

INVITED REVIEW

**VIRUS INDUCED RNA SILENCING AND SUPPRESSION:
DEFENCE AND COUNTER DEFENCE****J. Burgyán***Agricultural Biotechnology Center, Plant Biology Institute, P.O. Box 411, H-2101 Gödöllő, Hungary***SUMMARY**

In plants, the mechanism of RNA silencing has evolved to defend plants against viral infection as well as to regulate of gene expression for growth and development. However, viruses counteract this antiviral defence by expressing silencing suppressor proteins, which are potent weapons in the 'arms' race between plants and invading viruses. These proteins efficiently inhibit RNA silencing by interacting with various steps of the different silencing pathways and these mechanisms of suppression are being unravelled progressively. Our better understanding the molecular bases of the induction and the suppression of RNA silencing dramatically improved our basic knowledge about intimate plant-virus interactions and also provide valuable tools to unravel the diversity, regulation and evolution of RNA-silencing pathways.

Key words: RNA silencing, VIGS, plant virus silencing suppressors, mechanism of silencing suppression, siRNA.

INTRODUCTION

RNA silencing is a eukaryotic gene regulatory system that inhibits gene expression through RNA-mediated sequence-specific interactions. It is conserved across kingdoms and is manifested as quelling in fungi, RNA interference (RNAi) in animals and co-suppression or post-transcriptional gene silencing (PTGS) in plants. RNA silencing is a potent defensive system against parasitic nucleic acids such as transposons or viruses and operates as such in both plants and animals (Voinnet, 2005a).

In higher plants there are at least three RNA silencing pathways, which are involved in antiviral defence, regulation of plant gene expression and the condensation of chromatin into heterochromatin (Baulcombe,

2004). The antiviral arm of RNA silencing acts as an adaptive defence system and operates at cell-autonomous and non cell-autonomous levels, the latter probably being responsible for protection of the whole plant against virus invasion. RNA silencing is efficiently triggered by double-stranded RNA structures and the activation of RNA silencing by viruses leads to sequence-specific degradation of the genome of the inducer viral RNAs. Because the majority of known plant viruses have RNA genomes, replicate via dsRNA intermediates and single-stranded viral genome RNA forms secondary structures, it is not surprising that plant viruses are strong inducers as well as targets of Virus Induced Gene Silencing (VIGS, Fig. 1).

VIGS also operates against DNA viruses, which may form dsRNA by the annealing of overlapping complementary transcripts or by conversion of single-stranded viral RNAs to dsRNA by plant-encoded RNA-dependent RNA polymerase (RDR). In plants, virus induced gene silencing prevents virus accumulation and, in many instances, viruses replicating in plant cells have evolved a variety of strategies to counteract this antiviral defence mechanism. The most important counterdefensive strategy of plant viruses involves suppressor proteins of silencing, which are encoded in both RNA and DNA viral genomes (Silhavy and Burgyan, 2004; Voinnet, 2005a).

Many silencing suppressor proteins have been identified in almost all viral genomes, but these proteins are structurally diverse and often lack any common sequence motifs. In plants, RNA silencing pathways involved in antiviral defence and plant development intersect. Since silencing suppressor proteins expressed by viruses counteract with antiviral defence it is likely that viral suppressors impair, as a side effect, other gene silencing pathways involved plant gene regulations (Silhavy and Burgyan, 2004; Voinnet, 2005a). This perturbation of endogenous gene expression could lead to abnormal plant phenotypes (symptoms of virus infection), which would provide an answer at molecular level to the long-standing question of how viruses induce such symptoms.

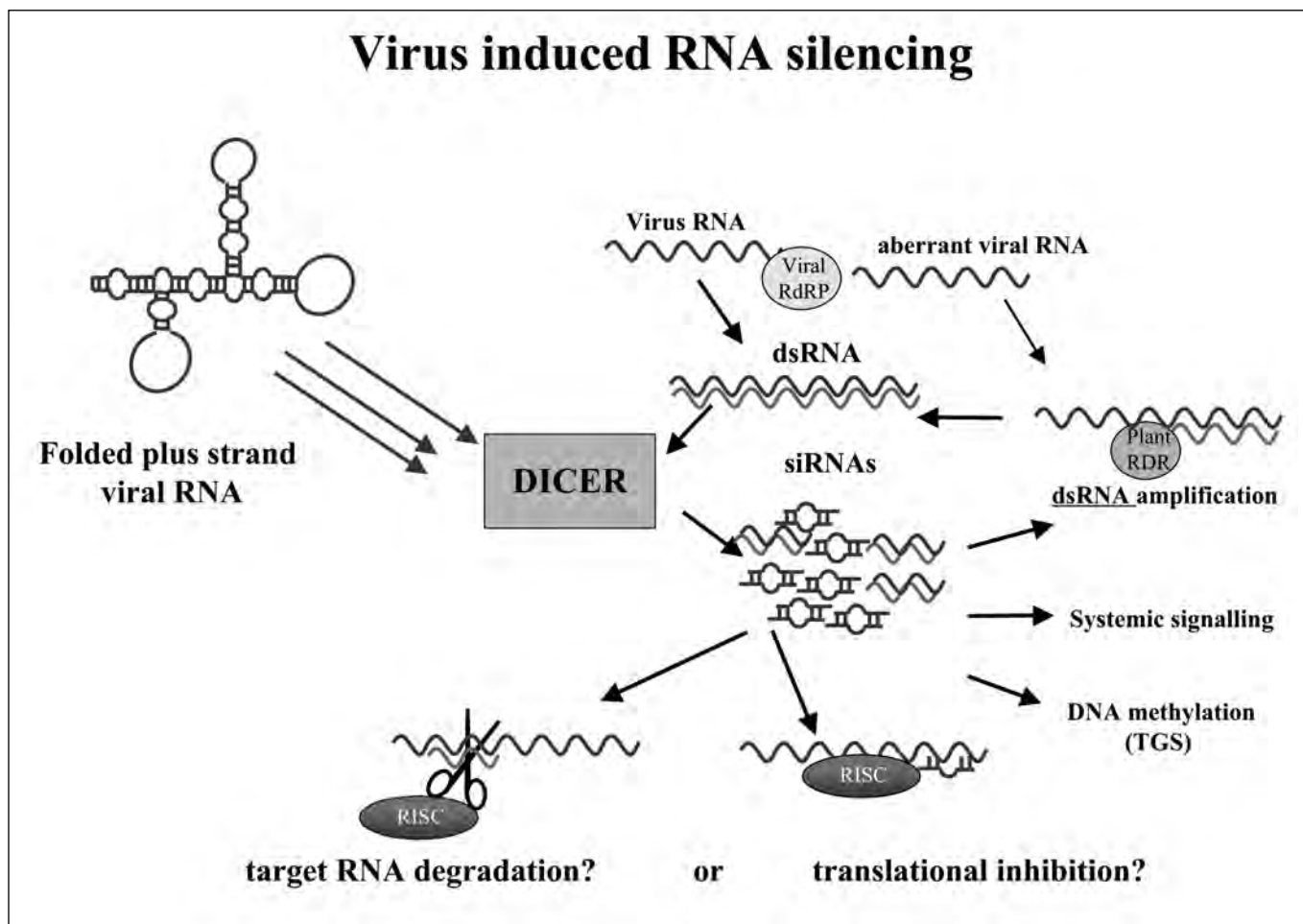


Fig. 1. Simplified model for plant RNA silencing. Virus induced RNA silencing is initiated by dicing of double-stranded (ds) or highly structured viral RNAs into 21-24 nt siRNAs. Ds viral RNAs can be produced by viral RdRP or plant RDR and then diced to small ds siRNAs. Viral siRNAs activate RISC complex for target cleavage or translational arrest and may also guide plant RDR to amplify dsRNA, which are diced again to siRNAs. These siRNAs are also responsible for systemic signalling and transcriptional gene silencing (TGS).

