RNA silencing is a conserved pathway and it may result in gene expression blockage in eukaryotic organisms. RNA silencing is also part of a highly adaptable immune system response against viruses in plants and animals. It is generally thought that virus-induced RNA silencing is that double-stranded replicative intermediates of RNA viruses, and/or double-stranded RNA produced from the viral RNA by host RNA-dependent RNA polymerases are recognized by Dicer-like proteins for the production of viral siRNAs (vsiRNAs). However, recent studies show that vsiRNAs originated predominantly from highly structured single-stranded viral RNAs is a general characteristic for RNA viruses, dsDNA virus as well as for sub-viral pathogens, e.g. viroid. Increasing lines of evidence has also shown that the plant antiviral response involves hierarchical action of DCLs. To counteract antiviral silencing, many viral genomes encode suppressor proteins to combat the defense pathway. The most common strategy for viral suppressors to inhibit RNA silencing is via binding to siRNAs. Some viral suppressor can also bind to long dsRNA and maybe compete with DCLs to access viral RNA substrates, or inhibit the activity of specific DCLs in the production of vsiRNAs. This review will give an update on the current view of these researches on antiviral silencing and defense in plants.

**INTRODUCTION**

RNA silencing (RNA interference, RNAi) is a conserved pathway and it may result in gene expression blockage in eukaryotic organisms in both the cytoplasm and the nucleus, which is involved in regulating sequence-specific gene expression, transposon control, chromatin modification, virus resistance and development (Baulcombe 2004; Meister and Tuschl 2004; Baulcombe 2005). Small RNAs, the unifying feature of RNA silencing, involved in these RNAi-mediated processes include microRNAs (miRNAs) and other small interference RNAs (siRNAs), while the key host protein components in these processes include RNAseIII-type enzymes called Dicers, RNA-dependent RNA polymerases and Argonaute protein-containing effector complexes called RNA-induced silencing complexes (RISCs). In Arabidopsis, there are four Dicer-like enzymes (DCLs), six RNA-dependent RNA polymerases (RDRs) and ten Argonautes (AGOs). In plant, the miRNA pathway generates 21-24 nt miRNAs from single-stranded hairpin RNA precursor by DCL1, regulating the sequence-specific gene expression, especially in some crucial developmental processes (Jones-Rhoades et al. 2006). miRNAs also control endogenous TAS-derived 21-nt trans-acting siRNAs (ta-siRNAs), which guide the target mRNA cleavage and function as regulators of gene expression as plant miRNAs do (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005; Yoshikawa et al. 2005). DCL4 is required for the 21-nt ta-siRNAs synthesis. RNA SUPPRESSOR OF GENE SILENCING3 (SGS3) and RDR6 synthesize another strand of the miRNA-dependent, AGO1-mediated single-stranded TAS primary cleavage products to form double-stranded RNA (dsRNA) for DCL4-mediated cleavage (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005). Heterochromatin-related siRNAs, a kind of 24-nt endogenous siRNAs, produced by DCL3, have important functions in DNA methylation and chromatin remodeling (Xie et al. 2004). RDR2, proposed to contribute to dsRNA conversion from heterochromatin-related transcripts (Xie et al. 2004), and RNA Pol IVa complex, proposed to function as the RDR in a self-amplifying loop (Kanno et al. 2005), are required for formation of these 24-nt siRNAs. Recently, a new class of endogenous 24-nt nat-siRNAs due to DCL2-mediated cleavage for natural antisense overlapping transcripts has been identified (Borsani et
Taken together, the production of these siRNAs is always triggered by dsRNAs, which is processed into 21-24nt RNA duplex by Dicer and its homologues. One strand of small RNA duplex is then incorporated into RISC and leads to sequence-specific RNA degradation, translation inhibition or DNA and/or histone modification.

