

Learning from the Enemy: Innate RNA Interference Renders Mosquitoes Asymptomatic to Arboviral Infection and Provides Researchers with New Approaches to Subdue its Effects

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Abstract

This paper examines arboviral infection with the goal of characterizing the innate immune response of human disease vectors such as mosquitoes. RNA interference and conserved innate immunity pathways allow mosquitoes to resist symptoms as they carry arboviruses. RISCs, piRNA, and p-bodies are of special interest not only due to their conservation across phyla but also due to the specificity with which they can be used to characterize an infection. For each of these interference mechanisms, I will discuss: 1) the essential molecules conserved, 2) how these molecules act to tolerate or resist pathogens, and 3) the positive feedback mechanisms which amplify the immune response. Understanding developments in the field of RNA interference would allow arboviral infection to be more easily recognized, distinguished, targeted, and treated at the molecular level in-field and at a lower cost.

Keywords: Arboviruses; Microfilariasis; Vertebrates; Phylogenetic; Urbanization

Introduction

Arboviruses rely entirely on the organisms they infect to complete their life cycle; in fact, the word “arbovirus” is an acronym for arthropod-borne virus. These viruses alternate between infecting vertebrates (known as “hosts”, such as birds and livestock) and arthropods (known as “vectors”, often mosquitoes or ticks). Horizontal transmission occurring between unrelated vector or host organisms spreads arboviruses through sexual contact and blood feeding. Vertical transmission from parent to child is more rare but does occur [1].

Arbovirus is, however, a term that you will not find in a Linnean taxonomy, as they cannot be characterized under a single phylogenetic clade. The most well-known arboviruses are classified in the Flaviviridae family, which includes Yellow Fever Virus, West Nile Virus, Dengue Virus, Japanese Encephalitis, Zika, and Hepatitis C virus. Other notable arboviruses belong to the families Bunyaviridae (Rift Valley Fever, Heartland Virus), Reoviridae (African Horse Sickness, Colorado Tick Fever virus) and Togoviridae (Alphaviruses, including the Chikungunya virus) [2,3]. All but one known arbovirus (African Swine Fever Virus, a ssDNA) are single-stranded RNA viruses, placing them into Groups IV and V of the Baltimore classification. The *Aedes* mosquito has been studied more extensively than most other arboviral vectors (such as *Culex* mosquitoes or ticks) as its two species, *Ae. aegypti* and *Ae. albopictus*, have become the major carriers of human mosquito-borne diseases [2].

Arboviruses continue to pose a significant health risk to humans globally due to climate change, urbanization, and the ease with which these viruses are able to adapt to new hosts and conditions [2]. Mosquito-borne diseases account for approximately half a million deaths worldwide each year [3]. Furthermore, climate change, mass

migrations, and urbanization allow for the expansion of zones in which humans are at risk for arboviral infection.

For example, the habitat for *West Nile Virus* (WNV) has expanded enormously in the last three decades. It alternately infects Passeriforme birds and *Culex* mosquitoes in order to reach its human hosts. *West Nile Virus* is especially difficult to attack due to its many serotypes, distinguishable strains of a virus found in different geographies. As climate change shifts the habitats of their vectors, serotypes overlap and infections become more difficult to characterize and treat. Furthermore, the introduction of the invasive house sparrow (*Passer domesticus*), a highly competent host for the virus, likely introduced WNV into the New World [2].

Japanese *Encephalitis Virus* has continued to spread throughout Asia and the South Pacific, and *Rift Valley Fever Virus* has seen increasing epidemics across East Africa due to increased global temperatures which have allowed vector populations to become more successful worldwide. For example, the El Niño effect promotes precipitation in east Africa, creating pools of standing water that serve as mosquito breeding grounds. Flash flooding also triggers the simultaneous hatching of *Aedes* eggs, leading to large-scale epidemics [2].

Arboviruses which infect ruminants (such as *Bluetongue Virus* and *Venezuelan Equine Encephalitis Virus*) have come to prominence due to human activity as well. An outbreak of VEEV in 1995 was most likely due to the unintentional leakage of an isolate from the 1966 epidemic. Strains of ruminant viruses are found to be highly adaptable and can often result from extremely subtle environmental changes [2].

