RNA Goes Mobile

In recent years, much has been learned about various related phenomena of gene silencing in plants and other eukaryotes. RNA silencing (also termed post-transcriptional gene silencing [PTGS]) is a sequence-specific RNA degradation process that is triggered by the formation of double-stranded (ds) RNA and can be induced by viruses or transgenes. Transcriptional gene silencing (TGS) is a related phenomenon in plants that is triggered by the formation of dsRNA homologous with gene promoter (and sometimes coding) regions, but it leads to methylation and chromatin remodeling, which cause inhibition of transcription rather than RNA degradation. Both types of gene silencing share the key features of dependence on dsRNA, which is cleaved into small (21 to 25 nucleotides) interfering RNAs. It is generally accepted that gene silencing is a defense mechanism against the activity of transposable elements (e.g., TGS in plants and PTGS in a variety of other eukaryotes) and viral infection (PTGS in higher plants) (Waterhouse et al., 2001).

RNA silencing signals can be transmitted systemically, as shown by the systemic spread of silencing after localized viral infection and graft-transmissible spread of silencing. The most likely candidates for the mobile silencing signal are dsRNA, small interfering RNAs, or aberrant RNA molecules (reviewed in Mlotshwa et al., 2002). Recent studies are beginning to suggest that RNA is commonly transported through the phloem (Ruiz-Medrano et al., 1999; Lucas et al., 2001) and that the regulation of RNA trafficking plays an important role in plant development in addition to its role in PTGS (reviewed in Vance and Vaucheret, 2001; Haywood et al., 2002).

In this issue of The Plant Cell, Foster et al. (pages 1497–1508) present evidence for a functional “surveillance system” that governs the selective entry of RNA into the shoot apex. This surveillance system appears to have the dual role of excluding viral RNA from the shoot apical meristem (thus protecting the germline by preventing meiotic transmission of the virus to the next generation) and, possibly, permitting the selective entry of endogenous RNA that is involved in organ development (Figure 1). Also in this issue, Llave et al. (pages 1605–1619) identify a set of 125 endogenous small RNAs in Arabidopsis and show that the accumulation of specific small RNAs is regulated developmentally. These authors propose that, as in animal systems, small RNAs play important roles in post-transcriptional and epigenetic events involved in both defense and development.

PTGS AS A VIRAL DEFENSE MECHANISM

The majority of plant viruses (>90%) are single-stranded RNA viruses. These viruses...
are replicated by a virus-encoded RNA-dependent RNA polymerase (RDRP), which produces a dsRNA intermediate. The requirement for a dsRNA intermediate in viral replication, which does not occur commonly in healthy plants, allows plants to use dsRNA as a trigger for the induction of sequence-specific degradation of viral RNA. Transgenic-induced gene silencing appears to be a corollary to this apparent antiviral adaptation in plants. It was discovered that transgene-induced silencing is also dependent on the formation of dsRNA, which can form in transgenic plants as a result of the integration of multiple transgenes as inverted repeats (Waterhouse et al., 1998). Transgenes designed for gene silencing now routinely are constructed to produce dsRNAs or hairpin RNAs (Smith et al., 2000; Wang and Waterhouse, 2000).

A current model for PTGS, which relies on information from animal as well as plant systems, is that dsRNA forms a complex with an enzyme similar to Escherichia coli RNase III called Dicer, which cleaves fragments of ~21 nucleotides of sense and antisense RNA. These fragments go on to hybridize with homologous single-stranded RNAs (e.g., viral genomic RNA, or in the case of transgene-induced silencing, plant RNA homologous with the transgene), which bind more Dicer complexes, leading to continued sequence-specific RNA degradation and viral suppression or gene silencing (Waterhouse et al., 2001).

The model includes mechanisms for the initiation, maintenance, and spread (systemic transmission) of PTGS and is supported by the identification of plant genes, such as Arabidopsis SGS2/SDE1 and AGO1, that are required for one or more of these phases. SGS2/SDE1 encodes a protein with similarity to RDRP proteins involved in PTGS in Neurospora crassa and Caenorhabditis elegans (Dalmay et al., 2000; Mourrain et al., 2000), and AGO1 encodes a translation initiation factor–like protein that also is homologous with proteins associated with PTGS in N. crassa and C. elegans. The DCR1 gene from Drosophila, which encodes the RNase III-type Dicer associated with PTGS in this organism, is homologous with Arabidopsis CAF1 (Waterhouse et al., 2001). The involvement of CAF1 in PTGS has not been examined, but a mutation in this gene was shown to cause meristem defects and abnormal development of leaves and floral organs in Arabidopsis (Jacobsen et al., 1999).

