Chapter 6

Plant Defense and Counter Defense by Viruses

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http://dx.doi.org/10.5772/intechopen.79114

Abstract

RNA silencing is a robust sequence-specific RNA degradation process triggered by the formation of double-stranded RNA (dsRNA). RNA silencing was first discovered in transgenic plants, where it was termed co-suppression or post-transcriptional gene silencing (PTGS). In plants, it serves as an antiviral defense, and small RNA pathways serve as a defense against viruses and other invading nucleic acids. This chapter focuses on the interactions between host small RNA pathways and viral suppressors of silencing. Invading viruses carry genetic material that controls the host cell's machinery and tricks it into producing proteins and new viruses. Through RNA silencing, plant cells recognize this viral genetic material, remember and copy it so that other cells in the organism can be warned to destroy the virus. All cells in microbes, fungi, plants and mammals employ RNA silencing. However, viruses are known to fight back using RNA silencing suppressors, proteins that inhibit this defense mechanism. RNA silencing suppressors have been reported recently in other forms of pathogens like bacteria and oomycetes, which suggest that these pathogens have this inherent capability of counter defense across various kingdoms. In this chapter, we discuss some of these phenomenal counter defense mechanisms by the viruses.

Keywords: RNA silencing, PTGS, antiviral defense, silencing suppressors

1. Introduction

1.1. The beginning of a story

As a matter of surprise, plant scientists, during the last decade of twentieth century, developed enthusiasm toward mechanism of gene silencing by virtue of plant transformation experiments, in which the introduction of a transgene into genome led to the silencing of transgene and homologous endogene [1, 2]. From these initial studies, plant biologists gained a wealth of information from gene silencing mechanisms and their complex pathways including their

mutual multiserial interactions that they express. Besides, the biologists have made prominent achievements in using RNA silencing as a powerful tool for studying gene expression and crop improvements. RNA silencing (also called as posttranscriptional gene silencing PTGS) refers to a family of gene silencing effects by which the expression of one or more genes is downregulated or entirely suppressed by the introduction of the antisense RNA molecule. The most common and studied representation is RNA interference (RNAi). RNAi is a biological process in which RNA molecules inhibit gene expression or translation by neutralizing targeted mRNA molecules. It also plays a crucial role in defending plants against viruses. Enzymes search double-stranded RNA (dsRNA) that is normally present in cells and digest it into small pieces that render them inefficient to cause disease. The phenomenon of RNAi process can be divided into three steps. First, a long double-stranded RNA (dsRNA) that is introduced into the cell is modified into small RNA duplexes by a ribonuclease III (RNAase III) enzyme known as DICER; second, these duplexes are unwound and one of the strands is advantageously loaded into a protein complex known as the RNA-induced silencing complex, and at this point, RISC binds to an ARGONAUTE (AGO) protein. Third, this complex essentially scans the transcriptome and locates target RNAs. The packed ssRNA called the gRNA (guide RNA) directs an endonuclease that is present in RISC (also known as slicer), now known to be an Argonaute protein to split mRNAs that contain a sequence homologous to the siRNA. The importance of siRNAs in antiviral defense is to direct RISC complex to viral genomic and sub-genomic RNAs, thereby targeting those molecules for destruction (**Figure 1** Courtesy Trends in Microbiology, Vol.16. No.5). Multifarious use of siRNAs as specificity factors has been demonstrated in antiviral defense. The dsRNA sequence source for various RNA and DNA viruses is not known, but it is likely that they could have originated during viral

