

Review

Helicases in Antiviral Immunity:
Dual Properties as Sensors
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Many helicases have a unique ability to couple cognate RNA binding to ATP hydrolysis, which can induce a large conformational change that affects its interaction with RNA, position along RNA, or oligomeric state. A growing number of these helicases contribute to the innate immune system, either as sensors that detect foreign nucleic acids and/or as effectors that directly participate in the clearance of such foreign species. In this review, we discuss a few examples, including retinoic acid-inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and Dicer, focusing on their dual functions as both sensors and effectors. We will also discuss the closely related, but less understood, helicases, laboratory of genetics and physiology 2 (LGP2) and Dicer-related helicase-1 and -3 (DRH-1 and -3).

Sensors and Effectors in Antiviral Immunity

Discrimination between self and non-self nucleic acid is a ubiquitous mechanism for detecting pathogen infection. Despite a universality of four nucleotide building blocks, most if not all organisms have a set of sensors that can distinguish between self and foreign nucleic acids on the basis of distinct primary sequence, secondary structure, post-transcriptional processing, and/or cellular locations. For these sensors to be functional as part of the host defense mechanism, all pathogen detection must be followed by activation of effector molecules and clearance of the foreign nucleic acids or infectious agents.

The sensors convey the 'pathogen detection signal' to the effector system through diverse mechanisms. In some cases, the receptor and effector systems are directly coupled in time and space by being physically associated with each other either in the form of a protein complex or within a single polypeptide chain. This has been seen with the bacterial restriction-modification system, in which recognition of bacteriophage DNA is immediately followed by restriction digestion [1]. Alternatively, pathogen detection can be followed by a series of signaling events without causing immediate changes in the foreign nucleic acids. The detection signal is amplified and transmitted to effector systems either in the same infected cells or in uninfected neighboring cells through secreted signaling molecules. This seems to be the more common mechanism in vertebrates, where viral nucleic acid detection by pattern recognition receptors (PRRs) activates signaling that induces expression and secretion of cytokines. The cytokines in turn establish antiviral responses in both infected and neighboring cells by upregulating antiviral genes and effector molecules. A growing number of classically defined sensors and effectors, however, have been found to display properties of both sensors and effectors. In this review, we discuss such examples in the family of DExD/H motif helicases, an emerging class of innate immune proteins.

Trends

Immune system function relies on the cooperation between sensors that recognize pathogen-associated nucleic acids and effectors that clear foreign nucleic acids and infectious agents.

Helicase represents an emerging class of antiviral proteins that function as foreign nucleic acid sensors and/or effectors.

RIG-I/MDA5 were initially thought to be viral RNA receptors that exclusively function through their downstream signaling pathways, but recent studies reported effector functions independent of downstream signaling.

Dicers also function as effectors and sensors, respectively, by cleaving viral double-stranded RNAs (dsRNAs) and by activating the downstream signaling or effector systems that generate more robust and amplified antiviral responses.

LGP2 in vertebrates and DRH-1/-3 in nematodes are close homologs of RIG-I/MDA5 and play important roles in antiviral immunity and antiviral RNAi, respectively.

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Helicases as an Emerging Class of Antiviral Receptors and Effectors

Helicases are ubiquitous and involved in various aspects of nucleic acid biology in all domains of life. Their functions range from DNA replication to mRNA splicing and antiviral defense [2,3]. The DExD/H motif helicases belong to the helicase superfamily 2 and constitute one of the largest families of helicases. They commonly contain the two RecA-like domains, between which resides the ATP binding and hydrolysis pocket [4]. They also share a number of sequence motifs (including Walker A and B motifs) that are important for ATP hydrolysis and nucleic acid binding [4]. ATP hydrolysis is induced by binding of an appropriate nucleic acid, and is often accompanied by a large conformational change in the helicase protein. This protein conformational change can be further propagated and cause structural changes in the bound nucleic acid, remodeling of protein–nucleic acid complexes, or translocation of the helicase along the bound nucleic acid [2,5]. While the term ‘helicase’ implicitly suggests a duplex unwinding activity, not all helicases or members of the DExD/H helicase family share this activity. Many do not unwind duplexes and some members of the DExD/H family can stably associate with the duplex RNA without unwinding, thus they are termed Double-stranded RNA-dependent ATPases (DRA) [6].

