

RNA interference and antiviral defense

Maartje R. Inklaar

Student number: s2474557

Master: Molecular Biology and Biotechnology

Date of submission: 25-12-2018

Supervised by Prof. P.J.M. van Haastert

Cell Biochemistry Dept., University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands

Abstract

One of the intracellular defense mechanisms against viral intruders is the production of virus-derived small silencing RNAs, that guide specific virus clearance by RNA interference (RNAi). This antiviral response by RNAi has been shown in plants, fungi and animals. Though, there is continuous debate about the antiviral function of small silencing RNA in mammals. Consistent with such an antiviral response in mammals are the reported virus-encoded suppressors of RNAi. Not only antiviral RNAi response in mammals is researched to a large extent, but also PIWI-interacting RNA (piRNA) and their associated PIWI clade of Argonaute proteins are subject of many studies. PiRNA has a main function in genome defense, by silencing transposons, but there is also an increasing amount of reports of piRNA and a role in antiviral defense. This review will summarize the current knowledge regarding small silencing RNAs and their biogenesis mechanisms. Furthermore, I will discuss recent finding concerning antiviral RNAi defense and piRNA is highlighted throughout the review.

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List of abbreviations

Abbreviation	Description
AGO	Argonaute; member of Argonaute protein family
Dicer	Endonuclease; part of ribonuclease III family
dsRNA	Double-stranded RNA
miRNA	Micro RNA
piRNA	PIWI-interacting RNA
piRISC	piRNA-induced silencing complex
ribonuclease	RNase
RISC	RNA-induced silencing complex
RNAi	RNA interference
siRNA	Small interference RNA
TE	Transposable element
VSR	Virus-encoded suppressor of RNAi
vpiRNA	Virus-derived PIWI-interacting RNA
vsiRNA	Virus-derived small interfering RNA

Introduction

Each living organism has developed multiple pathways and mechanisms to form a line of protection against certain threats, including invasion by exogenous microorganisms such as viruses. This capacity of defense against foreign material is essential for maintaining life. The human immune system uses for example cytotoxic T cells to recognize and destruct virally infected cells. Opposed to such cellular defense mechanisms, there are also potent intracellular mechanisms that play a part in the attempt of an organism to protect itself against a viral infection. Such a mechanism is the RNA silencing pathway, one of the many discoveries over the last decades concerning the field of RNA interference (RNAi). RNA silencing is a regulatory mechanism for controlling gene expression that is present in almost all eukaryotic organisms.¹ It causes the cleavage of double stranded RNA (dsRNA) and the resulting small RNA duplex can be complementary paired to a different strand of RNA to silence it.²

Fire *et al.*³ drew the attention to the phenomenon of RNA silencing in 1998 when they showed that dsRNA had the effect of potent and specific genetic interference after direct injection into adult *C. elegans*. Gene silencing as a result of the interference was also detected in the progeny of *C. elegans* and the term RNA interference was coined by the authors. Fire *et al.* were the first in explaining the causes behind these concepts of interference and silencing.^{3,4}

The discovery of RNA interference contributed to the great transformation that RNA molecular biology went through over the last decades. Small noncoding RNAs are an important topic within the RNA research field. These small RNAs regulate genes and genomes and can occur at different levels of genome function, such as transcription, RNA processing, RNA stability and translation. The term 'RNA silencing' indicates the inhibitory effect of small RNAs on gene expression and control.⁵ The overall concept is that the small RNAs guide effector proteins, with at the core a protein from the Argonaute family, to target nucleic acid molecules via base-pairing interactions.⁵ Small RNAs are largely responsible for making the silencing machinery addressable for research, due to possibilities for prediction and control. Hence, small RNAs are believed to not only be of great importance to improving theoretical understanding in this field but also for the more practical and applied aspects of RNA molecular biology.⁵