Urbanization is also a cause of the increasing frequency of arboviral epidemics. As humans live in closer proximity, we lose our status as a dead-end host and allow these viruses to spread among a larger population. In fact, humans are the only vertebrate host of most Dengue virus strains (DENV). All 4 serotypes have a wide range of symptoms from mild flu-like symptoms to a vascular fragility and

hemorrhagic fever. Due to the diversity and mild nature of early symptoms (often unified by a quickly rising fever), it can be difficult to identify the exact disease before it progresses to a late stage. Tissue research is often difficult to perform due to cultural, practical, and monetary limitations on performing autopsies in developing countries [2].

Zika has recently come to global prominence due to its correlation with microcephaly, Guillain-Barre syndrome, and other congenital abnormalities when detected in fetal brain tissue or amniotic fluid [3]. Its rapid spread through Brazil and the Americas likely resulted from either increased air travel from the Pacific and other affected regions. While in most cases Zika infection leads to mild flu-like symptoms, its effects can be catastrophic for developing fetuses. An effective vaccine or treatment has not been identified, and it is notably difficult to distinguish its most severe symptoms from those of other flaviviruses, hindering the ability of medical practitioners to effectively treat it [4].

Chikungunya virus is another arbovirus which has proven difficult to manage in India and tropical regions of the Americas. This alphavirus bears many similarities to other arboviruses in that it lacks a vaccine and easy to perform field test. As of now the only effective way to prevent infection is to avoid being bitten, an arduous task in developing tropical countries [2,3].

While initial infection is largely preventable in developed countries, the necessary strategies and equipment are often not available in the developing tropical countries where the viruses are most rampant. Molecular approaches such as vaccination vary in availability with the specific arbovirus and serotype [3]. In developing countries, blood samples and vaccines can be difficult to transport and keep cold until an assay can be performed to identify the infection. Therefore, a field medic must often be able to distinguish between multiple arboviruses and serotypes from symptoms alone, a task which would be made significantly easier by a low-cost field marker. However, no such marker is available today due to difficulty in finding a unifying yet strain-distinguishable sign of arboviral infection.

Ultimately, a new approach is needed in order to allow researchers to better characterize, treat, and respond to arboviral infection. And it may be useful to perform this sort of investigation at the vector level rather than focusing on the arboviral hosts. There are less ethical issues associated with testing mosquitoes and other arthropods than with humans, and the disease can be assessed quickly from a molecular analysis rather than by way of general symptoms. Examining the innate immunity that arthropods have adapted in order to protect themselves from the disease may be advantageous for attacking the global challenges posed by arboviruses.

Innate Immunity Strategies

Mosquitoes do not experience the negative symptoms of arbovirus infection as humans and other mammals do. Research has suggested that this asymptomatic response is due to a wide array of innate immune pathways that protect mosquitoes' cells from infections. Innate immunity is often achieved at the molecular level by interfering with the nucleic acids inserted by a viral pathogen to hijack the machinery of a cell. In the absence of an immune response, these viruses will utilize endogenous enzymes (as well as their own capsid proteins) in order to rapidly produce protein products. These proteins will allow assembly of new viruses that can be exported out of the cell, becoming available to infect new cells and continuing their life cycle. Arboviruses with negative sense RNA (Group V, such as

Bunyaviridae), must carry RNA polymerases in order to transcribe their genetic material into to a complementary positive sense strand to be transcribed by cellular proteins. However, carriers of arboviruses have evolved defenses to disrupt this cycle; foreign RNA sequences activate signaling pathways within the cell to inactivate viral RNA and prevent it from disrupting normal cellular functions. Furthermore, the products of these pathways (such as degraded mRNA, or small sliced siRNA) exert positive feedback on the beginning of the pathway, amplifying the response [5].

Studying the innate immunity of mosquito vectors is useful to humans attempting to better understand the virus and motivate medical applications. Many of these same mechanisms that have evolved in the *Aedes* mosquito (the vector genus whose genome is most well-characterized) most likely evolved in humans as well. Admittedly, there are important differences in the evolution of each species. The relationship between the virus and mosquito is largely commensal (+/0). The virus profits as the mosquito delivers the virus to a vertebrate host (thereby continuing its life cycle) and the mosquito's innate immunity mechanisms largely prevent the vector from experiencing any negative selection.