Viral infection would trigger a set of RNA silencing reactions, which is named as virus-induced gene silencing (VIGS). Different classes of viral siRNAs (vsiRNAs) derived from various viruses are detected in corresponding virus-infected host (Blevins et al. 2006; Deleris et al. 2006; Moissiard and Voinnet 2006). DCL2 is the first identified DCL which contributes to viral siRNAs biogenesis and functions in antiviral defense (Xie et al. 2004), and recently, emerging evidences indicate that all four DCLs in Arabidopsis have hierarchical contribution to viral siRNAs biogenesis (Voinnet 2005; Deleris et al. 2006; Moissiard and Voinnet 2006). Meanwhile, some RDRs, such as RDR6 and RDR1, are proposed to amplify VIGS signal and function in systemic virus silencing (Xie and Guo 2006). The Arabidopsis ago1 mutants and ago2-deficiency flies were shown to be hypersensitive to virus infection, respectively (Morel et al. 2002; van Rij et al. 2006). Therefore, besides its endogenous functions, RNA silencing is a part of a highly systemic response against viruses in plants and animals. Correspondingly, viruses have also evolved suppression mechanisms to counteract host RNA silencing system, and various virus-encoded proteins have been identified as RNA silencing suppressors to interfere with host silencing machinery. Induction, amplification and suppression of RNA silencing due to virus invasion have been well reviewed (Voinnet 2005; Xie and Guo 2006). This review will give an update on how vsiRNAs originate, how plant virus and plant RNA silencing antiviral system, especially DCLs, fight against each other.

TRIGGERS FOR RNA-MEDIATED PLANT ANTIVIRAL DEFENSE

In plant and animal, several possible primary sources of RNA silencing as elicited by viral and sub-viral pathogens have been well summarized (Voinnet 2005). Most plant viruses belong to positive single-stranded RNA virus. Dependence on viral RDR, dsRNAs intermediates are generated. Moreover, viral normal and aberrant RNAs could also be transformed into dsRNAs by host RDRs, for instance, RDR6 (Voinnet 2005). This makes the general thought that these dsRNA are probably processed into viral siRNAs, which trigger primary VIGS. Alternatively, siRNAs also could originate from highly structured region of viral single-stranded RNAs. Recent studies suggest that viral siRNAs originate predominantly from highly structured single-stranded viral RNAs (Silhavy et al. 2002; Landry and Perreault 2005; Molnar et al. 2005). On the assumption that viral dsRNAs intermediates are the only or main triggers for DCLs processing, the content of viral negative-strand-derived siRNAs should be closely equal to the one of viral positive-strand-derived siRNAs, and the length of target RNAs should probably determine the efficiency of the targeting step. However, the study of defective interfering RNAs (DI RNAs) of Cymbidium ringspot tombusvirus (CymRSV) has shown that is not really the case (Havelda et al. 1997; Silhavy et al. 2002). They show that the large DI RNAs containing a highly base paired structure could be efficiently targeted by RNA silencing machinery, and the target activity depends on specific sequence/structures rather than the length of target molecules (Silhavy et al. 2002). A highly base-paired structure in the DI RNAs is found to be sufficient to trigger viral RNA silencing and the mutation that increased the stability of this structure conferred higher accumulation of vsiRNAs (Havelda et al. 1997; Silhavy et al. 2002). Analysis of the population of the viral siRNAs in several RNA virus-infected plants also shows that the vsiRNAs are derived mainly from plus-stranded RNA, especially from the region thought to fold into base paired structures (Silhavy et al. 2002; Molnar et al. 2005). An in vitro dicer cleavage assay also indicates that the pre-miRNA-like hairpin from peach latent mosaic viroid (PLMVd) is sufficient to trigger Dicer cleavage (Landry and Perreault 2005). Potato spindle tuber viroid (PSTVd)-derived siRNAs have recently been cloned and identified to be derived from the secondary structure of viroid RNAs (Perreault et al. 2005). Moreover, a highly structured Caulimovirus, the siRNA northern blot assays on Cauliflower mosaic virus (CaMV)-infected plants showed that the ratio between 21-nt and 24-nt siRNAs in infected dcl4 mutants differed from different CaMV regions detected (Blevins et al. 2006). The differences might be due to the structure of viral RNA intermediates and the structure characteristics determine the specificity of DCLs. Recently, the 35S leader of CaMV genome, which likely forms a highly structured secondary structure, was found to be a major source of CaMV-derived siRNAs in CaMV-infected plants (Moissiard and Voinnet 2006). Therefore, it’s obvious that highly structured viral single-stranded RNAs could be accessible for DCLs and might be the main triggers for plant antiviral defense activity, at least for primary VIGS reaction.

