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MOLECULAR BIOLOGY

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Mobile small RNA in plants provides cell-to-cell communication that affects gene expression during development.

Rob Martienssen

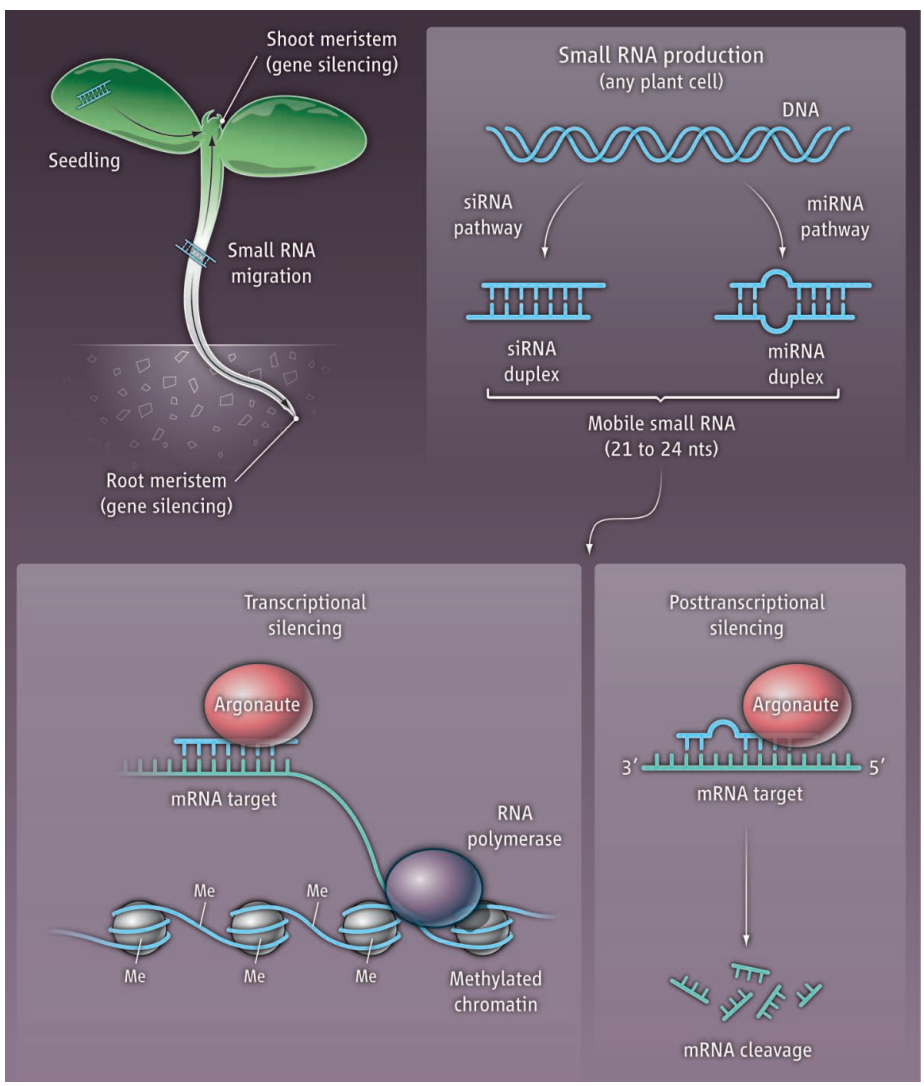
It has been known for almost 100 years that when a plant virus infects a leaf, mobile signals are transmitted through vessels in the stem to other leaves to confer resistance to subsequent infection. More recently, the silencing of exogenous transgenes has been shown to involve a mobile signal (*1*). Although RNA molecules have been implicated in systemic plant cell-to-cell communication, the nature of mobile RNA that silences gene expression has not been clear (*2*). Now, four studies—including those by Molnar *et al*. (*3*) and Dunoyer *et al*. (*4*) on pages 872 and 912 of this issue—report that small interfering RNA (siRNA) and microRNA (miRNA) are mobile signals that control gene expression during plant development.

