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Curr Opin Insect Sci. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:
Curr Opin Insect Sci. 2015 April 1; 8: 111–120. doi:10.1016/j.cois.2015.01.006.

RNA interference-mediated antiviral defense in insects

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Abstract

Small interfering RNA (siRNA)-mediated RNA interference (RNAi) pathways are critical for the detection and inhibition of RNA virus replication in insects. Recent work has also implicated RNAi pathways in the establishment of persistent virus infections and in the control of DNA virus replication. Accumulating evidence suggests that diverse double-stranded RNAs produced by RNA and DNA viruses can trigger RNAi responses yet many viruses have evolved mechanisms to inhibit RNAi defenses. Therefore, an evolutionary arms race exists between host RNAi pathways and invading viral pathogens. Here we review recent advances in our knowledge of how insect RNAi pathways are elicited upon infection, the strategies used by viruses to counter these defenses, and discuss recent evidence implicating Piwi-interacting RNAs in antiviral defense.

Introduction

Central to the survival of all organisms is a competent immune system capable of restricting or eliminating intracellular pathogens such as viruses. Although several innate immunity pathways (e.g. Toll, Imd, JAK-STAT etc.) play virus-specific antiviral roles (reviewed in [1–3]), the RNA interference (RNAi) pathway is the most broadly-acting [4] and robust antiviral pathway in insects (reviewed in [5–8]). RNAi is also a major antiviral system in plants [9] and nematodes [10], and recent evidence suggests that RNAi may also serve an antiviral role in mammals [11,12]. The finding that RNAi inhibitors are encoded by diverse insect RNA [13–25] and DNA [26,27] viruses further emphasizes the importance of RNAi in the evolutionary arms race between virus and host. RNAi pathways restrict virus replication (and also silence cellular gene expression) through the production of small non-coding RNAs called small interfering RNAs (siRNAs). These siRNAs associate with Argonaute (Ago) proteins to seek out and destroy viral (or cellular) single-stranded (ss) RNAs in a sequence-specific manner. Other eukaryotic small RNAs, such as microRNAs

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(miRNAs) and Piwi-interacting RNAs (piRNAs), which normally regulate cellular gene expression [28] and transposon activity [29], respectively, have also been implicated in antiviral defense recently [8]. These various small RNAs are often defined by their origin, size, interaction with specific Agos, and functions [8]. Here we focus on recent progress in understanding the role of RNAi/siRNAs and piRNAs in mediating antiviral immunity (for a review of miRNA-mediated antiviral defense, see [30,31] and Asgari, this issue). Given the wide availability of genetic tools in *Drosophila melanogaster* and the importance of other dipterans (e.g. mosquitoes) as vectors for arboviruses (viruses transmitted by arthropods to vertebrates), research in insect antiviral RNAi pathways is most advanced in *Diptera*. Here we review key aspects of antiviral RNAi in dipterans, but also draw on examples from studies of RNAi-based antiviral immunity in non-dipteran insects.

RNAi, siRNAs, and antiviral defense

Mechanism of the RNAi pathway

RNAi is initiated upon recognition and cleavage of long double-stranded (ds) RNA by Dicer-2, an RNAse III family dsRNA endonuclease, into ~19–25-nt long siRNA duplexes with characteristic 2 nt 3' overhangs [32] (Fig. 1). Dicer-2 can recognize dsRNA from endogenous (e.g. cellular transcripts with secondary structures) or exogenous (e.g. experimentally introduced or viral) sources [33,34]. Dicer-2 cleavage of viral dsRNA produces viral siRNAs (vsiRNAs). These siRNAs are then loaded into the Argonaute-2 (Ago2)-containing RNA-induced silencing complex (RISC) [35]. Upon loading into RISC, one of the siRNA strands (the passenger) is degraded in a process dependent upon Ago2 and the endoribonuclease C3PO [36]. The other strand (the guide) remains associated with Ago2 and is 2'-*O*-methylated on its 3'-terminal nt by the Hen1 methyltransferase, creating an active or mature RISC [37,38]. Base-pairing of the guide strand to a complementary target ssRNA leads to Ago2-mediated cleavage (slicing) of the target. In *Drosophila*, the biogenesis and loading of siRNAs derived from endogenous and experimentally-introduced dsRNA into RISC require the Dicer-2 cofactors and dsRNA-binding proteins Loquacious PD (Loqs-PD) and R2D2 [39,40]. Only R2D2, however, is required for loading of vsiRNAs into RISC [41]. Thus, invertebrate RNAi systems may recognize or process viral dsRNA differently than other exogenous dsRNAs. In support of this, the antiviral RNAi response in nematodes requires a Dicer-related DEx-H-box protein that is dispensable for the RNAi response to experimentally-introduced dsRNA [42]. Differences in structure or intracellular localization between viral and other exogenous dsRNAs may determine the specific host factors required for their processing [43]. Although RNAi responses initiate in infected cells, studies in dipteran insects have suggested that antiviral RNAi signals (as either vsiRNA or longer viral dsRNA) can travel to uninfected cells [44,45], creating a systemic RNAi response that blocks viral spread [7,44].

