Commentary

RNA traffics information systemically in plants

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A new paradigm for intercellular signaling in vascular plants is emerging that offers to revolutionize our understanding of these deceptively simple organisms and reveal the true level of their sophistication in information processing. The new conceptual understanding is based on directed trafficking of information-rich macromolecular signals (transcription factors and RNA molecules) between cells and systemically throughout the plant (for review, see ref. 1). Intercellular and systemic movement occurs via two specialized structures: (*i*) intercellular organelles known as plasmodesmata, which provide continuity of cytoplasm and endoplasmic reticulum between adjacent cells; and (*ii*) the phloem, which is responsible for translocation of photoassimilates from source leaves to sink tissues and, together with the xylem, forms the vascular system of higher plants.

In this issue of PNAS, Klahre *et al.* (2) explore the molecular nature of RNA molecules that induce systemic RNA silencing in plants. One role of RNA silencing is to defend against viral infections by the detection of double-stranded RNA (dsRNA) viral replication intermediates and their cleavage by an RNase III enzyme called Dicer that produces short (≈22-nt) RNA molecules, known as short interfering RNAs (siRNAs) (for review, see ref. 3). Recent discoveries in both plants and animals have established that similar small RNA molecules, known as microRNAs, are produced from endogenous dsRNA transcripts via Dicer (4–7). At least some microRNAs appear to be essential regulators of fundamental developmental processes, including stem cell proliferation in nematodes and meristem proliferation and establishment of organ polarity in flowering plants (4, 5, 8, 9).

The Dicer system for producing short regulatory RNAs is clearly ancient, having originated in a common ancestor of plants, animals, and fungi. Whether its original function in unicellular eukaryotes was viral resistance or regulation of endogenous gene expression is unknown. During the evolution of land plants, plasmodesmata and the vascular system evolved as a means to translocate water, nutrients, and photoassimilates throughout the plant, paving the way for the further evolution of sophisticated supracellular control mechanisms based on macromolecular signaling (1). Indeed, higher plants have been found to translocate select endogenous transcripts great distances from the site of transcription via the phloem and plasmodesmata (10). For instance, in an interspecific graft of cucumber to squash, transcripts originating in the squash rootstock are found to translocate to shoot apical meristems of the cucumber scion. Importantly, these mobile transcripts are not short RNAs but are hundreds of base pairs long; phloem-mediated trafficking of short endogenous RNAs still remains to be demonstrated. Plant viruses exploit this system by using viral-encoded "movement proteins" (MPs) that transmit the virus between cells and throughout the plant (11). Plants encode MPs that are homologs of viral MPs (12). It seems likely that during evolution, viruses captured plant MP coding sequences and incorporated them into their genomes to exploit the plant's macromolecular signaling system, although one cannot exclude the possibility that movement proteins first evolved in viruses and were then incorporated into plant genomes and used for macromolecular trafficking.

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Direct evidence that mobile transcripts can be functional derives from a dominant gain-of-function mutation in tomato called *Mouse ears* (*Me*). *Me* is due to a transcriptional fusion of a homeobox gene to another transcript, resulting in a phloem mobile gain-of-function transcript that converts genetically wild-type scions to the distinctive leaf morphology phenotype of the genetically *Me* stock (13). Fusion transcripts were localized in the shoot apical meristem, which was surprising because phloem-transported viruses generally cannot enter the meristem. This observation suggests that plant apices possess a surveillance system that accepts and regulates endogenous RNA signals and protects the meristem from viral infection. Strong support for the existence of a mechanism regulating selective entry of RNA is provided by the discovery of a viral protein that interferes with the ability of plants to exclude viral RNA and RNA silencing signals from the shoot apex (14).

RNA silencing can produce systemic signals that transmit the silencing state throughout the plant (15, 16). Systemic signals allow plants to track down and destroy viral genomes as they move through the vascular system and plasmodesmata and to establish resistance in distant cells in advance of the arrival of the virus. Not surprisingly, viruses have responded to this system by evolving a diverse variety of proteins that can block either RNA silencing or movement of the systemic silencing signal (17–19). Because the signal is sequence-specific, it is thought to be a nucleic acid, most likely an RNA, but the precise nature of the signal molecule is not known. A common suggestion is that the signal is siRNA itself.

Klahre *et al.* (2) approached this problem with biolistic delivery of RNA and DNA homologous to a reporter gene. Two reporter gene systems were used. One, based on the green fluorescent protein, allows real-time monitoring of the movement of silencing signals. The other is a positive reporter system based on a tetracycline inducible β-glucuronidase (GUS) gene. By silencing the tetracycline repressor (TetR) transcript, repression of the GUS reporter gene is relieved, resulting in expression of GUS, a histologically stainable activity. As in *Caenorhabditis elegans*, dsRNAs homologous to the full-length coding sequence of TetR were the most effective inducers of RNA silencing; single-stranded sense and antisense molecules were much less effective and their activity was destroyed by RNase treatment.

