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The Evolving World of Small RNAs from RNA Viruses

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Abstract

RNA virus infection in plants and invertebrates can produce virus-derived small RNAs. These RNAs share features with host endogenous small interfering RNAs (siRNAs). They can potentially mediate RNA interference (RNAi) and related RNA silencing pathways, resulting in specific antiviral defense. Although most RNA silencing components such as Dicer, Ago2, and RISC are conserved among eukaryotic hosts, whether RNA virus infection in mammals can generate functional small RNAs that act in antiviral defense remains under discussion. Here, we review recent studies on the molecular and biochemical features of viral siRNAs and other virus-derived small RNAs from infected plants, arthropods, nematodes, and vertebrates and discuss the genetic pathways for their biogenesis and their roles in antiviral activity.

INTRODUCTION

RNA-mediated gene silencing is an evolutionarily conserved mechanism in which the presence of intracellular double-stranded (ds) RNA triggers a pathway that leads to the production of small RNAs that mediate regulation of gene expression. Small RNAs, ~ 20–30 nucleotides (nt) in length, are non-coding and regulate gene expression by base pairing with their RNA targets to effect posttranscriptional and/or transcriptional silencing. In the past decade, virus-derived small RNAs have been discovered in plants, arthropods, nematodes, and mammals. These small RNAs share features with host endogenous small interfering RNAs (siRNAs) and can potentially mediate RNA silencing pathways against virus infection.

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In infected cells, replication of the virus genome generates dsRNAs. Viral dsRNA molecules are produced in cells infected with diverse types of RNA viruses: (i) dsRNA viruses such as Drosophila X virus (DXV) [1]; (ii) single-stranded RNA viruses that produce dsRNA as the result of the formation of secondary structures, such as Sindbis virus [2, 3], vesicular stomatitis virus [4], and enterovirus 71 (EV71) [5], and/or dsRNA replication intermediates such as Drosophila C virus (DCV) and Semliki Forest virus (SFV) [6, 7].

Long viral dsRNA molecules trigger the antiviral siRNA pathway [8]. The dsRNAs are processed by the dsRNA-specific endoribonuclease (RNase) Dicer into ~22 bp dsRNAs. One strand of the RNA duplex is loaded into the RNA-induced silencing complex (RISC). [9, 10]. The loaded viral siRNA binds a viral RNA (genome or transcript) by sequence complementarity leading to Ago-dependent degradation of the targeted RNA and restriction of virus replication [9, 10]. However, viruses have evolved numerous evasion strategies, including RNAi suppressors, to counteract the host's antiviral immunity. Recent studies indicate that in addition to siRNA, other related small RNAs, such as microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs), contribute to antiviral responses as well. This has led to a better understanding of small RNA-based antiviral immunity and its contribution to the control of viral spread and pathogenesis [11–15]. In this review, we summarize our current understanding of the biogenesis of RNA virus-derived small RNAs (vsRNAs) and their roles in antiviral defense in plants, arthropods, nematodes, and vertebrates. We will also discuss the controversy as to whether or not vsRNAs elicit an RNAi-type antiviral response in vertebrates. Finally, we will survey ways in which viruses short circuit the RNAi pathway.

RNA SILENCING PATHWAYS

Synthesis of siRNAs from long viral dsRNA and their mechanisms of action in posttranscriptional gene silencing are well characterized. In *Drosophila* for example, Dicer-2 cleaves long dsRNAs into a pool of 21-nt siRNA duplexes (Fig. 1A) [16]. Loquacious isoform PD (Loqs-PD), a dsRNA binding protein, and Arsenite resistant protein 2 (Ars2) increase the affinity of Dicer-2 for its substrates [17]. R2D2, another dsRBP, and Armitage, an RNA helicase, participate in siRNA loading with Ago2 and facilitate assembly of RISC [18, 19]. C3PO, an endoribonuclease, aids the cleavage of one strand (passenger strand) of the siRNA duplex by Ago2 [20]. The remaining strand (guide strand) is subsequently methylated at its 3′ end by Hen-1 (an S-adenosylmethionine methyltransferase) to facilitate the maturation of RISC [21]. RISC binds its RNA target by base pairing via the guide strand RNA. Ago2-mediated degradation of the target RNA ensues. siRNA synthesis and function is similar in plants (Fig. 1B). However, plants present the ability to produce secondary siRNAs via action of cellular RNA-dependent RNA polymerases (RDRP).

ANTIVIRAL FUNCTION OF siRNA

Plants

RNA silencing is a major antiviral mechanism in plants. Several lines of evidence underlie the antiviral role of siRNAs in virus-infected plants. (i) vsRNAs were discovered for the first time in potato virus X (PVX)-infected plants in 1999 [22]. Abundant antisense small RNAs complementary to viral RNA were detected in PVX-infected plants. These RNAs were about

25 nt in length and were derived from viral RNA, as their accumulation required virus replication. These antisense small RNAs exhibited antiviral activity as they base pair with the viral RNA to induce posttranscriptional gene silencing and inhibit virus replication. Subsequently, antiviral siRNAs derived from cucumber mosaic virus (CMV), cymbidium ringspot virus, tombusvirus, tobacco rattle virus, turnip crinkle virus, turnip mosaic virus, sugarcane mosaic virus, and Chinese wheat mosaic virus were discovered [23–30]. (ii) Many plant viruses encode suppressors of RNA silencing to overcome the host's RNAi-based defense system (discussed later). Plants infected by suppressor-deficient viruses rapidly recover from virus-induced disease [11, 31–34]. (iii) Plants harboring mutant components of the siRNA pathway are more susceptible to virus infection [31, 35].

Virus-derived siRNAs can also enter the plant RDRP-mediated amplification cycle to enhance the antiviral silencing response. For example, Wang et al. [23] and Garcia-Ruiz et al. [28] demonstrated that antiviral RNA silencing within *Arabidopsis thaliana* cells infected with cucumber mosaic virus (CMV) or turnip mosaic virus required the host's RDRP. RDRP serves to amplify the numbers of antiviral siRNA molecules initially generated during virus replication.

The diversity of Ago proteins in *Arabidopsis thaliana*, for example, lends flexibility to the siRNA-mediated antiviral response in this organism [36, 37]. *A. thaliana* has ten Ago genes, of which Ago1, Ago2, and Ago7 have been implicated in antiviral defense against various viruses. Ago3 and Ago5 also associate with virus-derived siRNAs and play roles in antiviral responses [38, 39]. Genetic analyses of *A. thaliana* revealed that Ago 1, Ago 2, and Ago10 enhance antiviral defense against turnip mosaic virus in various organs [39].

Arthropod invertebrates

The siRNA/RNAi pathway mediates the antiviral response in invertebrates, as indicated by the effects of mutations within the RNAi pathway on virus replication. Li et al. [40] showed that infection of Drosophila S2 cells with flock house virus (FHV) resulted in rapid accumulation of FHV-specific siRNAs of both positive- and negative-strand polarities. Accumulation of the siRNAs decreased the abundance of FHV RNAs at later stages of virus infection. Clearance of FHV genomic and subgenomic RNA from infected cells required Ago2, suggesting an antiviral, RNA silencing role for the siRNAs. Moreover, flies deficient for Dicer or Ago2 were unable to control virus replication, and as a consequence, were hypersensitive to FHV infection. This study provided the first experimental evidence that RNA silencing acts as an antiviral defense mechanism in animal cells.

An abundance of other discoveries followed regarding additional RNAi factors and viruses. For example, Ago2 is essential in order to block two other insect positive-strand RNA viruses, Drosophila C virus (DCV) and cricket paralysis virus (CrPV) [41, 42]. Vesicular stomatitis virus (VSV), a negative-sense, single-stranded RNA virus, replicates more efficiently in Dicer, Ago2, or R2D2 mutant flies [43]. Dicer protects flies against infection by Sindbis virus (SINV), an enveloped, positive-sense, single-stranded RNA virus [44]. Additionally, depletion of Ago2, R2D2, VIG, Armitage, Rem 62, or Ars2 in flies increases susceptibility to a diverse set of RNA viruses including Drosophila X virus (DXV), FHV, DCV, SINV, and VSV [45, 46].