Detection and inhibition of virus replication by the RNAi pathway

RNA and DNA virus replication is significantly higher in dipteran cells or animals that are deficient in RNAi pathway components such as Dicer-2, R2D2, and Ago2 [18,19,46–50]. Furthermore, virus infection of RNAi-deficient animals is often associated with higher mortality rates [18,19,47,51]. More recently, we [52] and others [53] have implicated

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Dicer-2 and Ago2 in the defense against RNA and DNA viruses in lepidopteran hosts [52,53]. These studies illustrate a critical role for insect RNAi pathways in controlling infection by diverse viruses.

Using next generation sequencing to identify vsiRNAs in infected cells and subsequently mapping vsiRNAs to corresponding viral genomes, recent studies have provided insights into the putative viral dsRNAs cleaved by Dicer-2 to generate vsiRNAs [6]. These studies have revealed that, depending on the virus, the source of viral dsRNA processed by Dicer-2 may be from: 1) viral genomes (e.g. for dsRNA viruses); 2) replication intermediates of ssRNA viruses; 3) structured elements in viral ssRNA (genomes or transcripts); and 4) overlapping viral transcripts that base-pair to form dsRNA (Fig. 1 and Table 1). Intriguingly, Dicer-2-mediated recognition of viral dsRNA not only elicits RNAi but can also promote expression of Vago, a secreted protein that activates the antiviral JAK-STAT pathway [54–56]. Therefore, identifying viral RNAs recognized by Dicer-2 may reveal how RNAi and JAK-STAT pathways are triggered during infection.

Putative Dicer-2 substrates in RNA virus infection

Replication of ssRNA viruses involves the production of an antigenome-an RNA strand of opposite polarity to the genome-that serves as a template for genome synthesis, and vice versa. Consequently, ssRNA virus replication results in dsRNA replication intermediates. Although genomic strands are present at higher levels than antigenome strands during ssRNA virus infection [57], vsiRNAs mapping to genome and antigenome strands are often present at similar levels and are typically distributed across the entire length of the genome or antigenome [41,50,58–60]. These observations suggest that during ssRNA virus replication, dsRNA replication intermediates are major Dicer-2 substrates for vsiRNA production [6]. In contrast, the genomic dsRNA itself is likely the major Dicer-2 substrate during infection with dsRNA viruses [21,58,61].

It is important to note that these general observations are by no means the rule for all RNA virus infections (Table 1). For example, ~87% of the vsiRNAs generated during infection of *Drosophila* cells with Drosophila C virus, a ssRNA virus, map to the genomic strand [62], suggesting that dsRNA structures within the viral genome are major Dicer-2 substrates. A bias for genome strand vsiRNAs has also been noted during infections of dipteran [48,63,64], and more recently, hemipteran hosts [61] with other ssRNA viruses.

Curiously, although vsiRNAs targeting ssRNA viruses are typically distributed across the entire length of the viral genome or antigenome, they may target certain regions termed "hot spots" more heavily than others (cold spots). Hot spots may occur because those particular regions are more accessible to Dicer-2 or because of highly structured RNA produced at these loci [8]. For example, vsiRNAs targeting Rift Valley fever virus, a tripartite ssRNA virus with three genomic strands (L, M, S), predominantly map in equal numbers to both genome and antigenome strands for L and M segments, but largely map to the antigenomic strand of the S segment in a specific hot spot region that produces an RNA hairpin structure [62]. Hot spots have also been observed during infection with dsRNA viruses from *Reoviridae,* although it has been suggested that these may result from either differential

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access of Dicer-2 to regions within genomic dsRNA [65] or because of panhandle structures encoded by reovirus mRNAs [61].

The hot and cold spots detected during vsiRNA profiling may actually reflect a "decoy" mechanism used by viruses to divert host RNAi responses away from targeting essential viral RNAs [8,60]. Indeed, it was hypothesized that the heavily targeted RNA hairpin of the antigenomic S segment of Rift Valley fever virus may in fact act as such a decoy [62]. Furthermore, a prior study found hot spot vsiRNAs targeting the ssRNA virus, Semliki forest virus, to be less effective than vsiRNAs derived from cold spot regions of the viral genome in restricting virus replication [60]. Similarly, hot spot vsiRNAs targeting vesicular stomatitis virus (a ssRNA virus) were found to largely derive from abundant defective interfering particles produced during viral replication and these vsiRNAs were not efficiently loaded into RISC [62]. Therefore, viruses may benefit from the preferential cleavage of abundant decoy RNA transcripts by Dicer-2 because it may prevent processing of more limited viral RNAs needed for replication and because vsiRNAs derived from decoy RNAs may be less competent for loading into RISC.