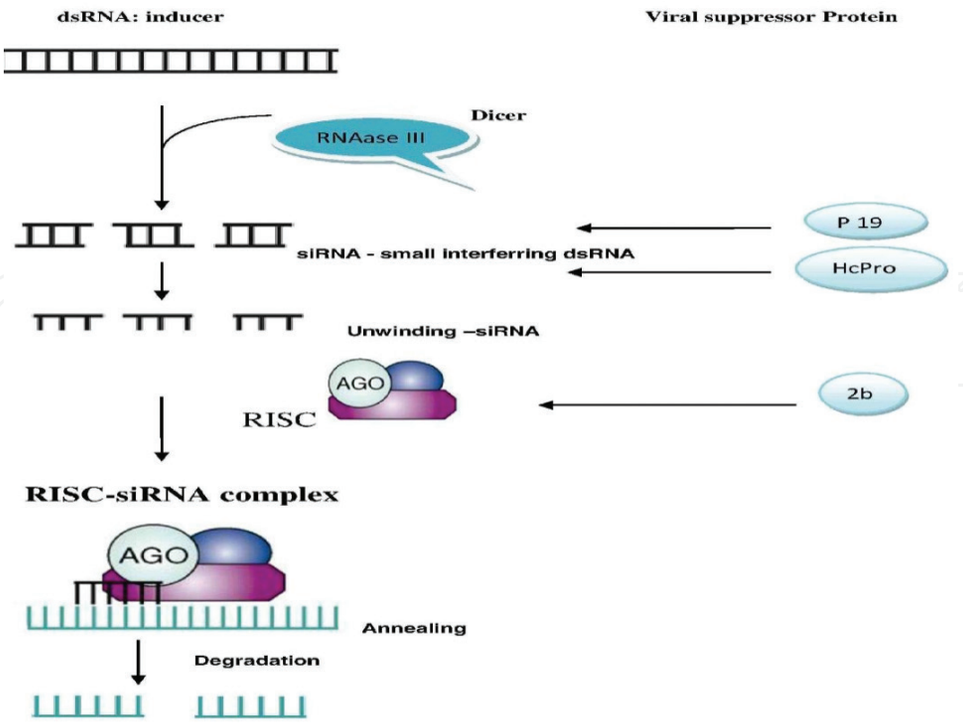

Figure 1. RNA-cleavage activity leading to the degradation of AGO1.

replication and/or from internal pairing of long RNA molecules [3]. RNA silencing is a highly complex system consisting of various proteins and processes [4]. This complexity makes the phenomenon of RNA silencing efficient for endogenous RNA expression during plant development and growth as well as for controlling viral infection. Viruses have adapted a robust mechanism for combating plant defense machinery by expressing suppressor proteins, which are capable of interfering in the process of RNA interference (RNAi) silencing pathway.

Features of RNAi

- Double-stranded RNA (dsRNA) rather than single-stranded antisense RNA is the intrusive agent.
- Silencing can be introduced in various progressive stages of the mechanism.
- RNAi is of high-degree specificity gene silencing mechanism.
- It circumvents the problems caused by knocked out genes in early stages (which could veil desired observations).
- Silencing actions are passed through ages. It means that some of the dsRNA molecules could not silence the genes in parent, but the same ds RNA molecule did silence genes in the offspring because the dsRNA sequence did not match any of the parent genes.

2. Viruses fight back

RNA silencing is predominantly a robust defense mechanism adapted by plants against pathogenic intruders especially viral pathogens. Viral pathogens have the ability to stay by suppressing RNA silencing. Plant viruses have developed tricky measures to overcome the host silencing response. One of these strategies is to overcome host-silencing response by producing proteins that target the signaling steps of RNA silencing [5]. Plants use RNA-silencing mechanism and produce short interfering RNA (SiRNA) molecules in a defense response against viral infection. To counter this defense response, virus produces suppressor proteins that can block the host silencing pathway or interfere with its function in plant cells [6]. The evidence of virus encoding RNA silencing suppressor proteins begins from experiments when silenced transgenes were again activated after virus inoculation. Silencing suppressors have been identified in DNA-containing viruses and from positive-strand RNA viruses [6]. Collectively, the plant viral silencing suppressors are diverse in sequence and evolutionary origin. Being functionally diverse, they target cell autonomous steps and systemic signaling steps of RNA silencing mechanism. Before discussing the specific silencing suppressors, it is important to consider the consequences. At the earliest, there would appear to be a conflict, and later, many viruses encode the protein suppressors that block the signaling steps of RNA silencing. From the pioneer experiments of first viral suppressors of silencing in 1998, the scientists have generated a wealth of information about plant viral proteins that block silencing pathway elucidating their mechanism of action [7, 8]. Virus-encoded RNA-silencing suppressors interfere with various steps of the different silencing pathways and the mechanisms of suppression are being unraveled more and more.