Further studies reported discovery of positive-, negative-, and double-stranded vsRNAs and their involvement in antiviral defense in *D. melanogaster*, mosquitoes, silkworms, and *C. elegans* [11, 14, 35]. An analysis of small RNAs produced during VSV infection of Drosophila cells revealed that VSV-derived siRNAs were approximately evenly distributed between the positive and negative strands; the majority of these siRNAs clustered in a 1.6 kb region at the 5′ end of the genome [4]. Rift Valley Fever virus (RVFV)-derived siRNAs were present in approximately equal ratios between the positive and negative strands, and were distributed evenly across the genome. Likewise, analyses of vsRNAs from Himetobi P virus (HiPV)-infected small brown planthoppers revealed virus-derived siRNAs 21- and 22-nt in length [47]. Nearly two-thirds of the siRNAs were derived from the HiPV genomic RNA strand and were evenly distributed across the genome. Silencing Ago2 enhanced HiPV accumulation, providing genetic evidence for siRNA-mediated defenses against HiPV in this insect.

Mosquitoes encode homologues of all the RISC components described in *Drosophila*. An abundance of evidence indicates that siRNA/RNAi provides a critical antiviral mechanism in mosquitoes. For example, replication of o'nyong-nyong virus (ONNV) in mosquitoes is inhibited upon introduction of dsRNAs that target the viral genome [48]. This was the first demonstration that siRNA acts as an anti-arboviral response in mosquitoes. Studies from Blair and colleagues demonstrated that replication of Sindbis virus (SINV) and dengue type 2 virus (DENV-2) in cultured mosquito cell lines and adult mosquitoes was inhibited by transient expression of, or cytoplasmic introduction of, a long dsRNA derived from the virus genome. vsRNAs were detected, suggesting that siRNAs target viruses in mosquitoes [49–52]. *Aedes aegypti* employs RNAi against Sindbis virus infection, and depletion of Ago2, Dicer, or R2D2 in adult mosquitoes increases virus load in cells infected with ONNV or DENV-2 [48, 49, 53].

Expression by viruses of proteins or RNAs that suppress RNAi in mosquitoes provide additional evidence for RNAi-based antiviral immunity. For example, suppressor protein B2 inhibits siRNA function during ONNV and SINV infection [2]. Recently, van Cleef et al. [54] demonstrated that, while the mosquito RNA machinery processes Culex Y virus (CYV) dsRNAs into siRNAs, the CYV VP3 protein promotes suppression of RNA silencing. These observations strongly suggest that mosquitoes use siRNA/RNAi as a major antiviral mechanism against arboviruses. Clearly, viruses that encode siRNA/RNAi suppressors will replicate and disseminate more effectively in the host. (A later section will provide more discussion of virus-encoded RNAi suppressors.)

We should note that the antiviral response in mosquitoes and other insects is a critical factor for viral transmission and dissemination [55, 56]. For example, arboviruses in the *Flaviviridae*, *Bunyaviridae*, *Togaviridae*, and *Reoviridae* families are transmitted to vertebrate hosts via arthropod vectors such as mosquitoes, ticks, and midges. Several mosquito-borne arboviruses, such as DENV and chikungunya viruses, cause worldwide epidemics and high mortality or morbidity rates in humans. The mosquito vectors of arboviruses must control viral infections to minimize pathology in the mosquito, and thereby allow persistent infection and lifelong virus transmission.

The antiviral siRNA response in midges resembles that of other insect vectors of arboviruses. Midges transmit bluetongue virus (BTV) (*Reoviridae*), Oropouche virus (*Bunyaviridae*), and Schmallenberg virus (*Bunyaviridae*) to humans and livestock. Using reporter gene-based assays, Schnettler et al. [57] demonstrated functional antiviral siRNAs in BTV-infected *Culicoides sonorensis*-derived KC cells. These siRNAs inhibited BTV replication. Sequencing of small RNAs from infected cells revealed 21-nt, virus-derived siRNAs, similar to the virus-derived siRNAs produced during arbovirus infections of mosquitoes. In addition, the BTV dsRNA genome is accessible to a Dicer-like nuclease.

Unlike mosquitoes and midges, tick antiviral siRNAs are less understood and present distinct differences. Recently, Schnettler et al. [58] reported identification of vsRNAs in Langat virus (flavivirus)-infected tick cells. The vsRNAs appeared to be a key factor in the antiviral response. However, the small RNAs were longer (22 nt) than those from other arbovirus vectors and were mainly derived from the termini of the viral genome. Apparently, the subgenomic RNA of tick-borne flavivirus interferes with the tick's siRNA-dependent, antiviral immunity. This study highlights an important difference in the antiviral RNAi response between two major classes of arbovirus vectors.

Non-arthropod invertebrates

The siRNA/RNAi pathway also operates in non-insect invertebrates such as *Caenorhabditis elegans*. Virus-derived siRNAs protect *C. elegans* against virus infection. *C. elegans* cells defective for RDE-1 (Ago2 homolog), DRE-4 (R2D2 homolog), or RRF-1 (RDRP, required for siRNA amplification) are more susceptible to VSV infection [59, 60]. In addition, *C. elegans* lacking RDE-1 is more sensitive to Orsay virus (a positive-strand RNA virus, related to nodavirus) infection [61].

In *C. elegans*, Dicer partners with the dsRNA binding protein RDE-4 to process virus dsRNA into siRNAs. Full antiviral activity also requires a number of worm-specific genes such as rsd-2 (RNAi spreading defective 2) [62]. The rsd-2 null mutants permit higher levels of viral RNA accumulation. Ectopic expression of RSD-2 in rsd-2 mutants reverses the effect. RSD-2 functions in a mechanism that is independent of RDE-4, but it requires the RDRP, rrf-1. Thus, it is involved in the amplification of siRNAs for transduction of siRNA signals throughout the organism. As such, antiviral defense in *C. elegans* depends on the collective actions of RDE-4-dependent and RDE-4-independent pathways to generate siRNAs [63]. We also note that the viral small RNA-mediated silencing effects can be transmitted to multiple generations. The virus-derived small RNAs are transmitted in a template-independent manner and silence viral genomes present in worms that are unable to produce their own virus-derived small RNAs. These inherited RNA molecules protect ensuing generations from the virus by silencing the viral genome [64].

Deep sequencing of viral small RNAs elucidated the Lepidopteran antiviral response against infection by cricket paralysis virus (CrPV; *Reoviridae*), a segmented dsRNA virus [65]. The analysis showed that the silkworm *Bombyx mori* generates virus-derived siRNAs when infected by Bombyx cytoplasmic polyhedrosis virus (BmCPV). Moreover, in addition to cleavage of viral dsRNAs by Dicer, an uncharacterized RNase cleaves the viral RNA to

generate vsRNAs. This RNase activity may be an important early factor for the host's antiviral defense in Lepidoptera.

Vertebrates

As discussed above, it is well established in plants and invertebrates that for antiviral RNAi, Dicer processes virus-derived dsRNA into siRNAs that guide Ago proteins to silence complementary viral RNA. As a counter measure, viruses encode suppressors that block RNA silencing [35]. However, whether mammals restrict virus infections by virus-derived siRNAs and RNAi is still controversial. Cullen et al. [66] and Weng et al. [12] have recently discussed findings that favor or challenge RNAi as an antiviral response in mammals. Below, we update and summarize recent reports on this topic.

1. Evidence against antiviral RNAi in mammals—Although several studies have provided evidence suggesting vsRNAs, including siRNAs, promote an antiviral response in infected mammals, other research groups have challenged the effectiveness of vsRNAs in antiviral defense. One rationale is simply that the abundance of vsRNAs generated in infected mammalian cells may not be sufficient to initiate an antiviral response. Estimates are that at least 100 copies per cell of a small RNA are required for it to be functional [67]. Parameswaran et al. [68] examined vsRNAs from mammalian host cells infected with five vertebrate viruses: hepatitis C virus (HCV), polio, DENV, VSV, and West Nile virus (WNV). In some cases they detected only "vanishingly rare" levels (i.e., < 0.1% of total cellular small RNAs) of certain vsRNAs in infected cells. However, other groups readily detected vsRNAs with Northern blots [12, 69–71]. These observations might be explained by a bias in vsRNA measurement by deep sequencing and Northern blotting, such that levels of vsRNA determined by deep sequencing might be underestimated.