Putative Dicer-2 substrates in DNA virus infections

Recent studies in *Drosophila* have demonstrated that dsDNA viruses also elicit vsiRNA production [4,51,62]. These vsiRNAs mostly mapped to hot spots in viral genomes where either convergent overlapping transcription and/or production of a structured transcript was predicted to generate dsRNA (Table 1). Interestingly, infection of the lepidopteran *Helicoverpa armigera* with *Helicoverpa armigera* single nucleopolyhedrovirus triggers the production of vsiRNAs that predominantly map to late viral genes required for virus replication and assembly. It has been suggested that preferential targeting of late genes by RNAi may be beneficial to the virus in regulating its own gene expression program and ensuring proper replication prior to host cell lysis [53].

RNAi and Persistent Virus Infection

In insects, arboviruses establish persistent infections in which they are not cleared but are restricted to a level that prevents more pathogenic (and potentially fatal) acute infections. Recently, Goic *et al.* [66] implicated RNAi in contributing to the establishment of persistent infections. Using *Drosophila* cells or animals, they showed that during infection with Flock house virus (FHV) endogenous reverse transcriptases copy FHV RNA into complementary DNAs (cDNA) forming FHV-retrotransposon cDNA chimeras. These chimeric cDNAs, which may be incorporated into the cellular genome under certain circumstances, are then transcribed to produce dsRNAs that are processed by Dicer-2 into vsiRNAs that restrict FHV replication. In FHV-infected cells treated with reverse transcriptase inhibitors, FHV-retrotransposon DNA chimeras are not made, persistent FHV infection is blocked, and instead, a more cytopathic FHV infection ensues [66]. These findings suggest that cDNA-derived vsiRNAs contribute to the initial control of viral replication and help to establish a persistent infection. These cDNA-derived vsiRNAs may also serve to amplify the canonical antiviral RNAi response (Fig. 1) in organisms such as *D. melanogaster*, which lack RNA-directed RNA polymerases (which amplify vsiRNA responses in other organisms such as plants) [7]. Recent studies in arthropods have shown that virus-specific dsRNA

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immunizations can invoke immunity to subsequent challenge with the corresponding virus, suggesting an RNAi-based immunological memory is created upon viral dsRNA inoculation [44,67,68]. Future studies will be needed to determine if virus-derived DNA chimeras (if integrated into the host genome) could provide a mechanism for RNAi-based immunological memory of virus infection.

Viral countermeasures to antiviral RNAi responses

Given the importance of RNAi in restricting broad classes of viruses, it is not surprising that diverse insect RNA and DNA viruses have evolved strategies to counter RNAi responses. Virus-encoded suppressor of RNAi (VSR) factors can inhibit the RNAi pathway at one or more steps (Fig. 1 and Table 2). For example, B2 proteins encoded by nodaviruses, such as FHV [69], Wuhan nodavirus [15,70], and Nodamura virus, bind both long dsRNAs and siRNAs [14,71], preventing dsRNA processing by Dicer-2 and siRNA loading into RISC. Thus, B2 dsRNA-binding activity may protect viral dsRNA replication intermediates from Dicer-2 cleavage [57]. In contrast, the B2 encoded by mosinovirus (MoNV), a mosquito-specific nodavirus, blocks RNAi triggered by long dsRNA but not by siRNA [16]. However, MoNV-infected mosquito cells are resistant to RNAi triggered by siRNA, suggesting that another MoNV factor suppresses RNAi at a step after siRNA biogenesis [16]. In addition to binding dsRNA, some B2 proteins may also inhibit RNAi through direct interactions with Dicer-2 [70,72], further highlighting the multi-faceted mechanism by which these VSRs inhibit RNAi.

Besides nodaviruses, dsRNA-binding VSRs have been identified in other virus families including *Dicistroviridae* (e.g. Drosophila C virus (DCV) 1A protein [17,19,73]) and, more recently, *Birnaviridae* (e.g. Culex Y virus and Drosophila X virus VP3 proteins [20,21]) and *Iridoviridae* (Invertebrate iridescent virus type 6 (IIV-6) 340R protein [27]). Each of these VSRs bind long dsRNA and likely inhibit Dicer-2 cleavage of viral dsRNA. In addition, birnavirus VP3 and IIV-6 340R proteins also bind siRNAs, and may block their loading into RISC. Interestingly, Heliothis virescens ascovirus-3e encodes an RNase III enzyme that may block RNAi initiation by competing with Dicer-2 for dsRNA substrates and/or by degrading siRNAs [26].

