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Mosquito immune responses to arbovirus infections

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The principal mosquito innate immune response to virus infections, RNA interference (RNAi), differs substantially from the immune response to bacterial and fungal infections. The exo-siRNA pathway constitutes the major anti-arboviral RNAi response and its essential genetic components have been identified. Recent research has also implicated the Piwi-interacting RNA pathway in mosquito anti-arboviral immunity, but Piwi gene-family components involved are not well-defined. Arboviruses must evade or suppress RNAi without causing pathogenesis in the vector to maintain their transmission cycle, but little is known about mechanisms of arbovirus modulation of RNAi. Genetic manipulation of mosquitoes to enhance their RNAi response can limit arbovirus infection and replication and could be used in novel strategies for interruption of arbovirus transmission and greatly reduce disease.

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Introduction

Insects, as do all metazoans, mount an innate immune response upon exposure to infectious agents. Innate immunity is the cellular-level first line of defense against infection and is initiated by detection of a pathogen-associated molecular pattern (PAMP) by a host pattern-recognition receptor (PRR). Insect genomes do not encode elements of protein-based adaptive immune responses, and thus must rely totally on innate immunity for protection. The fruit fly *Drosophila melanogaster* is a model organism for the study of anti-arboviral defense in mosquitoes; both are members of the order Diptera and can be infected with arboviruses, although Drosophila are not arbovirus vectors. In addition, experimental infections of Drosophila by intrathoracic injection with high viral doses frequently result in pathogenesis. Arbovirus infections of mosquitoes naturally occur by infectious blood-meal ingestion and are generally non-pathogenic and persistent, possibly due to the balance that has evolved between the mosquito innate anti-viral response to control pathogenesis and the arboviral evasion without complete suppression of this response. Availability of the Drosophila genome sequence [1] and use of facile genetic techniques have provided a framework for genetic comparisons with *Anopheles gambiae* [2,3], *Aedes aegypti* [4,5] and *Culex quinquefasciatus* [6,7••] as these mosquito genome sequences have been published.

Drosophila innate immunity

In the canonical Drosophila innate immune response following bacterial or fungal injection, transcriptional signaling cascades induced by detection of PAMPs by host PRRs result in activation of transcription factors from the NF-κB family (e.g., Dif, Relish and dorsal) and, ultimately, release of antimicrobial peptides (AMPs) into the insects' hemolymph. Two major signaling pathways with well-defined microbial PAMPs and insect PRRs are Toll, which responds to fungal and Gram-positive bacterial infections and Imd (immune deficiency), which is triggered by Gram-negative bacterial infections [8]. Several studies of the Drosophila transcriptional response to viral infection have implicated elements of Toll and Imd pathways in antiviral immunity, depending upon the virus used [9–13]. Viral PAMPs and identities and functions of host effectors for these pathways have not been characterized for the most part. Injection with Drosophila C virus (DCV) induces the JAK/STAT pathway, which also can be activated by septic injury [14], resulting in transcription of several genes with STAT-binding elements in their promoters [15]. One such STAT-regulated, DCV-responsive gene encodes a small protein called Vago that was shown to control DCV load in the fat body after infection. Intriguingly, induction of *vago* transcription was dependent on the DExD/H-box domain of Dicer 2, the initiator of the anti-viral RNAi response [16•].

Recently, Kemp *et al.* [17••] explored the role of the JAK/STAT pathway in Drosophila innate immunity to a diverse set of viruses. Each virus infection resulted in a unique pattern of gene induction; however, flies with a mutation in *hopscotch*, their sole Janus kinase (JAK) gene, were susceptible to significantly increased mortality only after infection with members of the *Dicistroviridae* insect virus family, and not with the arbovirus Sindbis (SINV, *Alphavirus*) or any of the other four viruses tested. In contrast, Drosophila with a mutation in the *dcr2* gene,

encoding the PRR for the exo-siRNA pathway of the RNAi response, exhibited increased mortality after infection with insect viruses of all families, including a DNA-containing iridovirus. Their study confirmed previous findings that RNAi, which is the Drosophila innate immune response unique to viral infections, is the most effective and wide-ranging antiviral response in insects [16•,18].