Another issue is whether vsRNAs in infected mammalian cells arise from Dicer cleavage or non-specific RNA degradation. Dicer produces siRNAs that are typically 22 ± 2 nt with two unpaired nucleotides at the 3′ termini of either strand, and derive in roughly equal proportions between the plus and minus strand of virus dsRNA [68, 72]. By contrast, vsRNAs from mammalian cells typically present a random size-distribution and strong strand bias; this suggests they result from non-specific degradation of viral genome RNA [5, 68]. A recent study by Bogerd er al. [72] using deep sequencing demonstrated that infection of human cells by DENV or WNV did not result in production of any virus-derived siRNA or miRNA. The reads from DENV-infected cells showed no length preference between 18–29 nt; most of the reads from WNV-infected cells were < 20 nt. Additionally, they used gene editing to generate human cell lines lacking Dicer activity (NoDice) and thus unable to produce small RNAs. Infection of these cells with a wide range of viruses, including DENV, WNV, yellow fever virus, Sindbis virus, Venezuelan equine encephalitis virus, measles virus, influenza A virus, reovirus, VSV, human immunodeficiency virus type 1 (HIV-1), or herpes simplex virus 1 (HSV-1) did not enhance virus replication. This overall uncertainty has led to the speculation that the type I interferon (IFN-I) response is sufficient to suppress virus replication [66, 73, 74]. In mammalian cells, the IFN-I response is an antiviral defense that recognizes viral dsRNA and triggers IFN-stimulated gene (ISG) expression to suppress virus replication [73, 74].

Work from Backes et al. [75] examined the antiviral contributions of both silencing by small RNAs and the IFN-I system in mammalian cells. Deep sequencing results showed that the two most abundant VSV-derived small RNAs were Dicer independent. To assess the contribution of small RNAs and IFN to the mammalian response to virus infection, they engineered mutant VSV strains that were able to disrupt small RNA-mediated silencing, or to disrupt IFN activity, or to disrupt both activities. They found that a lack of small RNAs did not affect virus titers in vitro or in vivo. By contrast, a virus strain with the capacity to inhibit the IFN system increased virus titers. This study indicated that small RNA-mediated silencing is not a physiological contributor to the IFN-triggered cellular response to RNA virus infection in mammalian cells. Their findings are consistent with an earlier report of Seo et al. [76], which showed that IFN signaling stimulated by virus infection inhibited mammalian RNAi by inducing poly-ADP-ribosylation of RISC. These results suggest that in uninfected cells, ISG expression is suppressed by miRNAs. Virus infection leads to inhibition of miRNA activity, thus releasing the suppression of ISG expression. Taken together, these data suggest that mammalian RNAi and miRNAs may not serve as antiviral effectors in mammalian cells.

tenOever and colleagues [77] have hypothesized that even though the IFN-I response provides the major antiviral defense in mammalian cells (and chordates in general), the RNAi machinery might nonetheless be sufficiently robust to provide antiviral immunity under certain conditions. To examine their hypothesis, they engineered an RNAi-like response in mammalian cells and assessed its efficacy against infection by Influenza A virus (IAV) in the presence or absence of the IFN-I response. Their strategy was to produce virus strains that would be targeted by species-specific miRNA (acting as a siRNA) or that would be self-targeted by virus-derived small RNA. The major finding from their study was that an RNAi response elicited by the host- or virus-derived small RNAs was highly effective at attenuating IAV in mammalian cells, even in the absence of the IFN-I response. Thus, RNAi can be engineered to act as an efficient antiviral defense in mammals. However, as they also concluded, an evolutionary event may have resulted in the transition from an RNAi-based to an IFN-I–based antiviral system since both systems can function in mammals.

2. Evidence for antiviral RNAi in mammals—By contrast, several studies suggest the existence of vsRNAs with antiviral activity in infected mammalian cells. Mammals have the necessary protein components such as Dicer, Ago2, and RISC to generate viral-derived siRNAs and load them into enzymatically active complexes. As well, even though the study by Parameswaran et. al. [68], noted above, found vanishingly rare quantities of some vsRNAs, others were found to be highly abundant, i.e., comparable in levels to abundant miRNAs.

Another issue noted above is whether vsRNAs in infected mammalian cells arise from Dicer cleavage or non-specific RNA degradation. Maillard et al. [78] reported that encephalomyocarditis virus (EMCV) or Nodamura virus (NoV) infection of undifferentiated mouse cells led to accumulation of ~22-nt RNAs with all the signature features of siRNAs, i.e., they were derived from viral dsRNA replication intermediates, associated with Ago2, were not produced in Dicer-knockout cells, and decreased in abundance upon cell differentiation. The study by Parameswaran et al. [68] also examined several characteristics of vsRNAs that would distinguish them from non-specific RNA degradation products. (i) For

HCV, polio, VSV, and WNV infected cells, the positive-to-negative strand ratios among vsRNAs were nearly 1:1 as would be expected for processing by Dicer or a different, dsRNA-specific endoribonuclease. (ii) There was evidence of two unpaired nucleotides at the 3′ termini of either strand within the total pool of vsRNAs. (iii) There was evidence that vsRNAs associated with the four Ago proteins in RISC and only one strand of the duplex was incorporated into these complexes.

Additional evidence consistent with RNAi-mediated antiviral immunity in mammals is that a number of mammalian viruses encode potent viral suppressors of RNA silencing (VSR). For example, the nucleocapsid N protein from mouse hepatitis virus (MHV, *Coronaviridae*) suppresses RNAi activity in infected cells and this requires its dsRNA-binding activity [79]. Knockdown of Dicer-1 or Ago2 enhances virus replication in mouse cells, as is expected for an RNA-mediated response. Likewise, genetic ablation of a NoV-encoded VSR, which antagonizes Dicer during infections, inhibits virus replication; viral replication is normal in RNAi-deficient mouse cells [78]. Finally, infection of hamster cells and suckling mice by NoV requires suppression of RNAi by the NoV B2 protein [80]. Loss of B2 activity leads to accumulation of viral siRNAs and rapid clearance of mutant viruses in mice. These studies provide compelling evidence for an antiviral RNAi response in mammals.

Recent studies from our groups showed that vsRNAs might also act through mechanisms beyond RNAi. Substantial levels of vsRNAs are generated in enterovirus 71 (EV71)-infected mammalian cells, and vsRNA accumulation is Dicer-dependent [5]. Overexpression of vsRNA inhibits internal ribosomal entry site (IRES)-dependent virus translation in infected cells. By contrast, blocking vsRNA function with antimers enhances virus yield and virus protein synthesis. At least a subset of vsRNAs blocks viral IRES activity by targeting stem-loop II within the viral IRES. Whether EV71 vsRNAs play an antiviral role in viral infection awaits further investigation. Subsequent studies found that vsRNA can enhance association of host regulatory RNA-binding proteins with the EV71 IRES [81]. The proteins identified so far include AUF1, HuR, KSRP/FBP2, and Ago2. Given the roles of these host proteins in translation and mRNA degradation, we speculate that vsRNAs may control virus translation, and replication, by affecting interaction of these regulatory proteins with the IRES [81].

In conclusion, one possible contributor to the controversy over an RNAi-mediated antiviral response in mammalian cells is that studies have employed different cell lines, viruses, and VSRs, yielding conflicting results. This suggests that certain cell types may support vsRNA generation and function, whereas others do not. It is perhaps noteworthy that ESCs and possibly other undifferentiated cell types appear to utilize RNAi for antiviral defense, but these are cells that do not mount an IFN-I response [66, 78]. This has led to the speculation that RNAi- and interferon-mediated antiviral responses play complementary roles [66, 77]. Verifying the specific cell niches that support vsRNA production and identifying potential roles of vsRNAs in mammalian hosts are essential. Clearly, additional work is needed to ascertain whether vsRNAs do significantly contribute to the mammalian antiviral response, particularly somatic cells.

VIRUS-ENCODED RNAi SUPPRESSORS

Viruses are both inducers and targets of gene silencing [82, 83]. Virus-induced gene silencing is elicited by dsRNA intermediates of replicating viruses or structured regions of viral RNAs [84]. The RNA silencing, antiviral defense mechanism is thus a major obstacle towards virus replication. As a counter defense, viruses encode protein suppressors of RNA silencing, or VSRs, that permit virus replication. Discovery of diverse viral proteins as suppressors of RNA silencing provides strong evidence for the effectiveness of vsRNAs for antiviral defense [11, 13].