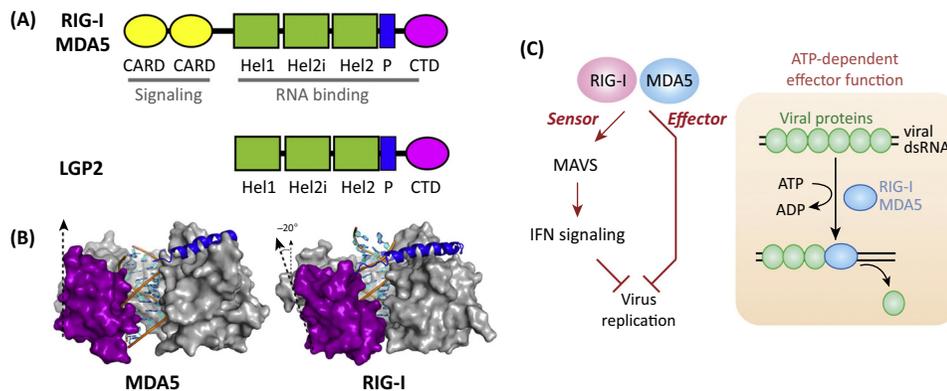
Antiviral functions of helicases are best exemplified by the RIG-I-like receptors, RIG-I and MDA5. They were first thought to function exclusively as canonical PRRs, which act through a downstream antiviral signaling pathway. However, more recent studies revealed effector-like functions that directly suppress viral replication independent of their downstream signaling pathway [7–9]. Another example of helicases with such dual properties as sensor and effector is Dicer, an RNase III component of the RNAi system. During viral infection, viral dsRNA is processed by Dicer into siRNAs, which then further silence viral genes through the action of the RNA-induced silencing complex (RISC) [10]. While dicing of viral dsRNA alone can in theory inhibit viral replication, it is insufficient in most cases, and RISC and additional amplification steps are necessary for effective antiviral defense. In this respect, Dicer plays dual roles both as an effector that directly cleaves viral dsRNA and as a sensor that generates the amplifiable ‘signal’ that activates more powerful downstream effector complex, RISC.

In this review, we will discuss RIG-I/MDA5 and Dicers, with the focus on their dual functions as both sensors and effectors. We will also discuss closely related, but less understood, helicases, LGP2 (a homolog of RIG-I/MDA5) and DRH-1 and -3. While a growing number of other helicases, such as DDX1 [11], DHX9 [12], and DDX17 [13], are reported to function in antiviral immunity by serving as either a viral nucleic acid sensor or antiviral effector, we will not discuss these helicases here.

Receptor Functions of RIG-I and MDA5

RIG-I and MDA5 are essential viral RNA sensors in vertebrates [14]. Unlike Toll-like receptors 3, 7, and 8, which sense viral RNAs in the endosome by being localized on the endosomal membrane, RIG-I and MDA5 are soluble receptors functional in the cytoplasm. Both RIG-I and MDA5 were initially discovered independent of their roles in antiviral immunity ([15] Y.W. Sun, thesis, Shanghai Second Medical University, 1997). In 2004, a cDNA library screen led to a pioneering discovery and identified RIG-I as a viral RNA receptor that upregulates the type I interferon (IFN) upon viral infection [16]. Subsequent studies identified MDA5 as another sensor for viral RNAs [16,17]. More detailed analysis showed that RIG-I and MDA5 display distinct RNA specificity and recognize largely different groups of viruses [18].

