citovsky V. Intercellular RNA transport in plants. Curr Top Plant Biol 2001;2:167–179.
3. Oparka KJ, Santa Cruz S. The great escape: phloem transport and unloading of macromoleculaes. Annu Rev Plant Physiol Plant Mol Biol 2000;51:323–347.
4. Santa Cruz S. Phloem transport of viruses and macromolecules—what goes in must come out. Trends Microbiol 1999;7:237–241.
5. Jorgensen RA, Atkinson RG, Forster RL, Lucas WJ. An RNA-based information superhighway in plants. Science 1998;279:1486–1487.
6. Fagard M, Vaucheret H. (Trans)gene silencing in plants: How many mechanisms? Annu Rev Plant Physiol Plant Mol Biol 2000;51:167–194.
7. Citovsky V, Zambryski P. Systemic transport of RNA in plants. Trends Plant Sci 2000;5:52–54.
8. Lucas WJ, Bouché-Pillon S, Jackson DP, Nguyen L, Baker L, Ding B, Hake S. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. Science 1995;270:1980–1983.
9. Kühn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB. Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science 1997;275:1298–1300.
10. Ruiz-Medrano R, Xoconostle-Cázares B, Lucas WJ. Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants. Development 1999;126:4405–4419.
11. Sasaki T, Chino M, Hayashi H, Fujiwara T. Detection of several mRNA species in rice phloem sap. Plant Cell Physiol 1998;39:895–897.
12. Chen JJ, Janssen BJ, Williams A, Sinha N. A gene fusion at a homeobox locus: alterations in leaf shape and implications for morphological evolution. Plant Cell 1997;9:1289–1304.
13. Palauqui JC, Elmayan T, Pollien JM, Vaucheret H. Systemic acquired silencing: transgene-specific post-translational silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J 1997; 16:4738–4745.
14. Voinnet O, Baulcombe DC. Systemic signalling in gene silencing. Nature 1997;389:553.
15. Leisner SM, Turgeon R. Movement of virus and photoassimilate in the phloem–a comparative analysis. Bioessays 1993;15:741–748.

16. Sessions A, Yanofsky MF, Weigel D. Cell–cell signaling and movement by the floral transcription factors LEAFY and APETALA1. Science 2000;289:779–782.
17. Perbal MC, Haughn G, Saedler H, Schwarz-Sommer Z. Non-cell-autonomous function of the *Antirrhinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell-to-cell trafficking. Development 1996;122:3433–3441.
18. Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 1999;286:950–952.
19. Waterhouse PM, Graham MW, Wang MB. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc Natl Acad Sci USA 1998;95:13959–13964.
20. Voinnet O, Vain P, Angell S, Baulcombe DC. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. Cell 1998;95:177–187.
21. Creager A, Scholthof K-B, Citovsky V, Scholthof H. Tobacco mosaic virus: Pioneering research for a century. Plant Cell 1999;11:301–308.
22. Lucas WJ, Gilbertson RL. Plasmodesmata in relation to viral movement within leaf tissues. Annu Rev Phytopathol 1994;32:387–411.
23. Tzfira T, Rhee Y, Chen M-H, Citovsky V. Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. Annu Rev Microbiol 2000;54:187–219.
24. Bloomer AC, Butler PJG, Van Regenmortel MHV, Fraenkel-Conrat H, ed; The Rod-Shaped Plant Viruses. New York: Plenum Press. 1986. 19–57.
25. Citovsky V, Knorr D, Schuster G, Zambryski P. The P30 movement protein of tobacco mosaic virus is a single-strand nucleic acid binding protein. Cell 1990;60:637–647.
26. Citovsky V, Zambryski P. Transport of nucleic acids through membrane channels: Snaking through small holes. Annu Rev Microbiol 1993; 47:167–197.
27. Xoconostle-Cázares B, Xiang Y, Ruiz-Medrano R, Wang HL, Monzer J, Yoo BC, McFarland KC, Franceschi VR, Lucas WJ. Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. Science 1999;283:94–98.
28. Citovsky V, Ghoshroy S, Tsui F, Klessig DF. Non-toxic concentrations of cadmium inhibit tobamoviral systemic movement by a salicylic acid-independent mechanism. Plant J 1998;16:13–20.
29. Ghoshroy S, Freedman K, Lartey R, Citovsky V. Inhibition of plant viral systemic infection by non-toxic concentrations of cadmium. Plant J 1998;13:591–602.
30. Ueki S, Citovsky V. Inhibition of post transcriptional gene silencing by non-toxic concentrations of cadmium. Plant J 2001;28:in press.
31. Béclin C, Berthomé R, Palauqui JC, Tepfer M, Vaucheret H. Infection of tobacco or *Arabidopsis* plants by CMV counteracts systemic post-transcriptional silencing of nonviral (trans)genes. Virology 1998;252:313–317.