Diverse RNA viruses encode protein suppressors of RNA silencing. In general, RNA silencing suppressors from different virus families that target the same step in the RNAi pathway do not share sequence identity or structural conservation. It is believed that these proteins have evolved independently in different viruses. Below, we provide a brief survey of virus-encoded suppressors to provide a sampling of the different steps and trans-acting factors within the RNAi pathway that viruses target (Fig. 1).

Suppressors targeting Ago proteins

Some suppressors target the Ago proteins and act via multiple mechanisms. The first suppressor shown to bind an Ago protein in vivo was the 2b protein of cucumber mosaic virus (CMV), a positive-strand RNA virus [85]. Interaction of the viral 2b protein with Ago1 in CMV-infected *A. thaliana* plants inhibits Ago1-mediated slicing of the target RNA in vitro. The 2b protein also interacts with long dsRNA and siRNA duplexes in vitro [86] and can inhibit host RDRP-dependent amplification of siRNAs [87]. Polerovirus encodes the F-box–containing P0 protein, which promotes degradation of Ago1 in tobacco plants [88, 89]. The binding of P0 to a component of RISC prevents siRNA-RISC assembly. Ago1 molecules not assembled into RISC are readily degraded. This occurs by P0 targeting Ago1 for ubiquitination and subsequent degradation by proteasomes.

Cellular proteins possessing glycine/tryptophan (GW/WG) repeats are important regulators of RNAi through their interactions with Ago proteins. For example, turnip crinkle virus (TCV) encodes the P38 protein, which contains two GW/WG repeats. P38 directly interacts with Ago1 and inhibits Ago1 activity [90]. Sweet potato mild mottle virus (SPMMV) encodes P1, which contains three GW/WG repeats. P1 binds to Ago1 and inhibits the silencing activity of RISC in two ways: by outcompeting an essential, endogenous GW/WG-containing component of RISC, or preventing recognition of target RNA by complementary siRNA loaded in RISC [91].

Some VSRs inhibit the slicing activity of Ago proteins. For example, cricket paralysis virus (CrPV) encodes protein 1A, which suppresses Ago2-mediated antiviral defense in Drosophila S2 cells [42]. 1A directly interacts with Ago2 and inhibits RNA silencing [92]. Likewise, Nora virus of *Drosophila melanogaster* (DmelNV) counteracts antiviral RNA silencing activity by encoding a host-specific VP1 protein. VP1 interacts with Ago2 and inhibits its slicer activity [93].

Suppressors targeting dsRNAs and/or siRNAs

VSRs also target the RNA components of RNA silencing. Functionally, these suppressors either bind long viral dsRNA to prevent processing into siRNAs or they bind siRNAs to block their activity. For example, the B2 protein encoded by FHV contains a dsRNA-binding domain. In FHV-infected *Drosophila* cells, B2 interacts with viral dsRNA intermediates and inhibits processing of long dsRNA into siRNAs [94]. The B2 protein encoded by NoV, like FHV, a member of the *Alphanodavirus* genus, inhibits RNA silencing by interacting with viral dsRNA and siRNA duplexes in mammalian cells [95]. Notably, B2-deficient NoV replicates slower than the wild-type virus in BHK-21 (baby hamster kidney) cells, suggesting B2-dependent resistance to the antiviral vsRNA mechanism in mammalian cells [80]. The DCV 1A proteins bind and shield long dsRNA from Dicer cleavage [96]. P19 encoded by tombusviruses promotes systemic infection by selectively binding to siRNA duplexes and preventing the siRNAs from being assembled into RISC [97, 98]. HC-Pro encoded by turnip mosaic virus is required to establish infection and systemic spread. HC-Pro acts by sequestering virus-derived siRNAs. HC-Pro may also function through its interaction with transcription factor RAV2, translation initiation factor eIF4E, Hsp90, and/or via downstream effects on silencing factors [39]. The mosquito Culex Y virus (CYV) VP3 protein binds dsRNA and siRNA and interferes with Dicer cleavage. This was the first virus-encoded suppressor reported in infected mosquito cells [54]. The tospovirus nonstructural NS protein sequesters siRNAs and prevents them from being loaded into RISC in infected plants [99, 100]. Moreover, as described above, MHV-encoded nucleocapsid N protein suppresses antiviral RNA silencing through its dsRNA-binding activity in mammalian cells [79].

RNase-like suppressors

Some RNA viruses that infect plants, insects, fish, or other animals encode class I RNase III-like proteins. Sweet potato chlorotic stunt virus (SPCSV) encodes an RNase III that contains an RNase domain and a dsRNA-binding domain. The viral RNase III cleaves long dsRNA, and 21–24–bp siRNA duplexes, into 14-bp fragments resulting in suppression of RNA silencing in SPCSV-infected plants [101]. Suppression requires the catalytic activity of the RNase III. Interestingly, even though most of the viral siRNA suppressor proteins identified so far do not share any conserved amino acid motifs or structural features, the RNase III enzymes are an exception, as they are homologous in unrelated RNA viruses [102].

Suppressors targeting Dicer

Flaviviruses generate a small, structured, non-coding RNA from the viral 3′UTR, called sfRNA. Recently, sfRNAs of WNV, DENV, Kunjin virus, tick-borne Langat virus, and tick-borne encephalitis virus (TBEV) were shown to suppress RNA silencing in insect and mammalian cells [58, 103–105]. sfRNAs co-immunoprecipitate with Dicer and Ago2 in infected cells. sfRNAs compete with Dicer substrates resulting in decreased Dicer activity. Furthermore, DENV non-structural protein 4B (NS4B) inhibits Dicer activity through an unknown mechanism [106]. Interestingly, in addition to binding dsRNAs and siRNAs, the FHV B2 protein can also interact with Dicer and inhibit its activity [107]. The Wuhan nodavirus (WhNV) B2 protein suppresses RNA silencing in *Drosophila* by interacting with

Dicer-2 and sequestering dsRNAs and siRNAs [108]. B2 directly binds to the RNase III and PAZ domains of Dicer-2 and blocks processing of dsRNAs by Dicer-2 and loading of siRNAs into RISC. RNA binding promotes the interaction of B2 and Dicer-2.

CONCLUSIONS AND PERSPECTIVES

RNA silencing is a primary defense mechanism against virus infection in plants, invertebrates, and possibly vertebrates. Viruses have evolved counter-defensive measures through the activity of virus-encoded, silencing-suppressor proteins. Understanding how antiviral silencing is controlled, and how suppressor proteins function, is essential for understanding how virus and host co-evolve, why some viruses are highly virulent in different hosts, and how sustainable antiviral strategies can be developed. Key challenges in the field are to characterize the molecular and biochemical features of vsRNAs, to identify the genetic pathway(s) for their biogenesis, and to determine their antiviral mechanisms. For example, what are the molecular/structural requirements for Dicer to recognize the substrates and initiate the siRNA pathway in infected cells? How do vsRNAs guide Ago2 and by what mechanisms? Do the vsRNAs in Ago-RISC turn over during virus infection? What downstream events are required for the most effective antiviral RNA silencing? As virus-derived small RNA research progresses further, it will surely advance our understanding of RNA silencing as an antiviral response.