Since the above-mentioned discovery of RNAi, there have been discoveries in this field that have broadened the diversity of regulatory RNAs. The small silencing RNAs, that have been categorized based on partnering with the Argonaute protein family and their processing mechanism, can be subdivided into three classes, microRNA (miRNA), small interfering RNA (siRNA) and PIWI-interacting RNA (piRNA). The distinction between the two categories of miRNA and siRNA was already established early in the 21st century. MiRNA was recognized as regulator of endogenous genes, and siRNA had the reputation of defender of genome integrity in response to viruses or transposons. One of the most prominent similarities between the two types of small silencing RNA can be found in their form of assembly, since they both associate with effector proteins to create RNA-induced silencing complexes (RISCs).⁵ During this period in the 21st century, there were hints that PIWI-interacting RNAs existed and later on Aravin *et al.*⁶ identified the piRNAs in the testis of *D. melanogaster*. After more discoveries it became clear that these specific RNAs were associated with the protein PIWI and that they are involved in organismal development, anti-viral defense and genome protection against transposal element activity.⁷ To date, the piRNAs and PIWI proteins have been found in a broad range of animals, with only a few exceptions, including several types of nematodes.⁸

Antiviral defense is an important part of RNAi research and the RNA silencing pathway was first established as the main antiviral pathway in the plant kingdom.⁹ Key components of this pathway are also shown to be evolutionary conserved in all eukaryotes and the mechanism is recognized as innate antiviral immunity not only in plants but also in fungi and animals.^{5,10} Important to note is that the role of RNAi in mammals remains hotly debated, especially when considered that they already have a

potent innate immune system and extensive adaptive immunity. In this field of RNA-based antiviral immunity it is therefore a key challenge to characterize the molecular and biochemical features of virus-derived small RNAs that are produced after infection. Researchers set out to identify the genetic pathway(s) for the small RNA biogenesis and to determine their effector mechanisms in antiviral immunity.¹¹

This review outlines the involvement of RNA interference in antiviral defense and provides examples from across the multiple kingdoms with a focus on the developments of the past years. The emphasis lies on piRNAs, since this particular subdivision of small interference RNA and its relation to antiviral defense is relatively new to the field. A process we will briefly shed light on is the defense against transposable elements, since this type of defense is important for the understanding of piRNA and their functions. Finally, we will provide insights regarding the mechanism of viruses to cope with RNAi and give a short overview of applications made possible by antiviral RNAi.

RNA interference

Knowledge in the field of RNA interference is continuously growing, which is partly due to the development of next-generation sequencing. This resulted in a large catalog of functional noncoding RNAs that have diverse roles in regulation of multiple key biological processes, including organismal development, anti-viral defense and genome protection against transposal element activity.⁷

Small silencing RNAs

Small RNAs are an important class of the functional noncoding RNAs that mediate effect on DNA. In general, they are derived from longer precursors that can have both endogenous and exogenous sources, including acute viral infections and transposable elements (TEs).¹² The small RNA are a crucial component of RNA silencing machinery where they specify the identity of the target. Therefore, the small RNAs involved in RNA silencing are collectively called 'small silencing RNAs'.¹²

As mentioned earlier on, the collection of small silencing RNAs can be subdivided into three categories, siRNA, miRNA and piRNA. There are many differences causing this division, though there are similarities, mostly between miRNA and siRNA. Their similarity in size and sequence-specific inhibitory functions suggest a relation in biogenesis and mechanism. Indeed, both classes depend upon the same families of proteins: Dicer enzymes and Argonaute proteins (AGO).⁵ Dicer is an endonuclease belonging to the ribonuclease (RNase) III family, which transforms precursors of siRNA and miRNA into their mature forms. Thereafter the RNAs are loaded onto one of the AGO proteins.¹ Unlike siRNA and miRNA, the piRNAs are processed by a mechanism independent of the Dicer enzyme and solely associate with the PIWI-clade of the Argonaute family. siRNA and miRNA together with an AGO protein form the RNA-induced silencing complex (RISC), whereas piRNA and PIWI proteins form a piRNA-induced silencing complex (piRISC).¹ How these different classes arise due to their biogenesis mechanisms, specifically for mammals, is portrayed in a simplified way in Figure 1.

Small interfering RNA and microRNA are alike in the sense that they are both derived from double-stranded RNA precursors. Primary miRNA is transcribed from miRNA genes on the genome by RNA polymerase II. These primary miRNAs always form hairpin structures, whereas for siRNA this is one out of three options; cis-dsRNA, trans-dsRNA or hairpin structure.¹ The main source of these siRNAs in mammals are genes transcribed in antisense orientation.