RIG-I and MDA5 share sequence similarity and the same domain architecture. They have the tandem caspase activation recruitment domains (CARDs) at the N terminus (Figure 1A), which interact with the downstream adaptor molecule, mitochondrial antiviral signaling (MAVS) protein, and are responsible for activation of the antiviral signaling pathway [19]. At the center is the



Trends in Biochemical Sciences

Figure 1. Antiviral Activities of Retinoic Acid-Inducible Gene-I (RIG-I)-like Helicases. (A) Domain architectures of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). CARD refers to the caspase activation recruitment domain. Hel1 and Hel2 refer to the two conserved RecA-like domains in the DExD/H helicase family. Hel2i is the helicase insertion domain. P stands for the pincer domain. CTD indicates the C-terminal domain. (B) Structures of the CARD-deletion mutants of MDA5 and RIG-I in complex with double-stranded RNA (dsRNA). The helicase domains, pincer, and CTD are colored gray, blue, and purple, respectively. The image is adapted from [21]. (C) The dual functions of RIG-I and MDA5 as canonical pattern recognition receptors (PRRs) that suppress viral replication through the downstream signaling pathway and effectors that directly act against viruses. For the effector functions, ATP-dependent protein displacement activity is summarized on the right side.

DExD/H motif helicase domain, which not only contains the two RecA-like domains (Hel1 and Hel2) that are conserved among all DExD/H helicases but also an insertion domain, Hel2i, which was first identified in the archaeal helicase Hef [20] (Figure 1A). The isolated helicase domain is inefficient in dsRNA binding and ATP hydrolysis, and these activities require the cooperation with the C-terminal zinc binding domain (to be referred to as CTD) [21,22].

A series of structural and biochemical studies showed that RIG-I and MDA5 bind dsRNA in slightly different ways that can explain their divergent RNA specificities. Both RIG-I and MDA5 form a ring-like structure and wrap around dsRNA upon binding, but differentially orient CTD to adopt distinct RNA recognition modes (Figure 1B) [21,23–25]. In MDA5, CTD is placed parallel to the axis of dsRNA, allowing MDA5 to adopt the stem-binding mode with little or no contact to the dsRNA end (Figure 1B) [25]. This MDA5 monomer then stacks head-to-tail along the length of dsRNA and cooperatively forms a filament [25,26], which is required for high-affinity interaction with dsRNA and its selective recognition of long dsRNA [27]. RIG-I, by contrast, adopts the dsRNA end-capping mode by having CTD tilted towards the dsRNA end (Figure 1B) [21,23,24]. This end-capping mode allows RIG-I to directly recognize the 5′-di- or triphosphate moiety and blunt end of dsRNA [28–31], which are features of many viral RNAs but are often removed from mammalian host RNAs during maturation.

What is the role of the helicase domain in RIG-I/MDA5 and their signaling activities? Both RIG-I and MDA5 hydrolyze ATP only upon binding to dsRNAs but the impact of ATP hydrolysis differs, particularly in its effects on their oligomerization states. While MDA5 filament is formed independent of ATP, during ATP hydrolysis the MDA5 filament undergoes a disassembly from the filament termini, which leads to a decrease in the filament stability on short dsRNA and selective accumulation of MDA5 molecules on long dsRNA [27,32,33]. Since filament formation brings together CARDs for their oligomerization and this oligomerization of CARDs is required for activation of MAVS, the ATP-mediated negative regulation of MDA5 filaments explains how it avoids recognition of short cellular dsRNA (for more details, see review in [33]).

RIG-I also requires oligomerization of CARDs to activate MAVS [34], but the mechanism by which it induces oligomerization of CARDs differs from that of MDA5. RIG-I can bind a dsRNA end as a monomer independent of ATP [24], but during ATP hydrolysis RIG-I translocates along dsRNA [35] and assembles short filaments near dsRNA termini [36,37]. This filamentous oligomerization in turn promotes CARD oligomerization through the proximity-induced mechanism similarly to MDA5 [36]. Thus, ATP hydrolysis provides a mechanism for RIG-I to oligomerize the helicase domain on dsRNA and promote its signaling activity.

RIG-I can also be activated by short dsRNA (~20 bp), where filaments cannot assemble [30,38]. In this case, CARD oligomerization is largely mediated by K63-linked polyubiquitin chain as ubiquitin chains bridges between neighboring subunits of CARDs [34,39]. Even in this case, the ATP hydrolysis activity was proposed to play an important role in RIG-I signaling. Multiple structures of RIG-I in complex with various ATP analogs [21,23,24,40] suggest that ATP binding and hydrolysis induce progressive closing of the helicase domain, which was proposed to trigger release of CARDs from the autoinhibited configuration [40].