Other VSRs, such as Cricket paralysis virus (CrPV) 1A protein, VP1 proteins encoded by *Drosophila melanogaster* Nora virus (DmelNV) and *Drosophila immigrans* Nora-like virus (DimmNV), physically interact with Ago2 and haven been shown to block target cleavage by pre-assembled RISC using *in vitro* slicer assays [17,22,23]. Intriguingly, recombinant DimmNV VP1 protein can interact with *D. immigrans* but not *D. melanogaster* Ago2 and thus can antagonize slicer activity in *D. immigrans* embryo extracts but not in *D. melanogaster* embryo extracts [23]. In contrast, recombinant DmeINV VP1 proteins interact with Ago2 and antagonize slicer activity when added to either *D. melanogaster* or *D. immigrans* embryo extracts [23]. Whether DmeINV can actually infect *D. immigrans* and, in turn, whether DimmNV can replicate in *D. melanogaster* is unknown. However, when Sindbis virus (SINV), an arbovirus that lacks a VSR, was engineered to encode DmeINV VP1, the virus replicated to higher levels than the parental virus in both *D. melanogaster* and *D. immigrans* animals, whereas recombinant SINV encoding DimmNV VP1 only displayed

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enhanced replication in *D. immigrans* and not in *D. melanogaster* [23]. These results suggest that some VSRs may be host species-specific and must therefore be identified and characterized using a relevant host [23].

Mosquito-borne arboviruses establish a persistent, non-pathogenic infection in mosquitoes despite being targeted by host RNAi machinery [50]. Therefore, it has been unclear whether arboviruses use VSRs to evade RNAi systems or whether they lack VSRs because viral suppression by RNAi ensures that infections remain non-pathogenic to the vector host. Indeed, engineering of Sindbis virus to encode FHV B2 enhances viral pathogenicity in mosquitoes [50]. However, recent evidence suggests that the flaviviruses, West Nile virus and Dengue virus, may produce a structured sub-genomic flavivirus RNA (sfRNA) to act as a decoy for Dicer proteins, preventing the processing of essential dsRNA replication intermediates [25]. Furthermore, the Dengue virus NS4B protein displays VSR activity and can block human Dicer cleavage of dsRNA through an unknown mechanism without binding dsRNA [24]. Future studies are needed to unravel the molecular functions of these flavivirus-encoded VSRs. Given that several insect-restricted viruses establish persistent infections yet encode VSRs, it is likely that multiple virus- and host-specific factors such as virus replication kinetics, VSR potency, and RNAi response efficiencies determine whether persistent infections will be established [74].

Collectively, these findings indicate that diverse insect viruses from diverse families have independently evolved strategies to counter the RNAi pathway. However, because most VSRs have been studied in isolation, future studies with VSR-deficient strains will be needed to determine their effect(s) on the replication and associated pathogenicity of the viruses that encode them.

piRNAs and antiviral defense

Insect piRNAs are 24–30 nts and are defined by their preference for uridine at their 5’ ends (U1 bias), 2’-*O*-methylation of their 3’ terminal nucleotide, and interaction with Piwi-clade Ago proteins, which include Piwi, Aubergine (Aub), and Ago3 in *Drosophila* [29]. piRNAs are initially processed from antisense ssRNA precursors transcribed from genomic loci termed piRNA clusters [75]. These “primary” piRNAs associate with Piwi or Aub. Primary piRNAs guide the cleavage of sense-stranded piRNA precursors, generating the 5’ ends of secondary piRNAs that associate with Ago3 and display an adenine bias at position 10 (A10 bias). In turn, the sense-stranded secondary piRNAs guide the Ago3-mediated cleavage of antisense piRNA precursors to generate the 5’ ends of antisense-stranded secondary piRNAs [76]. This self-enforcing loop of secondary piRNA biogenesis is known as the “ping-pong” amplification cycle. piRNAs are abundant in the germline, where they silence transposable elements and protect genomic integrity [29]. However, virus-derived piRNAs (vpiRNAs) were identified in a *Drosophila* ovarian somatic sheet (OSS) cell line harboring covertly-replicating RNA viruses, suggesting that the piRNA pathway might also function in antiviral defense in somatic cells surrounding ovarian germ cells [58].

vpiRNA-like small RNAs were also found during profiling of small RNAs in Dengue virus type-2- and cell fusing agent virus-infected cells derived from *Aedes aegypti* and *Ae.*