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References

1. Dobos P, Hill BJ, Hallett R, Kells DT, Becht H, Teninges D. Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded RNA genomes. J Virol. 1979; 32:593–605. [PubMed: 228080]
2. Myles KM, Wiley MR, Morazzani EM, Adelman ZN. Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes. Proc Natl Acad Sci U S A. 2008; 105:19938–19943. [PubMed: 19047642]
3. Fragkoudis R, Attarzadeh-Yazdi G, Nash AA, Fazakerley JK, Kohl A. Advances in dissecting mosquito innate immune responses to arbovirus infection. The Journal of general virology. 2009; 90:2061–2072. [PubMed: 19570957]
4. Sabin LR, Zheng Q, Thekkat P, Yang J, Hannon GJ, Gregory BD, Tudor M, Cherry S. Dicer-2 processes diverse viral RNA species. PLoS One. 2013; 8:e55458. [PubMed: 23424633]
5. Weng KF, Hung CT, Hsieh PT, Li ML, Chen GW, Kung YA, Huang PN, Kuo RL, Chen LL, Lin JY, et al. A cytoplasmic RNA virus generates functional viral small RNAs and regulates viral IRES activity in mammalian cells. Nucleic Acids Res. 2014; 42:12789–12805. [PubMed: 25352551]
6. Siu RW, Fragkoudis R, Simmonds P, Donald CL, Chase-Topping ME, Barry G, Attarzadeh-Yazdi G, Rodriguez-Andres J, Nash AA, Merits A, et al. Antiviral RNA interference responses induced by Semliki Forest virus infection of mosquito cells: characterization, origin, and frequency-dependent functions of virus-derived small interfering RNAs. J Virol. 2011; 85:2907–2917. [PubMed: 21191029]
7. Myles KM, Morazzani EM, Adelman ZN. Origins of alphavirus-derived small RNAs in mosquitoes. RNA Biol. 2009; 6:387–391. [PubMed: 19535909]

8. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998; 391:806–811. [PubMed: 9486653]
9. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol. 2009; 10:126–139. [PubMed: 19165215]
10. Kobayashi H, Tomari Y. RISC assembly: Coordination between small RNAs and Argonaute proteins. Biochim Biophys Acta. 2015
11. Ding SW. RNA-based antiviral immunity. Nat Rev Immunol. 2010; 10:632–644. [PubMed: 20706278]
12. Weng KF, Hsieh PT, Huang HI, Shih SR. Mammalian RNA virus-derived small RNA: biogenesis and functional activity. Microbes and infection/Institut Pasteur. 2015; 17:557–563.
13. Wu Q, Wang X, Ding SW. Viral suppressors of RNA-based viral immunity: host targets. Cell Host Microbe. 2010; 8:12–15. [PubMed: 20638637]
14. Nayak A, Tassetto M, Kunitomi M, Andino R. RNA interference-mediated intrinsic antiviral immunity in invertebrates. Curr Top Microbiol Immunol. 2013; 371:183–200. [PubMed: 23686236]
15. Blair CD, Olson KE. The role of RNA interference (RNAi) in arbovirus-vector interactions. Viruses. 2015; 7:820–843. [PubMed: 25690800]
16. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature. 2001; 409:363–366. [PubMed: 11201747]
17. Cenik ES, Fukunaga R, Lu G, Dutcher R, Wang Y, Tanaka Hall TM, Zamore PD. Phosphate and R2D2 restrict the substrate specificity of Dicer-2, an ATP-driven ribonuclease. Mol Cell. 2011; 42:172–184. [PubMed: 21419681]
18. Tomari Y, Matranga C, Haley B, Martinez N, Zamore PD. A protein sensor for siRNA asymmetry. Science. 2004; 306:1377–1380. [PubMed: 15550672]
19. Tomari Y, Du T, Haley B, Schwarz DS, Bennett R, Cook HA, Koppetsch BS, Theurkauf WE, Zamore PD. RISC assembly defects in the Drosophila RNAi mutant armitage. Cell. 2004; 116:831–841. [PubMed: 15035985]
20. Liu Y, Ye X, Jiang F, Liang C, Chen D, Peng J, Kinch LN, Grishin NV, Liu Q. C3PO, an endoribonuclease that promotes RNAi by facilitating RISC activation. Science. 2009; 325:750–753. [PubMed: 19661431]
21. Horwich MD, Li C, Matranga C, Vagin V, Farley G, Wang P, Zamore PD. The Drosophila RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC. Curr Biol. 2007; 17:1265–1272. [PubMed: 17604629]
22. Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. Science. 1999; 286:950–952. [PubMed: 10542148]
23. Wang XB, Wu Q, Ito T, Cillo F, Li WX, Chen X, Yu JL, Ding SW. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2010; 107:484–489. [PubMed: 19966292]
24. Szittya G, Moxon S, Pantaleo V, Toth G, Rusholme Pilcher RL, Moulton V, Burgyan J, Dalmay T. Structural and functional analysis of viral siRNAs. PLoS Pathog. 2010; 6:e1000838. [PubMed: 20368973]
25. Szittya G, Molnar A, Silhavy D, Hornyik C, Burgyan J. Short defective interfering RNAs of tombusviruses are not targeted but trigger post-transcriptional gene silencing against their helper virus. Plant Cell. 2002; 14:359–372. [PubMed: 11884680]
26. Donaire L, Barajas D, Martinez-Garcia B, Martinez-Priego L, Pagan I, Llave C. Structural and genetic requirements for the biogenesis of tobacco rattle virus-derived small interfering RNAs. J Virol. 2008; 82:5167–5177. [PubMed: 18353962]
27. Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. Science. 2006; 313:68–71. [PubMed: 16741077]
28. Garcia-Ruiz H, Takeda A, Chapman EJ, Sullivan CM, Fahlgren N, Brempelis KJ, Carrington JC. Arabidopsis RNA-dependent RNA polymerases and dicer-like proteins in antiviral defense and

small interfering RNA biogenesis during Turnip Mosaic Virus infection. Plant Cell. 2010; 22:481–496. [PubMed: 20190077]

29. Xia Z, Peng J, Li Y, Chen L, Li S, Zhou T, Fan Z. Characterization of small interfering RNAs derived from Sugarcane mosaic virus in infected maize plants by deep sequencing. PLoS One. 2014; 9:e97013. [PubMed: 24819114]
30. Yang J, Zheng SL, Zhang HM, Liu XY, Li J, Li JM, Chen JP. Analysis of small RNAs derived from Chinese wheat mosaic virus. Arch Virol. 2014; 159:3077–3082. [PubMed: 24997977]
31. Szittya G, Burgyan J. RNA interference-mediated intrinsic antiviral immunity in plants. Curr Top Microbiol Immunol. 2013; 371:153–181. [PubMed: 23686235]
32. Silhavy D, Molnar A, Lucioli A, Szittya G, Hornyik C, Tavazza M, Burgyan J. A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-nucleotide double-stranded RNAs. EMBO J. 2002; 21:3070–3080. [PubMed: 12065420]
33. Burgyan J, Havelda Z. Viral suppressors of RNA silencing. Trends Plant Sci. 2011; 16:265–272. [PubMed: 21439890]
34. Nakahara KS, Masuta C. Interaction between viral RNA silencing suppressors and host factors in plant immunity. Curr Opin Plant Biol. 2014; 20:88–95. [PubMed: 24875766]
35. Ding SW, Voinnet O. Antiviral immunity directed by small RNAs. Cell. 2007; 130:413–426. [PubMed: 17693253]
36. Pumplin N, Voinnet O. RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat Rev Microbiol. 2013; 11:745–760. [PubMed: 24129510]
37. Schuck J, Gursinsky T, Pantaleo V, Burgyan J, Behrens SE. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic Acids Res. 2013; 41:5090–5103. [PubMed: 23535144]
38. Carbonell A, Carrington JC. Antiviral roles of plant ARGONAUTES. Curr Opin Plant Biol. 2015; 27:111–117. [PubMed: 26190744]
39. Garcia-Ruiz H, Carbonell A, Hoyer JS, Fahlgren N, Gilbert KB, Takeda A, Giampetruzzi A, Garcia Ruiz MT, McGinn MG, Lowery N, et al. Roles and programming of Arabidopsis ARGONAUTE proteins during Turnip mosaic virus infection. PLoS Pathog. 2015; 11:e1004755. [PubMed: 25806948]
40. Li H, Li WX, Ding SW. Induction and suppression of RNA silencing by an animal virus. Science. 2002; 296:1319–1321. [PubMed: 12016316]
41. van Rij RP, Saleh MC, Berry B, Foo C, Houk A, Antoniewski C, Andino R. The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in Drosophila melanogaster. Genes Dev. 2006; 20:2985–2995. [PubMed: 17079687]
42. Wang XH, Aliyari R, Li WX, Li HW, Kim K, Carthew R, Atkinson P, Ding SW. RNA interference directs innate immunity against viruses in adult Drosophila. Science. 2006; 312:452–454. [PubMed: 16556799]
43. Mueller S, Gausson V, Vodovar N, Deddouche S, Troxler L, Perot J, Pfeffer S, Hoffmann JA, Saleh MC, Imler JL. RNAi-mediated immunity provides strong protection against the negative-strand RNA vesicular stomatitis virus in Drosophila. Proc Natl Acad Sci U S A. 2010; 107:19390–19395. [PubMed: 20978209]
44. Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL. Essential function in vivo for Dicer-2 in host defense against RNA viruses in drosophila. Nat Immunol. 2006; 7:590–597. [PubMed: 16554838]
45. Zambon RA, Vakharia VN, Wu LP. RNAi is an antiviral immune response against a dsRNA virus in Drosophila melanogaster. Cell Microbiol. 2006; 8:880–889. [PubMed: 16611236]
46. Sabin LR, Zhou R, Gruber JJ, Lukinova N, Bambina S, Berman A, Lau CK, Thompson CB, Cherry S. Ars2 regulates both miRNA- and siRNA- dependent silencing and suppresses RNA virus infection in Drosophila. Cell. 2009; 138:340–351. [PubMed: 19632183]
47. Xu Y, Huang L, Wang Z, Fu S, Che J, Qian Y, Zhou X. Identification of Himetobi P virus in the small brown planthopper by deep sequencing and assembly of virus-derived small interfering RNAs. Virus Res. 2014; 179:235–240. [PubMed: 24239755]
48. Keene KM, Foy BD, Sanchez-Vargas I, Beaty BJ, Blair CD, Olson KE. RNA interference acts as a natural antiviral response to O'nyong-nyong virus (Alphavirus; Togaviridae) infection of Anopheles gambiae. Proc Natl Acad Sci U S A. 2004; 101:17240–17245. [PubMed: 15583140]

