Molecular biology experienced a significant shift in thinking in recent years with growing evidence that microRNAs (miRNAs) play a major role in the control of eukaryotic gene expression during development. miRNAs and related small interfering RNAs (siRNAs) are 21- to 25-nucleotide long noncoding RNA molecules that regulate translation of protein-coding mRNAs, either by affecting degradation of target mRNAs or the attenuation or termination of translation without mRNA degradation. Both miRNAs and siRNAs are produced from longer RNA precursors through the activity of ribonuclease III–like nucleases called Dicer in animals and DICER-LIKE (DCL) in plants. The main difference between these two classes of small RNA is their point of origin: miRNAs are encoded by their own genes, which are distinct from recognized protein-coding genes, whereas siRNAs are processed from long double-stranded RNA (dsRNA) precursors arising from mRNAs, transposons, viruses, or heterochromatic DNA (Bartel and Bartel, 2003).

Interestingly, plants appear to have evolved numerous partially overlapping small RNA pathways, which is unlike the situation in many animals. Arabidopsis encodes four DCL proteins (DCL1-4), and Xie et al. (2004) have shown that DCL1, DCL2, and DCL3 function in biogenesis of miRNA, viral siRNA, and endogenous siRNA, respectively. Arabidopsis also contains at least three genes encoding RNA-dependent RNA polymerases, which are involved in the production of some siRNAs, and a family of 10 ARGONAUTE proteins, some of which are components of the RNA-induced silencing complex that performs small RNA-induced transcript degradation (Xie et al., 2004 and references therein). Biochemical and genetic evidence supports the existence of multiple small RNA pathways in plants that may have a wide variety of functions in plant growth and development (Dunoyer et al., 2004; Xie et al., 2004).

In plants, siRNAs have been studied for many years in relation to posttranscriptional gene silencing, also known as RNA silencing, which is believed to represent a natural defense mechanism against viral infection and the activity of transposable elements (Vance and Vaucheret, 2001; Waterhouse et al., 2001). dsRNA, which is not normally produced during routine transcription of host protein-encoding genes...
but often is produced during viral replication or by the activity of other aberrant or pathogenic nucleic acids, is recognized by one or more DCL proteins and processed into siRNAs, which then target the invading transcripts for destruction. Plant virus genomes often encode suppressors of host RNA silencing, such as the helper component–proteinase of potyviruses and the p25 protein encoded by potato virus X, providing support for the notion that RNA silencing is an antiviral mechanism in plants (reviewed in Li and Ding, 2001).

miRNAs appear to be another way that eukaryotes use Dicer/DCL recognition of dsRNA to produce small regulatory RNAs. In this case, miRNA precursors encoded within an organism’s own genome form a specific fold-back RNA structure creating a double-stranded region that is recognized by Dicer/DCL and cleaved into miRNAs directed against endogenous target mRNAs. miRNAs can act similarly to siRNAs and effect degradation of target mRNAs, or they may cause attenuation or termination of translation without mRNA degradation (Hutvágner and Zamore, 2002; Chen, 2004). miRNAs often show a high degree of evolutionary conservation across species, and there is growing evidence that they represent a major class of regulatory molecules having broad significance in a wide range of developmental processes in plants and animals (Carrington and Ambros, 2003; Bartel, 2004). Fifteen classes of miRNAs have been identified in Arabidopsis (Bartel and Bartel, 2003 and references therein). Rhoades et al. (2002) identified 49 predicted targets for 14 different Arabidopsis miRNAs, and the majority of these were transcription factor gene families associated with cell differentiation or developmental patterning. More recently, several miRNAs have been found to play specific roles in plant development, including the regulation of flowering time and floral organ identity (Aukerman and Sakai, 2003; Chen, 2004), and leaf polarity and morphology (Palatnik et al., 2003; Juarez et al., 2004; Kidner and Martienssen, 2004).