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albopictus. However, like the vpiRNAs observed in the Drosophila OSS cell line, the majority were of positive polarity, and there was no clear evidence for ping-pong amplification [77]. Interestingly, a recent study found vpiRNAs with characteristics of ping-pong amplification (e.g. U1 and A10 biases) in the head and thorax tissues of *Aedes albopictus* infected with Chikungunya virus [78]. The presence of ping-pong vpiRNAs in the soma of mosquitoes may be due to the broad expression of an amplified family of Piwi clade Agos (including Ago3 and 7–8 Piwi proteins) in mosquito tissues as opposed to *Drosophila*, where Ago3 and Aub appear to be restricted to the germline [5,78]. Several other recent studies have identified vpiRNAs in mosquitoes and mosquito-derived cell lines infected with RNA viruses (Table 3).

How viral transcripts enter the piRNA biogenesis pathway and a definitive role for piRNAs in antiviral defense remain to be determined. The strongest evidence implicating piRNAs in antiviral defense comes from studies showing increased susceptibility of dipteran hosts to infection upon inactivation or knockdown of Piwi clade Agos [48,79,80]. More recently, Schnettler *et al.* [81] have shown that knockdown of Ago2 or Piwi4 enhances Semliki Forest virus replication in *Aedes aegypti*-derived cells, suggesting roles for both RNAi and piRNA pathways in restricting virus replication. The use of genome-editing tools to inactivate Ago genes should help sort out the specific contributions of RNAi and piRNA pathways to antiviral defense in mosquitoes.

Concluding remarks

The findings that: 1) antiviral RNAi genes are among the most rapidly evolving immunity genes in insects [82,83]; 2) RNAi-deficient animals are hypersensitive to virus infection; and 3) divergent viruses encode VSRs, all point to a central role for RNAi in the evolutionary arms race between viruses and insect hosts. Recent profiling of vsiRNAs has provided insights into the viral signatures recognized by Dicer-2, but the relative effectiveness of different vsiRNAs in restricting virus replication is still largely unknown. This is an important point because vsiRNAs generated in hot spot regions, although more abundant, can be less effective than vsiRNAs from cold spot regions of the viral genome in restricting virus replication [60]. Furthermore, because vsiRNA and endogenous small RNA profiles might be altered by VSR activities [63,73,84], it will be important to both identify and characterize VSRs to understand how they modulate RNAi processes and contribute to viral pathogenesis. Important challenges for the future will be to understand the relative contribution of the siRNA, miRNA, and piRNA pathways in antiviral immunity and to characterize the potential effects of virus infection and virus-encoded factors on each pathway. Identifying viral factors that specifically inhibit the piRNA pathway would help to solidify a role for piRNAs in antiviral defense and may also provide new tools for answering many questions that remain regarding their biogenesis.

Acknowledgements

The authors thank Dr. Darryl Conte and members of the Mello Lab for their constructive comments on the manuscript. Work in the Mello Lab is supported by an NIH grant (GM058800) to C.C.M. C.C.M. is a Howard Hughes Medical Institute Investigator. Funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of this review; and in the decision to submit the manuscript for publication.

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Highlights

- Insect RNA interference (RNAi) pathways detect and restrict viruses
- Dicer-2 detects diverse viral dsRNA signatures and initiates the RNAi pathway
- RNAi may play a role in establishing persistent, non-pathogenic virus infections
- Both RNA and DNA viruses encode inhibitors of RNAi
- The RNAi-related piRNA pathway may also restrict virus replication in insects