The primary VIGS initiation is RDR6-independent (Schwach et al. 2005), and this was further supported by the study on CaMV 35S leader-derived vsiRNAs, whose accumulation level is not reduced in rdr6 mutant (Schwach et al. 2005; Moissiard and Voinnet 2006). However, RDR6 in Arabidopsis, functions as the amplifier of VIGS signals so that it’s important for secondary VIGS reaction and systemic virus silencing in antiviral defense (Voinnet 2005; Xie and Guo 2006). Long dsRNAs amplified by host RDRs will be targeted by DCLs for cleavage and confer dominant content of secondary vsiRNAs, one should detect more dispersed population of the vsiRNAs from viral genome. However, it was not really the case in studies on some viruses (Silhavy et al. 2002; Molnar et al. 2005; Moissiard and Voinnet 2006). The possible explanation is that RDRs have specificity for viral RNAs (Schwach et al. 2005), and RDR6 activity may be more dependent on the quality rather the mere concentration of template (Xie and Guo 2006). The extensive fold-back structure may be such template that is not suitable for RDR6 to synthesize new viral dsRNAs, resulting in a little or no de novo amplification of viral siRNAs. Such highly structured single-stranded viral RNAs are, therefore, dominant to trigger DCLs antiviral activities.

Whether the secondary structures formed by single-stranded viral RNA resemble the miRNA precursor hairpins that are recognized by DCL1, which DCL4 and DCL5, expressed in a highly structured RNA, and how DCLs cleave the respective secondary structure of viral RNAs remain to be further investigated. Therefore, more detailed analysis of population of vsiRNAs in virus-infected wild type plants and different dcl mutants, combined with structure biological analysis of RNA and Dicer proteins, will help us find the possible substrate of DCLs and the recognition specificity of different DCLs with antiviral activities.