The mosquito develops structures which allow it to successfully carry and transport the virus to mammalian hosts, while the mosquito neither benefit nor is harmed from being infected. Some experiments have suggested a mutualistic (+/+) relationship in which the mosquito draws some benefit from being infected with the virus [6].

RISC and Reward

RNA interference encompasses the numerous mechanisms through which RNAs inhibit the expression of genes. Endogenously triggered RNA interference for gene regulation was initially received with skepticism when discovered in *Arabidopsis* due to its blatant violation of molecular biology's Central Dogma [7].

However, the standard gene to transcript to protein pathway does not have the explanatory power to account for regulation of protein products. Furthermore, it became clear that any mechanism would need to be complex, versatile, and confer a large evolutionary advantage. The prevention of infection by viral parasites conferring foreign nucleotide sequences turned out to fulfill these criteria.

Double-stranded RNA was first recognized for its ability to produce responses at both the cellular and organismal level when inoculated into *C. elegans* [7]. The enzymes and other cellular mechanisms that work together to produce this response were later confirmed by independent studies in *Arabidopsis*, *C. elegans*, and *Neurospora*, demonstrating that the pathway is conserved across phyla [8].

As with many evolutionarily conserved phenomena, RNA interference mechanisms possess a number of central elements which are contained within the RNA-induced silencing complex (RISC). While accessory proteins vary, all known eukaryotic RISCs contain two key elements: Argonaute proteins and small interfering RNAs (siRNAs) [7].

Argonaute proteins function to bind the siRNAs and position them to interact with the target molecule (often viral or endogenous RNA). Then, Argonaute either directly discards the target sequence, or recruits outside enzymes in the cytoplasm to silence the gene at the chromosomal, transcriptional, or translational level. Multiple RISC complexes often exist within a cell; each is named after its core Argonaute protein (AGO1, AGO2, AGO3, Aub).

A RNA-induced transcriptional silencing complex (RITS) contains a similar set of mechanisms (including Argonautes) but is localized to the nucleus. The form and function of the Argonaute proteins allow the RISC and RITS to accept a diverse clade of RNAs. Different subdomains of the Argonaute tertiary structure accept different types of RNA (such as PIWI-interacting RNA, siRNA, etc.) enzymes [9,10] (Figures 1 and 2).

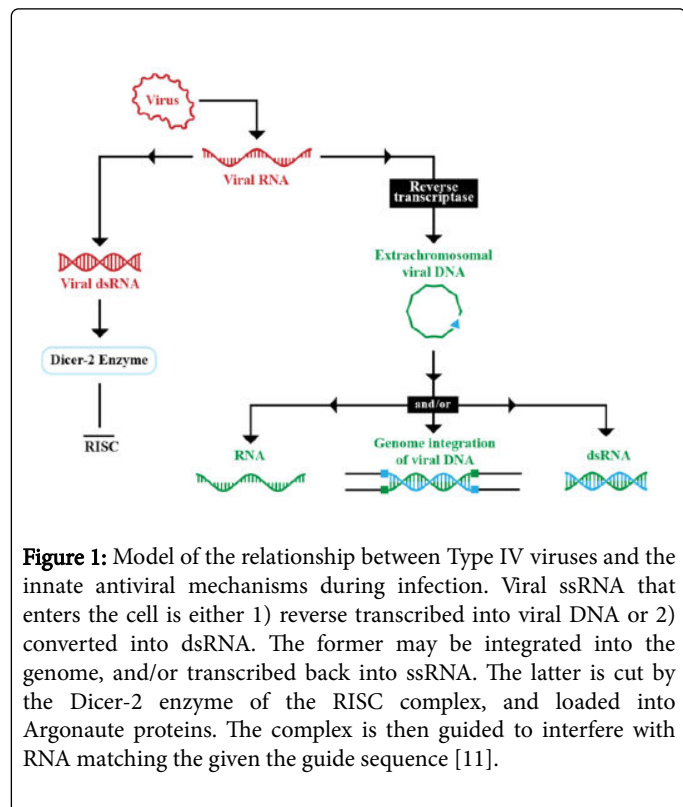


Figure 1: Model of the relationship between Type IV viruses and the innate antiviral mechanisms during infection. Viral ssRNA that enters the cell is either 1) reverse transcribed into viral DNA or 2) converted into dsRNA. The former may be integrated into the genome, and/or transcribed back into ssRNA. The latter is cut by the Dicer-2 enzyme of the RISC complex, and loaded into Argonaute proteins. The complex is then guided to interfere with RNA matching the given the guide sequence [11].