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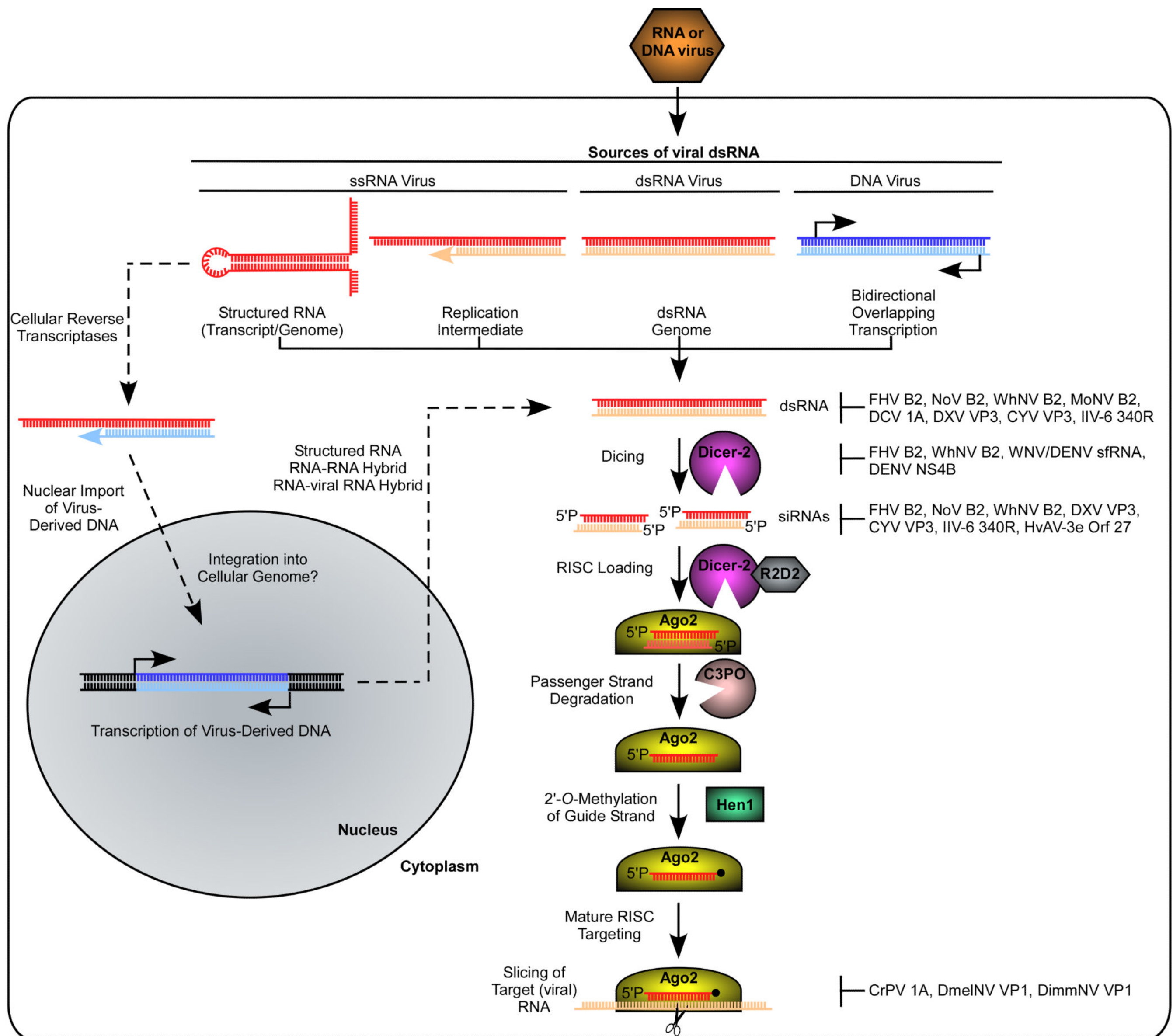

Figure 1.

Antiviral RNAi pathway in insects (adapted from [90]). Dicer-2 recognizes and cleaves viral dsRNA arising from a variety of sources (see also Table 1), into predominantly 21 or 22 nt long small interfering RNA (siRNA) duplexes. These siRNA duplexes are loaded into the RNA-induced silencing complex (RISC) containing Ago2, the passenger strand is degraded, and the guide strand is 2'-*O*-methylated at the 3' end. This mature RISC then targets viral RNA complementary to the guide strand for cleavage (slicing) by Ago2, thereby restricting virus replication. Recent work suggests that cellular reverse transcriptases can convert viral RNA into DNA forms early in infection [66]. Transcription of virus-derived DNA produces dsRNAs containing viral sequences that can enter the RNAi pathway, resulting in siRNA production that serves to dampen virus replication, allowing for the establishment of a persistent infection [66]. Both RNA and DNA viruses encode RNAi suppressors (shown at right) that target the RNAi pathway at one or more steps (see also Table 2). Virus

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abbreviations: Flock house virus (FHV); Nodamura virus (NoV); Wuhan Nodavirus (WhNV); mosinovirus (MoNV); Drosophila C virus (DCV); Drosophila X virus (DXV); Culex Y virus (CYV); Invertebrate iridescent virus type 6 (IIV-6); West Nile virus (WNV); Dengue virus (DENV); Heliothis virescens ascovirus-3e (HvAV-3e).

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Table 1

vsiRNA profiles from virus-infected insects and putative Dicer-2 substrates. Adapted from [6,8].