DCLS ACTIVITIES IN PLANT ANTIVIRAL RESPONSE

DCLs function as the key enzymes of diverse RNA silencing pathway. As mentioned above, plant such as Arabidopsis have evolved more diverse sRNAs and RNA silencing pathway (Vaucheret 2006; Vazquez 2006). Coordinated or redundant by functions of DCLs in different endogenous sRNAs pathways and transgene-induced silencing pathway (Brodersen and Voinnet 2006; Vaucheret 2006; Vazquez 2006) suggest the complex action of DCLs in the plant antiviral response. In numerous studies made with RNA virus infection of Arabidopsis del1 single mutant, neither viral RNA accumulation nor the extent and/or consistency of VIGS was altered in any of the single dcl mutants in comparison with WT-infected plants (Blevins et al. 2006; Deleris et al. 2006), which suggest redundancy among DCLs in
mediating antiviral silencing. Recent studies made with all possible combination of Arabidopsis dcl double and triple mutant strengthen the conclusion that plant antiviral response involves hierarchical action of DCLs (Blevins et al. 2006; Deleris et al. 2006; Fusaro et al. 2006). Deleris et al. (2006) have shown that the respective 21-nt and 22-nt siRNAs products of DCL4 and DCL2 guide an antiviral RISC to promote defense against Tobacco rattle virus (TRV) and DCL4 and DCL2 belong to Dicer-related proteins (DCAPs) is required for DCLs non-siRNAs (Gasciolli et al. 2005), but also can substitute DCL3 in processing miR173 and the other three DCLs) (Hiraguri et al. 2006). Therefore, DRB proteins might be one of the candidates of DCAPs. The patterns of vsiRNAs products of DCL4 and DCL2 double mutant, suggesting that DCL2 not only can substitute DCL4, as in production of endogenous siRNAs (Gasciolli et al. 2005; Xie et al. 2005; Yoshioka et al. 2005), but also can substitute DCL3 in production of DNA vsiRNAs (Blevins et al. 2006). DCL1, thought to process hairpin RNAs in miRNA pathway, appears to process hairpin RNAs and generate 21-nt siRNAs in triple dcl mutant lacking DCL2, DCL3. DCL1 can transform with hairpin RNA-mediated silencing constructs (Fusaro et al. 2006). Similarly, DCL1 appears to generate 21-nt vsiRNAs in the triple dcl mutant infected with DNA viruses (Blevins et al. 2006; Moissiard and Voinnet 2006), to a limited extent, in which, the production of miR173 seems to be decreased at the same time (Blevins et al. 2006). A supposed explanation is that DCL1 takes over the role of DCLs while other DCLs are related to the other TRV targeting the dsDNA intermediates and/or minichromosome of DNA viruses, while have no obvious targets and become the by-products of silencing machinery in anti-RNA-virus response. The nuclear localization of DCL1, DCL3 and DCL4 (Papp et al. 2003; Xie et al. 2004; Hiraguri et al. 2005) does not seem to be consistent with their actions on processing of RNA and DNA virus siRNAs. However, relocalization of DCLs during infection is possible. In fact, previous study has reported that DCL2 has a nucleocytoplasmic distribution, and the DCL2-GFP fusion protein has predominant accumulation in the nucleus of N. benthamiana cells in the transient assay system (Xie et al. 2004). The preference might be also important for DCLs cleavage properties. For instance, given that the interaction with some associated proteins (name them as Dicer-Associated Proteins, DCAPs) is required for DCLs cleavage, the cleavage properties of DCLs might be modulated by the properties of DCAPs: the subcellular localization of DCAPs might determine the subcellular localization of functional DCLs; the affinity of Dicer-DCAP interaction might modulate the accessibility and specificity of Dicer to different substrates; even that, the Dicer-DCAP interaction might determine the size of siRNAs products, considering that DCL2 can produce both 22-nt vsiRNAs and 24-nt nat-siRNAs (Borsani et al. 2005). Based on a well-developed biochemical system using Arabidopsis protein extract for Dicer activity assay, dsRNA substrates could be cleaved into 21- and 24-nt siRNAs by Arabidopsis total protein extract or purified DCL1/DCL3 complexes (Qi et al. 2005). Meanwhile, size-exclusion chromatography has indicated that DCL1 and DCL3 reside in >660 kDa complex conferring 21-nt siRNA-generating activity and ~440 kDa complex conferring 24-nt siRNA-generating activity, respectively (Qi et al. 2005). These observations indicate that some different factors associate with DCL1 and DCL3, respectively, conferring different siRNA products from the same dsRNA substrates. Furthermore, considering that the molecular weight of DCL1 and DCL3 are 188.3 kDa, respectively, it also raises a question whether DCL4, which is thought to be the preferred Dicer for 21-nt siRNAs production from dsRNA substrates (Brodersen and Voinnet 2006), also resides in the >660 kDa complex and confers 21-nt siRNA-generating activity rather than DCL1. DRB family proteins might be essential for Dicer function, some cases of Dicer-DRB interaction in Drosophila, Caenorhabditis elegans and Mus musculus are required for miRNA processing (Vazquez 2006). Specificity and redundancy in DCL-DRB interaction and specificity of subcellular localization of DRBs have been recently reported (Hiraguri et al. 2005). Therefore, DRB proteins might be one of the candidates of DCAPs. The patterns of vsiRNAs in infected hy1-2, mutant of a member of Arabidopsis DRB protein HYL1, are shown no change with wild type plant, consistent with Arabidopsis between trv1 trv1 DCL1 (about 50-fold stronger than those between HYL1 and the other three DCLs) (Hiraguri et al. 2005), suggesting HYL1 might be not essential for vsiRNAs biogenesis. Recently, DRB4 was found to interact with DCL4 in vivo and function in the ta-siRNA pathway (Nakazawa et al. 2007). Responses of other DRB mutants in viral infection should be of interest in future works. Meanwhile, DCL-AGO interaction could not be excluded in this issue.
VIRAL SUPPRESSORS WITH RNA-BINDING ACTIVITY