Dicer enzymes break down the dsRNA into small interfering/regulatory RNAs~21 bp in length [7]. RISC and RITS then apply siRNAs to disable the target RNA in four distinct ways: 1) heterochromatin formation, 2) slicing of target RNAs, 3) transcriptional inactivation, 4) DNA elimination. These methods are summarized in Figure 3 [5].

These proteins broadly represent the group of cellular elements which are activated by the presence of dsRNA [12]. For (-)-sense ssRNA viruses (a group which encompasses the vast majority of arboviruses), viral enzymes allow single-stranded RNA to become dsRNA and activate the RISC (thereby helping the mosquito to protect itself and remain asymptomatic, as discussed below). (+)-sense ssRNA is first converted into (-)-sense RNA by other viral at the chromosomal level, the RITS (loaded with siRNA) sorts through transcripts as they are generated in the nucleus and attempts to match them to the guide sequence. If a match is found, histone methyltransferases increase the density of chromatin packaging on the respective DNA, preventing transcription [13].

If such a match is found outside of the nucleus, the RISC will initiate a slicing of the target RNA. The “Slicer” enzyme which performs this function remained until Song et al. examined its piwi-domain and tertiary structure, demonstrating that it is in fact a catalytically Argonaute protein [14]. In humans and mosquitoes, only AGO2 is catalytically active [10].

At the protein level, RISC recruits miRNAs to repress translation. Resulting from endogenous transcripts being folded into hairpin like structures, they are loaded into Argonaute proteins in order to guide the complex to its target [15]. At most 7 bases of miRNA need to be matched to the target for translational repression to occur. In *Drosophila*, AGO1 promotes de-adenylation and removal of the 5' cap by recruiting outside complexes. AGO2 blocks binding of proteins to eukaryotic initiation factors, further repressing translation [5].

Lastly, RISC can potentially work genome wide, eliminating harmful DNA from the genome. This method is by far the rarest, only having been identified in *Tetrahymena*, a protozoan that has evolved a complex life cycle in order to defend against DNA and RNA parasites (such as viruses).