Both siRNA and miRNA are assisted by RNases in the maturation process. For miRNA this comprises of first the creation of pre-miRNA from primary miRNA by Drosha and then the cleavage by Dicer to mature miRNA.⁸ As for siRNA, it is only Dicer that assists in the process of transforming long dsRNA into mature siRNAs.⁸ The resulting RNA bears one of the hallmarks of RNase III enzyme products, namely 5' monophosphate and 2',3' hydroxyl 2-nucleotide overhanging 3' ends. These hydroxy termini are found on all animal miRNAs and many animal siRNAs, but some arthropod siRNAs have been found to contain 2'-O-methylated termini.¹³

The type of transportation to cytoplasm within the biogenesis pathways differs between siRNA and miRNA. The hairpin shaped primary miRNA is cropped by the microprocessor Drosha–DGCR8 (DiGeorge syndrome critical region 8) complex before it is transported by Exportin 5 and RanGTP. The transport of siRNA and piRNA does not require any assistance.^{1,8}

Argonaute proteins

After the duplex miRNA or siRNA has been loaded into an AGO, the guide strand has to be chosen. This choice is dependent of the relative thermodynamic stability and the first nucleotide composition of its 5' end. Followed by this decision the passenger strand is eliminated by unwinding or cleavage by the AGO, depending whether it is the miRNA or siRNA pathway respectively (Fig.1 A).^{8,14}

The piRNAs are derived from long single-stranded precursor transcripts and have 5' monophosphate and 2'-O-methyl 3' on their termini, like some exceptions within the class of siRNAs.⁸ The AGO proteins consist of three typical domains, PAZ, MID and PIWI, where PAZ is the domain that differs between the PIWI clade and the other AGO proteins.⁸ This PAZ domain is specific for binding one of the different small silencing RNAs, since it resides at the amino terminus and functions as a binding pocket for the 3' end of the RNA.¹⁴ The 5' side of the RNA is placed in the MID domain of the protein and the cleavage occurs in the PIWI domain (Fig. 1).⁸ Part B of Figure 1 shows the mechanism of cleavage where hydrolysis of the phosphodiester bond takes place between nucleotides t10 and t11.

The mechanisms of biogenesis of piRNAs have been proposed to vary among different cell types, tissue and animals. Though, a recent publication by Gainetdinov et al.¹⁵ presents results in favor of a single model to explain the piRNA production in most animals. They propose that piRNA start the biogenesis process by guiding the PIWI proteins to slice the precursors transcripts to then direct the fragmentation of these sliced precursors. This stepwise process yields tail-to-head strings of pre piRNAs.¹⁵ Gainetdinov et al.¹⁵ found evidence for this universal mechanism in many animals, including humans, and they place the PIWI proteins at the center of piRNA biology.