To counteract plant host RNA silencing, many plant viruses have encoded proteins that suppress different steps of the RNA silencing machinery (Silhavy and Burgyan 2004; Voinnet 2005; Bisaro 2006). Elucidating the mechanism of viral suppression of RNA silencing is significant for understanding the mechanism of host RNA silencing machinery. Till now, more than 30 viral suppressors have been identified in plant viruses (Silhavy and Burgyan 2004; Cao et al. 2005; Dunoyer et al. 2005; Bisaro 2006; Zrzacha et al. 2007). Recently, based on multiple in vivo and in vitro approaches, increasing evidences suggest that many of viral suppressors are RNA-binding proteins, either size-selective or size-independent, though they evolve independently and show low sequence and/or structure homology. For instance, results of in vivo and in vitro assays show that viral suppressors of Tobacco etch virus (TEV) HC-Pro, Tombusvirus p19 and Closterovirus p21 have size-selective ds-siRNA binding activity (Lakatos et al. 2004; Lakatos et al. 2006; Merai et al. 2006). Through direct competition target cleavage assays and RISC formation direct competition assays using the Drosophila embryo extracts, Lakatos et al also showed that TEV HC-Pro, p19 and p21 uniformly inhibited the siRNA-triggered RISC assembly through sequestering siRNA, and none of them inhibit preassembled RISC activity in vitro or in vivo (Lakatos et al. 2006). P19-defective tombusvirus infected plants recovered from infection resulting from viral RNA degradation induced by vsiRNA-containing RISC antiviral activity has recently been confirmed by biochemical assays (Omarov et al. 2007; Pantaleo et al. 2007). The presence of an active p19 in wild-type tombusvirus inhibited ssRNA-specific rebonuclease activity (Omarov et al. 2007). Moreover, these suppressors are also shown to bind miRNA/miRNA* intermediates in vivo, as a consequence, inhibiting miRNA pathway. These are strengthened by the 3D structure of p19 (Vargason et al. 2003; Ye et al. 2003) and the octameric ring structure of p21 (Ye and Patel 2005). However, in transgenic plant system, HC-pro is shown to prevent the accumulation of siRNA (Mallory et al. 2002) and not to interact directly with microRNA/miRNA*, but do interfere with the incorporation of siRNAs into RISC (Chapman et al. 2004). The diverse results might be due to different experiment conditions in these independent studies, and might also reflect multiple suppression functions for HC-pro and only dominantive functions display in a certain experiment system. For other size-selective ds-siRNAs binding suppressors, like Peanut stunt virus p15 and tombusvirus p19, the presence of the overhangs of ds-siRNAs is required for their binding activities (Merai et al. 2006). The 3' overhangs ds-siRNAs also increase the TEV HC-pro binding efficiency. Nevertheless, siRNA duplex sequestration seems to be the most common strategy for viral suppressors to inhibit RNA silencing (Lakatos et al. 2006; Merai et al. 2006; Uhrig 2006; Wang et al. 2006).

Other viral suppressors such as Turnip crinkle virus (TCV) p38, Pothos latent virus (PoLV) p14, are shown to have size-independent ds-siRNA and long dsRNA binding activity (Thomas et al. 2003; Merai et al. 2005, 2006). Sequestration of wider range of RNA targets by p38 and p14 suggests that some viral suppressors might target multiple silencing steps, probably not only inhibiting the maintenance/amplification step, but also the other, such as the silencing-triggering step. Sequestring long dsRNAs may prevent certain DCL/DCLs cleavage, and/or prevent RDRs to yield more dsRNA substrates for DCLs. In addition, several viral suppressors are shown to bind ssRNA. A geminivirus-encoded suppressor AC4 from African cassava mosaic virus Cameroon Strain (ACMV), was reported to bind single-stranded form of miRNAs and siRNAs but not dsRNAs in in vitro binding assays (Chellappan et al. 2005), suggesting that ACMV-AC4 interfere with RISC loading at the downstream step of small RNA biogenesis and dsRNA unwinding. Therefore, long dsRNA, ds-sRNA and/or ssRNA in the silencing pathway are possible targets for most viral suppressors.