Not surprisingly, some viruses appear to have evolved a counterdefense strategy against PTGS. Potexviruses, such as Potato virus X (PVX) and White clover mosaic virus (WCIMV), have simple genomes that include just five open reading frames. These encode RDRP, coat protein, and three proteins collectively termed the triple gene block (TGB) proteins, designated TGBp1 to TGBp3. TGBp1 was identified as a viral movement protein capable of effecting an increase in the size exclusion limit of plasmodesmata, but all three TGB proteins and the coat protein are required for effective cell-to-cell transmission of the virus (Lough et al., 1998, 2000).

Lough et al. (2000) presented a model in which TGBp2 and TGBp3 are membrane-associated proteins that function to deliver TGBp1 complexed with coat protein and viral RNA to the plasmodesmata. The presence of TGB proteins, in particular TGBp1, also is associated with an inhibition of the systemic transmission of the PTGS signal (Voinnet et al., 2000), thus enhancing the ability of the virus to establish systemic infection.

Certain other viruses have similar anti-PTGS activity. For example, the cucumovirus Cucumber mosaic virus 2b protein is similar to TGBp1 in its ability to inhibit the systemic transmission of PTGS, and the potyvirus HC-Pro (helper component proteinase) can suppress PTGS in nonvascular tissue (Vance and Vaucheret, 2001).

**THE SHOOT APEX SURVEILLANCE SYSTEM**

Interestingly, plants appear to have evolved a special adaptation for the protection of the shoot apex against viral invasion. Despite the ability of viruses for systemic infection, viral RNA and viral protein often are excluded from shoot and root apical meristem regions, and meristem tip culture is an effective means of obtaining virus-free plants (Matthews, 1992). This has the important effect of preventing the spread of the virus to progeny via meiotic transmission, because plant germ cells arise from the shoot apical meristem.

In some cases, the PTGS signal apparently can enter the shoot apex, but the virus itself does not gain entry and is not transmitted to the next (meiotic) generation. Allowing entry of the PTGS signal to the shoot apex could, in effect, serve as a double layer of protection against meiotic transmission of the virus. As a first layer of protection, the virus is excluded from the shoot apex. However, if it happens to gain entry, the PTGS signal is ready to activate the second line of defense.

Foster et al. (2002) made use of the TGBp1 movement protein from WCIMV to examine the movement of virus and PTGS signals into the shoot apex. Transgenic Nicotiana benthamiana plants were created that expressed TGBp1 under the control of the 35S promoter of Cauliflower mosaic virus. The transgenic plants showed a “spikey” phenotype, characterized by the production of radially symmetric bladeless lateral organs (as opposed to the asymmetric production of leaves in the wild type; see Figure 1), the severity of which was correlated with the levels of TGBp1 transcript produced.

Because lateral organs initiate from the shoot apical meristem, the group used in situ hybridization to examine both the level of TGBp1 transcript and the expression of a marker of organ polarity (the YABBY domain of genes that are expressed primarily in abaxial cells of normally developing leaves) in the shoot apices of transgenic and wild-type plants. The YABBY gene FILAMENTOUS FLOWER, which showed high homology with the marker probe, has been shown to specify abaxial cell fate in Arabidopsis (Sawa et al., 1999; Siegfried et al., 1999). The TGBp1 transcript was present at high levels in the shoot apical
region of transgenic plants showing a severe spikey phenotype, and YABBY marker expression was reduced and distributed throughout abaxial and adaxial cells, in contrast to the high expression only in abaxial cells in the wild type.