Based on these models of RIG-I/MDA5 described earlier, one may conclude that ATP hydrolysis negatively regulates the signaling activity of MDA5, while it is required for the RIG-I signaling activity. However, this turned out to be not so simple. For both RIG-I/MDA5, some ATPase-deficient mutants show gain-of-function phenotype while others show loss-of-function phenotype [41,42]. The limitation in the current model of RIG-I/MDA5 reflects the challenge of understanding how the dynamics of the helicase domain are propagated to CARDs and allow its signaling function. This remains as a key outstanding question in the field (see Outstanding Questions).

Effector Functions of RIG-I and MDA5

Until recently, it has been thought that RIG-I and MDA5 function as canonical PRRs in that their functions are mediated exclusively by MAVS and the downstream signaling pathways to induce type I and III IFN or other cytokines. IFN in turn induces IFN-stimulated genes (ISGs), which establishes the antiviral state in infected and neighboring cells while activating the appropriate adaptive immune response. In 2011, a large-scale antiviral ISG screen identified RIG-I and MDA5, in addition to a few other proteins, as antiviral effector-like molecules that can function in the absence of IFN signaling [43]. While this study clearly showed the IFN-independent activities of RIG-I and MDA5, it did not exclude the possibility that the observed antiviral activities are as a result of IFN-independent, but signaling-dependent functions of RIG-I/MDA5.

In 2015, three independent studies [7–9] identified signaling-independent, direct antiviral functions of RIG-I and MDA5 (Figure 1C). Among these, the first two studies [7,8] are distinct from the third [9] in that the effector functions of RIG-I do not require ATP. During infection by hepatitis B virus (HBV), RIG-I detects the 5' end of pre-genomic RNA [7], which contains the encapsidation sequence that recruits the viral polymerase, P, for initiation of the reverse transcription. Interestingly, association between P and the 5' end of the pre-genomic RNA and the subsequent viral replication was found to be interfered by RIG-I or its mutants defective in ATP hydrolysis or signaling [7]. A similar effect of RIG-I was observed in another study with influenza A [8], which revealed that RIG-I can recognize the fully encapsidated genome of influenza A, likely by competing with or removing the capsid molecules from the genomic RNA. This view was further supported by the observation that a mutation in the capsid component (PB2) that reduced its affinity for RNA also increased the efficiency with which RIG-I recognizes the nucleocapsid [8].

Another recent study identified effector functions of RIG-I and MDA5 that, in contrast to the studies above, are critically dependent on their ATPase activities [9]. This study showed that, during ATP hydrolysis, RIG-I and MDA5 efficiently displace a wide range of roadblocks, including

viral proteins such as NS1 from influenza A, from their positions on dsRNA. This activity can restrict replication of a broad range of viruses, although not all of them. Intriguingly, RIG-I and MDA5 increased, rather than decreased, the activity of the dsRNA-dependent kinase protein kinase R (PKR) by transiently clearing dsRNA of viral inhibitor proteins [9]. These observations raise the question of whether the signaling-independent antiviral activities of RIG-I and MDA5 are mediated by the increased activity of other effector molecules, such as PKR or 2',5'-oligoadenylate synthetase 1 (OAS1). Alternatively, RIG-I and MDA5 may directly interfere with the core viral replication steps by displacing key viral proteins, such as replication machineries or capsids, from viral RNAs. Future investigation is necessary to identify viral proteins that the displacement activities of RIG-I/MDA5 target during infection, and to understand why certain viruses are resistant to the displacement activity of RIG-I/MDA5 while others are not (see Outstanding Questions).