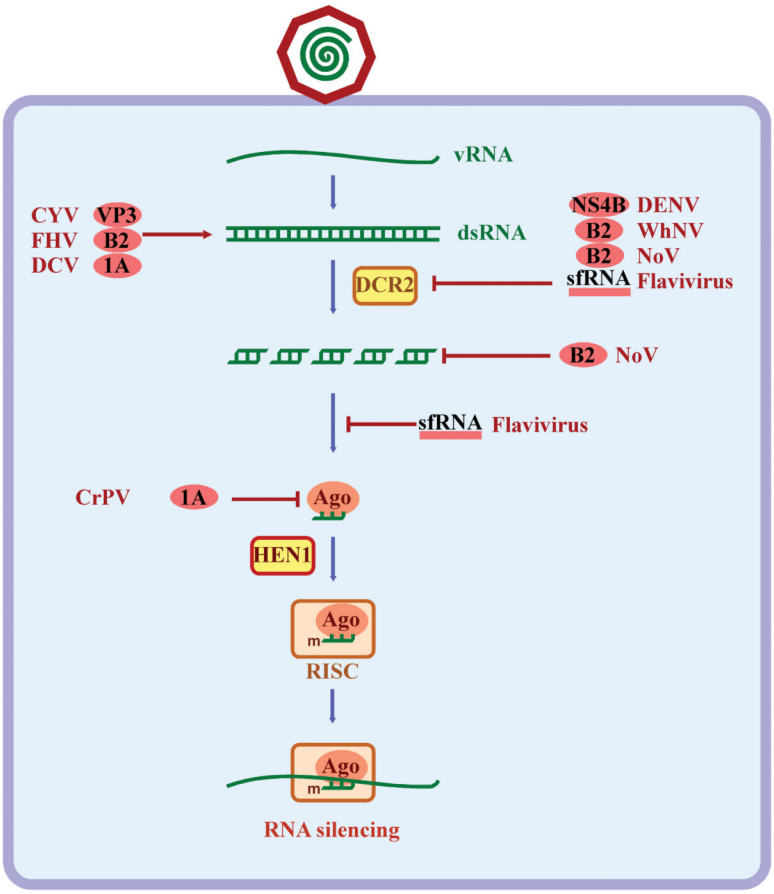
49. Sanchez-Vargas I, Scott JC, Poole-Smith BK, Franz AW, Barbosa-Solomieu V, Wilusz J, Olson KE, Blair CD. Dengue virus type 2 infections of Aedes aegypti are modulated by the mosquito's RNA interference pathway. PLoS Pathog. 2009; 5:e1000299. [PubMed: 19214215]
50. Gaines PJ, Olson KE, Higgs S, Powers AM, Beaty BJ, Blair CD. Pathogen-derived resistance to dengue type 2 virus in mosquito cells by expression of the premembrane coding region of the viral genome. J Virol. 1996; 70:2132–2137. [PubMed: 8642634]
51. Olson KE, Higgs S, Gaines PJ, Powers AM, Davis BS, Kamrud KI, Carlson JO, Blair CD, Beaty BJ. Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. Science. 1996; 272:884–886. [PubMed: 8629025]
52. Adelman ZN, Blair CD, Carlson JO, Beaty BJ, Olson KE. Sindbis virus-induced silencing of dengue viruses in mosquitoes. Insect Mol Biol. 2001; 10:265–273. [PubMed: 11437918]
53. Campbell CL, Black WCt, Hess AM, Foy BD. Comparative genomics of small RNA regulatory pathway components in vector mosquitoes. BMC Genomics. 2008; 9:425. [PubMed: 18801182]
54. van Cleef KW, van Mierlo JT, Miesen P, Overheul GJ, Fros JJ, Schuster S, Marklewitz M, Pijlman GP, Junglen S, van Rij RP. Mosquito and Drosophila entomobirnaviruses suppress dsRNA- and siRNA-induced RNAi. Nucleic Acids Res. 2014; 42:8732–8744. [PubMed: 24939903]
55. Blair CD. Mosquito RNAi is the major innate immune pathway controlling arbovirus infection and transmission. Future Microbiol. 2011; 6:265–277. [PubMed: 21449839]
56. Karlikow M, Goic B, Saleh MC. RNAi and antiviral defense in Drosophila: setting up a systemic immune response. Dev Comp Immunol. 2014; 42:85–92. [PubMed: 23684730]
57. Schnettler E, Ratinier M, Watson M, Shaw AE, McFarlane M, Varela M, Elliott RM, Palmarini M, Kohl A. RNA interference targets arbovirus replication in Culicoides cells. J Virol. 2013; 87:2441–2454. [PubMed: 23269795]
58. Schnettler E, Tykalova H, Watson M, Sharma M, Sterken MG, Obbard DJ, Lewis SH, McFarlane M, Bell-Sakyi L, Barry G, et al. Induction and suppression of tick cell antiviral RNAi responses by tick-borne flaviviruses. Nucleic Acids Res. 2014; 42:9436–9446. [PubMed: 25053841]
59. Schott DH, Cureton DK, Whelan SP, Hunter CP. An antiviral role for the RNA interference machinery in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2005; 102:18420–18424. [PubMed: 16339901]
60. Wilkins C, Dishongh R, Moore SC, Whitt MA, Chow M, Machaca K. RNA interference is an antiviral defence mechanism in Caenorhabditis elegans. Nature. 2005; 436:1044–1047. [PubMed: 16107852]
61. Felix MA, Ashe A, Piffaretti J, Wu G, Nuez I, Belicard T, Jiang Y, Zhao G, Franz CJ, Goldstein LD, et al. Natural and experimental infection of Caenorhabditis nematodes by novel viruses related to nodaviruses. PLoS Biol. 2011; 9:e1000586. [PubMed: 21283608]
62. Lu R, Yigit E, Li WX, Ding SW. An RIG-I-Like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in Caenorhabditis elegans. PLoS Pathog. 2009; 5:e1000286. [PubMed: 19197349]
63. Guo X, Zhang R, Wang J, Lu R. Antiviral RNA silencing initiated in the absence of RDE-4, a double-stranded RNA binding protein, in Caenorhabditis elegans. J Virol. 2013; 87:10721–10729. [PubMed: 23885080]
64. Rechavi O, Minevich G, Hobert O. Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 2011; 147:1248–1256. [PubMed: 22119442]
65. Zografidis A, Van Nieuwerburgh F, Kolliopoulou A, Apostolou-Karampelis K, Head SR, Deforce D, Smagghe G, Swevers L. Viral small RNA analysis of Bombyx mori larval midgut during persistent and pathogenic cytoplasmic polyhedrosis virus infection. J Virol. 2015
66. Cullen BR, Cherry S, tenOever BR. Is RNA interference a physiologically relevant innate antiviral immune response in mammals? Cell Host Microbe. 2013; 14:374–378. [PubMed: 24139396]
67. tenOever BR. RNA viruses and the host microRNA machinery. Nat Rev Microbiol. 2013; 11:169–180. [PubMed: 23411862]
68. Parameswaran P, Sklan E, Wilkins C, Burgon T, Samuel MA, Lu R, Ansel KM, Heissmeyer V, Einav S, Jackson W, et al. Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. PLoS Pathog. 2010; 6:e1000764. [PubMed: 20169186]

