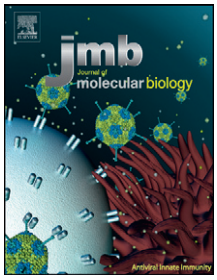

Insect Antiviral Innate Immunity: Pathways, Effectors, and Connections

Megan B. Kingsolver[†], **Zhijing Huang**[†] **and Richard W. Hardy**

Department of Biology, *Indiana University, Bloomington, IN 47405, USA*

Correspondence to Richard W. Hardy: *212 South Hawthorne Drive, Bloomington, IN 47405, USA.*
rwhardy@indiana.edu
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Abstract

Insects are infected by a wide array of viruses some of which are insect restricted and pathogenic, and some of which are transmitted by biting insects to vertebrates. The medical and economic importance of these viruses heightens the need to understand the interaction between the infecting pathogen and the insect immune system in order to develop transmission interventions. The interaction of the virus with the insect host innate immune system plays a critical role in the outcome of infection. The major mechanism of antiviral defense is the small, interfering RNA pathway that responds through the detection of virus-derived double-stranded RNA to suppress virus replication. However, other innate antimicrobial pathways such as Imd, Toll, and Jak-STAT and the autophagy pathway have also been shown to play important roles in antiviral immunity. In this review, we provide an overview of the current understanding of the main insect antiviral pathways and examine recent findings that further our understanding of the roles of these pathways in facilitating a systemic and specific response to infecting viruses.

Introduction

Viruses are obligate intracellular pathogens with a limited coding capacity mandating the sequestration of cellular resources to promote their replication. Viruses that infect insects have huge consequences economically and medically. Insect transmitted arboviruses, such as the dengue viruses (DENV) and the yellow fever virus (YFV), place billions of people across the globe at risk of life-threatening diseases. Obtaining a deeper understanding of the biology of the virus within the insect host and the host response to the virus provides the potential for the development of novel transmission interventions.

In insects, the innate response plays the major role in the control and clearance of pathogens following infection, although there is some evidence for an immune response that resembles the vertebrate adaptive response [1,2]. The innate immune system is characterized by the activation of pattern recognition receptors (PRRs) capable of binding pathogen-associated molecular patterns (PAMPs), molecules present in the pathogen but not found in the host. Binding of PAMPs leads to the activation of signaling pathways resulting in the production of effector molecules capable of suppressing pathogen replication. This system provides the first line of defense against invading pathogens. In insects, the innate response is robust and may function to clear infection in the case of true insect pathogens; however, in the case of arbovirus infection, the insect innate response limits pathogenesis but does not clear the infection allowing transmission of the virus to a vertebrate host. In fact, it can be seen that arbovirus infection of insects in which the innate immune system has been compromised can result in increased viral load, morbidity, or mortality, suggesting that the innate immune system is engaged and necessary for vector survival [3–9].

When challenged with viruses, the most robust insect response is through the RNA interference (RNAi) pathway that utilizes virus-generated double-stranded RNA (dsRNA) to produce small, interfering RNAs (siRNAs) that function to target viral RNA for degradation and hence inhibit replication [9–12]. Additionally, signal transduction pathways resulting in