VIRAL SUPPRESSORS INTERACTING WITH HOST SILENCING COMPONENTS

Increasing line of evidences has shown that, besides targeting RNA components, some viral suppressors also interact with plant host protein constituent of silencing machinery. The plant protein rgs-CaM, which was found to interact with HC-Pro in a two hybrid system, is identified as the first endogenous suppressor of PTGS (Anandalakshmi et al. 2000). Not only by sequestering ds-sRNAs, HC-Pro was shown to inhibit silencing by activating rgs-CaM, suggesting that HC-Pro may activate an endogenous mechanism that negatively regulates RNA silencing (Anandalakshmi et al. 2000). Studies on TuMV suggest HC-Pro may inhibit multiple steps downstream from DCL1-processing and maturation of miRNAs, probably suppresses the assembly and activity of RISC (Kasschau et al. 2003). In a RISC formation direct competition assay with HC-Pro, it is striking that the addition of Drosophila embryo extract significantly increased the affinity of HC-Pro to siRNA and led to form a new complex containing labeled siRNAs, which did not happen without adding Drosophila embryo extract (Lakatos et al. 2006). It suggests that one or more cellular factor may interact with HC-Pro and increase the affinity of HC-Pro to siRNA and provides a clue for researchers to find the endogenous HC-Pro-interacting factor/factors.

DCL4 has been identified as a key component of non-cell autonomous RNA silencing in a genetic screen using the SUC-SUL system, which suggests cell-to-cell signaling requires DCL4-dependent 21-nt siRNAs (Dunoyer et al. 2005). Virus-derived 21-nt siRNAs are also shown to be DCL4-dependent (Blevins et al. 2006; Deleris et al. 2006; Fusaro et al. 2006). DCL4-dependent 21-nt siRNAs accumulate in suppressor p38-deficient TCV-GFP/pAp38 infected plants but not in TCV-GFP infected plants, suggesting that the production of DCL4-dependent 21-nt is inhibited by the p38 (Dunoyer et al. 2005). Moreover, TCV-GFP/pAp38 has movement defects in wild type infected plant, which can be rescued in p38 transgenic plants and dcl1dcl4 double mutant (Deleris et al. 2006). All of these evidences suggest that DCL4 plays an important antiviral role in producing 21-nt cell-to-cell silencing signals and restricting viral systemic infection, while its activity could be suppressed by TCV p38.

According to the RNA binding activity of p38, the suppression of DCL4 is probably via competing with DCL4 to interact with the passenger strand of the ds siRNAs. Alternatively, p38 may direct or indirect interact with DCL4 to inhibit its activity in the production of 21-nt siRNAs.

AGO1-containing RISC plays an important role in endogenous RNA silencing pathway. It is shown that AGO1 associates with endogenous miRNAs and ta-siRNAs but not virus-derived siRNA (Baumberger and Baulcombe 2005). However, recently, Zhang et al. (2006) showed that AGO1 also associates with CMV-derived siRNAs. The 2b protein encoded by CMV was shown to inhibit the activity of long range silencing signals (Guo and Ding 2002). In vivo and in vitro assays showed that CMV 2b suppressor directly interacted with AGO1, and the interaction occurred primarily on one surface of the PAZ-containing module and part of the PIWI-box of AGO1(Zhang et al. 2006). RISC reconstitution assay also showed that 2b specifically blocks the AGO1 cleavage action resulting in accumulation of passenger strand of tasiRNA and star strand of miRNA, as a consequence, attenuating miRNA-mediated RNA silencing and increasing of accumulation of miRNA target mRNA (Zhang et al. 2006). Moreover, CMV-infected plant and 2b transgenic plant show the similar developmental abnormality with ago1 mutant (Zhang et al. 2006). All these results suggest that CMV 2b interacts with AGO1 and AGO1-containing RISC is also important for VIGS. As PAZ-containing module is sufficient for the interaction with CMV 2b, it
could be anticipated that DCLs, which also contain PAZ domain, probably could interact with CMV 2b as well. Hopefully, more endogenous protein components of RNA silencing interacting with viral proteins would be identified in the near future.

