Virology 281, 1–5 (2001)
doi:10.1006/viro.2000.0812, available online at http://www.idealibrary.com on IDEAL®

MINIREVIEW

Activation and Suppression of RNA Silencing by Plant Viruses

James C. Carrington,[1] Kristin D. Kasschau, and Lisa K. Johansen

Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340

Received November 3, 2000, returned to author for revision December 4, 2000; accepted December 27, 2000

Plants have a number of ways to resist viruses. Innate responses, such as those triggered by dominant resistance gene products, include local cell death and systemic acquired resistance and have been studied for decades. Adaptive responses in plants, on the other hand, were recognized only a few years ago. This brief review focuses on RNA silencing, a fascinating adaptive response to invasive or mobile RNAs, and the counter-defensive strategies used by viruses to overcome the silencing response.

RNA silencing refers to the related processes of posttranscriptional gene silencing (PTGS) in plants, RNA interference in animals, and gene quelling in fungi. A unifying principle of RNA silencing is that a target RNA is inactivated by degradation in a sequence-specific manner. RNA silencing can be triggered efficiently by nuclear transgenes that form a high degree of double-stranded (ds) structure or that express a highly abundant or aberrant transcript. RNA silencing in plants can also be triggered by replicating RNA- and DNA-containing viruses. In some cases, RNA silencing during infection results in a "recovery" phenotype, in which new leaves that emerge are substantially free of virus and lack symptoms of infection. Additionally, RNA silencing in response to one virus may cross-protect against infection by another closely related virus. If a recombinant virus containing sequences homologous to a nuclear gene is used for infection, then silencing triggered by the virus will target the transcript of the nuclear gene. Conversely, RNA silencing triggered by a nuclear gene will target an incoming virus if that virus contains genome sequences homologous to the nuclear gene. Thus, viruses can be both triggers and targets of RNA silencing.

[1] To whom correspondence and reprint requests should be addressed. Fax: 509-335-2477. E-mail: carrington@wsu.edu.

MECHANISMS OF RNA SILENCING

A general mechanistic model for the cell autonomous steps of the RNA silencing pathway(s) derives from plant, animal, and fungal systems, as well as from *in vitro* systems using Drosophila cell extracts (Fig. 1). This model explains induction of silencing by diverse types of nuclear transgenes, dsRNA constructs, and viruses. Nuclear transgenes or DNA viruses that encode functional or aberrant transcripts may activate RNA silencing to various degrees, although the efficiency with which silencing is triggered may depend on the extent of secondary structure in the RNA transcript. The initiator mRNA is likely recognized by a cellular RNA-dependent RNA polymerase (RdRp)-like protein (termed SDE1/SGS2 in plants). *Arabidopsis thaliana* plants with *sde1/sgs2* mutations are RNA silencing-defective using mRNA-producing transgenes. Although exactly how the inducer RNA is recognized, transcribed, or processed by the RdRp is not clear, it is postulated that the complementary RNA product anneals with the template to form limited amounts of dsRNA. In fact, the critical role of dsRNA in the process is underscored by the high initiator activity of dsRNA in virtually all RNA silencing systems examined in detail. DsRNA-containing intermediates are likely recognized by a dsRNA-specific nuclease. Bass (2000) proposed that this dsRNase might belong to a class of proteins containing RNaseIII-like, helicase, and dsRNA binding domains. This nuclease would process the dsRNA-containing initiator molecules to small RNAs of 21–23 nucleotides in length. Such small RNAs that correspond to sense and antisense polarities of RNA silencing targets are consistently found in silencing cells and can be produced in the *in vitro* silencing systems that are programmed by dsRNA. The small RNAs are proposed to associate with either the initiator-specific dsRNase or a different RNase-like protein, converting the enzyme into a sequence-specific nuclease that recognizes targets via hybridization to the small RNAs. Thus, initiation of silenc-