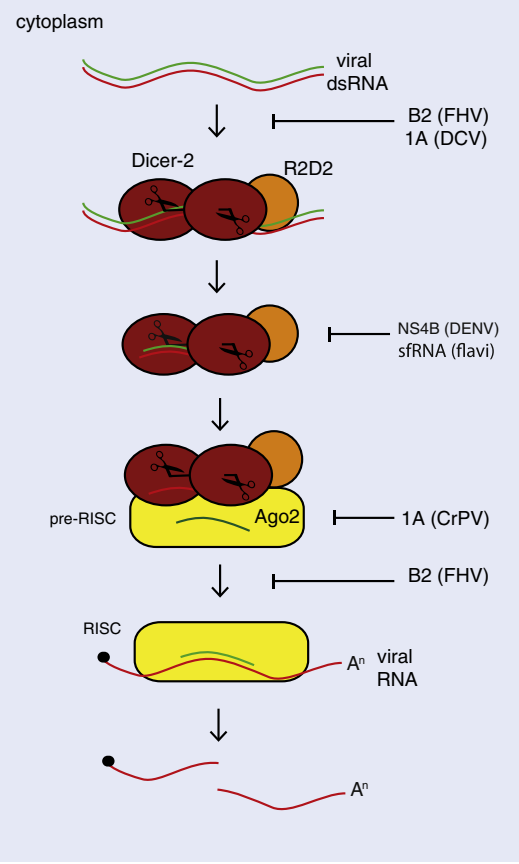

Fig. 1. siRNA pathway. Cytoplasmic, virus-derived dsRNA is recognized by Dicer-2 in complex with R2D2. Dicer-2 cleaves the dsRNA into 21-nt siRNAs. The siRNA is loaded into the pre-RISC complex where duplex unwinding and selection of a guide strand occurs. The guide strand functions to direct RISC to the viral RNA target through base pairing. The Ago-2 protein in the RISC cleaves the viral RNA target inhibiting virus replication. Points in the pathway at which viral suppressors of RNAi function are shown.

changes in cellular gene expression are an important component of the antiviral innate immune system. Nf-κB pathways, Toll and Imd, have been well characterized as essential in the immune response to bacteria and fungi (reviewed by Lemaitre and Hoffmann [13]). Evidence from studies in *Drosophila* and mosquitoes indicates that these pathways also play a role in antiviral defenses [14–17]. There is also growing evidence that the Jak-STAT pathway may be functionally analogous to the mammalian interferon system [18]. This pathway is typically activated in uninfected bystander cells resulting in alterations in cellular transcription and downstream antiviral activity [4].

In vertebrates, the innate immune system signals for an immediate response to infection that potentiates a systemic and specific adaptive response resulting in immune memory. While insects lack an orthologous adaptive response, it is becoming apparent that innate immune pathways are connected and give rise to a systemic antiviral immune response that is specific and has the potential to last beyond the duration of a given viral infection. In this review, we will provide an overview of the current understanding of these antiviral pathways and examine evidence for the connectivity of pathways and a systemic, specific antiviral response.

RNAi Antiviral Response

NAi in insects plays a significant role in limiting and controlling virus infection [9–12]. There are currently three well-characterized RNAi-related pathways (reviewed by Kim *et al.* [19]): (i) siRNA pathway in which siRNAs are generated from dsRNA either derived from exogenous sources such as virus infection or encoded by the cell genome, (ii) micro-RNA (miRNA) pathway in which miRNAs are generated from cell-encoded transcripts and ultimately function to regulate gene expression generally at the level of translation, and (iii) PIWI-interacting RNA (piRNA) pathway in which piRNAs are transcribed from the cellular genome and do not require processing to function in the epigenetic control of genomic elements in the germ line. The pathway predominantly responsible for antiviral activity in insects is the siRNA pathway, and this will be the main focus of the following section.

siRNA pathway components

Virus-derived siRNAs are generated through the recognition and processing of dsRNAs produced during virus infection (reviewed by Ding [20]). The dsRNA can occur as a replication intermediate for single-stranded RNA viruses, due to secondary structure in viral RNAs, or as a consequence of base pairing of convergent transcripts. Recognition of the dsRNA by Dicer proteins (members of the RNase III family of endoribonucleases) results in the production of siRNA (Fig. 1). These are loaded into the pre-RNA-induced silencing complex (RISC). Unwinding of the duplex occurs along with guide strand selection that defines target specificity on the basis of complementarity. Targeted RNA is degraded through the RNase activity of Argonaute [21].

Most of the characterization of the virus-derived siRNA pathway in insects has been performed in *Drosophila*. Flies possess two Dicer proteins; Dicer-1 is required for the production of miRNA, whereas Dicer-2 is necessary for processing dsRNA in order to generate siRNA [22,23]. Dicer-2 functions as the activating PRR for the siRNA pathway binding to dsRNA and cleaving it to small 21-nt siRNAs with 2-nt 3′ overhangs. For exogenous dsRNA (such as virus-derived dsRNA), processing by Dicer-2 requires