Virus	Family	Host[a]	Viral Genome[b]	Putative Dicer-2 Substrates[c]	Refs.
RNA virus					
Sindbis virus	*Togaviridae*	*D. melanogaster; Ae. aegypti; Ae. albopictus*	(+)ssRNA	dsRNA RIs; structured ssRNA	[4,50,87,88]
O'nyong-nyong virus	*Togaviridae*	*An. gambiae*	(+)ssRNA	dsRNA RIs; structured ssRNA	[59]
Semliki Forest virus	*Togaviridae*	*Ae. albopictus; Ae. aegypti*	(+)ssRNA	dsRNA RIs	[60,81]
Chikungunya virus	*Togaviridae*	*Ae. aegypti; Ae. albopictus*	(+)ssRNA	dsRNA RIs	[78]
Drosophila C virus	*Dicistroviridae*	*D. melanogaster*	(+)ssRNA	dsRNA RIs; structured ssRNA	[58,62]
Homalodisca coagulata virus-1	*Dicistroviridae*	*H. vitripennis*	(+)ssRNA	dsRNA RIs; structured ssRNA	[61]
Dengue virus	*Flaviviridae*	*Ae. aegypti; Ae. albopictus*	(+)ssRNA	dsRNA RIs	[77,86]
West Nile virus	*Flaviviridae*	*D. melanogaster; Ae. albopictus; C. quinquefasciatus*	(+)ssRNA	dsRNA RIs; structured ssRNA	[64,87]
Cell fusing agent virus	*Flaviviridae*	*Ae. aegypti; Ae. albopictus*	(+)ssRNA	dsRNA RIs	[77]
Flock House virus	*Nodaviridae*	*D. melanogaster*	(+)ssRNA	dsRNA RIs; structured ssRNA	[57,58, 63]
American nodavirus	*Nodaviridae*	*D. melanogaster*	(+)ssRNA	dsRNA RIs; defective RNAs	[58]
Drosophila A virus[d]	*Tetraviridae*	*D. melanogaster*	(+)ssRNA	dsRNA RIs	[58]
Nora virus	*Unassigned*	*D. melanogaster*	(+)ssRNA	dsRNA RIs	[58]
Rift Valley fever virus	*Bunyaviridae*	*D. melanogaster; Ae. aegypti; Ae. albopictus*	(−)ssRNA	dsRNA RIs; intergenic RNA hairpin	[62,89]
La Crosse virus	*Bunyaviridae*	*D. melanogaster; Ae. albopictus*	(−)ssRNA	dsRNA RIs; structured ssRNA	[87]
Schmallenberg virus	*Bunyaviridae*	*Ae. aegypti; Cu. sonorensis*	(−)ssRNA	dsRNA RIs	[65]
Vesicular stomatitis virus	*Rhabdoviridae*	*D. melanogaster*	(−)ssRNA	dsRNA RIs; viral genometranscript hybrids	[41,47, 62]
Bluetongue virus	*Reoviridae*	*Ae. aegypti; Cu.sonorensis*	dsRNA	Genomic dsRNA	[65]
Homalodisca vitripennis reovirus	*Reoviridae*	*H. vitripennis*	dsRNA	Genomic dsRNA; structured ssRNA	[61]
Culex Y virus[e]	*Birnaviridae*	*C. tarsalis*	dsRNA	Genomic dsRNA	[21,85]
Drosophila X virus	*Birnaviridae*	*D. melanogaster*	dsRNA	Genomic dsRNA	[58]
Drosophila birnavirus	*Birnaviridae*	*D. melanogaster*	dsRNA	Genomic dsRNA	[58]
Drosophila totivirus	*Totiviridae*	*D. melanogaster*	dsRNA	Genomic dsRNA	[58]
DNA Virus					

Virus	Family	Host[a]	Viral Genome[b]	Putative Dicer-2 Substrates[c]	Refs.
Invertebrate iridescent virus type 6	*Iridoviridae*	*D. melanogaster*	dsDNA	convergent overlapping transcripts	[4,51]
Vaccinia virus	*Poxviridae*	*D. melanogaster*	dsDNA	Convergent overlapping transcripts; RNA hairpins encoded by genomic termini; structured ssRNA	[62]
Helicoverpa armigera single nucleopolyhedrovirus	*Baculoviridae*	*He. armigera*	dsDNA	convergent overlapping transcripts; structured ssRNA	[53]

[a]Either cell line or whole organism.

Genus abbreviations: Ae., *Aedes*; An., *Anopheles*; C., *Culex*; Cu., *Culicoides*;D., *Drosophila*; H., *Homolodisca*; He., *Helicoverpa*.

[b]Positive and negative polarity ssRNA virus genomes are indicated by (+) and (−), respectively.

[c]RIs, replication intermediates.

[d]Drosophila tetravirus referred to in [58] is believed to be a strain of Drosophila A virus [6].

[e]Mosquito X virus referred to in [85] is believed to a variant of Culex Y virus [6].