MECHANISM OF RNA SILENCING

The common feature of RNA silencing is the presence of 21- to 24-nucleotide (nt) small interfering (si) RNAs (Hamilton and Baulcombe 1999; Hamilton *et al.*, 2002; Plasterk, 2002). Biochemical and genetic analyses have shown that the core mechanisms of RNA silencing are shared among different eukaryotes (Plasterk, 2002; Voinnet, 2002; Zamore, 2002; Baulcombe, 2004; Hannon and Conklin, 2004; Meister and Tuschl, 2004). RNA silencing is triggered by ds or self-complementary foldback RNAs that are processed into 21-24 nt siRNA or microRNA (miRNA) duplexes by the RNase III-type DICER enzymes (Bernstein *et al.*, 2001; Nykanen *et al.*, 2001). These small RNAs guide the sequence-specific inactivation of a target mRNA *via* an RNA-induced silencing complex (RISC) (Hammond *et al.*, 2000). As a general rule, RISC mediate cleavage of target mRNA when there is perfect or near perfect base pairing be-

tween the mRNA and the short guide RNA or, when there is partial complementarity, translation repression (Hutvagner and Zamore, 2002; Aukerman and Sakai, 2003; Chen, 2003; Doench *et al.*, 2003). SiRNAs can also guide another effector complex, the RNA-induced transcriptional gene silencing (RITS) complex to direct the chromatin modification of homologous DNA sequences (Verdel *et al.*, 2004).

Regardless of its origin, the appearance of dsRNA in the cytoplasm of plant cells induces RNA silencing. It has been demonstrated previously that an RNA-dependent RNA polymerase (RDR6) is involved also in RNA silencing (Dalmay *et al.*, 2000; Mourrain *et al.*, 2000), presumably by converting target ssRNA into dsRNA, which are then processed by DICER to generate 21 nt siRNAs. These siRNAs program RISC for target cleavage and are also involved in both the short-range and long-range spread of RNA silencing (Himber *et al.*, 2003; Voinnet, 2005b).

MicroRNA (miRNA) are another class of small endogenous regulatory RNAs that control gene expression in plants and animals. These approximately 21-nucleotide RNAs are processed by a Dicer-like 1 enzyme (DCL1) from stem-loop regions of long primary transcripts. Then miRNAs are loaded into RISC, which generally cleaves the complementary mRNAs thereby modulating – in a sequence-specific manner – the expression of these transcripts, a process that has been implicated in the control of cell differentiation, development and probably many other cellular functions (Bartel, 2004).

A third class of small RNAs, the termed “*trans*-acting (ta-) siRNAs”, are processed by DCL4 from long ds RNA produced by RDR6. These ta-siRNAs mainly target the expression of other genes rather than their own expression (Peragine *et al.*, 2004; Vazquez *et al.*, 2004; Allen *et al.*, 2005; Dunoyer *et al.*, 2005; Gascioli *et al.*, 2005).

In plants, the biogenesis of small RNAs (siRNA, miRNA, and ta-siRNA) has an additional step whereby they are methylated on the ribose of the last nucleotide by the methyltransferase HEN1 (Chen, 2005).

DISCOVERY OF VIRUS INDUCED GENE SILENCING AND VIRAL SILENCING SUPPRESSORS

Probably the first report of virus induced gene silencing was published as long ago as 1929 by McKinney. In this paper, McKinney reported that tobacco plants infected with the “green” strain of *Tobacco mosaic virus* (TMV) were protected against infection by a closely related second virus (TMV “yellow” strain). This phenomenon was later described as “cross-protection” and several models have been developed in the past to explain this mechanism (Hull, 2002). However, a well supported explanation of cross-protection was given less than a decade ago when it was shown that virus infection prevents infection by a second virus if there are homologous sequences between the two viruses. Importantly, this virus RNA-mediated cross-protection was functionally equivalent to post-transcriptional gene silencing (Ratcliff *et al.*, 1999).

Interestingly, it is turned out that synergism (when one virus infection enhances the severity of another virus infection), another well known but poorly understood phenomenon in plant virology, is also related to virus induced gene silencing. More precisely, it is related to the suppression of virus induced gene silencing. Study of the potexvirus–potyvirus synergistic interaction led to the identification of the potyviral Helper component proteinase (HC-Pro) as the synergism determinant in this interaction (Pruss *et al.*, 1997). Subsequently HC-Pro and another viral protein (2b of *Cucumber mosaic virus*) were identified by different labora-

tories (Anandalakshmi *et al.*, 1998; Brigneti *et al.*, 1998; Kasschau and Carrington, 1998) as the first silencing suppressor proteins.

The discovery of silencing suppressor activity of HC-Pro and 2b strongly suggested that potyviruses and cucumoviruses induce an RNA silencing response that could generally restrict virus accumulation in infected plants. HC-Pro and 2b had been previously characterized as pathogenicity determinants required for efficient virus accumulation in infected plants, although they were not required for virus replication. This link between silencing suppressors and pathogenicity determinants inspired the reinvestigation of other pathogenicity determinants leading to the discovery of many other silencing suppressor proteins encoded by both RNA and DNA viruses (Table 1) (Voinnet *et al.*, 1999; Li and Ding, 2001; Silhavy and Burguán, 2004).

MECHANISM OF VIRUS INDUCED GENE SILENCING

Plant viruses are known to be strong inducers, as well as targets, of virus induced gene silencing (Fig. 1). The first observation which provided evidence that viruses are not only targets of transgene induced RNA silencing but also elicit silencing themselves was reported by Lindbo *et al.* (1993). The authors showed that transgenic plants expressing a truncated version of the coat protein of *Tobacco etch virus* (TEV) were initially susceptible to TEV infection and showed symptoms. However, a few weeks after the transgenic plants recovered from the TEV infection, newly developed leaves were symptomless and virus-free. Strikingly the recovered leaves were resistant against a second TEV infection but were susceptible to infection by the heterologous *Potato virus Y* (PVY).

The recovery of a plant from natural virus infection had been well-known for long time, however, the explanation for the phenomenon responsible was first provided only in the late 1990s. Ratcliff *et al.* (1997) elegantly demonstrated that the recovered leaves of nepovirus-infected plants are resistant to infection by a second virus bearing sequences homologous to sequences in the first virus and the mechanism of resistance was shown to be similar to transgene-induced gene silencing. They also suggested that viruses are potentially both initiators and targets of gene silencing.

The discovery of 21 nt siRNAs that provide the sequence specificity of RNA silencing was one of the most important steps in the exploration of RNA silencing. The accumulation of virus derived siRNAs – the hallmark of gene silencing – in virus-infected tissues was also demonstrated (Hamilton and Baulcombe, 1999; Szittyá *et al.*, 2002) indicating the activation of VIGS. High levels of siRNA correlate with the activity of VIGS

Table 1. RNA silencing suppressor proteins encoded by plant viruses.