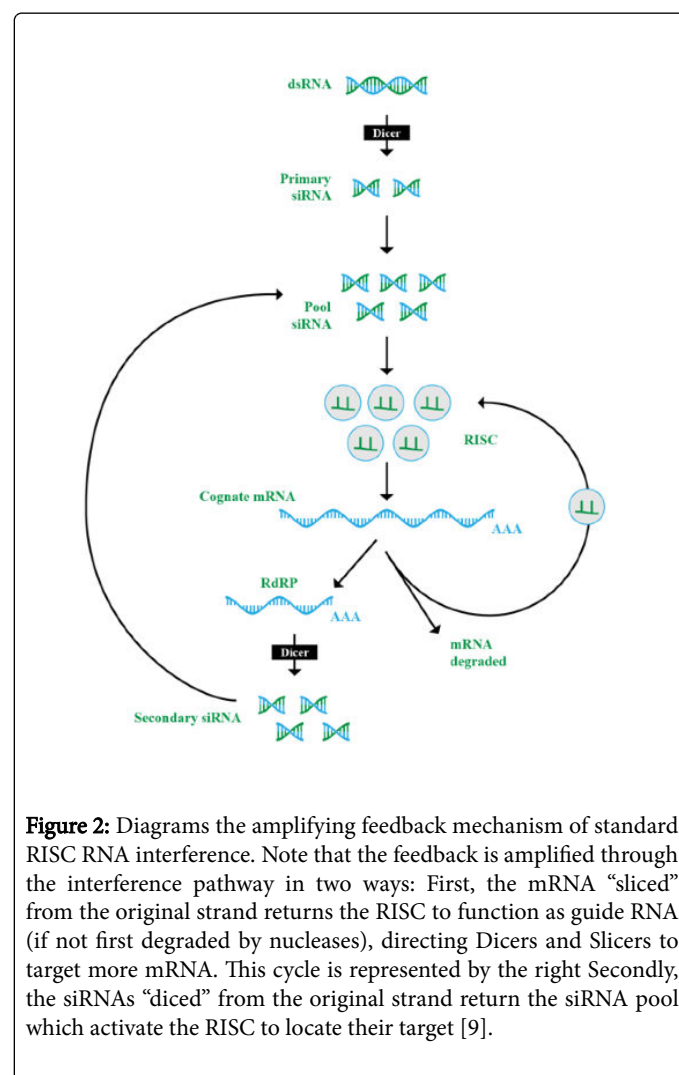


Figure 2: Diagrams the amplifying feedback mechanism of standard RISC RNA interference. Note that the feedback is amplified through the interference pathway in two ways: First, the mRNA “sliced” from the original strand returns the RISC to function as guide RNA (if not first degraded by nucleases), directing Dicers and Slicers to target more mRNA. This cycle is represented by the right Secondly, the siRNAs “diced” from the original strand return the siRNA pool which activate the RISC to locate their target [9].

RISCs search through the transcribed RNAs of the germ line nucleus or foreign sequences, upon which the corresponding DNA is blocked from rejoining the genome [5]. While such a mechanism is rare, it does provide insight into the number of ways that a host might attempt to eliminate a virus. The moral of this case study: researchers should be cautious not to limit themselves to a single paradigm; life forms have evolved a surprisingly complex variety of ways to adapt to being taken advantage of by others.

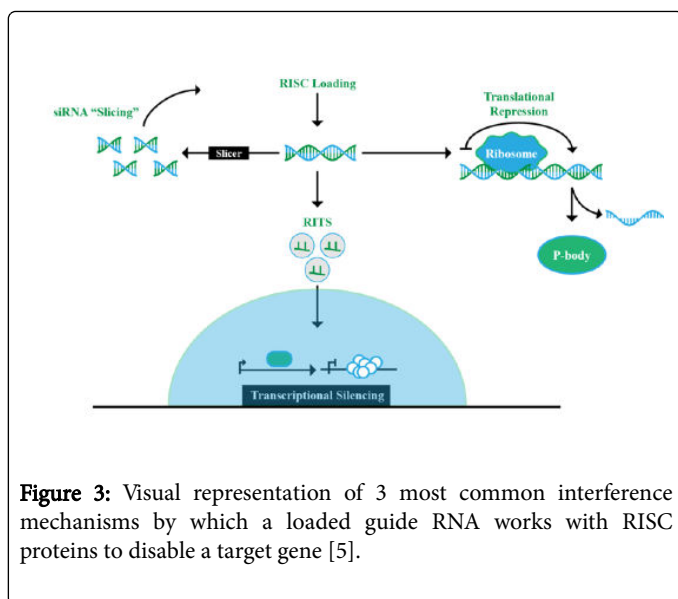


Figure 3: Visual representation of 3 most common interference mechanisms by which a loaded guide RNA works with RISC proteins to disable a target gene [5].

Resistance and Tolerance

Due to their lengthy coexistence and coevolution, mosquitoes and arboviruses have evolved a unique relationship. In fact, the way in which mosquitoes undergo arboviral infection is fundamentally distinct from that of other carriers at the molecular level. Mosquitoes have adapted to tolerate arboviral infection of nearly all its cells for extended periods of time, while most organisms must resist the spread of infection. In a resistance model, a host prevents the virus from hijacking new cells and infecting new areas of the body. In a tolerance model, a host prevents a virus that has hijacked its cells from shutting down key cellular machinery [12].

In a series of publications [11,12], Goic et al. investigate DNA of endogenous RNA origin, and use their results to clarify the resistance vs. tolerance distinction for arthropods. Reverse-transcribed RNA plays a significant role in facilitating the innate immune response of mosquitoes to the viruses that they asymptotically carry and spread. Goic et al. first investigate the presence of “vDNA”, endogenous DNA that interferes with viral DNA, observed after simulating a viral (+)-sense, ssRNA infection in a *Drosophila* model [11]. They activate RISC using dsRNA, which was then cut by a Dicer into ~21 bp fragments. Upon finding that RNAi inhibited viral particles, preventing them from completing a full replication cycle, they propose that *Drosophila* resisted, rather than tolerated the arboviral infection [11].