LGP2, the CARD-less Homolog of RIG-I and MDA5

LGP2 is a close homolog of RIG-I and MDA5, but lacks CARDs (Figure 1A), and thus the MAVS-mediated signaling activity. While LGP2 appears to negatively regulate RIG-I through both RNA-dependent and -independent mechanisms [44–48], it has conversely been shown to upregulate the signaling activity of MDA5 in a dose-dependent manner [47,49,50]. Biochemical studies showed that LGP2 binds dsRNA more efficiently in the presence of ATP [51], and utilizes this tight binding to somehow facilitate nucleation of the MDA5 filament, and thus its antiviral activity [52]. Intriguingly, LGP2 knockout mice displayed reduced, but still significant antiviral responses [53], suggesting that its role in antiviral innate immunity is not essential. Instead, LGP2 deficiency leads to a severe defect in antigen-specific CD8⁺ T cell expansion and consequent T cell-mediated antiviral immunity [53]. This defect in T cell immunity was independent of MAVS [53], suggesting another unknown function of LGP2 beyond modulating the signaling activities of RIG-I/MDA5. Whether this activity of LGP2 is mediated by its regulation of signaling-independent functions of RIG-I and MDA5, or through a yet uncharacterized biochemical mechanism (perhaps involving protein displacement similarly to RIG-I/MDA5 [7–9]) remain to be tested (see Outstanding Questions).

Dicers in the RNA Interference Pathway

Dicer is a conserved RNase III and a key component of RNAi. Dicer cleaves dsRNA and pre-miRNA to generate siRNA and miRNA, respectively, which then guide RISC to the complementary mRNA for cleavage and/or gene silencing. In metazoans and plants, Dicer proteins generally contain a DEXD/H motif helicase domain that is highly homologous to those of RIG-I/MDA5/LGP2. The two RecA-like domains (Hel1 and Hel2) are conserved across all of the DEXD/H family members, but in this case the insertion domain (Hel2i) between Hel1 and Hel2 (Figure 2A) and overall 3D shape are also conserved with RIG-I/MDA5/LGP2 [54].

The antiviral function of RNAi and gene silencing was first discovered in plants, but later confirmed in nematodes and insects [10]. Their antiviral activities were evidenced by an increased viral susceptibility upon knockout or knockdown of any of the core RNAi components and the presence of viral suppressors against the host RNAi system. The potential antiviral role of RNAi in mammals has been difficult to prove due, in part, to the dominating role of the IFN-based immune system, including the components RIG-I and MDA5. Recently, two independent studies [55,56] identified the antiviral activity of RNAi in mouse embryonic stem cells, baby hamster kidney (BHK) cells, and in suckling mouse, where RIG-I-like receptors and the IFN system are suppressed. Intriguingly, the importance of RNAi in mammalian antiviral immunity diminishes during development, which could be possibly explained by the emergence of IFN-based immunity and its negative impact on RNAi [57].

The antiviral function of RNAi is typically mediated in two steps: first, Dicer recognizes and processes viral dsRNA into siRNA (Dicer as an effector, Figure 2B), and second, siRNA silences

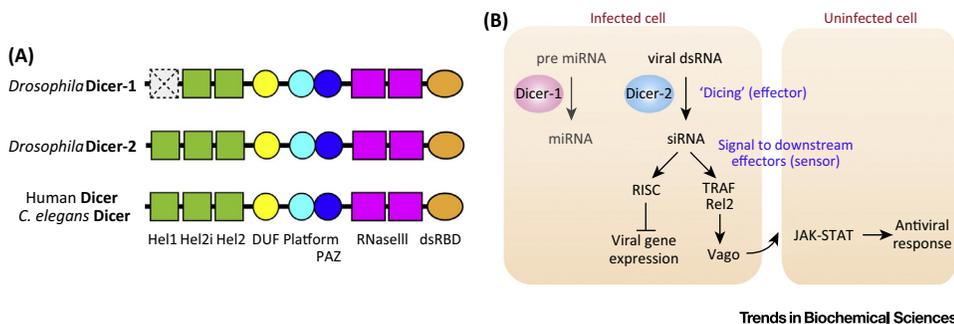


Figure 2. Antiviral Activities of Dicers. (A) Domain architectures of Dicers from *Drosophila melanogaster* and *Caenorhabditis elegans*, and human. Dicer-1 has the truncated Hel1 (indicated by X), thus lacking the functional helicase property. (B) Antiviral activities of *Drosophila Dicer-2*. Dicer-2 functions as an antiviral effector molecule by cleaving viral double-stranded RNA (dsRNA) upon recognition. At the same time, it functions as a pattern recognition receptor (PRR)-like sensor by having the cleavage product, siRNA, act as a signal to activate the downstream effector, RNA-induced silencing complex (RISC), for gene silencing and the TRAF–Rel2 signaling pathway for expression of Vago. Vago is then secreted and systemically spread to uninfected cells to establish the antiviral response.