Virus genera	Viruses	Suppressors and mechanism of action	Other functions	References
Positive-strand RNA viruses				
<i>Carmovirus</i>	<i>Turnip Crinkle virus</i>	P38, DRB	Coat protein	Merai, 2006 Thomas <i>et al.</i> , 2003
<i>Cucumovirus</i>	<i>Cucumber mosaic virus</i> ; <i>Tomato aspermy virus</i>	2b, NI	Host-specific movement	Brigneti <i>et al.</i> , 1998
<i>Closterovirus</i>	<i>Beet yellows virus</i>	P21, SRB	Replication enhancer	Chapman <i>et al.</i> , 2004
	<i>Citrus tristeza virus</i>	P20, NI P23, NI CP	Replication enhancer Nucleic-acid binding Coat protein	Reed <i>et al.</i> , 2003 Lu <i>et al.</i> , 2004
<i>Comovirus</i>	<i>Cowpea mosaic virus</i>	S protein	Small coat protein	Liu <i>et al.</i> , 2004b
<i>Hordeivirus</i>	<i>Barley yellow mosaic virus</i>	γb, SRB	Replication enhancer; movement; Seed transmission; pathogenicity determinant	Merai, 2006 Yelina <i>et al.</i> , 2002
<i>Pecluvirus</i>	<i>Peanut clump virus</i>	P15, SRB	Movement	Dunoyer <i>et al.</i> , 2002 Merai, 2006
<i>Polerovirus</i>	<i>Beet western yellows virus</i> ; <i>Cucurbit aphid-borne yellows virus</i>	P0, PPI	Pathogenicity determinant	Pazhouhandeh <i>et al.</i> , 2006 Pfeffer <i>et al.</i> , 2002
<i>Potexvirus</i>	<i>Potato virus X</i>	P25	Movement	Voinnet <i>et al.</i> , 2000
<i>Potyvirus</i>	<i>Potato virus Y</i> ; <i>Tobacco etch virus</i> ; <i>Turnip yellows virus</i>	HC-Pro, SRB	Movement; polyprotein processing; aphid transmission; pathogenicity determinant	Anandalakshmi <i>et al.</i> , 1998; Brigneti <i>et al.</i> , 1998; Kasschau and Carrington, 1998; Lakatos, 2006; Voinnet <i>et al.</i> , 1999
<i>Sobemovirus</i>	<i>Rice yellow mottle virus</i>	P1, NI	Movement; pathogenicity determinant	Voinnet <i>et al.</i> , 1999
<i>Tombusvirus</i>	<i>Tomato bushy stunt virus</i> ; <i>Cymbidium ringspot virus</i> ; <i>Carnation Italian ringspot virus</i>	P19, SRB	Movement; pathogenicity determinant	Silhavy and Burgyan, 2004 Silhavy <i>et al.</i> , 2002
<i>Tobamovirus</i>	<i>Tobacco mosaic virus</i> ; <i>Tomato mosaic virus</i>	P30, NI	Replication	Kubota <i>et al.</i> , 2003
<i>Tymovirus</i>	<i>Turnip yellow mosaic virus</i>	P69, NI	Movement; pathogenicity determinant	Chen <i>et al.</i> , 2004
Negative-strand RNA viruses				
<i>Tospovirus</i>	<i>Tomato spotted with virus</i>	NSs, NI	Pathogenicity determinant	Bucher <i>et al.</i> , 2003
<i>Tenuivirus</i>	<i>Rice hoja blanca virus</i>	NS3, NI	Unknown	Bucher <i>et al.</i> , 2003
Double-strand RNA viruses				
<i>Phytoreovirus</i>	<i>Rice dwarf virus</i>	Pns10, NI	Unknown	Cao <i>et al.</i> , 2005
DNA viruses				
<i>Begomovirus</i>	<i>African cassava mosaic virus</i>	AC4, miRB AC2	Putative synergistic genes Transcriptional activator protein	Chellappan <i>et al.</i> , 2005 Chellappan <i>et al.</i> , 2005
	<i>Tomato yellow leaf curl virus</i>	C2	(TrAP)	Voinnet <i>et al.</i> , 1999

(a)DRB, dsRNA binding; SRB, siRNA binding; miRB, miRNA binding; NI, not identified.

resulting in lower viral titre and in some cases, immunity or recovery in upper non-inoculated leaves (Ratcliff *et al.*, 1997; Szittyta *et al.*, 2002). Thus VIGS acts as an

RNA-mediated defence response to protect plants against viral infection (Moissiard and Voinnet, 2004; Voinnet, 2005a).

APPLICATION OF VIGS

Shortly after the discovery of virus induced gene silencing this phenomenon was refined and adapted to develop a technology for high throughput functional genomics (Ruiz *et al.*, 1998; Baulcombe, 1999). This new approach employs virus vectors that carry sequences from endogenous plant genes. The infection of plant with these recombinant viruses results in the silencing of not only the viral genomes but also host genes highly similar to the inserted endogenous plant gene sequences. The consequence of silencing a plant gene is the development of a phenocopy of a mutant plant deficient in the corresponding plant gene/s. Using this technology one can rapidly obtain information about the function of the silenced gene.

The first plant virus based gene vectors were TMV (Donson *et al.*, 1991) and *Potato virus X* (PVX) (Chapman *et al.*, 1992). These vectors were initially developed to express foreign proteins in plants and only several years later were used as potent tools to induce silencing in functional genomics studies (Ruiz *et al.*, 1998; Faivre-Rampant *et al.*, 2004). The limitation of VIGS technology is that a virus vector can be used only in plants that are hosts of the virus used. For example the first VIGS vectors (e.g.: PVX) do not infect the model plant *Arabidopsis thaliana*. Therefore new vectors were developed to overcome this difficulties such as the *Tobacco rattle virus* (TRV)-based vector (Ratcliff *et al.*, 2001), which efficiently infects many dicotyledonous plant species including tomato, potato, pepper and *A. thaliana* (Liu *et al.*, 2002a; Lu *et al.*, 2003; Brigneti *et al.*, 2004; Chung *et al.*, 2004). VIGS vector was also developed for monocotyledonous plants (Holzberg *et al.*, 2002b) and used successfully to identify genes associated with powdery mildew resistance in barley (Hein *et*

al., 2005). In addition to RNA virus-based vectors, DNA virus-based VIGS vectors have also been developed using *Tomato golden mosaic virus* (TGMV) (Kjemtrup *et al.*, 1998) and *Cabbage leaf curl virus* (CaLCuV) (Turnage *et al.*, 2002).

The most important VIGS vectors are listed in Table 2. More detailed description of these VIGS vectors and their applications were recently reviewed by Burch-Smith *et al.* (Burch-Smith *et al.*, 2004) and Muangsan & Robertson (Muangsan and Robertson, 2004). Interestingly, the technology based on VIGS was more frequently used to analyse the functions of genes associated with disease resistance than those of other plant genes implicated in other cellular processes. This may be due to the fact that scientists working on plant resistance genes are more willing to use plant viruses as tools.

VIRAL SILENCING SUPPRESSORS AND THE MOLECULAR BASES OF SILENCING SUPPRESSIONS

For successful infection, plant viruses have to evade or suppress RNA silencing. Indeed, one of the most compelling pieces of evidence for the antiviral function of RNA silencing is that many of plant viruses have evolved proteins that suppress various steps of the silencing machinery (Voinnet *et al.*, 1999; Li and Ding, 2001; Silhavy and Burguán, 2004; Silhavy *et al.*, 2002; Voinnet, 2005a).

Theoretically, viral suppressor proteins can counteract RNA silencing-mediated defence at three steps (i) preventing the generation of siRNAs, (ii) inhibiting the incorporation of siRNAs into effector complexes, (iii) interfering with one of the effector complexes.