69. Girardi E, Chane-Woon-Ming B, Messmer M, Kaukinen P, Pfeffer S. Identification of RNase L-dependent, 3′-end-modified, viral small RNAs in Sindbis virus-infected mammalian cells. MBio. 2013; 4:e00698–00613. [PubMed: 24255120]
70. Morazzani EM, Wiley MR, Murreddu MG, Adelman ZN, Myles KM. Production of virus-derived ping-pong-dependent piRNA-like small RNAs in the mosquito soma. PLoS Pathog. 2012; 8:e1002470. [PubMed: 22241995]
71. Hussain M, Asgari S. MicroRNA-like viral small RNA from Dengue virus 2 autoregulates its replication in mosquito cells. Proc Natl Acad Sci U S A. 2014; 111:2746–2751. [PubMed: 24550303]
72. Bogerd HP, Skalsky RL, Kennedy EM, Furuse Y, Whisnant AW, Flores O, Schultz KL, Putnam N, Barrows NJ, Sherry B, et al. Replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. J Virol. 2014; 88:8065–8076. [PubMed: 24807715]
73. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. Nat Rev Immunol. 2008; 8:559–568. [PubMed: 18575461]
74. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011; 472:481–485. [PubMed: 21478870]
75. Backes S, Langlois RA, Schmid S, Varble A, Shim JV, Sachs D, tenOever BR. The Mammalian response to virus infection is independent of small RNA silencing. Cell Rep. 2014; 8:114–125. [PubMed: 24953656]
76. Seo GJ, Kincaid RP, Phanaksri T, Burke JM, Pare JM, Cox JE, Hsiang TY, Krug RM, Sullivan CS. Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. Cell Host Microbe. 2013; 14:435–445. [PubMed: 24075860]
77. Benitez AA, Spanko LA, Bouhaddou M, Sachs D, tenOever BR. Engineered Mammalian RNAi Can Elicit Antiviral Protection that Negates the Requirement for the Interferon Response. Cell Rep. 2015; 13:1456–1466. [PubMed: 26549455]
78. Maillard PV, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW, Voinnet O. Antiviral RNA interference in mammalian cells. Science. 2013; 342:235–238. [PubMed: 24115438]
79. Cui L, Wang H, Ji Y, Yang J, Xu S, Huang X, Wang Z, Qin L, Tien P, Zhou X, et al. The Nucleocapsid Protein of Coronaviruses Acts as a Viral Suppressor of RNA Silencing in Mammalian Cells. J Virol. 2015
80. Li Y, Lu J, Han Y, Fan X, Ding SW. RNA interference functions as an antiviral immunity mechanism in mammals. Science. 2013; 342:231–234. [PubMed: 24115437]
81. Lin JY, Brewer G, Li ML. HuR and Ago2 Bind the Internal Ribosome Entry Site of Enterovirus 71 and Promote Virus Translation and Replication. PLoS One. 2015; 10:e0140291. [PubMed: 26451954]
82. Rahman J, Karjee S, Mukherjee SK. MYMIV-AC2, a geminiviral RNAi suppressor protein, has potential to increase the transgene expression. Appl Biochem Biotechnol. 2012; 167:758–775. [PubMed: 22592775]
83. Ma X, Nicole MC, Meteignier LV, Hong N, Wang G, Moffett P. Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. J Exp Bot. 2015; 66:919–932. [PubMed: 25385769]
84. Voinnet O. Induction and suppression of RNA silencing: insights from viral infections. Nat Rev Genet. 2005; 6:206–220. [PubMed: 15703763]
85. Zhang X, Yuan YR, Pei Y, Lin SS, Tuschl T, Patel DJ, Chua NH. Cucumber mosaic virus-encoded 2b suppressor inhibits Arabidopsis Argonaute1 cleavage activity to counter plant defense. Genes Dev. 2006; 20:3255–3268. [PubMed: 17158744]
86. Goto K, Kobori T, Kosaka Y, Natsuaki T, Masuta C. Characterization of silencing suppressor 2b of cucumber mosaic virus based on examination of its small RNA-binding abilities. Plant Cell Physiol. 2007; 48:1050–1060. [PubMed: 17567638]
87. Diaz-Pendon JA, Li F, Li WX, Ding SW. Suppression of antiviral silencing by cucumber mosaic virus 2b protein in Arabidopsis is associated with drastically reduced accumulation of three classes of viral small interfering RNAs. Plant Cell. 2007; 19:2053–2063. [PubMed: 17586651]

88. Bortolamiol D, Pazhouhandeh M, Marrocco K, Genschik P, Ziegler-Graff V. The Polerovirus F box protein P0 targets ARGONAUTE1 to suppress RNA silencing. Curr Biol. 2007; 17:1615–1621. [PubMed: 17869109]
89. Csorba T, Lozsa R, Hutvagner G, Burgyan J. Polerovirus protein P0 prevents the assembly of small RNA-containing RISC complexes and leads to degradation of ARGONAUTE1. Plant J. 2010; 62:463–472. [PubMed: 20128884]
90. Azevedo J, Garcia D, Pontier D, Ohnesorge S, Yu A, Garcia S, Braun L, Bergdoll M, Hakimi MA, Lagrange T, et al. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 2010; 24:904–915. [PubMed: 20439431]
91. Giner A, Lakatos L, Garcia-Chapa M, Lopez-Moya JJ, Burgyan J. Viral protein inhibits RISC activity by argonaute binding through conserved WG/GW motifs. PLoS Pathog. 2010; 6:e1000996. [PubMed: 20657820]
92. Nayak A, Andino R. Slicer activity in Drosophila melanogaster S2 extract. Methods Mol Biol. 2011; 721:231–244. [PubMed: 21431689]
93. van Mierlo JT, Overheul GJ, Obadia B, van Cleef KW, Webster CL, Saleh MC, Obbard DJ, van Rij RP. Novel Drosophila viruses encode host-specific suppressors of RNAi. PLoS Pathog. 2014; 10:e1004256. [PubMed: 25032815]
94. Chao JA, Lee JH, Chapados BR, Debler EW, Schneemann A, Williamson JR. Dual modes of RNA-silencing suppression by Flock House virus protein B2. Nat Struct Mol Biol. 2005; 12:952–957. [PubMed: 16228003]
95. Sullivan CS, Ganem D. A virus-encoded inhibitor that blocks RNA interference in mammalian cells. J Virol. 2005; 79:7371–7379. [PubMed: 15919892]
96. Aliyari R, Wu Q, Li HW, Wang XH, Li F, Green LD, Han CS, Li WX, Ding SW. Mechanism of induction and suppression of antiviral immunity directed by virus-derived small RNAs in Drosophila. Cell Host Microbe. 2008; 4:387–397. [PubMed: 18854242]
97. Lakatos L, Csorba T, Pantaleo V, Chapman EJ, Carrington JC, Liu YP, Dolja VV, Calvino LF, Lopez-Moya JJ, Burgyan J. Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. EMBO J. 2006; 25:2768–2780. [PubMed: 16724105]
98. Li F, Ding SW. Virus counterdefense: diverse strategies for evading the RNA-silencing immunity. Annu Rev Microbiol. 2006; 60:503–531. [PubMed: 16768647]
99. Schnettler E, Hemmes H, Huismann R, Goldbach R, Prins M, Kormelink R. Diverging affinity of tospovirus RNA silencing suppressor proteins, NSs, for various RNA duplex molecules. J Virol. 2010; 84:11542–11554. [PubMed: 20739523]
100. Hedil M, Sterken MG, de Ronde D, Lohuis D, Kormelink R. Analysis of Tospovirus NSs Proteins in Suppression of Systemic Silencing. PLoS One. 2015; 10:e0134517. [PubMed: 26275304]
101. Cuellar WJ, Kreuze JF, Rajamaki ML, Cruzado KR, Untiveros M, Valkonen JP. Elimination of antiviral defense by viral RNase III. Proc Natl Acad Sci U S A. 2009; 106:10354–10358. [PubMed: 19515815]
102. Weinheimer I, Jiu Y, Rajamaki ML, Matilainen O, Kallijarvi J, Cuellar WJ, Lu R, Saarma M, Holmberg CI, Jantti J, et al. Suppression of RNAi by dsRNA-degrading RNaseIII enzymes of viruses in animals and plants. PLoS Pathog. 2015; 11:e1004711. [PubMed: 25747942]
103. Schnettler E, Sterken MG, Leung JY, Metz SW, Geertsema C, Goldbach RW, Vlak JM, Kohl A, Khromykh AA, Pijlman GP. Noncoding flavivirus RNA displays RNA interference suppressor activity in insect and Mammalian cells. J Virol. 2012; 86:13486–13500. [PubMed: 23035235]
104. Pijlman GP. Flavivirus RNAi suppression: decoding non-coding RNA. Curr Opin Virol. 2014; 7:55–60. [PubMed: 24793201]
105. Moon SL, Dodd BJ, Brackney DE, Wilusz CJ, Ebel GD, Wilusz J. Flavivirus sfRNA suppresses antiviral RNA interference in cultured cells and mosquitoes and directly interacts with the RNAi machinery. Virology. 2015; 485:322–329. [PubMed: 26331679]
106. Kakumani PK, Ponia SS, SRK, Sood V, Chinnappan M, Banerjea AC, Medigeshi GR, Malhotra P, Mukherjee SK, Bhatnagar RK. Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. J Virol. 2013; 87:8870–8883. [PubMed: 23741001]