Next Klahre *et al.* (2) bombarded their

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reporter plants with synthetic siRNA molecules. Double-stranded siRNAs were highly effective inducers of silencing, whereas sense and antisense siRNAs were not able to induce silencing at all, showing directly, for the first time in plants, that siRNAs can trigger RNA silencing, just as in animals. Short DNA molecules of the same sequence as siRNA molecules were incapable of triggering silencing. Using real-time monitoring of green fluorescent protein expression, siRNAs were also found to be able to induce silencing systemically in leaves distant from the original site of induction. Silencing resulted in new siRNAs that correspond to regions both 5′ and 3′ to the siRNA trigger sequence, as has also been shown recently for virus-induced silencing (20). In the latter case, this ''spreading'' of RNA silencing to adjacent sequences has been shown to require transcription of the target gene and a functional gene encoding an RNA-directed RNA polymerase (RdRP) homolog (20).

In RNA interference in animals, siRNAs are incorporated into RISC (the RNA-induced silencing complex) as guide RNAs that allow RISC to target homologous transcripts (3). Production of new siRNAs in animals spreads only toward the 5′ end of the targeted transcript, not 3′ to the inducer sequence, suggesting that siRNAs may act as primers for RdRP to produce dsRNA corresponding to the 5′ end of the target (21, 22). These long dsRNA molecules are substrates for Dicer, which produces siRNAs corresponding to the entire length of the dsRNA. To account for the fact that spreading occurs both 5′ and 3′ to the target region in plants, Klahre *et al.* (2) suggest the possibility that a circular RNA might be the template for RdRP. Alternatively, interaction of RISC with the targeted RNA might trigger recognition and primer-independent copying by RdRP from the 3′ end of the transcript (2, 20). Characterization of the tomato RdRP enzyme has shown that its activity is primer independent (23).

So what is the nature of the systemic silencing signal in plants? As Klahre *et al.* (2) suggest, it is either siRNA itself or some intermediate in the silencing process, perhaps related to the spreading phenomenon. Given that plants can traffic long functional transcripts great distances via the phloem (14), it is clearly possible that the systemic silencing signal is a long RNA. Interestingly, the Vance laboratory found a viral inhibitor of RNA silencing, HC-Pro, to block the production of siRNA in source tissues but not to prevent the production and movement of the systemic silencing signal through graft unions to silence target genes in sink tissues (24). This observation constrains the hypothesis that the signal is siRNA such that it must be argued that low concentrations of siRNA, undetectable in source tissues, are sufficient for long-distance transmission of silencing. Thus, a major challenge now facing plant scientists is to differentiate between the possibilities that low concentrations of siRNA comprise the signal and the signal is a longer RNA molecule, possibly copied from the target RNA by RdRP. In the latter case, the role of siRNA would be to mark the target RNA for copying by RdRP. Mutants exist in *Arabidopsis* that block the ability of transgenes to trigger silencing, and yet these do not block the ability of RNA viruses to trigger silencing. These genes encode RdRP and RNA helicase homologs, which are presumed to be involved in recognition of sense transcripts and their conversion to dsRNA molecules (25, 26). If it could be shown that in such mutants double-stranded siRNAs could trigger systemic silencing, the case for low abundance siRNAs being the systemic signal would be strengthened considerably. An important complication for such experiments, however, is that RdRP may be necessary for amplifying siRNA to sufficient levels to act as a systemic signal. Perhaps bombardment with sufficient quantities of siRNA might overcome this problem.

Determination of the molecular nature and whole-plant behavior of systemic silencing signals potentially has broad implications for endogenous gene regulation in plants (27), as is evidenced by the abundance of microRNAs encoded by the *Arabidopsis* genome (6, 7). Assuming that some of these microRNAs do indeed have a regulatory function, the possibility exists that they could be trafficked systemically to distant organs and contribute to long-distance regulation in response to environmental cues perceived by only part of the plant. It has long been known that a flowering signal (known as ''florigen'') can be induced by appropriate treatment of a single leaf, which then transmits florigen to the vegetative shoot apex, converting it to a reproductive meristem and initiating inflorescence development. Conceivably, florigen may exist among the long phloem-mobile RNAs already identified by Lucas and colleagues in cucurbits (11, 28). Alternatively, florigen could be a microRNA, and candidates for florigen may lie in the microRNAs recently identified in *Arabidopsis* (6, 7). We should expect bombardment of plants with siRNAs (2) to be followed soon by similar experiments using microRNAs to investigate systemic gene regulation. Demonstration that microRNAs traffic systemically and selectively in plants would significantly enrich the emerging paradigm positing an ''RNA information superhighway'' that facilitates communication and coordination of vital information for plant growth, development, and responses to the environment.

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