So far, more than a dozen silencing suppressors (Table 1) have been identified from different types of

Table 2. The most often used VIGS vectors.

^(a) Vectors	Plant Species	Vector induced symptoms	Developed for large scale analysis	Reference
TVM	<i>N. benthamiana</i>	Variable	Yes	Lacomme <i>et al.</i> , 2003 Fitzmaurice <i>et al.</i> , 2002
PVX	<i>N. benthamiana</i>	variable	Yes	Escobar <i>et al.</i> , 2003
TRV	<i>N. benthamiana</i> , <i>Arabidopsis</i>	Mild	Yes	Liu <i>et al.</i> , 2002b; Ratcliff <i>et al.</i> , 2001
	Tomato	Mild	Yes	Liu <i>et al.</i> , 2002b
SVISS	Tobacco	Mild	No	Gossele <i>et al.</i> , 2002
BSMV	Barley	Moderate	Yes	Holzberg <i>et al.</i> , 2002a; Lacomme <i>et al.</i> , 2003 Fitzmaurice <i>et al.</i> , 2002
CbLCV	<i>Arabidopsis</i>	variable	No	Turnage <i>et al.</i> , 2002
TGMV	<i>N. benthamiana</i>	Variable	No	Peele <i>et al.</i> , 2001

^(a)TMV, *Tobacco mosaic virus*; PVX, *Potato virus X*; TRV, *Tobacco rattle virus*; SVISS, TMV satellite virus-induced silencing system; BSMV, *Barley stripe mosaic virus*, CbLCV, *Cabbage leaf curl virus*; TGMV, *Tomato golden mosaic virus*.

viruses, including positive strand RNA, negative strand RNA and ss DNA viruses. Strikingly, no sequence homology has been detected between distinct silencing suppressors. This diversity of silencing suppressor proteins suggests that these viral proteins – having different other functions in the virus life cycle (Table 1) – probably evolved independently in different virus groups. Consistently, it was suggested that different suppressors inhibit silencing mechanisms at different steps.

Although our knowledge is still limited about the mechanism of the silencing suppression by different suppressors, few members of silencing suppressor protein families have been studied in detail. An early report showed that the potyvirus-encoded helper component proteinase (HC-Pro) enhances the replication of unrelated viruses (Pruss *et al.*, 1997). Indeed, HC-Pro was the first viral protein identified as a suppressor of transgene- and virus-induced RNA silencing. Analyses of data from various experimental systems led to the development of several different models for the mechanism of HC-Pro silencing suppression.

It was proposed that HC-Pro reverses established RNA silencing by acting on RISC (Anandalakshmi *et al.*, 1998; Brigneti *et al.*, 1998; Voinnet *et al.*, 1999) involving rgs-CaM, a calmodulin-related protein that is a cellular negative regulator of silencing (Anandalakshmi *et al.*, 2000). Other observations suggested that HC-Pro acts downstream of an RNA-dependent RNA polymerase impairing the DICER activity (Mallory *et al.*, 2001; Dunoyer *et al.*, 2004).

A recent comparative study of different silencing suppressor proteins predicted that RISC activation was suppressed through interaction between HC-Pro and a protein or complex required for siRNA duplex unwinding (Chapman *et al.*, 2004).

The tombusviral 19kDa protein (p19) is one of the best-studied silencing suppressors so far. Indeed recent advances in understanding the molecular mechanism underlying p19 suppressor activity revealed that p19 specifically binds 21 nt ds siRNAs *in vitro* and *in vivo*, preventing siRNA incorporation into effector complexes such as RISC (Silhavy *et al.*, 2002; Lakatos *et al.*, 2004). This model was well supported by the three-dimensional X-ray crystal structure of a p19-siRNA complex, which revealed that a p19 homo-dimer acts as a ds RNA calliper binding the ends of the siRNA duplex while measuring its length (Vargason *et al.*, 2003; Ye *et al.*, 2003). Moreover, Lakatos *et al.* (2006) (Lakatos *et al.*, 2006) showed that p19 can only prevent the assembly of RISC by sequestering ds siRNA or miRNA duplexes, however once the RISC complex assembled, p19 had no more effect on it. The reason for this is that p19 is not able to bind the ss siRNA or miRNA that guide the assembled RISC for target cleavage.

The third well-studied suppressor is p21 of *Beet yellows virus* (BYV). The molecular base of the mechanism

of p21 silencing suppression was found to be very similar to that of p19. It was shown that p21 inhibits silencing pathways by binding siRNAs or ds miRNA intermediates (Chapman *et al.*, 2004; Voinnet, 2005a).

Our very recent *in vivo* and *in vitro* studies have further clarified the mode of action of these silencing suppressors (HC-Pro, p19 and p21). It was demonstrated unequivocally that HC-Pro, like p19 and p21, impairs RNA silencing by si- and miRNA sequestration, which results in the inhibition of siRNA guided RISC assembly (Lakatos *et al.*, 2006).

Since these suppressors bind si and miRNA duplexes, one might expect that they interfere with the 3' methylation of si- and miRNAs. Indeed, recent results support this hypothesis. Transgenic expression of the HC-Pro results in a marked decrease in the 3' terminal modification of viral siRNAs but does not significantly affect the modification of endogenous miRNAs (Ebhardt *et al.*, 2005). Surprisingly, the effects of p19 and HC-Pro on 3' terminal methylation were clearly different and may reflect subtle differences as to how and perhaps where these suppressors bind and sequester small RNA duplexes (L. Lakatos *et al.*, unpublished information).

Silencing inhibition through siRNA sequestration seems advantageous, as production of siRNAs is a conserved element of the antiviral silencing in any host. p19, p21 and HC-Pro are structurally and evolutionarily unrelated proteins, each representing a small protein family specific to its respective viral taxon (Koonin *et al.*, 1991; Reed *et al.*, 2003; Vargason *et al.*, 2003; Ye *et al.*, 2003; Dolja *et al.*, 2006). Although only a limited number of silencing suppressors have been proved unequivocally to bind small RNA duplexes (Chapman *et al.*, 2004; Dunoyer *et al.*, 2004; Lakatos *et al.*, 2004; 2006), there are several other silencing suppressor proteins that are suggested to be siRNA-binding proteins, such as p14 of *Pothos latent virus*, 2b protein of *Cucumber mosaic virus*, p38 of *Turnip crinkle virus*, p15 *Peanut clump virus* and γ B3 of *Barley stripe mosaic virus* (Méraï, 2006). Thus the siRNA duplex-binding mechanism represents a recurring strategy that has evolved independently in several virus families (e.g.: *Tombusviridae*, *Potyviridae*, *Bromoviridae* *Closteroviridae*) within the positive-strand RNA viruses.

Although, it seems that siRNA sequestration is a widely used strategy to suppress RNA silencing there are also other known or predicted mechanisms of silencing suppression. P0 RNA silencing suppressor protein of the polerovirus *Beet western yellows virus* (BWYV) acts as an F-box protein that targets an essential component of silencing machinery. It was suggested that P0 interacts with its substrate protein to assign it for ubiquitination and then for degradation (Pazhouhandeh *et al.*, 2006).

The coat protein of *Turnip crinkle virus* (TCV) was suggested to inhibit DICER activities (Qu *et al.*, 2003), which was confirmed by Méraï *et al.*, (2006) who showed

that the CP of TCV is a ds RNA binding protein, which probably interacts with either short or long virus-derived ds RNA. A more recent report demonstrated that p38 specifically inhibits DCL4 activity (Deleris *et al.*, 2006).