Upon inoculating *Drosophila* lines with a dsRNA virus, they successfully detect vDNA using PCR. However, the addition of AZT, a reverse transcriptase inhibitor, block production of vDNA. This result suggests that vDNA is likely of RNA origin, as reverse transcriptase is required for its production. Upon further genomic analysis, they find that the RNA transcript is coded for by retrotransposons. Goic et al. also present hypotheses as to how the vDNA travels through *Drosophila* upon infection. It is plausible that 1) new vDNA is produced in each cell after infection occurs and the cell’s machinery is hijacked, or that vDNA is transported across cell boundaries in an organism-wide immune response. The first hypothesis proved correct, confirming a resistance model for *Drosophila* [11].

In a later publication [12], Goic et al. repeat a similar set of experiments but using the *Aedes* mosquito and the Chikungunya virus (an alphavirus, Togoviridae family). vDNA was identified in the wings and legs of mosquitoes even when infectious viral particles were not found there, suggesting a tolerance model for mosquitoes. Furthermore, while mosquitoes can normally tolerate arboviral infection for their entire life, mosquitoes inoculated with AZT died after precisely 9 days (unlike *Drosophila* whose life expectancy highly variable). This result indicates 1) an inability to tolerate arboviral infection without vDNA (whose production is inhibited blocked by AZT), and 2) an inability to resist arboviral infection once it begins to spread through the body, as evidenced by the consistent life expectancy).

This difference provides insight into the distinct evolutionary relationship of the two organisms with viruses and provides evidence for a mutualistic relationship between mosquitoes and arboviruses [10]. Mosquitoes tend to tolerate the presence of the virus, allowing it to insert its molecular machinery into most all cell types, while resisting negative symptoms. Conversely, other arthropods (such as *Drosophila*) which have not evolved a sophisticated ecological relationship with the virus prefer to resist the infection, preventing it from hijacking new cells at all costs.

The distinction between resistance and tolerance models is not merely a conceptual one. Rather, it elucidates the mutualism between mosquitoes and arboviruses, suggesting that tolerating arboviral infection confers a selective advantage to the vector. While not currently well understood [6], the evolutionary history should prove useful to researchers seeking to characterize an effective vector. With this information, we can more effectively target vectors at the cellular level to eliminate the spread of arboviruses.

piRNAs

Beyond the standard interference pathways provided by the RISC, piwi-interacting RNAs (piRNAs) and p-bodies provide further evidence of a conserved innate antiviral response that can be harnessed by researchers to more easily distinguish and attack arboviral infection. Research in alphavirus infections first demonstrated that ping-pong-dependent piRNAs (a term referring to the pathway that amplifies their effect) act similarly to siRNA in allowing interference against viral infection. piRNAs are often transcribed from lncRNA genes, and have been associated with DNA transposable elements. To refute a common misconception: they do not necessarily arise from dsRNA being broken down [16].

The piwi-clade is a subregion of tertiary protein structure. Its presence has been evolutionarily conserved and correlated with the emergence of specialized germ cells in primitive animals. In *Drosophila*, the Aubergine and AGO3 proteins both contain a piwi-clade. The piwi pathway is largely independent from the previously discussed RNA interference pathways, and therefore presents unique opportunities for researchers to manipulate it at the molecular level.

First identified in *Drosophila*, the piwi-clade is found in all known animals. After early developmental separation of somatic and gametic cells, its expression becomes limited to the gametes. This localization presents an important limitation in studying piRNA *in vivo*. *Ex vivo* studies often cannot replicate the environmental conditions needed to facilitate all aspects of the pathway [16].