107. Singh G, Popli S, Hari Y, Malhotra P, Mukherjee S, Bhatnagar RK. Suppression of RNA silencing by Flock house virus B2 protein is mediated through its interaction with the PAZ domain of Dicer. FASEB J. 2009; 23:1845–1857. [PubMed: 19193719]

108. Qi N, Zhang L, Qiu Y, Wang Z, Si J, Liu Y, Xiang X, Xie J, Qin CF, Zhou X, et al. Targeting of dicer-2 and RNA by a viral RNA silencing suppressor in Drosophila cells. J Virol. 2012; 86:5763–5773. [PubMed: 22438534]



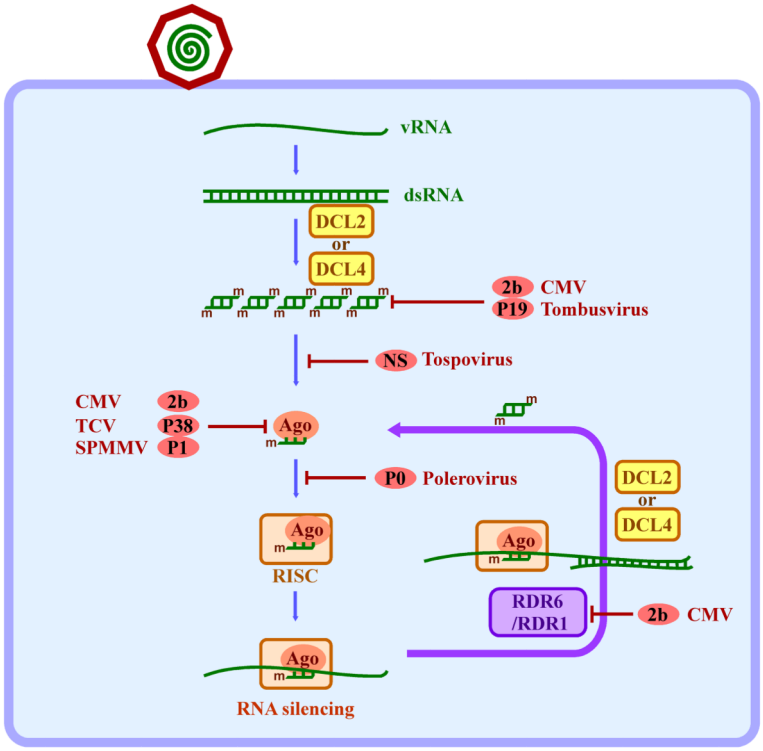


Fig 1. General antiviral RNAi pathways and virus-encoded RNAi suppressors

RNA virus-encoded RNAi suppressors target steps in the arthropod siRNA pathway (A) and the plant siRNA pathway (B). During viral replication, double-stranded RNAs (dsRNAs) are targeted by Dicer-2 (DCR2) in arthropod cells and Dicer-like protein (DCL) in plant cells and are cleaved into small fragments of dsRNA. The 3′-end of a siRNA is methylated (m) at the indicated steps in arthropod and plant cells. One strand of the small dsRNA is loaded into Argonaute protein (Ago) complexes and combined with other proteins to form the RNA-induced silencing complex (RISC). The guide siRNA within RISC forms base pairs with viral RNA to elicit RNA silencing via RNA degradation. The plant host can amplify siRNAs by the activity of cellular RNA-dependent RNA polymerase (RDRP). Viruses and their respective RNAi suppressor proteins (red ovals) target the indicated steps within the RNAi pathways depicted. For arthropods, these include the following viruses: cricket paralysis virus (CrPV), Culex Y virus (CYV), Drosophila C virus (DCV), dengue virus (DENV), flock house virus (FHV), Nodamura virus (NoV), sfRNA from flavivirus infection, and Wuhan nodavirus (WhNV). For plants, these include the following viruses: cucumber mosaic virus (CMV), polerovirus, sweet potato mild mottle virus (SPMMV), tombusvirus, tospovirus, and turnip crinkle virus (TCV).

Table 1

siRNAs or virus-derived small RNAs from virus-infected hosts.

Host species	Virus family	Viral genome	Virus (abbr.)	Experimental system	reference
Plant	*Alphaflexiviridae*	ssRNA(+)	potato virus X (PVX)	*in vivo*: *Nicotiana benthamiana*	22
			cymbidium ringspot virus (CymRSV)	*In vivo*: *Nicotiana benthamiana; Nicotiana clevelandii*	24, 25
	Bromoviridae	ssRNA(+)	cucumber mosaic virus (CMV)	*In vivo*: *Arabidopsis thaliana*	23
	Tombusviridae	ssRNA(+)	turnip crinkle virus (TCV)	*In vivo*: *Arabidopsis thaliana*	27
	Virgaviridae	ssRNA(+)	tobacco rattle virus (TRV)	*In vivo*: *Arabidopsis thaliana*	26, 27
			Chinese wheat mosaic virus (CWMV)	*In vivo*: wheat cv. Yannong 15	30
	Potyviridae	ssRNA(+)	turnip mosaic virus (TuMV)	*In vivo*: *Arabidopsis thaliana*	28
			sugarcane mosaic virus (SCMV)	*In vivo*: Maize (*Zea mays* L.)	29
Arthropod	*Nodaviridae*	ssRNA(+)	flock house virus (FHV)	Cell: *Drosophila*S2	40
	Dicistroviridae	ssRNA(+)	Himetobi P virus (HiPV)	*In vivo*: small brown planthopper (*Laodelphax striatellus*)	47
			cricket paralysis virus (CrPV)	Cell: *Drosophila*S2 *in vivo*: *Drosophila melanogaster*	41, 42
	Togaviridae	ssRNA(+)	o'nyong-nyong virus (ONNV)	*In vivo*: *Anopheles gambiae*	48
	Togaviridae	ssRNA(+)	Sindbis virus (SINV)	*in vivo*: *Aedes aegypti*	53
			Semliki Forest virus (SFV)	Cell: *Aedes albopictus* U4.4 and *Aedes aegypti* Aag2 cells	6
	Flaviviridae	ssRNA(+)	West Nile virus (WNV)	Cell: Aedes albopictus C6/36 cells	71
			dengue virus (DENV)	Cell: *Aedes albopictus* U4.4	76
			Langat virus (LGTV)	Cell:	58