The A-AC4 protein of a geminivirus was shown to bind mature miRNAs and it was predicted that A-AC4 recruits the mature miRNAs by interacting with one or more cellular factors that are associated with the RISC-loading complex or RISC (Chellappan *et al.*, 2005).

These alternative ways of suppressing silencing demonstrate the complexity of how viruses have evolved distinct mechanisms to modify the cell system to allow virus replication in plants.

ROLE OF VIRAL SUPPRESSORS IN VIRUS INDUCED SYMPTOMS

Although many viral suppressors (Table 1) were previously identified as pathogenesis determinants that are largely responsible for virus-induced symptoms, the molecular basis for virus-induced disease in plants has been a long-standing mystery. Recent advances in our understanding of the mechanism of silencing suppression provide a better insight into the molecular mechanism of virus-induced symptoms. It is well established that the antiviral and endogenous silencing pathways share common elements (e.g.: endogenous small regulatory RNAs such as si-, tasi- and ds miRNA intermediates) and silencing suppressors often interact with these common elements. Thus, it was reasonable to predict that many virus-induced symptoms are the consequences of the interaction of silencing suppressors and endogenous RNA silencing-mediated developmental pathways that share components with the antiviral RNA silencing. Indeed, HC-Pro of *Turnip mosaic virus* (TuMV) was shown to inhibit both endogenous miRNA-, and siRNA-mediated gene regulations, which resulted in the overexpression of miRNA targeted genes in transgenic plants expressing HC-Pro resulting in a phenotype resembling TuMV-infected plants (Kasschau *et al.*, 2003; Dunoyer *et al.*, 2004).

THE ROLE OF SILENCING SUPPRESSION IN SYSTEMIC VIRAL INFECTION

The discovery of molecular bases of silencing suppression also helped the understanding of the impact of silencing suppression in virus biology. Previous study using mutants of *Cymbidium ringspot virus* (CymRSV) demonstrated that the lack of p19 suppressor did not affect most of the basic viral functions such as genome replication, cell-to-cell movement and phloem long-distance transport (Dalmy *et al.*, 1993). In contrast, the systemic infection of plants inoculated with a silencing

suppressor mutant of CymRSV was seriously compromised and led to the development of a recovery phenotype (Szittyá *et al.*, 2002), suggesting that p19 suppressor targets a noncell-autonomous step of RNA silencing (Silhavy *et al.*, 2002). Indeed, *in situ* studies further demonstrated that the lack of p19 does not alter the phloem-dependent movement and the replication of CymRSV in and around the vascular bundles of systemic leaves (Fig. 2) (Havelda *et al.*, 2003). However, virus induced silencing prevents further cell-to-cell viral invasion of the leaf tissues, which are resistant to infection by a second virus with sequence homology to CymRSV (Szittyá *et al.*, 2002). The observation that p19 inhibits RNA silencing by siRNA sequestration and that p19 prevents the movement of a mobile virus-induced silencing signal, strongly suggest that the mobile signal is the siRNA itself, as was suggested for transgene short range silencing (Himber *et al.*, 2003; Dunoyer *et al.*, 2005). Thus when the p19 silencing suppressor is absent, the systemic signal moves faster than the virus in the infected plant, thereby establishing antiviral silencing in cells ahead of the infection front and as a result any virus entering such cells will be immediately destroyed by silencing-mediated RNA degradation (Fig. 2). Therefore the presence of the silencing suppressor is essential for the development of systemic virus infection.

CONCLUSION AND FURTHER DIRECTIONS

During the last few years dramatic progress has been made in the understanding of biological roles and pathways involved in RNA silencing. A large number of new silencing suppressor proteins have been described and the discovery of the molecular bases of silencing suppression has inspired new concepts about the molecular bases of symptoms caused by viruses. It is likely that viral symptoms are the consequence of different interactions between the silencing suppressors and the regulatory pathways of endogenous RNA silencing. However, viral symptoms could also be the consequence of casual targeting of plant mRNAs by virus-derived siRNA (Wang *et al.*, 2004), exemplifying the complexity of the role of RNA silencing in plant virus interaction.

The major function of RNA silencing is to protect plants against viral invasion, but surprisingly it seems that viruses may exploit this defence response to keep the virus titre at tolerable levels in plant tissues preventing the detrimental effects of virus over-accumulation.

Thus the action of RNA silencing ensures the survival of both the virus and the plants.

Silencing suppressors are probably involved in this fine-tuning of plant-virus interplay for joint survival, however our knowledge is very limited about the orchestration of this intimate host-parasite interplay.