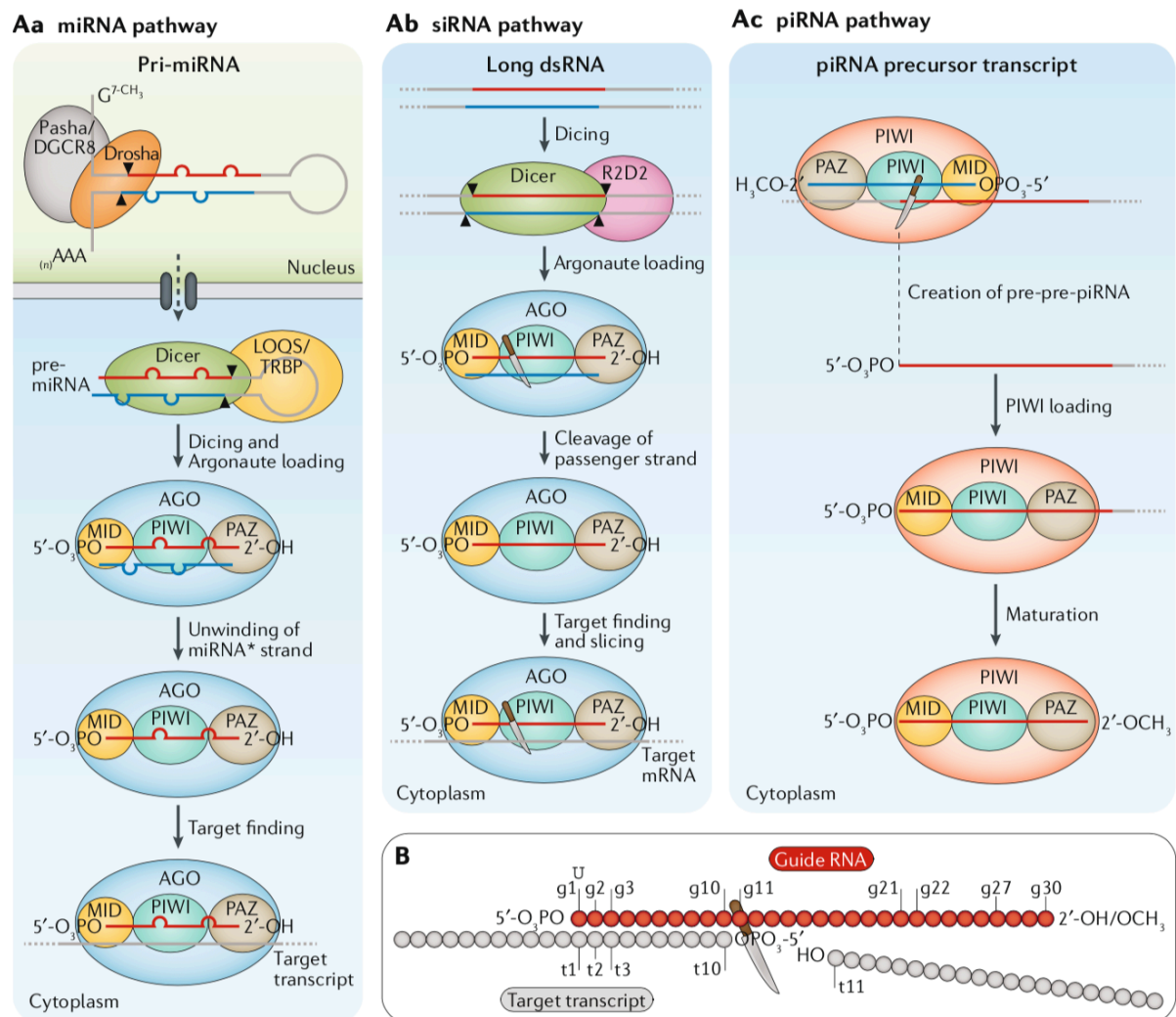


Figure 1. Argonaute protein family. A. Overview of the different pathways of small silencing RNAs. **Aa.** miRNA pathway. **Ab.** siRNA pathway. **Ac.** piRNA pathway showing the PIWI protein in the process of creating a mature piRNA. **B.** Figure portrays the cleavage between t10 and t11. The g-numbers indicate the nucleotide positions of the guide RNA while the t-numbers indicate the equivalent paired positions on the target RNA. (Figure from Ozata et al.⁸)

piRNA genomic source and biogenesis

There are several questions regarding the genomic source of piRNAs. It remains unsolved what the definition is of piRNA producing genes and how exactly the transcripts are marked for piRNA production.⁸ It is known that in flies¹⁶ piRNA precursors originate from heterochromatic loci, which is not the case for mammals, where the precursors appear to come from euchromatic RNA polymerase II transcription units¹⁷. The uni-strand clusters that have been found in all piRNA producing animals, are transcribed in the conventional and unidirectional way. The dual-strand variety has been found in certain arthropods and are believed to likely be present in others as well.⁸ These dual-strand clusters seem to require a specific transcriptionally repressive mark, H3K9me3, which is a trimethylation on a specific histone.⁸ A clear explanation for this finding still has to be established. Another interesting finding is that transcription of the dual-strand piRNAs has been found to correlate to Rhino occupancy of chromatin, where this germ-line specific H3K9me3 binding protein would perhaps have a regulatory function. The Rhino proteins seem to bypass the need for promotor sequences.¹⁸