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Table 2

Known/putative RNAi suppressors encoded by insect viruses and arboviruses. Adapted from [6].

Virus	Family	RNAi suppressor	Proposed mechanism of RNAi suppressor	References
RNA virus				
Flock House virus	*Nodaviridae*	B2	Binding long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC; Dicer-2 binding	[13,14,57,69, 72]
Nodamuravirus	*Nodaviridae*	B2	Binding of long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC; inhibition of Dicer-2 activity[a]	[14,57,71]
Wuhan Nodavirus	*Nodaviridae*	B2	Binding long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC; Dicer-2 binding	[15,70]
Mosinovirus	*Nodaviridae*	B2	Binding long dsRNA prevents cleavage by Dicer-2	[16]
Drosophila C virus	*Dicistroviridae*	1A	Binding long dsRNA prevents cleavage by Dicer-2	[17,19]
Cricket paralysis virus	*Dicistroviridae*	1A	Inhibition of AGO2 slicer (endonuclease) activity	[17,18,22]
Drosophila X virus	*Birnaviridae*	VP3	Binding long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC	[20,21]
Culex Y virus	*Birnaviridae*	VP3	Binding long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC	[21]
Nora Virus	Unassigned	VP1	Inhibition of Ago2 slicer (endonuclease) activity	[22,23]
Dimm Nora-like virus	Unassigned	VP1	Inhibition of Ago2 slicer (endonuclease) activity	[23]
Dengue virus	*Flaviviridae*	NS4B	Inhibition of Dicer-2 activity [a]	[24]
West Nile virus	*Flaviviridae*	sfRNA	Inhibition of Dicer-2 activity [a]	[25]
Dengue virus	*Flaviviridae*	sfRNA	Inhibition of Dicer-2 activity [b]	[25]
DNA virus				
Heliothis virescens ascovirus-3e	*Ascoviridae*	Orf 27 (RNase III)	Degradation of siRNA	[26]
Invertebrate iridescent virus type 6	*Iridoviridae*	340R	Binding long dsRNA prevents cleavage by Dicer-2; Binding siRNA prevents incorporation into RISC	[27]

[a] Experimental data obtained using human Dicer, inhibition of Dicer-2 in insects is presumed.

[b] Presumed function based on similarity to WENV sfRNA and ability to inhibit RNAi in insect cell assays.

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Table 3

Viruses for which vpiRNA-like small RNAs have been rep orted in insect host infections.

Virus	Family	Viral Genome[a]	Insect Host (cell line or *in vivo*)	References
Drosophila X virus	*Birnaviridae*	dsRNA	*D. melanogaster* (cell line)	[58]
Drosophila birnavirus	*Birnaviridae*	dsRNA	*D. melanogaster* (cell line)	[58]
American nodavirus	*Nodaviridae*	(+)ssRNA	*D. melanogaster* (cell line)	[58]
Drosophila A virus[b]	*Tetraviridae*	(+)ssRNA	*D. melanogaster* (cell line)	[58]
Nora virus	Unassigned	(+)ssRNA	*D. melanogaster* (cell line)	[58]
Drosophila C virus	*Dicistroviridae*	(+)ssRNA	*D. melanogaster* (cell line)	[58]
Dengue virus	*Flaviviridae*	(+)ssRNA	*Ae. aegypti* (cell line and *in vivo*); *Ae. albopictus* (cell line)	[77,86]
Cell fusing agent virus	*Flaviviridae*	(+)ssRNA	*Ae. aegypti* (cell line); *Ae. albopictus* (cell line)	[77]
Sindbis virus	*Togaviridae*	(+)ssRNA	*Ae. aegypti* (cell line); *Ae. albopictus* (cell line)	[87,88]
Chikungunya virus	*Togaviridae*	(+)ssRNA	*Ae. albopictus* (cell line and *in vivo*); *Ae. aegypti* (cell line and *in vivo*)	[78]
Semliki Forest virus	*Togaviridae*	(+)ssRNA	*Ae. albopictus* (cell line); *Ae. aegypti* (cell line)	[81]
La Crosse virus	*Bunyaviridae*	(−)ssRNA	*Ae. albopictus* (cell line); *Ae. aegypti* (cell line)	[87,88]
Schmallenberg virus	*Bunyaviridae*	(−)ssRNA	*Ae. aegypti* (cell line)	[65]
Rift Valley fever virus	*Bunyaviridae*	(−)ssRNA	*Ae. albopictus* (cell line); *Ae. aegypti* (cell line)	[89]

[a]Positive and negative polarity ssRNA virus genomes are indicated by (+) and (−), respectively.

[b]Drosophila tetravirus referred to in [58] is believed to be a strain of Drosophila A virus [6].