VIGS has become a powerful technology used in

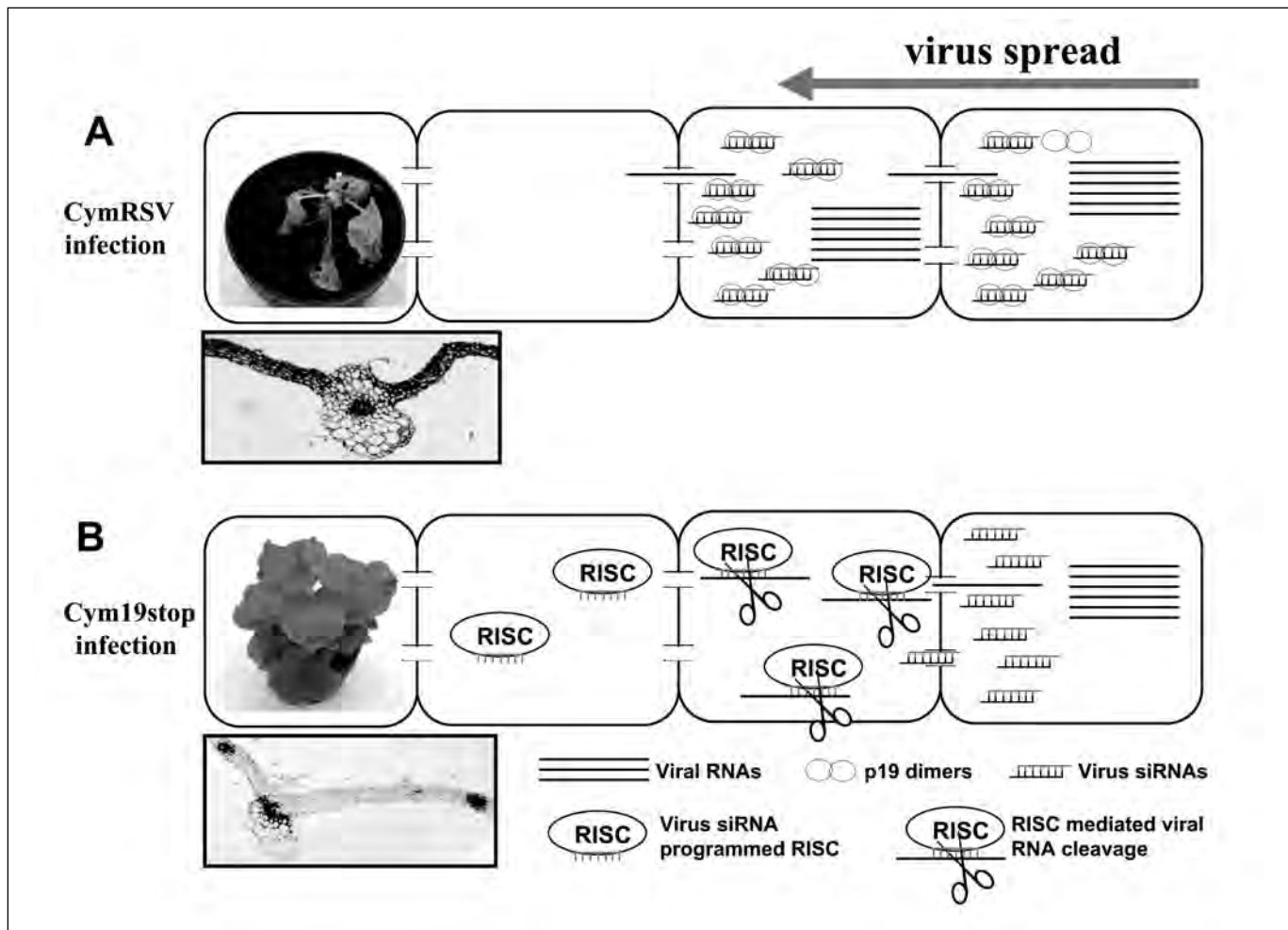


Fig. 2. Model of systemic invasion of tomosvirus virus infected plant. (A) In wt CymRSV infection, virus rapidly spread from the initially infected cells either cell-to-cell or long distance through the vascular system. This could happen because p19 silencing suppressor inhibits the RNA silencing based antiviral response by siRNA sequestration. Since no siRNA available for viral RNA targeting RISC assembly virus can invade the whole plant tissue (shown by the in situ analysis), which finally culminates in the plant death. (B) In the silencing suppressor deficient virus (Cym19stop) infected plant the virus replicates efficiently in the primary infected or cells around the vascular system (see in situ analysis). However, the further invasion of leaf tissue is inhibited since the silencing signal (virus specific siRNAs), establishing antiviral silencing in cells ahead of the infection front and the entering virus into such a cells will be immediately destroyed by virus siRNAs programmed RISCs. Leaf cross sections were hybridised with CymRSV specific probes.

functional genomics, because VIGS vectors are now available for most plant species, including model plants. Viral vectors are also used to express heterologous proteins for different purposes including animal or human vaccination against viruses. Silencing suppressor proteins of plant viruses such as p19 and HC-Pro, which target the most conserved elements of silencing pathways can be applied as powerful tools to dissect the RNA silencing pathways not only in plants but also in human cells (Lecellier *et al.*, 2005).

ACKNOWLEDGEMENTS

I am grateful to Gábor Giczey for critical reading of the manuscript. This research was supported by grants

from the Hungarian Scientific Research Fund (OTKA T46728 and OTKA NK60352) and "RIBOREG" EU project (LSHG-CT-2003503022).

REFERENCES

- Allen E., Xie, Z., Gustafson A.M., Carrington J.C., 2005. microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* **121**: 207-221.
- Anandalakshmi R., Marathe R., Ge X., Herr J.M., Mau C., Mallory A., Pruss G., Bowman L. 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., Mallory A.C., Smith T.H., Vance V.B., 1998. A viral suppressor of

- gene silencing in plants. *Proceeding of National Academy of Sciences USA* **95**: 13079-13084.
- Aukerman M.J., Sakai, H., 2003. Regulation of Flowering Time and Floral Organ Identity by a MicroRNA and Its APETALA2-Like Target Genes. *Plant Cell* **15**: 2730-2741.
- Bartel D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281-297.
- Baulcombe D., 2004. RNA silencing in plants. *Nature* **431**: 356-363.
- Baulcombe D.C., 1999. Fast forward genetics based on virus-induced gene silencing. *Current Opinion of Plant Biology* **2**: 109-113.
- Bernstein E., Caudy A.A., Hammond, S.M., Hannon G.J., 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**: 363-366.
- Brigneti G., Martin-Hernandez A.M., Jin H., Chen J., Baulcombe D.C., Baker B., Jones, J.D., 2004. Virus-induced gene silencing in Solanum species. *Plant Journal* **39**: 264-272.
- Brigneti G., Voinnet O., Li W.X., Ji L.H., Ding S.W., Baulcombe D.C., 1998. Viral pathogenicity determinants are suppressors of transgene silencing in Nicotiana benthamiana. *Embo Journal* **17**: 6739-6746.
- Bucher E., Sijen T., De Haan P., Goldbach R., Prins M., 2003. Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. *Journal of Virology* **77**: 1329-1336.
- Burch-Smith T.M., Anderson J.C., Martin G.B., Dinesh-Kumar S.P., 2004. Applications and advantages of virus-induced gene silencing for gene function studies in plants. *Plant Journal* **39**: 734-746.
- Cao X., Zhou P., Zhang X., Zhu S., Zhong X., Xiao Q., Ding B., Li Y., 2005. Identification of an RNA silencing suppressor from a plant double-stranded RNA virus. *Journal of Virology* **79**: 13018-13027.
- Chapman E.J., Prokhnovsky A.I., Gopinath K., Dolja V.V., Carrington J.C., 2004. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. *Genes and Development* **18**: 1179-1186.
- Chapman S., Kavanagh T., Baulcombe D., 1992. Potato virus X as a vector for gene expression in plants. *Plant Journal* **2**: 549-557.
- Chellappan P., Vanitharani R., Fauquet C.M., 2005. MicroRNA-binding viral protein interferes with Arabidopsis development. *Proceeding of National Academy of Sciences USA* **102**: 10381-10386.
- Chen J., Li W.X., Xie D., Peng J.R., Ding S.W., 2004. Viral virulence protein suppresses RNA silencing-mediated defense but upregulates the role of microRNA in host gene expression. *Plant Cell* **16**: 1302-1313.
- Chen X., 2003. A MicroRNA as a Translational Repressor of APETALA2 in Arabidopsis Flower Development. *Science* **11**: 11.
- Chen X., 2005. MicroRNA biogenesis and function in plants. *FEBS Letter* **579**: 5923-5931.
- Chung E., Seong E., Kim Y.C., Chung E.J., Oh S.K., Lee S., Park J.M., Joung Y.H., Choi D., 2004. A method of high frequency virus-induced gene silencing in chilli pepper (*Capsicum annuum* L. cv. Bukang). *Molecules and Cells* **17**: 377-380.
- Dalmay T., Hamilton A., Rudd S., Angell S., 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.
- Dalmay T., Rubino L., Burgyan J., Kollar, A., Russo M., 1993. Functional analysis of cymbidium ringspot virus genome. *Virology* **194**: 697-704.
- Deleris A., Gallego-Bartolome J., Bao J., Kasschau K.D., Carrington J.C., Voinnet O., 2006. A molecular framework for induction and suppression of antiviral RNA silencing in plants. *Science* **313**: 68-71.
- Doench J.G., Petersen C.P., Sharp P.A., 2003. siRNAs can function as miRNAs. *Genes and Development* **17**: 438-442.
- Dolja V.V., Kreuze J.F., Valkonen J.P., 2006. Comparative and functional genomics of closteroviruses. *Virus Research* **117**: 38-51.
- Donson J., Kearney C.M., Hilf M.E., Dawson W.O., 1991. Systemic expression of a bacterial gene by a tobacco mosaic virus-based vector. *Proceeding of National Academy of Sciences USA* **88**: 7204-7208.
- Dunoyer P., Himber C., Voinnet O., 2005. DICER-LIKE 4 is required for RNA interference and produces the 21-nucleotide small interfering RNA component of the plant cell-to-cell silencing signal. *Nature Genetics* **37**: 1356-1360.
- Dunoyer P., Lecellier C.H., Parizotto E.A., Himber C., Voinnet O., 2004. Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. *Plant Cell* **16**: 1235-1250.
- Dunoyer P., Pfeffer S., Fritsch C., Hemmer O., Voinnet O., Richards K.E., 2002. Identification, subcellular localization and some properties of a cysteine-rich suppressor of gene silencing encoded by peanut clump virus. *Plant Journal* **29**: 555-567.
- Ebhardt H.A., Thi E.P., Wang M.B., Unrau P.J., 2005. Extensive 3' modification of plant small RNAs is modulated by helper component-proteinase expression. *Proceeding of National Academy of Sciences USA* **102**: 13398-13403.
- Escobar C., Hernandez L.E., Jimenez A., Creissen G., Ruiz M.T., Mullineaux P.M., 2003. Transient expression of Arabidopsis thaliana ascorbate peroxidase 3 in Nicotiana benthamiana plants infected with recombinant potato virus X. *Plant Cell Report* **21**: 699-704.
- Faivre-Rampant O., Gilroy E.M., Hrubikova K., Hein I., Millam S., Loake G.J., Birch P., Taylor M., Lacomme C., 2004. Potato virus X-induced gene silencing in leaves and tubers of potato. *Plant Physiology* **134**: 1308-1316.
- Fitzmaurice W.P., Holzberg S., Lindbo J.A., Padgett H.S., Palmer K.E., Wolfe G.M., Pogue G.P., 2002. Epigenetic modification of plants with systemic RNA viruses. *Omic* **6**: 137-151.
- Gascioli V., Mallory A.C., Bartel D.P., Vaucheret H., 2005. Partially redundant functions of Arabidopsis DICER-like enzymes and a role for DCL4 in producing trans-acting siRNAs. *Current Biology* **15**: 1494-1500.