The biogenesis pathway for piRNA requires a long, single stranded, 5' monophosphorylated RNA and thus a mandatory step is the cleavage that generates this particular end. Two pathways appear to be in place to make piRNA 5' ends, one of them is the ping-pong cycle for creating secondary piRNAs. In this cycle a PIWI protein, guided by initiator piRNA, cleaves a target RNA transcript to transform this into a pre-pre-piRNA with a 5' monophosphorylated end. Then there is a responder piRNA that is produced from the 5' end and is cleaved by an endonuclease at its 3' end. The intriguing part of this pathway is that the new responder RNA can act as initiator RNA, which in turn produces a new responder that will be identical to the original initiator piRNA. You could describe this pathway as a loop, one that could potentially amplify unlimited if only there was indefinite availability of precursor piRNA.^{8,17} An important consequence is that this cycle causes higher diversity in the sequence that is part of the piRISC complexes.¹⁹

The other pathway to create 5' ends is the phased piRNA pathway or primary piRNA biogenesis pathway. Here, the responder pre-piRNA 3' end is created by an endonuclease that is independent of piRNA. This endonuclease is part of a complex of proteins and this complex is also responsible for fragmenting the remaining part of the responder pre-pre-piRNA. The results of this fragmenting is a string of tail-to-head RNA we call phased trailing pre-piRNAs.^{8,17}

Importantly, the two pathways described above work together. There is an interconnection between the ping-pong cycle and the phased primary piRNAs, that has been shown in fairly recent publications. The ping-pong pathway will generate 5' monophosphorylated pre-pre-piRNAs by slicing and these are capable of entering the phased piRNA pathway.¹⁷ The amplification by the ping-pong cycle will increase the amount of piRNAs and the phased processing mechanism creates small RNA molecules that are diverse and spread into downstream transcript regions. This collaboration therefore makes complex populations of piRNAs, as is suggested for most animals.^{8,17}

Transposon defense mechanism

A transposable element (TE) is a DNA element capable of changing its position within the genome, thereby can create mutations and eventually destabilize the genome. It is therefore no wonder that molecular systems have evolved to control this TE activity to provide protection for the genome. Though on the contrary, these TEs play an important role in fueling the development of new genes, the introduction of novel immune strategies and rewiring of gene regulatory circuits.²⁰ Naturally it is crucial for host cells to keep control of the TEs to prevent deleterious effects, especially when it comes to protecting the germ line. It can be said that as a evolutionary result of this need for retrotransposon control, the metazoan germline exercises the small silencing RNA pathways to mediate recognition of TE transcripts.²⁰ This defense taking place in the germ line of animals against the incorporation of transposable elements, or transposons, is called transposon silencing.⁸ Interestingly, there are TE silencing piRNAs that have been detected outside the germline, in the soma in a few animals. It has

been suggested after analysis of 20 species across the arthropod phylum that these somatic piRNAs were present in the ancestral arthropod an astounding 500 million years ago.¹³ The publication suggests that somatic piRNAs and their pathway would have active in the soma of the last common ancestor of the arthropods in order of keeping TEs under control.

The working mechanism of TE silencing by PiRNA first requires a PIWI protein loaded with a piRNA. This complex is capable of recognizing TE transcription sites by the presence of nearby TE transcripts and consequently recruits histone methyltransferases such as Su(var)3–9 and histone H1. The methylation of H3K9 by Su(var)3–9 causes the binding of histone protein 1a to the modified histones, thereby silencing the TEs.²¹

Not only piRNAs, but also endogenous siRNAs, have been reported to function as suppressors of TE expression in both gonadal and non-gonadal tissues.²¹ It is also possible for the two subtypes of RNA to work together in silencing transposons. For example, in worms the piRNA provokes a response by siRNA. This is unusual since in most animals piRNA causes silencing by slicing the mRNA transcripts or by preventing the mRNA to be formed, repressing the transcription at the start of the process.⁸ Another exceptional example is the presence of telomeric retrotransposons in *Drosophila*. *Drosophila* lack the enzyme telomerase for maintaining chromosomal ends. The retrotransposons provide the same result by silencing with piRNA to help maintaining a stable telomere length.⁸

