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Molecular Insights into MicroRNA-Mediated Control of Insect Host Defense Pathways

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Abstract: -

Insects are among the most successful animal groups on Earth, due in part to their highly efficient immune defenses against microbial attack. Their immune system relies on both cellular and humoral responses, along with RNA interference (RNAi). Small non-coding RNAs (snRNAs) generated through RNAi play crucial roles in regulating gene expression across nearly all living organisms, influencing key processes such as development, differentiation, immunity, and interactions between hosts and microbes. The major snRNAs involved in RNAi include small interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs). Besides host-derived snRNAs, some microorganisms also produce snRNAs that can modulate the dynamics of host–pathogen interactions. This review summarizes recent advances in understanding the role of microRNAs in insect pathogen relationships and highlights current perspectives in this rapidly expanding field.

Introduction:

Throughout evolution, hosts and pathogens have continually adapted strategies of attack and counter defense, shaping a long-running biological arms race. Animals typically protect themselves from microbial threats through two main immune strategies: innate and acquired immunity. Innate immunity relies on germline-encoded components that recognize and eliminate pathogens, whereas acquired immunity involves the production of antigen-specific effectors and the development of immunological memory.

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In insects, immunity is entirely innate and can be grouped into three major components:

(1) pathogen immobilization,

(2) core immune signaling pathways (Toll, Imd, and JAK–STAT), and

(3) the RNA interference (RNAi) pathway.

Pathogen immobilization represents the cellular response to bacterial and fungal infections and includes processes such as phagocytosis, engulfment, melanization, coagulation, and autophagy. These mechanisms help restrict invading microbes, which are subsequently eliminated either extracellularly through antimicrobial peptides (AMPs) or intracellularly. During systemic infection, insects synthesize and release AMPs into the hemolymph, where they directly kill pathogens. Once microbes invade, immune signaling pathways become activated. In insects, the principal pathways are Toll, Imd, and JAK/STAT. The Toll pathway is triggered by the endogenous cytokine Spätzle (Spz), which undergoes proteolytic activation. Spz binds the Toll receptor, inducing conformational changes that initiate downstream signaling. This interaction integrates signals from three upstream recognition pathways: those responding to microbial proteases, fungal cell wall components, and lysine-type peptidoglycan from Gram-positive bacteria. After receptor activation, MyD88 binds the Toll receptor, recruiting the kinase Pelle, which phosphorylates and degrades the inhibitor Cactus. The release of NF-κB transcription factors Dorsal or Dif allows them to enter the nucleus and regulate numerous immune-related genes. The Imd pathway detects meso-diaminopimelic acid (DAP) type peptidoglycan characteristic of Gram-negative bacteria and certain Gram-positive Bacilli. Recognition occurs via the membrane-bound receptor PGRP-LC and the intracellular receptor PGRP-LE. Flies lacking both receptors fail to induce AMPs against Gram-negative bacteria and become highly vulnerable to infection. The JAK/STAT pathway also contributes to insect immunity, particularly in antiviral defense and hemocyte development. Although its mode of activation during viral infection remains unclear, the pathway involves Unpaired (Upd1–3) ligands, the Domeless receptor, the JAK kinase, and the transcription factor STAT. Once activated, STAT becomes phosphorylated and moves into the nucleus to initiate transcription of immune-responsive genes.

miRNA biogenesis at a glance

Since the first microRNA (miRNA) was identified in *Caenorhabditis elegans* a little over twenty years ago, knowledge about how miRNAs are produced and how they

interact with their targets has expanded dramatically and continues to advance. Besides the well-known or canonical miRNA biogenesis pathway, several alternative non-canonical routes have also been documented. In the canonical pathway, miRNA genes are transcribed in the nucleus by RNA polymerase II, generating a primary miRNA (pri-miRNA) transcript. The length of this pri-miRNA can vary, but it contains one or more characteristic stem loop structures. A nuclear RNase III enzyme, Drosha, together with its partner Pasha (in insects), processes this pri-miRNA to release a ~70-nucleotide precursor miRNA (pre-miRNA). Exportin-5 then transports this hairpin-shaped pre-miRNA into the cytoplasm. Once in the cytoplasm, the pre-miRNA is further cleaved by the RNase Dicer to create a short RNA duplex. In insects, two Dicer enzymes are present: Dicer-1, which primarily produces miRNAs, and Dicer-2, which generates siRNAs. The resulting miRNA duplex initiates assembly of the RNA-induced silencing complex (RISC), with Argonaute (Ago) proteins as core components. Typically, miRNAs associate with Ago1-containing RISC, whereas siRNAs load into Ago2 complexes. However, studies show that miRNAs can also be incorporated into Ago2, particularly in older insects.