- Gossele V., Fache I., Meulewaeter F., Cornelissen M., Metzclaff M., 2002. SVISS - a novel transient gene silencing system for gene function discovery and validation in tobacco plants. *Plant Journal* **32**: 859-866.
- Hamilton A., Voinnet O., Chappell L., Baulcombe D., 2002. Two classes of short interfering RNA in RNA silencing. *Embo Journal* **21**: 4671-4679.
- Hamilton A.J., Baulcombe D.C., 1999. A species of small anti-sense RNA in posttranscriptional gene silencing in plants. *Science* **286**: 950-952.
- Hammond S.M., Bernstein E., Beach D., Hannon G.J., 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**: 293-296.
- Hannon G.J., Conklin D.S., 2004. RNA interference by short hairpin RNAs expressed in vertebrate cells. *Methods in Molecular Biology* **257**: 255-266.
- Havelda Z., Hornyk C., Crescenzi A., Burgyan J., 2003. *In Situ* Characterization of Cymbidium Ringspot Tombusvirus Infection-Induced Posttranscriptional Gene Silencing in *Nicotiana benthamiana*. *Journal of Virology* **77**: 6082-6086.
- Hein I., Barciszewska-Pacak M., Hrubikova K., Williamson S., Dinesen M., Soenderby I.E., Sundar S., Jarmolowski A., Shirasu K., Lacomme C., 2005. Virus-induced gene silencing-based functional characterization of genes associated with powdery mildew resistance in barley. *Plant Physiology* **138**: 2155-2164.
- Himber C., Dunoyer P., Moissiard G., Ritzenthaler C., Voinnet O., 2003. Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *Embo Journal* **22**: 4523-4533.
- Holzberg S., Brosio P., Gross C., Pogue G.P., 2002. Barley stripe mosaic virus-induced gene silencing in a monocot plant. *Plant Journal* **30**: 315-327.
- Hull R., 2002. *Matthews' Plant Virology*. Academic Press, San Diego, CA, USA.
- Hutvagner G., Zamore P.D., 2002. RNAi: nature abhors a double-strand. *Current Opinion in Genetic and Development* **12**: 225-232.
- Kasschau K.D., Carrington J.C., 1998. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. *Cell* **95**: 461-470.
- Kasschau K.D., Xie Z., Allen E., Llave C., Chapman E.J., Krizan K.A., Carrington J.C., 2003. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with Arabidopsis development and miRNA function. *Developmental Cell* **4**: 205-217.
- Kjemtrup S., Sampson K.S., Peele C.G., Nguyen L.V., Conkling M.A., Thompson W.F., Robertson D., 1998. Gene silencing from plant DNA carried by a Geminivirus. *Plant Journal* **14**: 91-100.
- Koonin E.V., Choi G.H., Nuss D.L., Shapira R., Carrington J.C., 1991. Evidence for common ancestry of a chestnut blight hypovirulence-associated double-stranded RNA and a group of positive-strand RNA plant viruses. *Proceeding of National Academy of Sciences USA* **88**: 10647-10651.
- Kubota K., Tsuda S., Tamai A., Meshi T., 2003. Tomato mosaic virus replication protein suppresses virus-targeted post-transcriptional gene silencing. *Journal of Virology* **77**: 11016-11026.
- Lacomme C., Hrubikova K., Hein I., 2003. Enhancement of virus-induced gene silencing through viral-based production of inverted-repeats. *Plant Journal* **34**: 543-553.
- Lakatos L., Szittyá G., Silhavy D., Burgyan J., 2004. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. *Embo Journal* **23**: 876-884. Epub 2004 Feb 19.
- Lakatos L., C.T., Pantaleo V., Chapman E.J., Carrington J.C., Liu Y.P., Dolja V.V., Fernández Calvino L., López-Moya J.J., Burgyán J., 2006. Comparative study of viral encoded silencing suppressors: small RNA binding is a common strategy to suppress RNA silencing. *Embo Journal* **25**: 2768-2780.
- Lecellier C.H., Dunoyer P., Arar K., Lehmann-Che J., Eyquem S., Himber C., Saib A., Voinnet O., 2005. A cellular microRNA mediates antiviral defense in human cells. *Science* **308**: 557-560.
- Li W.X., Ding S.W., 2001. Viral suppressors of RNA silencing. *Current Opinion in Biotechnology* **12**: 150-154.
- Lindbo J.A., Silva-Rosales L., Proebsting W.M., Dougherty W.G., 1993. Induction of a Highly Specific Antiviral State in Transgenic Plants: Implications for Regulation of Gene Expression and Virus Resistance. *Plant Cell* **5**: 1749-1759.
- Liu L., Grainger J., Canizares M.C., Angell S.M., Lomonosoff G.P., 2004. Cowpea mosaic virus RNA-1 acts as an amplicon whose effects can be counteracted by a RNA-2-encoded suppressor of silencing. *Virology* **323**: 37-48.
- Liu Y., Schiff M., Dinesh-Kumar S.P., 2002a. Virus-induced gene silencing in tomato. *Plant Journal* **31**: 777-786.
- Liu Y., Schiff M., Marathe R., Dinesh-Kumar S.P., 2002b. Tobacco Rar1, EDS1 and NPR1/NIM1 like genes are required for N-mediated resistance to tobacco mosaic virus. *Plant Journal* **30**: 415-429.
- Lu R., Folimonov A., Shintaku M., Li W.X., Falk B.W., Dawson W.O., Ding S.W., 2004. Three distinct suppressors of RNA silencing encoded by a 20-kb viral RNA genome. *Proceeding of National Academy of Sciences USA* **101**: 15742-15747.
- Lu R., Martin-Hernandez A.M., Peart J.R., Malcuit I., Baulcombe D.C., 2003. Virus-induced gene silencing in plants. *Methods* **30**: 296-303.
- Mallory A.C., Ely L., Smith T.H., Marathe R., Anandalakshmi R., Fagard M., Vaucheret H., Pruss G., Bowman L., Vance, V.B., 2001. HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. *Plant Cell* **13**: 571-583.
- McKinney H.H., 1929. Mosaic diseases of the Canary Islands, West Africa and Gibraltar. *Journal of Agricultural Research* **39**: 557-578.
- Meister G., Tuschl T., 2004. Mechanisms of gene silencing by double-stranded RNA. *Nature* **431**: 343-349.
- Mérai Z., Kerényi Z., Kertész S., Magna M., Lakatos L., Silhavy D., 2006. Double-stranded RNA binding could be a general plant RNA viral strategy to suppress RNA silencing. *Journal of Virology* **80**: 5747-5756.