0042-6822/01 $35.00

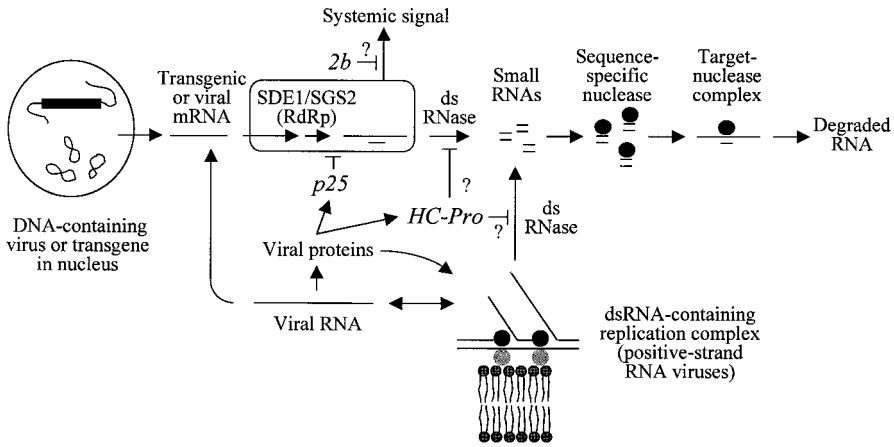

FIG. 1. RNA silencing pathways in plants. The key steps in RNA silencing of nuclear or viral RNAs are shown schematically. The steps that are proposed to be inhibited by the virus-encoded silencing suppressors 2b, p25, and HC-Pro (italicized) are shown. The boxed segment indicates the SDE1/SGS2-dependent, p25-sensitive part of the silencing pathway that also leads to production of the systemic silencing signal. A positive-strand RNA virus replication complex on a membrane surface exposed to the cytoplasm is shown. Double-stranded RNAs produced by either an SDE1/SGS2-dependent reaction or viral RNA replication are processed to small RNAs of 21–23 nucleotides in length by a dsRNase. These RNAs associate with the dsRNase or another nuclease-like protein to form the sequence-specific RNase that degrades target sequences.

ing with one inducer will result in sequence-specific cleavage of all RNAs containing high levels of sequence homology with the inducer.

RNA silencing triggered by replicating positive-strand RNA viruses shares several of the features described for silencing activated by nuclear genes. For example, small RNAs of 21–25 nucleotides corresponding to the viral genome are formed during infection, and sequence-specific RNA degradation occurs to inactivate RNAs with high homology to sequences within the viral genome. Positive-strand RNA viruses are particularly efficient triggers of RNA silencing. Indeed, recombinant viruses containing host gene sequences have been used effectively as functional genomics tools to induce knockout phenotypes in plants. Recent evidence suggests that silencing of RNA viruses may be initiated through a branch pathway that differs from that used for silencing of nuclear genes. At least one virus (tobacco rattle virus) can induce RNA silencing in Arabidopsis plants containing a *sde1/sgs2* mutation that inactivates RNA silencing of a nuclear gene. Also, a virus-encoded silencing suppressor (p25) appears to specifically affect cell autonomous silencing of a nuclear gene, but not a replicating virus (see below). These data suggest that replicating RNA viruses can trigger the effector stages (sequence-specific RNA degradation) of RNA silencing, but through a mechanism that bypasses the SDE1/SGS2-dependent steps. A reasonable hypothesis to explain these observations states that dsRNA formed during genome replication on cytoplasm-exposed surfaces of membranes serves directly as the substrate for the dsRNase that catalyzes formation of the small RNAs. In effect, the viral RdRp may functionally substitute for the cellular RdRp.

Plants also have a systemic signaling component to RNA silencing. Silencing induced in one part of a plant can trigger silencing in another part or in tissues across a graft union. Because silencing at distant sites is dependent on sequence homology with the inducer, a systemic, mobile signal containing nucleic acid corresponding to the silencing target is probably produced. Dissemination of the signal occurs by cell-to-cell movement through plasmodesmata linking adjacent cells and through the phloem which carries photoassimilates and macromolecules to distal tissues. Furthermore, transport of the signal appears to use the same intercellular route (plasmodesmata and phloem) as do viruses for cell-to-cell and long-distance movement (Fig. 2). Although the composition of the systemic signal has yet to be identified, analysis of a silencing suppressor protein from potato virus X (PVX) suggests that formation of the signal requires the SDE1/SGS2-dependent branch of the RNA silencing pathway.