Antiviral defense mechanisms

All living organisms need a form of antiviral defense to stay protected from viral infections. Mammal innate immunity for example uses the well-known interferon response. The mechanism discussed in this review is that of RNA interference, on which especially plants, worms and insects seem to depend.⁴ One of the hallmarks of RNAi involvement in antiviral defense is virus-derived small interfering RNA (vsiRNA). For generating these antiviral RNAs, first the viral dsRNA replicative intermediate needs to be recognized by Dicer. These intermediates, that are produced during the process of virus replication, are subsequently processed by Dicer into pieces of 21 to 23 nucleotide siRNAs.¹¹ The result is a virus-derived siRNA which is transferred by Dicer into AGO, together forming the core components of RISC. RISC, guided by the vsiRNA, is then capable of directing the cleavage of viral RNAs.^{11,22} Over the past decades there has been a substantial amount of research regarding the antiviral function of the RNAi pathway and multiple arguments came forth that will be discussed in the following section of this review.

The infection of fungi, plants and insects with either DNA or RNA viruses has been shown to trigger the production and subsequently accumulation of virus-derived siRNA (vsiRNA). Furthermore, these vsiRNAs are produced by a Dicer enzyme, providing evidence it is indeed small interference RNA that is generated. Also supporting of the antiviral function are the findings that when RNAi is compromised, the viruses from fungi, plants and invertebrates are replicating at a higher frequency. This is also true for defects in the biogenesis of specifically the vsiRNAs. When looking at the concept of antiviral function from the other side, the occurrence of virus-encoded suppressors of RNAi (VSRs) is also a favorable argument.²³

RNAi has been confirmed to play crucial roles in antiviral defense of invertebrates and all the components of the machinery required for the antiviral response are conserved through evolution in mammals.⁴ Even though the components are present, it is still under hot debate whether the small silencing RNAs serve the function of antiviral defense in mammalian cells. Recent studies have shown that viruses actively suppress the response by mammalian cells with VSRs.²⁴ There has been more and strong evidence for the production of vsiRNAs during authentic infection of mammalian cells by two

positive strand RNA viruses, Nodamura virus and encephalomyocarditis virus.²³ It therefore seems more plausible that RNAi would also play part in human antiviral defense mechanism. Overall, despite of the ongoing debate, there is accumulating evidence pointing in the direction of RNAi antiviral function in mammals.

VsiRNAs have been convincingly documented in mosquitoes and other insects after infection with diverse RNA viruses, indicating that the induction of antiviral RNAi could be a conserved feature of insect virus infection.^{16,22,25} In *Drosophila*, specific vsiRNAs are produced and distributed across the whole genome. In addition, elimination of key pathway proteins in *Drosophila* or derived cells resulted in improved virus replication and higher pathogenicity, supporting the concept of antiviral activity.^{16,26}

Aguado *et al.*²⁷ looked into a certain aspect of host antiviral defense, the dynamics and the biology of the virus itself. They used host microRNAs to recreate an RNAi-like environment in vertebrates. Furthermore, they set out to compare positive- and negative-stranded RNA viruses in their capacity to deal with this pressure posed by the difference in virus biology. The study revealed that evading defense by four distinct viral families was defined by the viral capacity to undergo homologous recombination. It was shown that positive-stranded RNA viruses could escape by undergoing strand switching and thereby quickly excising genomic material. Oppositely the negative-stranded viruses could be targeted by RNA interference and consequently were cleared after this targeting was executed effectively.²⁷

Overall, there have been studies that detected virus-derived small RNAs and RNA-based antiviral immunity in fungi, plants, *D. melanogaster*, mosquitoes, silkworms, *C. elegans* and mammals. In addition, there is more research claiming that the initial development of RNA interference was to create a defense against viruses.^{7,11}

piRNAs and antiviral defense

PIWI-interacting RNAs are known to have multiple functions, including a role in viral defense mechanisms, though there are substantial differences between all sorts of organisms.²² There are also differences concerning the either primary or secondary function of piRNAs in respect to the adaptive immune system of that particular organism.²⁵ Petit *et al.*⁷ suggest that *D. melanogaster* does not require piRNA for antiviral defense. On the other hand, this has been proven for mosquitos, which have virus-derived piRNA (vpiRNA) specific for several viruses, including flaviviruses such as the Zika virus.^{25,28} The function of these piRNAs is not yet known, though antiviral activity is one of the logical explanations.