- Moissiard G., Voinnet O., 2004. Viral suppression of RNA silencing in plants. *Molecular Plant Pathology* **5**: 71-82.
- 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., Vaucheret H., 2000. Arabidopsis SGS2 and SGS3 genes are required for post-transcriptional gene silencing and natural virus resistance. *Cell* **101**: 533-542.
- Muangsan N., Robertson D., 2004. Geminivirus vectors for transient gene silencing in plants. *Methods in Molecular Biology* **265**: 101-115.
- Nykanen A., Haley B., Zamore P.D., 2001. ATP requirements and small interfering RNA structure in the RNA interference pathway. *Cell* **107**: 309-321.
- Pazhouhandeh M., Dieterle M., Marrocco K., Lechner E., Berry B., Brault V., Hemmer O., Kretsch T., Richards K.E., Genschik P., Ziegler-Graff V., 2006. F-box-like domain in the polerovirus protein P0 is required for silencing suppressor function. *Proceeding of National Academy of Sciences USA* **103**: 1994-1999.
- Peele C., Jordan C.V., Muangsan N., Turnage M., Egelkrout E., Eagle P., Hanley-Bowdoin L., Robertson D., 2001. Silencing of a meristematic gene using geminivirus-derived vectors. *Plant Journal* **27**: 357-366.
- Peragine A., Yoshikawa M., Wu G., Albrecht H.L., Poethig R.S., 2004. SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes and Development* **18**: 2368-2379.
- Pfeffer S., Dunoyer P., Heim F., Richards K.E., Jonard G., Ziegler-Graff V., 2002. P0 of beet Western yellows virus is a suppressor of posttranscriptional gene silencing. *Journal of Virology* **76**: 6815-6824.
- Plasterk R.H., 2002. RNA silencing: the genome's immune system. *Science* **296**: 1263-1265.
- Pruss G., Ge X., Shi X.M., Carrington J.C., Bowman Vance V., 1997. Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* **9**: 859-868.
- Qu F., Ren T., Morris T.J., 2003. The coat protein of turnip crinkle virus suppresses posttranscriptional gene silencing at an early initiation step. *Journal of Virology* **77**: 511-522.
- Ratcliff F., Harrison B.D., Baulcombe D.C., 1997. A Similarity Between Viral Defense and Gene Silencing in Plants. *Science* **276**: 1558-1560.
- Ratcliff F., Martin-Hernandez, A.M. and Baulcombe, D.C., 2001. Technical Advance. Tobacco rattle virus as a vector for analysis of gene function by silencing. *Plant Journal* **25**: 237-245.
- Ratcliff F.G., MacFarlane S.A., Baulcombe D.C., 1999. Gene silencing without DNA. rna-mediated cross-protection between viruses. *Plant Cell* **11**: 1207-1216.
- Reed J.C., Kasschau K.D., Prokhnovsky A.I., Gopinath K., Pogue G.P., Carrington J.C., Dolja V.V., 2003. Suppressor of RNA silencing encoded by Beet yellows virus. *Virology* **306**: 203-209.
- Ruiz M.T., Voinnet O., Baulcombe D.C., 1998. Initiation and maintenance of virus-induced gene silencing. *Plant Cell* **10**: 937-946.
- Silhavy D., Burgyan J., 2004. Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends in Plant Sciences* **9**: 76-83.
- Silhavy D., Molnar A., Lucioli A., Szittyta G., Hornyik C., Tavazza M., Burgyan J., 2002. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide double-stranded RNAs. *Embo Journal* **21**: 3070-3080.
- Szittyta G., Molnar A., Silhavy D., Hornyik C., Burgyan J., 2002. Short defective interfering RNAs of tombusviruses are not targeted but trigger post-transcriptional gene silencing against their helper virus. *Plant Cell* **14**: 359-372.
- Thomas C.L., Leh V., Lederer C., Maule A.J., 2003. Turnip crinkle virus coat protein mediates suppression of RNA silencing in *Nicotiana benthamiana*. *Virology* **306**: 33-41.
- Turnage M.A., Muangsan N., Peele C.G., Robertson D., 2002. Geminivirus-based vectors for gene silencing in Arabidopsis. *Plant Journal* **30**: 107-114.
- Vargason J., Szittyta G., Burgyan J., Hall, T.M., 2003. Size selective recognition of siRNA by an RNA silencing suppressor. *Cell* **115**: 799-811.
- Vazquez F., Vaucheret H., Rajagopalan R., Lepers C., Gascioli V., Mallory A.C., Hilbert J.L., Bartel D.P., Crete P., 2004. Endogenous trans-acting siRNAs Regulate the Accumulation of Arabidopsis mRNAs. *Molecular Cell* **16**: 69-79.
- Verdel A., Jia S., Gerber S., Sugiyama T., Gygi S., Grewal S.I., Moazed D., 2004. RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* **303**: 672-676.
- Voinnet O., 2002. RNA silencing: small RNAs as ubiquitous regulators of gene expression. *Current Opinion in Plant Biology* **5**: 444.
- Voinnet O., 2005a. Induction and suppression of RNA silencing: insights from viral infections. *Nature Reviews Genetics* **6**: 206-220.
- Voinnet O., 2005b. Non-cell autonomous RNA silencing. *FEBS Letters* **579**: 5858-5871.
- Voinnet O., Lederer C., 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 Y.M., Baulcombe D.C., 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *Proceeding of National Academy of Sciences USA* **96**: 14147-14152.
- Wang M.B., Bian X.Y., Wu L.M., Liu L.X., Smith N.A., Isenegger D., Wu R.M., Masuta C., Vance V.B., Watson J.M., Rezaian A., Dennis E.S., Waterhouse P.M., 2004. On the role of RNA silencing in the pathogenicity and evolution of viroids and viral satellites. *Proceedings of National Academy of Sciences USA* **101**: 3275-3280.
- Ye K., Malinina L., Patel D.J., 2003. Recognition of small interfering RNA by a viral suppressor of RNA silencing. *Nature* **426**: 874-878.
- Yelina N.E., Savenkov E.I., Solovyev A.G., Morozov S.Y., Valkonen J.P., 2002. Long-distance movement, virulence, and RNA silencing suppression controlled by a single pro-

tein in hordei - and potyviruses: complementary functions between virus families. *Journal of Virology* **76**: 12981-12991.

Zamore P.D., 2002. Ancient pathways programmed by small RNAs. *Science* **296**: 1265-1269.

Received May 19, 2006
Accepted May 25, 2006