viral gene expression through RISC (Dicer as a sensor, Figure 2B). Components of RISC are required for full antiviral immunity [58–61], suggesting that the effector function of Dicer alone is not sufficient and that the downstream action of RISC is necessary for the robust suppression of viral replication. Interestingly, the activity of Dicer in sensing viral dsRNA and generating the ‘pathogen detection signal’ is further enhanced in *Drosophila melanogaster* and *Caenorhabditis elegans*, through a mechanism that allows cell-to-cell spreading of the signal [62–66]. This is analogous to the downstream effect of the PRR functions of RIG-I/MDA5. These PRR-like functions of the Dicer enzymes and their associated helicases are discussed in detail in the following sections.

Dicer-2 in *Drosophila*

While mammals and nematodes have a single Dicer enzyme for biosynthesis of both miRNAs and siRNAs, plants and *Drosophila* have multiple Dicer proteins with distinct RNA specificities. In *Arabidopsis thaliana*, there are four Dicer-like proteins (DCL1–4), with DCL1 being the only one responsible for miRNA biogenesis [67]. DCL2–4 can process viral dsRNAs and thus are involved in plant antiviral RNAi [68,69]. In *Drosophila*, two Dicers (Dicer-1 and -2) partition their functions for miRNA and siRNA, respectively [70]. Dicer-2, but not Dicer-1, processes long viral dsRNAs, thus playing a unique role in antiviral immunity [71,72]. Coincidentally, Dicer-2, but not Dicer-1, has a functional helicase domain (Figure 2A).

As mentioned earlier, dicing of viral dsRNA alone is insufficient for the antiviral RNAi in *Drosophila*, and additionally requires the action of the downstream effector RISC, in particular its nuclease component Argonaute 2 (AGO2) [59]. In addition to the canonical AGO2-dependent antiviral function, however, Dicer-2 can restrict viral replication independent of AGO2 by inducing expression of the antiviral gene Vago (Figure 2B) [62]. In *Culex quinquefasciatus* (the southern house mosquito), a Vago homolog is induced by Dicer-2 upon viral infection [63], and is secreted from infected cells to activate the antiviral signaling pathway of uninfected cells (Figure 2B) [73]. This property of Vago in mediating cell-to-cell spread of antiviral signal is analogous to the mode of action of mammalian IFN.

What is the role of the helicase domain in Dicer-2 and its antiviral function? Just like RIG-I/MDA5, the helicase domain of Dicer-2 (or any other Dicers) is thought to lack the duplex unwinding activity (although this would have to be demonstrated). Instead, its helicase domain was shown to modulate the RNA substrate specificity. Studies have shown that ATP hydrolysis by the

helicase domain of Dicer-2 is required for generation of siRNA from long dsRNA [74,75]. More detailed studies showed that the helicase domain enables processive production of multiple siRNAs from a single long dsRNA [76,77], possibly by allowing translocation of Dicer-2 along dsRNA. The helicase domain also contributes to its preference for siRNA precursors by overriding its intrinsic preference for the 3' overhang structure that is commonly present in pre-miRNAs [77,78]. Consistent with the role of the helicase domain in efficient recognition of long (viral) dsRNA, a mutation in the helicase domain of Dicer-2 impairs Vago induction upon infection with *Drosophila* C virus [62]. While the requirement for ATP and the ATP hydrolysis-competent helicase domain in long dsRNA processing was also seen in *C. elegans* and *Schizosaccharomyces pombe* [79,80], it does not appear to be conserved in all organisms, and may not necessarily indicate the requirement for the helicase domain. Human Dicer processes long dsRNAs better in the absence of the helicase domain [81], and an isoform of Dicer in mouse oocytes and *C. elegans* that lacks the helicase domain is more efficient in processing long dsRNA [82,83], suggesting an inhibitory role of the helicase domain in antiviral RNAi. Whether there is a common molecular principle underlying these seemingly disparate observations is an important area of future research (see Outstanding Questions).