As mentioned, antiviral defense by RNAi in somatic tissues is typically ascribed to siRNAs. However, some invertebrates make use of piRNAs to form a line of protection against viral infection in the soma. In particular mosquitoes seem to fight RNA viruses using the ping-pong pathway. The two mosquito PIWI proteins, Piwi5 and Ago3, participate in the ping-pong cycle by consuming viral positive- and negative-stranded RNAs to produce piRNAs (Fig. 2).²⁵ The ping-pong cycle is activated by piRNAs produced from loci where viral integrations have taken place. The derived piRNAs most likely allow the recognition of viral RNA as a result of the mosquito piRNA pathway. How this process of recognizing and clearing viral RNAs would occur in other animals is at the moment unknown, partly due to currently no sign of the ping-pong pathway being involved in the process of generating vpiRNAs.⁸

A question that arises is why some animals have antiviral responses bases on piRNA, when other animals entirely rely on RNA interference based on siRNA for their defense against viruses. It could be explained by the differences between these two subclasses of small silencing RNAs, specifically the precursors that enter the two pathways. The siRNA pathway is triggered by dsRNA whereas the piRNA biogenesis pathway is entered by single-stranded RNA (Fig. 1).⁸ It is possible that these differences in pathways and precursors and their ability to target RNA from a variety of viruses and stages of viral

infection is beneficial to the organism. This could provide a boost for the overall antiviral response and providing more insights into this concept seems important for the small RNA field.⁸

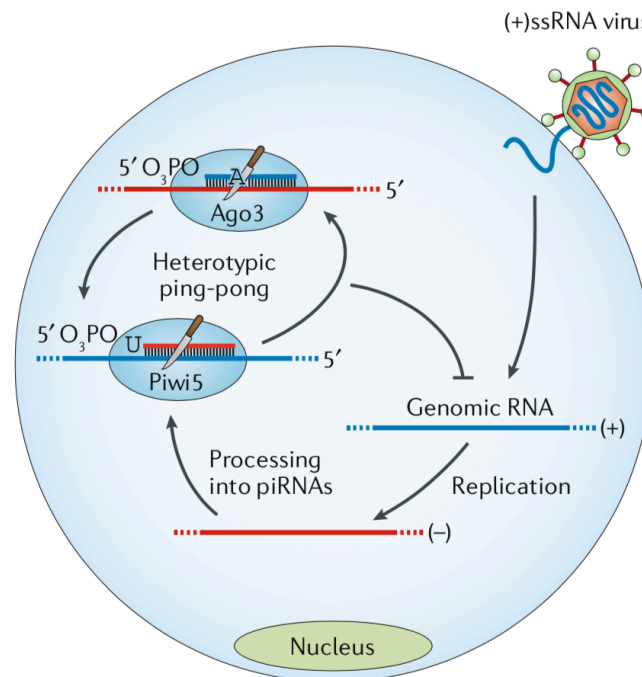


Fig. 2. Viral defense by piRNA. In some animals, somatic piRNAs fight viruses. When infected by a positive and single-stranded RNA virus, mosquitoes engage in an antiviral piRNA-based response. Upon viral replication, Piwi5 and Argonaute3 (Ago3) participate in the ping-pong cycle, consuming viral RNAs. (Figure from Ozata et al.⁸)

Viral suppressors of RNAi

Viruses have developed a counter attack in the form of proteins to fight the antiviral RNAi response by the infected cell. These proteins, called virus-encoded suppressor proteins of RNAi (VSRs), actively interfere with distinct steps of the RNAi machinery to ensure high virus production and efficient viral spread.²⁹ The many functions of VSRs include shielding the virus genome from RNAi, changing sequences in the viral genome, virus regulation of cellular miRNA expression and adaptation of the virus to cellular RNAi to escape its restriction.¹⁹

The accumulation of VSRs supports the view that RNAi has antiviral activity. Especially findings showing that viruses deficient in VSRs are not able of infecting wild-type hosts. The opposite also appears to be true since host mutants defective in RNAi have elevated levels of viruses that are VSR-deficient and results show infected host cells.²³ The VSRs seem to be the viral escape for forms of RNA interference and most likely play a part in the existence of resistant viruses.¹⁶