Studies in zebrafish (an important model organism for piRNAs due to its role in sex determination) have shown that the piwi pathway is not dependent on a Dicer protein. piRNA genes are often repetitive, and therefore can be linked to numerous loci within the genome. All piRNA locations within the genome are noncoding and contain many transposons. piRNA “clusters” are often packaged tightly within heterochromatin, suggesting that piRNAs play a role in determining chromatin structure. Although they tend to cluster near or within RISC complexes, piRNA can also form its own packages within the cytoplasm. Its average length is anywhere between 2 and 200 kb [16].

There are two separate models for how piRNA arises. Given that clusters can spawn both (+)-sense and (-)-sense strands, it is plausible that 1) they arise from long-stranded transcribed RNA, or 2) that they are transcribed directly from the genome. Although it is not known which model is correct, there is considerable evidence for the former: inserting a “P-element transposon”, which can not be transcribed, upstream of a piRNA locus resulted in lack of piRNA. It is apparent, however, that specialized polymerases and helicase variants are needed to produce and unwind the piRNA before it can perform its function. Piwi processing determinants are molecules which may hold potential for artificially regulating piRNA activity. Their ambiguous name suits them. they have not yet been isolated or well characterized and are a subject of ongoing investigation [17].

The ping-pong cycle is a feedback loop which serves to generate new piRNAs while at the same time cleaving new targets. It can be summarized as following: transposons produce an assortment of piRNAs of both senses which are loaded by piwi-clade proteins. When encountering a target RNA that is complementary to either strand, a Slicer (often Aubergine) cleaves the 5' end, thus inactivating the RNA as well as creating a new piRNA. The polynucleotide is then exported from the nucleus and reloaded into a new piwi-clade protein where they will guide them to slice and inactivate new target RNAs. Throughout this process, a number of auxiliary proteins prevent the RNA from degrading [18].

As with RISC-dependent molecular interference mechanisms, the piwi pathway likely evolved as a response to molecular parasites such as transposons (as well as their transcribed RNA form). Transposons are essentially similar to RNA viruses in many important ways. Robert et al. [10] found both mechanisms to be capable of independently silencing and co-suppressing many of the same genes in *C. elegans*. Furthermore, many of these genes code for Argonaute proteins, helicase, and proteins that regulate RNA polymerase. These products are all proteins which have been previously found to regulate piRNA activity [10]. These genealogical links form a solid connection between piRNA pathways and the innate immune pathways which attempt to defend against foreign polynucleotide sequences, such as those employed by arboviruses to hijack their hosts.

Stress Granules

Like other evolutionarily conserved antiviral interference mechanisms, processing bodies (p-bodies, also known as stress granules), were first discovered in *Arabidopsis*. In plants, mRNAs that have not been translated or spliced can localize together to form granules under stressful conditions (such as high temperatures). While inactivating most cellular functions, these clusters protect important genetic material from denaturation in a stressful environment. While in all phyla p-bodies accumulate functionally inactive nucleic acids, they have evolved a distinct antiviral purpose in vertebrates

(importantly, in mammals and arthropods due to their role as arboviral carriers) [19].

In humans, AGO1 ships siRNAs to locations where they can accumulate in the cell and form p-bodies. Stress granules are more structurally organized than RISCs; two proteins, TIA-1 and TIAR, are found at the core and contain specific epitopes to bind siRNAs containing relevant nucleic acids. As with heterochromatin, the tight packing prevents transcription of certain genes while preserving their sequence information [19].

P-bodies also hold potential as a recognizable marker of arboviral infection. First, as they appear when an organism is under stress, their presence is generally indicative of a infected cell. Furthermore, they localize infectious molecules into a single cluster; infection-specific proteins and nucleotides tend to be bind TIA-1 and TIAR epitopes on the outside, allowing for easy detection and classification of the infection [20].

Silva et al. detected the presence of an “RNA pseudoknot”, (closely resembling a stress granule in form and function) *in vitro* upon 1) simulating stress conditions and 2) exposing the cell to yellow fever flaviviral RNA [20]. Although much research remains to be done in this area [19], studies in *Drosophila* suggest that they are active in arthropods and their conserved nature suggests that a detectable marker is plausible.