Dicer-Related RNA Helicases 1 and 3 (DRH-1 and DRH-3) in *C. elegans*

Unlike *Drosophila*, *C. elegans* has a single Dicer enzyme (DCR-1) that is responsible for both siRNA and miRNA biogenesis. During viral infection, DCR-1 processes viral dsRNA into the primary siRNAs, which are then used to generate secondary siRNAs against the same target gene (Figure 3B) [84,85]. The secondary siRNA amplifies the gene silencing effect and is a unique feature shared among fungi, plants, and nematodes but not in insects or mammals. The silencing signal is amplified through at least two distinct steps, first by increasing the number of siRNAs against the target RNA within the same cell [86], and second by systemic spreading of the silencing signal (Figure 3B) [64,65]. For antiviral RNAi, both primary and secondary siRNAs are necessary, and involve another family of RIG-I homologs, DRH-1 and -3 [84]. DRH-1 and -3 share high sequence similarity with RIG-I not only in the helicase domain (Hel1, Hel2, and Hel2i) but also in the CTD (Figure 3A). DRH-1 and -3 also have a worm-specific N-terminal domain (NTD), which plays an important role in their functions.

DRH-1 was initially identified as a protein associated with RDE-4, a key dsRNA-binding protein involved in the biogenesis of siRNA, but not miRNA [87]. Subsequent studies showed that DRH-1 is

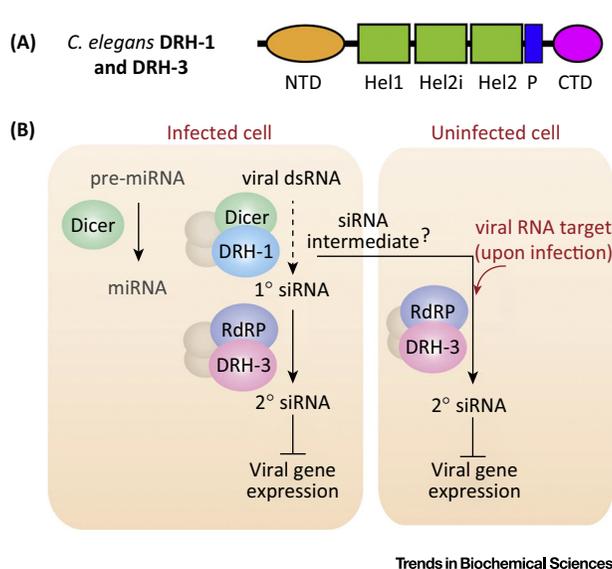


Figure 3. Antiviral Activities of Dicer-Related Helicase 1 and 3 (DRH-1 and DRH-3). (A) Domain architectures of DRH-1 and DRH-3 from *Caenorhabditis elegans*. NTD stands for the N-terminal domain. (B) Distinct antiviral activities of *C. elegans* DRH-1 and DRH-3. DRH-1 assists in Dicer-mediated cleavage of viral double-stranded RNA (dsRNA) and biosynthesis of primary siRNA (1° siRNA) against viral RNA. DRH-3, by contrast, participates in amplification of the primary siRNA signal by cooperating with RNA-dependent RNA polymerase (RdRP) in secondary siRNA (2° siRNA) production. The 2° siRNA response can be further amplified by cell-to-cell spread, allowing uninfected cells to bypass primary siRNA production and facilitating secondary siRNA production upon infection (i.e., upon introduction of the template for RdRP).

essential for primary siRNA production and antiviral defense (Figure 3B) [88,89]. Interestingly, DRH-1 is only required for RNAi against exogenous RNAs, such as viral RNA, whereas it is dispensable for RNAi against endogenous RNA [88]. While the molecular mechanisms of how DRH-1 achieves specificity for exogenous RNAs and contributes to the primary siRNA biogenesis are yet unclear, a few lines of evidence suggest that it may act as a viral RNA sensor, much like RIG-I. Experiments involving domain swap between DRH-1 and RIG-I revealed that the helicase domain and CTD of RIG-I can functionally replace those of DRH-1 [88]. Furthermore, the conserved sequence motif in CTD that is involved in dsRNA specificity of RIG-I is required for antiviral activities of both DRH-1 and the CTD-swap mutant [88]. These similarities suggest that DRH-1 and RIG-I may even share the same RNA specificity or other biochemical properties, such as translocation along dsRNA and filamentous oligomerization during ATP hydrolysis (see Outstanding Questions).