2.1. Suppressor and small RNA function

The prodigy of accumulation of primary siRNAs to considerable level in the presence of some suppressors and that the target RNA degradation blockage indicates that primary siRNAs are not functional. These findings have suggested viral suppressors of RNA silencing bind to small RNA duplexes, after they bind properly to small RNA duplexes, they separate them and prevent their entry into RNA-induced silencing complex or RISC effector complex (**Figure 1**) [9, 10] This separation of small RNA duplexes has been suggested as a usual mode of action for RNA silencing suppression. The suppressors of RNA silencing can also change the biochemical structure of siRNAs, thereby blocking their function. Earlier findings have suggested that plant endogenous small RNA and transgene siRNAs have methylated group at their 3′ termini, this being an HUA ENHANCER$_1$ (HEN$_1$) relying process in their synthesis this step of methylation of viral siRNAs has been shown for DNA or RNA virus-infected plants. It has been demonstrated that many viruses and viral suppressors interfere with both siRNA and/or miRNA methylation [11–13]. Moreover, the virus, alteration of host miRNA accumulation and function is believed to underlie at least some symptoms of plant virus infection [14, 15]. Despite the fact that maximum such research has focused on the role of viral suppressors, a recent study has shown that expression of other viral proteins can also affect miRNA accumulation and function [16]. Previous studies which considered plant viral suppressor's role in transgene induced silencing did not differentiate between primary and secondary siRNAs, and this led to topsy-turvies in the literature about whether a given suppressor did or did not block siRNA production. This ambiguity in results has been purposefully resolved, with the findings that some viral suppressors (i.e., P15 and P25) obstruct accumulation of primary siRNAs, whereas other viral suppressors (i.e., P1/Hc-Pro, P39, P19 siRNAs) leave primary siRNA accumulation unimpaired, [10, 12, 17]. This specialized obstruction in secondary siRNA accumulation might be produced simply by suppressing primary siRNA function.

3. RNA silencing suppressors

Plants defend themselves against viruses by RNA silencing; however, plant viruses spoil this defense machinery by expressing proteins that act as RNA silencing suppressors. Plants react to pathogens using elaborate networks of genetic interactions. Evidential progress has been made in understanding RNA silencing and how viruses counter this apparently ubiquitous antiviral defense. The best example of a viral suppressor that uses host factors that are not direct components of the silencing machinery to block silencing is the HC-Pro suppressor encoded by potyviruses. Two such factors have been reported so far [18, 19], and these will be discussed in more detail subsequently. The first is a calmodulin-like protein called regulator of gene silencing calmodulin-like (rgs-CaM). Tobacco rgs-CaM was identified as an HC-Pro-interacting protein in a yeast two-hybrid screen, and subsequent experiments showed that overexpression of rgs-CaM interfered with virus-induced gene silencing (even in the absence of HC-Pro). Plants encode a large family of calmodulin-like proteins, which are characterized by the presence of a calmodulin domain with either amino-terminal or carboxy-terminal extensions. Experiments to determine if rgs-CaM is required for HC-Pro suppression of

silencing await identification of the *Arabidopsis* homolog of the tobacco gene, which would open up numerous genetic approaches available in that model plant. Many viral protein suppressors of RNA silencing have been described so far, and extensive research was focused on a selection of these below mentioned proteins (suppressors).

3.1. Cucumoviral 2b

The 2b protein of the cucumovirus was recognized as a silencing suppressor at about the same time as P1/HC-Pro of potyviruses. The CMV 2b protein, a nuclear protein that is required for long distance movement of the virus, functions as the silencing suppressor [20]. Viral-suppressor protein 2b interact directly with components of the RNA-induced silencing complex RISC machinery, 2b interact with AGO1, by inhibiting its RNA-cleavage activity leading to the degradation of Argonaute protein AGO1 (**Figure 1**). 2b specifically inhibits AGO1 cleavage activity in RISC reconstitution assessment. In addition, AGO1 recruit's virus-derived small interfering RNAs (siRNAs) *in vivo*, suggesting that AGO1 is a major factor in defense against CMV infection. Viral suppressors of RNA silencing (VSRs) counter act RNAi based viral immunity. Many VSRs proteins have been reported, which play diverse functions in addition to suppressing RNA silencing, like viral replication, movement, coating and pathogenesis. Mostly plant viruses use VSRs as a tool to counter host defense machinery.