Two of the studies examined the genetic pathway required for the mobile silencing signal by making grafts between wild-type and mutant roots and shoots of the model plant *Arabidopsis thaliana*. Molnar *et al*. used next-generation sequencing to detect mobile small RNA in grafted roots defective in DCL2, DCL3, and DCL4, the Dicer-like enzymes in *Arabidopsis* that cleave long precursor RNA into small RNA. Thus, these roots cannot generate 22- and 24-nucleotide (nt) siRNA. Nonetheless, the roots accumulated these forms, indicating siRNA movement from the shoot. Small RNA generated from hundreds of loci was found in the grafted roots, mostly from transposons (DNA sequences that can move around the genome). By contrast, in another study, Dunoyer *et al*. (*5*) identified small RNA in grafted roots that derived from inverted repeats in the shoot.

Dunoyer *et al*. (*4*) further show that 21-nt siRNA is mobile by introducing small RNA directly (by particle bombardment) into *Arabidopsis* cells lacking RNA-dependent RNA polymerase. They observed the spread of targeted gene silencing along with the movement of labeled small RNA. The authors demonstrate that it is the double-stranded duplex, rather than the single-stranded form, of siRNA that is mobile. Dunoyer *et al*. (*5*) also report similar results with 24-nt siRNA duplexes.

All 3 classes of siRNA (21, 22, and 24 nt) are mobile and use components of both the posttranscriptional and the transcriptional gene silencing pathways of RNA interference (RNAi) to silence their targets (*1*). These components include the plant-specific RNA polymerase Pol IV, two RNA-dependent RNA polymerases (RDR2 and RDR6), and the chromatin-remodeling protein CLASSY (*1*). The key nucleases required for RNAi—Dicer and Argonaute—are encoded by multigene families, and act redundantly to some extent in generating these mobile small RNA types. Dunoyer *et al*. (*5*) further demonstrate that 24-nt mobile siRNA generated from inverted repeats depends on the exportin protein HASTY for their transport out of the nucleus. Importantly, Molnar *et al*. show that the targets of mobile siRNA signals are methylated by RNAi-dependent DNA methylation. This suggests that mobile RNA can have long-lasting effects on DNA silencing.

The rules for selecting which loci in the *Arabidopsis* genome generate mobile siRNA are still unclear. Naturally occurring inverted repeats are a major source of



Move to silence. A model for silencing gene expression during plant development involves mobile small RNA molecules (siRNA) that move systemically through the plant vascular system, as well as miRNA that may move over shorter distances. Small RNA move from source cells (such as in the shoot) into dividing cells in the root and shoot meristems where they silence targets, including transposons and genes.

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mobile siRNA (*5*), and some of these have known silencing functions, such as the phosphoribosylanthranilate isomerase (PAI1-4) inverted repeat (*3*), which silences a functional copy of the same gene (*6*). These inverted repeats are polymorphic, indicating that they arise and disappear rapidly during evolution, presumably responding to selection for target gene expression. In maize, a naturally occurring, polymorphic inverted repeat comprises the promoter and part of the coding region of the *Mutator* (*Mu*) transposon gene and is expressed in the shoot tip. It generates 24- and 25-nt small RNA, which are found in the surrounding leaves, suggesting their mobility (*7*). Once silenced by these small RNA, *Mu* elements remain silent in subsequent generations in the presence of RDR2 and Pol IV.

Endogenous mobile small RNA may be generated by many potential cellular sources in plants, and act as signals during development of various target cells. For example, 21-nt trans-acting siRNA (tasiRNA) can move across young leaves to reach their targets, which are silenced posttranscriptionally (*1*, *4*). Carlsbecker *et al*. now report that some 21-nt miRNA generated in root cells are mobile within the root, and posttranscriptionally silence multiple transcription factor genes in adjacent cells (*8*). These same miRNA appear to move in the shoot in the absence of the nuclease Argonaute1 (*9*), consistent with a mobile duplex form of small RNA.

Analogous to the transport of photosynthetic sugars, roots may act as a "sink" for mobile small RNA that originate in a "source" tissue, such as leaves of the shoot (*3*, *5*). However, only lateral roots are affected, which emerge after silencing is initiated by shoot grafting (*3*). Similarly, when shoots are the recipient of silencing signals, only leaves that arise after root grafting are affected (*2*). This indicates that gene silencing occurs predominantly in undifferentiated stem cells (dividing cells in the shoot and root meristems) that give rise to new organs in plants (*2*). Perhaps this is because RNAi-mediated transcriptional silencing is most efficient in dividing cells (*10*).