A proposed mechanism for VSRs is that the expression of three types, B2, NS1 and 3A, suppresses the biogenesis of the vsiRNAs and thus prevents the targeting of the cognate viruses during the infection of mammalian cells. Presumably, VSRs B2, NS1 and 3A all bind to the viral dsRNA replicative intermediates and inhibit Dicer processing of the vsiRNA precursors as has been demonstrated in vitro.²³ Low amounts of vsiRNA have been detected in infected mammalian cells, which could be explained by incomplete viral suppression of vsiRNA biogenesis.²⁴

Applications

For some time now, multi-resistant viruses pose a serious threat to public health. Thus, a decade ago RNAi was welcomed as a new and potentially powerful tool to target these viruses. Partly due to the fact that RNAi therapeutics can be administered in low dosage and still provide high effectiveness. But using RNAi for therapeutics is not a simple task. The large molecular weight is a disadvantage, which taken together with a net negative charge and hydrophilicity lowers the efficiency of crossing plasma membrane with siRNA significantly.³⁰

In the last two decades, RNAi has been used in an attempt to change interaction with viruses. Insects and especially mosquitoes have been engineered, hoping to provide better defense against viruses, in particular the multi-resistant viruses. Not only organisms such as mosquitoes are subject to engineering, viruses can also be engineered by using the RNAi pathways. Tsetsarkin *et al.*³¹ have succeeded in genomic insertion of recognition sides for mosquito-specific miRNAs. This resulted in a virus that was incapable of replication in mosquito cells and live mosquitoes but could replicate in mammalian cells. The outcome being that the virus can no longer infect human cells through a mosquito bite. Engineered viruses such as these could have extremely useful applications such as a vaccine.

In general, better understanding of mammalian immunology received by a new mammalian antiviral immunity would lead to many opportunities. One possibility could be that such a RNAi mechanism provides a genetic pathway to get rid of the virus without the need for killing the infected cell.

Conclusions

There is a dynamic relationship between viruses and cells, where RNA interference plays a part in fighting viruses on behalf of the cells, and where viruses try to evade this RNAi to achieve effective replication. Small silencing RNAs are an important component of RNAi and there is large collection of recent publications regarding one or more of its three classes; siRNA, miRNA and piRNA. The piRNA pathway, highlighted in this review, involved piRNAs that form a complex with PIWI proteins and is a conserved silencing pathway that also exhibits antiviral defense function in some organisms. This pathway is clearly distinct from the other small silencing RNA pathways in its mechanism, including the ping-pong cycle and its collaboration with the primary piRNA biogenesis pathway.

Although the discoveries and knowledge in the field of piRNAs have evolved and certainly have not been standing still, there are still many aspects of the piRNA biology that remain poorly understood. This could be due to the complex piRNA biogenesis and silencing machineries when compared to those of miRNA and siRNA. Besides, piRNA is mostly restrained to gonadal tissues which for a long time has stood in the way of a systematic analysis *ex vivo*.

Importantly, there are aspects of the piRNA pathway that make it attractive for future research and applications. This can be explained by addressing several factors, including the fact that piRNA is from single-stranded RNA, a substrate that is abundant in cells. Moreover, the intrinsic imprecision of piRNA biogenesis machinery produces enormously diverse piRNA guide sequences. This diversity could cause a drift in evolution, perhaps providing piRNA with new targets over time. When regulation of these targets would prove to be a selective advantage, this might result in new function of the piRNAs. The current known functions that deviate far from transposon silencing are in favor of this idea, so unexpected roles of piRNA might be waiting to be discovered. Another attractive aspect are the mechanisms and consequences of epigenetic changes caused by the piRNA pathway. This currently is a very booming area of research and is likely to gain insights into reprogramming.

As discussed, the application of engineering insects provides a possibility in defense against viruses. Engineering and editing genomes of not only insects but all organisms is an extremely important method and lies at the root of many important discoveries. Though it is crucial for such approaches to prove effective in the field of RNAi and antiviral response to first understand the interaction between viruses and RNAi. This interaction includes the specifics regarding the defense itself and not to forget, the counter defense. I believe that there could be a bright future for RNA interference in relation to antiviral defense. A great deal is unknown, but with ongoing improvement of techniques such as engineering and sequencing, a lot could be accomplished. There is definitely a need for effective strategy against multi-resistant viruses, RNAi could be a good contender.

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