RNAi in mosquitoes

RNAi is an antiviral defense mechanism in invertebrates and plants

Silencing of gene expression by introduction of long double-stranded (ds)RNA with cognate sequence to the silenced gene was described in both the nematode *Caenorhabditis elegans* [19] and Drosophila [20] in 1998. It was soon recognized that a similar gene silencing phenomenon had been previously observed in plants, and ultimately that RNA-mediated gene silencing was an antiviral defense mechanism in both plants and invertebrates [21,22,23•]. Modeling plant virus studies, we described inhibition of arbovirus replication in mosquitoes and mosquito cells resulting from expression of both virus genome-derived positive-sense and negative-sense RNA from alphavirus transduction vectors (presumably expressed as dsRNA during alphavirus replication) and from expression of virus genome-derived inverted repeat RNA from a plasmid, which formed dsRNA in the mosquito cell nucleus [24,25•, 26–28]. Following the description of dsRNA-mediated gene silencing (RNAi) in *C. elegans* and *D. melanogaster*, we proceeded to characterize the mechanism and machinery of mosquito RNAi in *An. gambiae* [29•] and *Ae. aegypti* [30,31••].

Components and mechanisms of mosquito antiviral RNAi

RNAi in mosquitoes is now known to comprise three major pathways, named for the effector RNAs that are their end products: small interfering (si)RNA, micro (mi)RNA, and Piwi-interacting (pi)RNA pathways. Each has a distinct role in either antiviral defense, regulation of development and gene expression, or defense of the genome against transposon mobilization and expression, although in Drosophila some interconnections have been noted [32]. The mosquito genes that encode major participants in each pathway have been identified by homology to Drosophila genes [33••]. The exogenous (exo)-siRNA pathway represents the major antiviral innate immune response in mosquitoes [34] and this antiviral response will be the focus of this review. The potential role of piRNAs in antiviral defense is less clear and will be discussed in section 'The piRNA pathway'.

The exo-siRNA pathway

The exo-siRNA response in arbovirus-infected mosquitoes can be triggered by dsRNA >150 bp in length [35] (Figure 1). In virus-infected cells, the source of dsRNA is thought to be genome replication intermediates, intra-strand RNA secondary structures [30,36•,37••,38•], or convergent transcription of DNA virus genomes [17••]. Other virus-specific RNA structures might also serve as PAMPs, since it has been shown that viruses with negative-sense RNA genomes generate little dsRNA during replication [39], yet infection of insect cells with vesicular stomatitis virus (VSV, *Vesiculovirus*), with a non-segmented negative-sense genome, and La Crosse and Rift Valley fever bunyaviruses, with three negative sense and/or ambisense genome segments, generates typical virus-specific small RNAs [40–43].

Figure 1

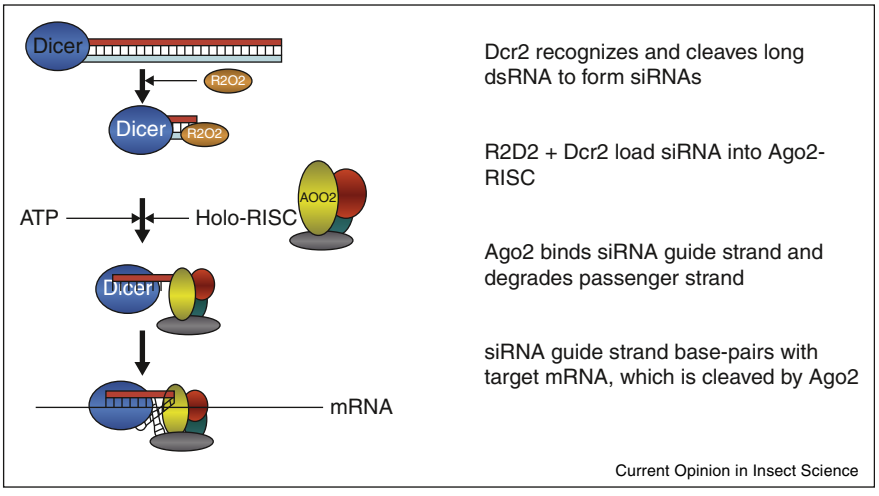

Diagram showing the essential components of the mosquito exo-siRNA pathway that is the major anti-viral immune response: long virus-specific dsRNA, which serves as the PAMP; Dicer 2 (Dicer), which serves as the PRR; R2D2, the dsRNA-binding protein; Argonaute 2 (Ago2), which acts as effector in cleaving target viral mRNA.