Mobile 24-nt siRNA may account for some curious properties of transposon silencing during plant development. For example, silencing of *Mu* transposons in maize occurs in the shoot meristem, giving rise to clonal regions in leaves. As the plant matures, the number of sectors in each leaf increases (*11*), suggesting that the developmental transition between early and late leaves may be controlled by a silencing process, and that a mobile signal silences stem cells as the meristem ages (*12*). These cells give rise to flowers, and so silencing is inherited in the next generation (*11*).

Flowers are also well-known sink tissues. Translocation of small RNA into flowers could affect the inheritance of epigenetic alleles. Sperm cells are loaded with mobile 21-nt transposon-targeting siRNA from the surrounding pollen grain, whereas the ovule and embryo sac have predominantly maternal 24-nt siRNA, which are required to silence transposons (*13*, *14*) and inhibit germ cell fate in adjacent cells (*13*). Those small RNA derived from the plant body could find their way into ovules and pollen grains, which are physiological sinks, just like meristems and roots.

Small RNA silencing in eukaryotes is uniquely sensitive to temperature and potentially other environmental signals (*10*), providing a potential mechanism for the environment to modify the germ line. The inheritance of acquired characters was envisioned not only by Lamarck, but also by Darwin, who proposed the existence of gemmules, somatic particles that entered the germ line and contributed such characters to the next generation (*15*). It remains to be seen if mobile small RNA will live up to that extraordinary vision.

References

1. P. Dunoyer, O. Voinnet, *Trends Plant Sci.* **14**, 643 (2009).
2. C. A. Brosnan *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14741 (2007).
3. A. Molnar *et al*., *Science* **328**, 872 (2010); published online 22 April 2010 (10.1126/science.1187959).
4. P. Dunoyer *et al., Science* **328**, 912 (2010); published online 22 April 2010 (10.1126/science.1185880).
5. P. Dunoyer *et al*., *EMBO J.* 10.1038/emboj.2010.65 (2010).
6. J. Bender, G. R. Fink, *Cell* **83**, 725 (1995).
7. R. K. Slotkin, M. Freeling, D. Lisch, *Nat. Genet.* **37**, 641 (2005).
8. A. Carlsbecker *et al*., *Nature* 10.1038/nature08977 (2010).
9. C. A. Kidner, R. A. Martienssen, *Nature* **428**, 81 (2004).
10. A. Kloc, R. Martienssen, *Trends Genet.* **24**, 511 (2008).
11. R. Martienssen, A. Baron, *Genetics* **136**, 1157 (1994).
12. R. S. Poethig, *Science* **250**, 923 (1990).
13. V. Olmedo-Monfil *et al*., *Nature* **464**, 628 (2010).
14. R. K. Slotkin *et al*., *Cell* **136**, 461 (2009).
15. C. Darwin, *The Variation of Animals and Plants Under Domestication* (John Murray, London, 1868).

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PHYSICS

Managing Multistate Quantum Entanglement

Christoph Wildfeuer

A general route for creating entangled states of large numbers of photons may be relevant to other applications of interferometry, such as gravity wave detection.

Good experiments minimally perturb the sample under study, but in the quantum realm, measurement cannot be separated from the system. Before the measurement, all possible outcomes can form an entangled state; measurement then causes this entangled state to collapse to one outcome. In 1935, Schrödinger presented a thought experiment to show how strange this situation really is. A cat is hidden in a box along with a sample of radioactive nuclei. If a nuclear decay occurs, poison is released and the poor cat dies. However, until we look in the box, the state of the cat entangles both a live and a dead cat. Experiments with Schrödinger-cat states that entangle several particles continue to provide deep insights into the measurement process. One such state, called a NOON state, entangles a fixed number of photons N, all of which are in one of two possible states (if these were coins, they would be all heads or all tails). On page 879 of this issue, Afek *et al*. (*1*) achieved a record by making NOON states in an interferometer with five entangled photons. Their approach may have implications for other implementations of interferometry, such as gravity wave detectors.

The NOON state was first discussed in 1989 by Sanders, who was particularly interested in the Schrödinger-cat aspect and quantum decoherence—how entangled states decay over time (*2*). These states can be the different optical paths, or modes, in an interferometer (see the figure). In the NOON state, all of the photons are mode a or mode b. However, we cannot tell which mode is occupied. In 2000, Dowling's group rediscovered this state in the context of quantum imaging, particularly for using quantum interference to improve the res-

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