In plants, the long-distance transport of protein and RNA through the phloem plays a critical role in non-cell-autonomous signaling that contributes significantly to plant development (Haywood et al., 2002). The systemic spread of RNA silencing via a phloem-transmissible signal is a well-characterized phenomenon that can be readily observed after localized viral infection or with heterografting experiments (Palaauqui et al., 1997; Voinnet and Baulcombe, 1997). Small RNAs have been viewed as a likely candidate for the systemic silencing signal (Hamilton and Baulcombe, 1999; Himber et al., 2003), but there has been no direct evidence of their transport through the phloem, and the nature of the mobile silencing signal has remained elusive (Motschwa et al., 2002). The extent to which miRNA function requires long-distance transport through the phloem is unknown and has not been explored previously.

In this issue of The Plant Cell, Yoo et al. (pages 1979–2000) show that small RNA (18 to 25 nucleotides in length) corresponding to authentic regulatory RNAs (siRNA and miRNA) can enter and move through the phloem of several plant species. Furthermore, these authors identify a novel protein, *Cucurbita maxima* PHLOEM SMALL RNA BINDING PROTEIN1 (CmPSRP1), and show that it likely plays a role in trafficking of small RNA through the phloem. Also in this issue, Sunkar and Zhu (pages 2001–2019) present a library of small RNAs isolated from Arabidopsis seedlings exposed to dehydration, salinity, cold stress, or abscisic acid treatment. They identify 15 new miRNA families that include 26 new miRNAs corresponding to 34 loci and show that some of these miRNAs are expressed in specific tissues and several are upregulated or downregulated in response to abiotic stress. Together, these two reports provide strong support for the idea that small regulatory RNAs are induced in response to a variety of external stimuli and are transported via the phloem to exert non-cell-autonomous control over diverse processes in plant growth and development (Figure 1).

Yoo et al. identified small single-stranded RNA 18 to 25 nucleotides in length in the phloem sap of *C. maxima* (pumpkin), *Cucumis sativus* (cucumber), *Lupinus albus* (white lupin), *Ricinus communis* (caster bean), and *Yucca filammentosa* (yucca). The group chose these species because of the relative ease of collecting phloem sap and methods that have been established with cucurbits. A library was constructed from *C. maxima* phloem sap RNA, and sequence comparisons revealed the presence of small RNAs corresponding to several putative target genes, including *Transposon-like 1* (TnL1) and TnL2, a small RNA identical to Arabidopsis miR159 proposed to target a MYB transcription factor, and genes encoding a bifunctional endonuclease and an RNA helicase. In addition, RNA gel blot analysis revealed the presence of small RNAs complementary to four previously identified plant miRNAs. Thus, it was determined that phloem sap contains bona fide small regulatory RNAs and not merely, for example, artifacts of RNase contamination or RNA shearing during sample preparation.

Subsequent experiments provided additional proof that small regulatory RNAs are transported through the phloem. In one set of experiments, the authors made use of transgenic *Cucurbita pepo* (yellow crookneck squash) lines expressing a viral coat protein (CP) gene that were either spontaneously silenced or nonsilenced for CP gene expression. siRNA ~23 nucleotides in length complementary to CP gene sequence was detected in the phloem sap of silenced, but not nonsilenced, *CP* transgenic lines. *CP* siRNA also was detected in phloem sap collected from wild-type cucumber scions grafted onto silenced *CP* transgenic squash lines, but was not detected in phloem sap collected from wild-type scions grafted onto either wild-type squash or nonsilenced transgenic CP squash lines. In another set of experiments, siRNA complementary to viral genomic RNA was detected in the phloem sap of *C. maxima* infected with *Cucumber yellows closterovirus*. These experiments provided direct evidence that both viral and transgene siRNA can enter and move through the phloem.