Discussion

Disease is often detected by analyzing the immune response of the host. RNA interference and its complex pathways allow a cell to inactivate foreign DNA or RNA before it poses a significant threat. Of practical importance is that they allow researchers to understand how organisms resist, tolerate, or otherwise respond to infection. It is hypothesized that highly conserved elements such as piRNA, p-bodies, and stress granules not only act in the *Aedes* mosquito and model organisms, but also in novel ways in other arboviral carriers and hosts. Therefore, studying the innate immunity of arthropods provides a pathway by which researchers can test for arboviral infection in any individual. It is plausible that humans inherited or independently evolved many similar mechanisms to arthropods in order to tolerate infection. And while innate immune pathways are often generalized, the polynucleotides targeted by the response remain infection-specific. Hybridization and other methods for detecting intracellular nucleic acids allow researchers to recognize and classify infection more effectively (even to the serotype). What is known about RNA interference can lead to cheaper and more effective options and approaches to testing and treating arbovirus infection in mammals.

RNA mechanisms are only somewhat effective in defending *Drosophila*, an arthropod which does not serve as an arboviral vector from molecular parasites, while very effective in defending *Aedes* from the same foreign polynucleotides. While only a portion of the surveyed research [11,12,15] studied these pathways in mosquitoes, the conservation of these mechanisms across species indicate that many of the same molecules and mechanisms can be studied to understand the human immune reaction to arboviruses. Researchers should be aware that RNA interference provides them with the tools to interfere and manipulate how arboviruses interacts with cellular mechanisms. The classical pathways of the RISC, as well as the complex piwi ping-pong dependent pathways, are dependent on a number of enzymes which either can be artificially targeted through mechanisms that have proved successful in inhibiting other pathways which employ the same

enzymes or in other organisms which naturally inhibit these pathways to perform a necessary response in the organism.

Much work remains to be done in this field to study and characterize the proteins in these pathways and open new possibilities for targeted assays and treatments. It is possible that RNA mechanisms become an integral part of the story of how researchers attempt to study, treat, and eradicate mosquito-borne disease on a global scale.

While the amount of detail involved in these mechanisms may seem like a disadvantage, the complexity is in fact an advantage and gives us numerous angles from which we might attempt to approach the problem. A complex signaling pathway is reliant on each component; inhibiting one from performing its function is enough to cause the entire network to fail. Of course, the mechanisms of evolution are powerful, and just as these pathways evolved to help the organism defend itself against parasites, human input may cause arboviruses to evolve further and put preventing their casualties out of our reach. But if we want to lower the half a million lives that mosquito-borne illness take each year (mostly in developing countries) [2], all possibilities are worth careful consideration. Testing for arboviral infection in both humans and mosquitoes today is an arduous process which requires laboratory sterile conditions to guarantee results. In order to diagnose Zika, Dengue, and Chikungunya, cerebrospinal fluid is preferable to any other substances, although blood and urine also are acceptable. An RT-PCR method is currently used to diagnose such viruses, and it takes considerable time to perform [21]. The results also must be tested against a control sample which could be potentially dangerous to relocate if attempting to perform the assay in tropical environments in which these viruses are endemic.

Conclusion

Many suggestions for eradicating arboviruses pose direct threats to humans, and a number have been recently enacted by some cities and jurisdictions. Mosquito control methods often involve introducing mosquitoes which confer a lethal or sterile gene into the population. This method is useful when there is a single target species in a particular region, but can be more limiting when there are multiple vector species or isolated populations. Paratransgenesis, a term which refers to the debilitation of a host parasite by first preventing the spread of its symbiont (mosquitoes), has been invoked by an up and coming method that has proved successful. Genetically modified bacteria (or in rare cases, fungi) have the advantage of spreading between multiple species of mosquitoes more easily; they produce molecules which could not only debilitate and sterilize the mosquitoes, but also could interfere with the arbovirus that it carries [22].

Such methods have been the subject of much ethical dispute, and may ultimately prove fruitless because arboviruses can evolve to find other suitable hosts, by making use of less endogenous mechanisms or providing its own anti-viral machinery to vectors where necessary [10]. However, they are still useful to explore, as they may lead us to new ways through which we can interact with the molecular machinery of the virus and the vector. Understanding more about the innate

immunity of mosquitoes to the viruses that they carry would prove useful in overcoming the many challenges facing the scientific community in confronting epidemics of mosquito-borne pathogens across the globe.

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