RNA SILENCING SUPPRESSORS

Early evidence that viruses encode RNA silencing suppressor proteins came from experiments in which silenced transgenes in plants were reactivated after virus infection or after introduction of genes encoding candidate suppressor proteins using virus vectors or additional transgenes. Silencing suppressors have been identified from positive-strand RNA viruses and DNA-containing viruses. In a number of cases, silencing suppressors have general pathogenicity-enhancing activities. As a group, the plant viral silencing suppressors are diverse in sequence and evolutionary origin. They are also functionally diverse, with some targeting cell auton-

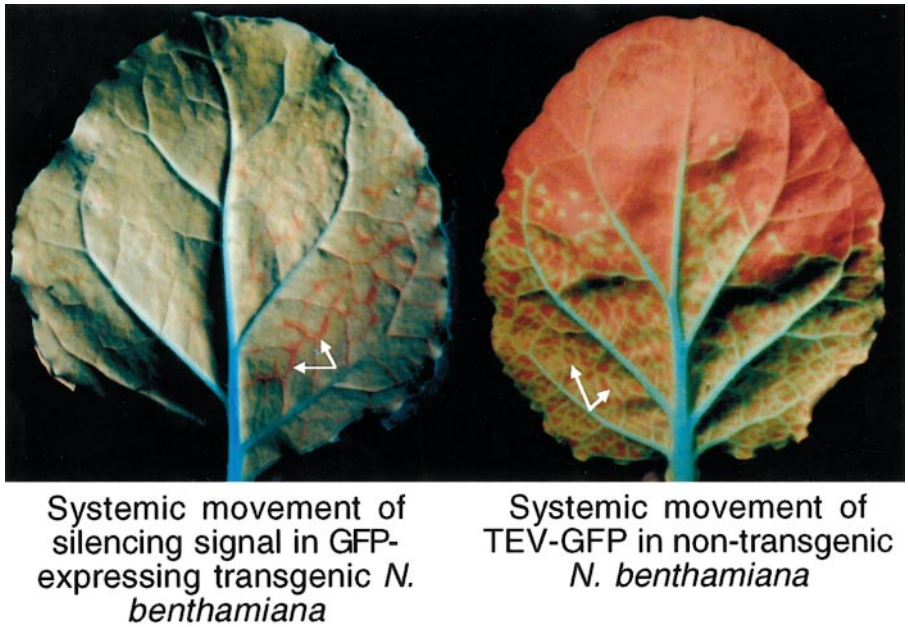

FIG. 2. Systemic signaling of RNA silencing and long-distance movement of viruses occur through the same intercellular route. Leaves from GFP-expressing transgenic or tobacco etch virus–GFP (TEV–GFP)-infected plants were photographed under long-wavelength UV illumination. Regions of the leaves that contain GFP fluorescence appear pale green, whereas regions lacking GFP appear red due to chlorophyll autofluorescence. (Left) Systemic RNA silencing of the GFP sequence in an upper leaf from a GFP-expressing transgenic plant was triggered by localized injection of a dsRNA inducer in lower leaves. The leaf shows uniform GFP expression, except in areas proximal to major and minor veins (arrows) due to RNA silencing that was triggered by systemic movement of a silencing signal. (Right) Systemic accumulation of TEV–GFP in an upper leaf from a nontransgenic plant that was inoculated in lower leaves. Note that both TEV–GFP and the silencing signal show similar patterns of systemic spread.

omous steps and others targeting systemic signaling steps.

Before focusing on specific silencing suppressors, it is important to consider the biological consequences of viral silencing suppression. At first glance, there would appear to be a conflict. On the one hand, many viruses encode suppressor proteins that arrest cell autonomous or signaling steps. On the other hand, many viruses that encode functional suppressors will trigger RNA silencing during infection. This apparent paradox may be explained, in some cases, by the step(s) in the silencing pathway affected by a particular suppressor. For example, a suppressor may interfere specifically with systemic signaling (see below) but not the cell autonomous reactions. In other cases, the suppressor may be shut off late in the infection cycle, or the silencing apparatus may simply overcome the suppressor if sufficient inducer RNA is produced. In any event, it seems reasonable to propose that silencing suppression might be a transient event during the infection process. A transient window of suppression might be sufficient to allow a virus to establish a systemic infection.