3.2. Potexviral P25

The P25 of the potexvirus, Potato virus X (PVX) is one of three cell-to-cell movement proteins (MPs) required for transport of virus from one cell to the next, the effects of P25 on cell autonomous and systemic silencing have been tested. 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 [14] 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 system. Studies have shown that the P25 protein encoded by potato virus X inhibits either the assembly or the function of the effector complexes of antiviral defense. Viruses counter the RNA silencing based counter defense by expressing VSRs. These VSRs are in turn recognized by host as avirulence (avr) factors to induce R- mediated resistance (Plant genomes carry many R genes that recognize specific pathogens and induce resistance against them).

3.3. Helper-component proteinase (HC-Pro)

Helper-component proteinase (a pathogenicity regulator of potyviruses) is a necessary, multifunctional protein of the family *Potyviridae* initially identified as a mediator of synergistic viral disease, acts to suppress the establishment of both transgene-induced and virus-induced gene silencing, and the Hc-Pro protein product is required for suppression. Hc-Pro binds to ds siRNA intermediates and has been suggested to function by sequestering ds siRNAs or by inhibiting their unwinding to ss siRNAs [15, 21] **Figure 1** Courtesy Trends in Microbiology, Vol.16. No.5. HC-Pro in association with p25 or 2b targets intracellular and intercellular silencing respectively. This discovery regarding possible mechanism of silencing suppression was shown by interaction

between P1/HC-Pro of TEV (Tobacco etch virus) and rgs-CaM a tobacco calmodulin like protein [18]. It was demonstrated that rgs-CaM suppresses itself RNA silencing mechanism upon over-expression in the plants points to the role of gene silencing as a natural antiviral defense system in plants and offer different approaches to explain the molecular basis of gene silencing.

3.4. Tombusvirus P19

From the time of its discovery, the Tombusvirus encoded P19 protein (P19) in the late 1980s, the status of this potent suppressor changed from being thought obsolete to its identification a decade later as an important viral pathogenicity factor. A recent study also has confirmed that *Pothos latent virus* (PLV) encode p14 silencing suppressor, although the genome of Tombusvirus is similar to PLV, its suppressor (p14) is smaller than P19 with higher affinity to long dsRNAs. Tombusvirus P19 is a protein encoded by *Tomato bushy stunt virus* and related tombusvirus. Studies have demonstrated that P19 and p14 are RNA silencing suppressors (RSS) in plant cells [22]. P19 was reported to suppress PTGS mainly along the vein tissue and in newly emerging leaves, whereas HC-Pro reversed PTGS in a non-tissue- specific manner [10, 23]. A study confirmed [10, 24] that

- P14 binds to long and short dsRNA including the siRNA duplex.
- P19 is a potent suppressor of PTGS
- P19 is a suppressor of viral induced gene silencing (VIGS), P19 can also fetter to ds siRNA by inhibiting their untwining to ss siRNAs, thereby counter the silencing mechanism.

3.5. V2 suppressor

The suppression of silencing is a key mechanism for successful viral entry The V2 protein of *Tomato yellow leaf curl China virus* (TYLCCNV) was identified as an RNA silencing suppressor by *Agrobacterium*-mediated co-infiltration. The V2 protein could inhibit local RNA silencing [25].