The authors wondered next what would happen if transgenic TGBp1 plants were inoculated with WClMV. They predicted that TGBp1 expression in the shoot apex would remain unaffected, and the spikey phenotype would be maintained, because it was previously shown that PTGS could not be induced in the shoot apex of N. benthamiana (Ruiz et al., 1998; Voinnet et al., 1998). Interestingly, infection of spikey plants resulted in the reversion of the phenotype to normal patterns of leaf development from the shoot apex, and over time, the virus-infected spikey plants were observed to cycle back and forth between the production of normal and spikey leaves (Figure 1).

It appeared that PTGS was activated in the shoot apex, because reversion of the phenotype was correlated with targeted degradation of the TGBp1 transcript. Furthermore, the spikey transgenic plants were found to be compromised in their ability to exclude WCIMV or PVX from the shoot apex. Thus, the presence of the TGBp1 transcript in the shoot apex was correlated with the development of abnormal organ polarity, the disruption of normal patterns of endogenous gene expression in the apex, and the inability to exclude viral RNA and PTGS signal from the apical region.

The work of Foster et al. (2002) is significant because it clearly shows that a surveillance system operates in the shoot apex (as proposed by Lucas et al., 2001) that not only excludes viral RNA from the apical meristem but also controls the entry and/or distribution of endogenous RNAs that affect cell differentiation and organ development. But the study also raises many questions. For example, how does TGBp1, whether alone or in conjunction with coat protein and the other TGB proteins, function both to enhance viral transmission and to prevent transmission of the PTGS signal, as was shown by Voinnet et al. (1998, 2000) for PVX TGBp1? It might be envisioned that the association of the (as yet unidentified) PTGS signal with the TGBp1-containing complex inactivates the signal (perhaps by marking it for degradation) while the TGBp1-containing complex moves through the phloem and effects systemic transmission of the virus.

What components make up the shoot apex surveillance system, and how does it work? Why does the phenotype of transgenic TGBp1 plants cycle back and forth between normal and spikey developmental meristems? The cycling phenomenon suggests that reverse-phase oscillations of TGBp1 transcript and PTGS signal occur. This might be explained by the continuous ectopic expression of TGBp1 disrupting the shoot apex surveillance system on the one hand and by the presence of the viral TGBp1-containing complex on the other hand, but more experiments are necessary to fully explain this phenomenon.

**SMALL RNAs IN PLANT AND ANIMAL DEVELOPMENT**

The work of Foster et al. (2002) suggests that phloem-mobile RNA functions to orchestrate organ development in plants. Recent research in animal systems has shown the existence of a class of micro-RNAs (miRNAs) that is conserved among C. elegans, Drosophila, and human and appears to function in diverse ways in the regulation of development (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). Among this class of miRNAs, lin-4 and let-7 are repressors of translation that have definitive roles in the timing of developmental transitions in C. elegans (Reinhart et al., 2000). Distinct patterns of expression of numerous miRNAs during the development of C. elegans, and the high degree of conservation with sequences from other animals, suggest that miRNAs may have diverse regulatory functions in euakaryotes.

Llave et al. (2002) show that a similar class of small RNAs occurs in Arabidopsis. These small RNAs showed distinct patterns of developmental expression and tissue specificity, and they resembled short interfering RNAs produced by the induction of PTGS in N. benthamiana. One difference between the plant small RNAs and animal miRNAs is that the Arabidopsis small RNAs appear to come from transposon-like regions and predicted protein-coding regions in addition to intergenic regions (IGRs), whereas animal miRNAs were identified only in IGRs. However, the majority of Arabidopsis small RNAs (90% of 125 sequences) arose from IGRs. The derivation of some IGR small RNAs from sequence clusters having both sense and antisense orientations is suggestive of amplification involving the activity of an RDRP and Dicer-like cleavage of the dsRNA product.

Thus, a situation is envisioned in which dsRNAs and/or small RNAs play various specialized regulatory roles in plant defense and development. For example, dsRNA arising from a “foreign” source (e.g., viruses, transposons, or transgenes) is targeted for destruction via PTGS or TGS, whereas dsRNA arising from a class of endogenous IGR sequences may be involved in epigenetic or post-transcriptional events that have a role in normal plant development. Small RNAs also could play dual roles in PTGS and development, as suggested by the association of developmental abnormalities and defects in PTGS in plants and animals. It appears that RNA is on the move and actively involved in plant defense and development.

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