DRH-3 appears to be involved in *C. elegans* RNAi in multiple ways, but in the context of antiviral RNAi, DRH-3 participates in biosynthesis of secondary siRNAs [90], thereby playing an important role in viral restriction (Figure 3B) [88]. The secondary siRNAs, also called 22G-RNAs due to their typical size of ~22 nt and guanosine at the 5' end, are produced through the action of RNA-dependent RNA polymerases (RdRPs), DRH-3, and other components, such as EKL-1 [90]. These proteins likely form a complex and generate the secondary siRNAs on the RNA targeted by the primary siRNA [90]. Mutations in the helicase domain of DRH-3 that are predicted to abrogate the ATPase activity impair secondary siRNA biogenesis [90] and its ability to restrict viral replication [88]. As with DRH-1, the mechanistic and functional details of the role of DRH-3 in RNAi are yet unclear. Mapping the secondary siRNAs against target transcripts showed that while the secondary siRNAs are normally well represented across the target gene, DRH-3-deficiency leads to a skewed distribution towards the 3' end [90]. This led to a model that DRH-3 may facilitate the movement of RdRP, and thus spreading of the secondary siRNA, from the 3' to 5' end of the target RNA. Biochemical analysis showed that ATP hydrolysis accelerates dissociation of DRH-3 from dsRNA [91], a result expected for (but insufficient to be suggestive of) a translocating motor. DRH-3 was also shown to form a dimer on 22-bp dsRNA [91], which may contribute to the secondary siRNA length specificity. Future research is required to understand the mechanism by which DRH-3 participates in the secondary siRNA production in the context of the RdRP complex (see Outstanding Questions).

Concluding Remarks

Many helicases have a unique ability to couple cognate RNA binding to ATP hydrolysis, and in turn to a large conformational change that can be propagated to target RNA or the helicase protein itself, including its interaction with RNA, position along RNA, and oligomeric state. These abilities are expected to be utilized for viral RNA sensor and/or effector functions of RIG-I/MDA5, Dicers, and DRH-1 and -3. In many cases, however, the exact role of the helicase domain and its ATP binding and hydrolysis in antiviral functions remain elusive or only partly known (see Outstanding Questions). This limitation reflects the challenges in working with these proteins, dissecting their conformational dynamics, and linking the dynamics to their biological functions. These challenges underscore the importance of combining multiple approaches, from biophysical and biochemical analysis to functional studies at cellular and organismic levels.

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Outstanding Questions

The helicase domain of RIG-I and MDA5 plays an important role not only in recognition of viral RNAs but also in coupling RNA binding to antiviral signaling. How does the conformational change or assembly architecture of the helicase domain propagate to the N-terminal signaling domains, the CARDs? How does ATP binding and hydrolysis contribute to this process?

Recent studies showed that RIG-I and MDA5 not only function as canonical PRRs that exert antiviral activity through the downstream signaling pathway but also as effector molecules that directly exert viral restriction by altering viral protein–RNA interactions. What are the targets of this remodeling activity of viral protein–RNA complexes, and why are certain viruses more susceptible than others to these activities?

LGP2 is a conserved paralog of RIG-I/MDA5, but its functions and mechanisms remain poorly understood. What are the proteins and RNAs that interact with LGP2 in the context of antiviral immunity and does LGP2 have other functions beyond the immune system, for example, in RNAi as previously suggested?

The helicase domain of Dicer affects its specificity for pre-miRNA versus long dsRNA, but its effect appears to be unconserved. How exactly does the helicase domain affect the RNA substrate specificity, and are there common molecular principles that underlie this seemingly divergent effect on RNA selectivity?

DRH-1 and DRH-3 are other close homologs of RIG-I/MDA5 in *C. elegans* and are involved in biogenesis of the primary and secondary siRNA, respectively. How do these helicases participate in siRNA biogenesis, and how do their helicase domains and their interactions with ATP contribute to their functions?

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