V2 suppressor of *Tomato yellow mosaic virus* binds the coiled-coil protein suppressor of the gene-silencing SGS3 homolog. These reports provide novel insight into the mechanisms developed by viruses to target the defense system of the plant [26]. DNA viruses from the Geminiviridae family encode several proteins namely C2, C4 and V2 which suppress transcriptional and post-transcriptional gene silencing (TGS/PTGS). In Begomovirus, the most abundant genus of this family, three out of six genome-encoded proteins, namely C2, C4 and V2, have been shown to suppress PTGS, with V2 being the potent PTGS suppressor. Beet curly top virus (BCTV), the model species for the Curtovirus genus, is able to infect the widest range of plants among Geminiviruses. In this genus, C2/L2 protein has been described as inhibiting post-transcriptional gene silencing [27].

3.6. P0 protein

The P0 protein of the Polerovirus (Polerovirus is a genus of viruses, in the family Luteoviridae, plants serve as natural hosts) and P1 protein of the Sobemovirus (Sobemovirus is a genus of

viruses, plants serve as natural hosts) suppress the plant's RNA silencing machinery. Here authors [28–30] identified a silencing suppressor protein (SSP), $P0^{PE}$, in the genus *Enamovirus* with only one species *Pea enation mosaic virus*-1 (PEMV-1) and showed that it and the P0s of the Polerovirus *Potato leaf roll virus* and *Cereal yellow dwarf virus* have strong local and systemic SSP activity, while the P1 of genus *Sobemovirus* type species *Southern bean mosaic virus* suppresses systemic silencing. The nuclear localized $P0^{PE}$ has no observable sequence conservation with known SSPs, but proved to be a strong suppressor of local silencing and a moderate suppressor of systemic silencing. Like the P0s from the Polerovirus $P0^{PE}$ destabilizes AGO1 and this action is mediated by an F-box-like domain. Therefore, despite the lack of any sequence similarity, the Poleroviral and Enamoviral SSPs have a conserved mode of action on the RNA silencing mechanism.

4. Conclusion

RNA silencing suppressors (RSSs) are very important factors for virus biology. Virus encodes RSS irrespective of their genome size, for example the geminivirus genome with only 2.7 kb genome size encodes three suppressors (AL2/AC2, AC4 and possibly βC1), whereas CTV with large genome size of 40 kb encodes three RSS. Therefore, the size of the genome is not an indicator of number RSS. Through their evolution, plants and pathogens have adapted and evolved a wide variety of sophisticated strategies to attack, defend, and counterattack. Plants have acquired abilities to sense and defend against invading pathogens by utilizing pre-existing and/or induced barriers to stop infection. In parallel, plant pathogens have evolved diverse ways to counter or overcome host disease resistance. One of the common pathogen strategies involves the production of plant defense suppressors. Viruses evolve rapidly to their host organism and adapt themselves. In contrast, the cellular organisms evolve and adapt to a lesser extent. Keeping in view the fact that viruses possess antiviral defense system suggests that viruses and their host have coevolved. This interdependence among the life forms cannot be fully understood except in an evolutionary frame work. RNAi is a resistance defensive mechanism in plants which targets viral genomes and transcripts to degradation, several findings have revealed viral suppressors that target plant proteins and the possible actions that viruses take during their interference with the defense systems of the host: there remain many unanswered questions for example, the type of proteolysis machinery used by P0 to degrade its plant interactor AGO1 is a matter of debate and the mechanism by which V2 disrupts the RNAi- silencing system of the plant is unknown. The more we dig into the ongoing battle between viruses and their hosts, the more we come to know about the intriguing defense and counter-defense strategies that enable plants and viruses to coexist. To conclude, it can be stated that the interactions between antiviral RNA silencing and the counter measures viruses have evolved to frustrate such process is a continuously evolving action in the continuously evolving microbial world. On the one hand, a very important topic in virology, and on the other hand, a strong starting point for breakthroughs in other fields of research such as functional genomics and development. In an application environment, RNA silencing has allowed us to develop efficient and broad virus resistance in plants, which plays a crucial role to the reliable production of food. RNAi suppression holds the potential of unearthing many unexpected surprises and this promising field is the object of intense investigation.

Acknowledgements

The author is thankful to Professor Qamar Abbas Naqvi, Department of Botany, Aligarh Muslim University, Aligarh, for providing necessary help in writing this chapter.

Conflict of interest

The author declares that there is no conflict of interest.

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