Finally, the authors identified CmPSRP1 as a small RNA binding protein that facilitates movement of small RNA across plasmodesmata. Phloem sap previously has been found to contain proteins...
involved in mRNA trafficking (Xoconostle-Cázares et al., 1999), which prompted Yoo et al. to look for phloem proteins that specifically bind to small RNA. Phloem sap protein blots (wherein proteins were first fractionated with fast protein liquid chromatography and further separated by PAGE) from pumpkin, cucumber, and lupin probed with different RNA species revealed the presence of a 27-kD protein that bound strongly to small RNA. The corresponding CmPSRP1 protein was purified from pumpkin. Conceptual translation of the gene encoding this protein and DNA gel blot analysis indicated that CmPSRP1 is a single copy gene in pumpkin that is not highly homologous to any other gene in plant databases, and RT-PCR using sequence-specific primers showed accumulation of CmPSRP1 transcripts in pumpkin phloem. Importantly, Yoo et al. conducted additional experiments that showed that CmPSRP1 not only preferentially binds ss-small RNA, it also selectively mediates trafficking of ss-small RNA through plasmodesmata. However, the general significance of PSRP1 in miRNA trafficking in plants remains to be determined, considering the apparent lack of conservation among other plant families.

Sunkar and Zhu focused on the discovery of new small regulatory RNAs in Arabidopsis and on investigating a potential role for small RNAs in plant response to abiotic stress. They first constructed a library of small RNAs (15 to 26 nucleotides in length) by size fractionation of RNA isolated and pooled from Arabidopsis seedlings exposed (separately) to cold, dehydration, salinity, and abscisic acid treatment. Approximately 9% of the 2500 cloned sequences corresponded to putative small regulatory RNAs (the remainder appeared to represent breakdown products of rRNA, tRNA, and nucleolar RNAs). Putative miRNAs were identified based on criteria established by Ambros et al. (2003) that the 200- to 300-nucleotide sequence surrounding an miRNA is capable of folding into a hairpin structure that is the miRNA precursor. The authors identified 13 of 15 previously characterized miRNA families and 15 new families of miRNA. They next analyzed various databases for Arabidopsis genes with sequence complementarity to the miRNAs to predict potential miRNA target genes. Fifty-one potential targets were identified within protein-coding regions of miRNAs, including genes encoding metabolic enzymes, transcription factors, signal transduction components, protein synthesis, and RNA processing proteins and enzymes in the ubiquitination pathway. Some of the miRNAs and their targets were found to be highly conserved in other plant species, including rice, Lotus, Medicago, and Populus. In addition to miRNAs, the authors also identified 102 other small RNAs that may play important roles in gene regulation.

Next, Sunkar and Zhu examined the potential for miRNA involvement in regulating plant stress responses. They found that the expression of several miRNAs was either upregulated or downregulated by one or more abiotic stress treatments, suggesting that they might be associated with regulation of gene expression in response to stress. One of the interesting observations in this regard was the increase in abundance of miRNA393 after cold, dehydration, and treatment with high salinity or abscisic acid. This miRNA is predicted to target the F-box protein TIR1, which functions as a regulator of auxin signaling through its role in targeting Aux/IAA proteins for degradation via the ubiquitin/proteasome pathway. Aux/IAA proteins repress the activity of auxin response factors and may function as negative or positive regulators of gene transcription (Dharmasiri and Estelle, 2004). Abiotic stress induction of miRNA393 could impose another level of regulation on TIR1-mediated responses. A similar investigation by Jones-Rhoades and Bartel (2004) identified some of the same new Arabidopsis miRNA families as Sunkar and Zhu and similarly showed that miRNAs can be induced by environmental stress. They showed that miR395, which targets mRNA encoding ATP sulfurylase enzymes that catalyze the first step of inorganic sulfate assimilation, was found to increase in response to sulfate starvation, suggesting that this miRNA might play a role in regulating sulfate metabolism in response to sulfate availability. Both of these studies show that several metabolic and cellular processes, in addition to distinct developmental pathways, may be regulated by miRNAs and that miRNAs can themselves be induced and repressed, adding another layer to the complexities of gene regulation. Moreover, the new miRNAs identified in both of these studies provide a valuable resource for future investigations on the roles of miRNA in the regulation of gene expression during development and in response to environmental conditions.

Together, the work of Yoo et al. and Sunkar and Zhu opens new avenues of research into small regulatory RNAs and propels us several steps forward in understanding the biological functions and mechanism of action of this fascinating and important class of regulatory molecules.

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REFERENCES


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