Potyviral HC-Pro

Numerous functions have been assigned to the potyviral HC-Pro protein. It has a cysteine-type proteinase domain, which catalyzes autoproteolytic cleavage between HC-Pro and the neighboring protein within the large viral polyprotein. Among other functions, HC-Pro is required for long-distance movement through the phloem and for maintenance of genome amplification. Tobacco etch potyvirus mutants with defects in the central region of HC-Pro are able to move cell to cell, but are restricted to initial infection foci in inoculated leaves. At the single cell level, these mutants are unable to maintain genome amplification, which shuts down prematurely. In addition, HC-Pro enhances pathogenicity and amplification levels of heterologous viruses. Each of these properties is likely the consequence of the RNA silencing suppressing function of HC-Pro.

HC-Pro targets a cell autonomous step that is necessary for maintenance of silencing triggered by both replicating viruses and transgenes. RNA silencing is reversed in cells that express HC-Pro, regardless of whether HC-Pro is delivered by injection of a transgene, by a virus vector, or by a genetic cross. The silencing-associated small RNAs from a silenced transgene are absent when HC-Pro is introduced, suggesting that HC-Pro targets a step coincident with, or upstream of, production of small RNAs. One possibility is that HC-Pro inhibits the dsRNase, or a factor required for dsRNase activity, that is required for small RNA production from both silenced transgene and viral RNAs (Fig. 1). Recently, HC-Pro was shown to interact with a calmodulin-related

cellular protein, which itself functions as an RNA silencing suppressor. This raises the possibility that HC-Pro functions indirectly by influencing a calcium-dependent regulator of RNA silencing. The finding of a cellular protein with suppressor activity also provokes the idea that viral suppressor genes originated in host organisms and were captured through host–virus recombination.

Cucumoviral 2b

Infection of plants containing a silenced reporter transgene with cucumber mosaic virus (CMV) results in silencing suppression, but only in newly emerged tissues that develop after infection. In contrast to plants infected by potyviruses, mature tissues that are silenced at the time of CMV infection remain silenced after infection, indicating that potyviruses and cucumoviruses likely target different components of the silencing response. The CMV 2b protein, a nuclear protein that is required for long-distance movement of the virus, functions as the silencing suppressor. The fact that 2b suppresses silencing only in newly emerged tissue after infection suggests that it targets either an initiation or a signaling step in the silencing pathway (Fig. 1). Mechanistic details for how 2b suppresses silencing are lacking.

Potexviral p25

The p25 protein of the potexvirus, PVX, is one of three cell-to-cell movement proteins (MPs) required for transport of virus from one cell to the next. Transport occurs through plasmodesmata, intercellular channels that traverse the cell wall and provide cytoplasmic and endomembrane continuity between cells. The three MPs are proposed to coordinate the interaction between encapsidated PVX and plasmodesmata, to modulate the aperture or permissivity of the channel, and to facilitate movement of virus to the adjacent cell. Fusion proteins containing the p25 sequence linked to a GFP reporter are able to move cell to cell after introduction (by microprojectile bombardment) into leaves, suggesting that p25 possesses plasmodesmal transport functions. The p25 protein catalyzes hydrolysis of ATP *in vitro* and contains motifs that resemble superfamily I-type RNA helicases.

The effects of p25 on cell autonomous and systemic silencing events have been tested using two types of inducers, a GFP gene under the control of a strong 35S promoter (35S-GFP) and replicating PVX–GFP recombinant viruses, after injection into lower leaves of transgenic *Nicotiana benthamiana* plants expressing a functional GFP transgene. Interestingly, p25 suppresses local, cell autonomous silencing (in the infiltration zone) with the 35S-GFP gene, but not with replicating PVX–GFP. Further, p25 suppresses systemic silencing triggered by both 35S-GFP and PVX–GFP inducers. These data suggest that production of the systemic silencing signal is a p25-sensitive step and that the signal requires the transgene inducer pathway regardless of whether the inducer is a transgene or a replicating virus. Perhaps viral genomic RNA or viral mRNA can trigger silencing (both local and systemic) through the SDE1/SGS2-dependent pathway, whereas the dsRNA-containing viral replication intermediates can trigger silencing (local only) through the direct route (Fig. 1). Whether p25 suppresses production of the systemic signal or a step upstream from signal production is not known. However, the fact that a viral protein inhibits the pathway leading to systemic signaling strongly implies that the systemic arm of the silencing response is part of the antiviral defense mechanism.