**OTHER VIRAL ACTIONS AGAINST RNA SILENCING**

Besides the direct targeting silencing pathway, escape of antiviral silencing is also an effect counter-defense by viral suppressors to protect viral RNA inside the plant cells (Xie and Guo 2006). Additionally, actions of some other viruses to combat RNA silencing are also notable, which might provide us more clues to investigate the plant host antiviral response. A recent study show that a potential silencing suppressor Polerovirus P0 (Pfeffer et al 2002), requires an F-box-like motif for its suppressor function (Pazhouhandeh et al. 2006). This suggests that P0 might act as an F-box protein that targets an essential component of the host RNA silencing defense pathway; or P0 might function as an antagonist of a cellular F-box protein, which normally degrades a negative regulator of the silencing pathway (Pazhouhandeh et al. 2006). The ubiquitination activity and the target of P0 will be important for the demonstration of this suppression, and will open a door for studying the correlation between the ubiquitination pathway and the RNA silencing pathway in plant. Interestingly, Red clover necrotic mosaic virus (RCNMV), a positive single-stranded RNA virus, suppresses sense-transgene-mediated RNAi by using multiple viral components required for viral RNA replication (Takeda et al. 2002). The requirement of DCL1 or its homologues for RCNMV infection and leading to inhibit miRNA biogenesis and host RNA interference (Takeda et al. 2002) suggest that DCL1, as one of components of host silencing machinery, becomes not a safeguard anymore but an accessory of viral invasion. Several different protein components encoded by different geminivirus genome, have been identified as the viral suppressor. Although their silencing mechanisms have not been fully understood, it provides us new sight of silencing suppression. The transcription activator protein AC2 is proposed to suppress RNA silencing by controlling the expression of host silencing effector genes (Trinks et al 2005), while Tomato golden mosaic virus AC2 interacts and inactivates adenosine kinase (ADK), a cellular enzyme shown to be required for supporting RNA silencing. Another identified suppressor protein, the βC1 protein of Tomato yellow leaf curl China virus-Y10 (TYLCCV) is shown to bind ssDNA and dsDNA without sequence specificity and its nuclear localization is required for silencing suppression activity (Cui et al. 2005a). Developmental defects in its transgenic plant suggest that it target at the silencing step overlapping the miRNA pathway (Cui et al. 2005b).

**SUMMARY**

Increasing experimental evidences indicate that not only dsRNA replicative intermediates and dsRNA generated by host RDRs, but also single-stranded highly structured region of RNA genome triggers DCLs antiviral activity. DCLs function as the sensors for viral RNAs and cleave them into respective siRNAs in different sizes, leading to further actions of plant RNA silencing machinery against viruses. It becomes clearer that DCLs act cooperative and hierarchical. Moreover, DCLs combine with other silencing components, such as RDRs, DRBs, AGOs or even themselves, seem to form an even complex network in antiviral silencing pathway.

On the other hand, viruses have evolved diverse strategies to suppress host RNA silencing. Most common strategy is via targeting the viral RNAs, such as long dsRNAs, ds-sRNAs and ssRNA, dependent on their RNA binding activity, to compete with DCLs, RDRs or AGOs for substrates. Targeting the protein component, such as DCLs and AGOs, is another efficient counteraction to plant antiviral response. Some of viral suppressors may also inhibit multiple steps of RNA silencing pathway. Moreover, some new suppression mechanisms are also proposed. The divers suppression mechanisms also reflect that the complexity of antiviral defense in plant. In fact, the RNA-silencing and other defense pathways can act together to limit virus infection. This is supported by the discovery that both CMV 2b and TEV P1/HC-pro can interfere with SA-defense pathway, and it can act as an enhancer of the RNA-silencing antiviral defense in plant (Ji and Ding 2001; Alamillo et al. 2006). The discovery of multiple functions of viral suppressors will help us to better understand the plant complex network on antiviral defense.

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