Given the large number of viruses in plants, there is a high likelihood that additional classes of silencing suppressors will be identified. The major challenges now lie in understanding how they function to interdict the silencing pathways. Finally, viewing RNA silencing as a process that occurs in animals as well as plants begs the following question: To what extent does RNA silencing contribute to antiviral defense in animals? The next few years promise some excitement as the roles of RNA silencing and silencing suppression in plants and animals are further defined.

Note added in proof. Bernstein *et al.* (*Nature* **409,** 363–366) recently identified Dicer, a silencing-associated dsRNase that catalyzes production of 22-nucleotide RNAs, using an extract prepared from *Drosophila* embryos. Dicer is biochemically distinct from the mRNA-degrading activity. Dicer contains helicase-like and RNase III signature domains and has similarity to the Zwille/ARGONAUTE/Piwi family of proteins.

REFERENCES

Review of RNA Silencing Mechanisms

Bass, B. L. (2000). Double stranded RNA as a template for gene silencing. *Cell* **101,** 235–238.

Factors Involved in RNA Silencing in Plants

Dalmay, T., Hamilton, A., Rudd, S., Angell, S., and Baulcombe, D. C. (2000). An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* **101,** 543–553.

Fagard, M., Boutet, S., Morel, J., Bellini, C., and Vaucheret, H. (2000). AGO1, QDE-2, and RDE-1 are related proteins required for posttranscriptional gene silencing in plants, quelling in fungi and RNA interference in animals. *Proc. Natl. Acad. Sci. USA* **97,** 11650–11654.

Hamilton, A. J., and Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286,** 950–952.

Mourrain, P., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Jouette, D., Lacombe, A. M., Nikic, S., Picault, N., Remoue, K., Sanial, M., Vo, T. A., and Vaucheret, H. (2000). Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* **101,** 533–542.

Waterhouse, P. M., Graham, M. W., and Wang, M. B. (1998). Virus

resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci. USA* **95,** 13959–13964.

Systemic RNA Silencing

Palauqui, J. C., Elmayan, T., Pollien, J. M., and Vaucheret, H. (1997). Systemic acquired silencing: Transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J.* **16,** 4738–4745.

Voinnet, O., and Baulcombe, D. C. (1997). Systemic signaling in gene silencing. *Nature* **389,** 553–555.

Silencing of Viruses and Viral Silencing Suppressors

Al-Kaff, N. S., Covey, S. N., Kreike, M. M., Page, A. M., Pinder, R., and Dale, P. J. (1998). Transcriptional and posttranscriptional plant gene silencing in response to a pathogen. *Science* **279,** 2113–2115.

Anandalakshmi, R., Marathe, R., Ge, X., Herr, J. M., Mau, C., Mallory, A., Pruss, G., Bowman, L., and Vance, V. B. (2000). A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. *Science* **290,** 142–144.

Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Smith, T. H., and Vance, V. B. (1998). A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. USA* **95,** 13079–13084.

Brigneti, G., Voinnet, O., Wan-Xiang, L., Ding, S. W., and Baulcombe, D. C. (1998). Viral pathogenicity determinants are suppressors of transgene silencing. *EMBO J.* **17,** 6739–6746.

Kasschau, K. D., and Carrington, J. C. (1998). A counter-defensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. *Cell* **95,** 461–470.

Ratcliff, F., Harrison, B. D., and Baulcombe, D. C. (1997). A similarity between viral defence and gene silencing in plants. *Science* **276,** 1558–1560.

Ratcliff, F. G., MacFarlane, S. A., and Baulcombe, D. C. (1999). Gene silencing without DNA. RNA-mediated cross-protection between viruses. *Plant Cell* **11,** 1207–1216.

Voinnet, O., Lederer, C., and Baulcombe, D. C. (2000). A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana. Cell* **103,** 157–167.

Voinnet, O., Pinto, V. M., and Baulcombe, D. C. (1999). Suppression of gene silencing: A general strategy used by diverse DNA and RNA viruses of plants. *Proc. Natl. Acad. Sci. USA* **96**(24), 14147–14152.