During RISC loading, one strand of the duplex (the miRNA or passenger strand) is usually degraded, while the guide strand forms the mature miRNA–RISC complex. In some cases, the miRNA strand is also retained and loaded into Ago2, where it may exert regulatory functions. The mature miRNA directs the complex to complementary target mRNAs, resulting in post-transcriptional regulation of gene expression. In certain situations, the miRNA–RISC complex may re-enter the nucleus and bind promoter regions to influence gene expression at the transcriptional level. Importantly, a single miRNA can regulate multiple mRNA targets, and individual mRNAs can be controlled by several different miRNAs.

MicroRNAs' function as host-pathogen interaction modifiers

Although miRNAs are well studied in the context of insect development, their involvement in host–pathogen interactions is still not fully understood. However, growing research over recent years indicates that miRNAs influence host–microbe relationships and even enable communication across kingdoms. For instance, both host-derived and viral miRNAs have been shown to either promote or inhibit viral replication. A recurring observation across different host–pathogen systems is that infection alters the expression levels of host miRNAs, and these shifts vary depending on the infection stage and the type of pathogen. At the same time,

many host genes are either upregulated or downregulated, potentially under the control of miRNAs or other small RNAs. Such changes in miRNA and mRNA expression may represent part of the host's defense strategy or may result from pathogen-driven manipulation, including that mediated by virus-encoded miRNAs. The following sections outline key examples highlighting how miRNAs contribute to regulating host–pathogen interactions.

miRNAs' function in controlling insect immunity

Although numerous studies have clarified the role of miRNAs in vertebrate immunity, their involvement in insect immune responses is still poorly understood, with most findings relying on computational predictions or correlations between miRNA and mRNA expression. For instance, Fullaondo et al. used bioinformatics tools to identify more than 70 miRNAs that might regulate key immune pathways such as Toll, Imd, JAK/STAT, melanization and JNK. Likewise, miRNAs in *Anopheles gambiae* including aga-miR-2304 and aga-miR-2390 were predicted to target genes like suppressin and prophenoloxidase, but these predictions still require experimental validation. Experimental studies have shown changes in miRNA expression following microbial exposure or stress. In *Tribolium castaneum*, immune stimulation with peptidoglycan altered miRNA profiles, with 59 miRNAs showing differential expression and 21 being upregulated. *Manduca sexta* also exhibited shifts in miRNA abundance after immune challenge, affecting processes such as pattern recognition, prophenoloxidase activation, AMP production, and conserved immune signalling pathways. In *A. gambiae*, depletion of Dicer-1 or Ago1 increased susceptibility to *Plasmodium berghei*, implying a possible role for miRNAs in anti-Plasmodium defense, although specific miRNAs were not identified.

One of the few miRNAs whose immune role is experimentally confirmed is miR-8. In *Drosophila melanogaster*, miR-8 suppresses antimicrobial peptide (AMP) genes such as *Drosomycin* and *Diptericin*, helping maintain low basal AMP levels under non-infected conditions. In miR-8 mutants, AMP transcripts increase markedly. Predicted targets include GNBP3, an activator of the Toll pathway during fungal infection, and Pvf, a regulator of the JNK pathway. Similar negative regulation of AMPs by miR-8 was shown in *Plutella xylostella* larvae, where miR-8 boosts serpin-27 levels, inhibiting Toll activation. Infection reduces miR-8 abundance, lowering serpin-27 levels and enabling Toll pathway activation. In *Aedes albopictus* (C6/36) cells, levels of the mosquito-specific miRNA aae-miR-2940 drop

after infection with the flavivirus WNVKUN. Since this miRNA enhances expression of the metalloprotease *ftsh*, which facilitates WNVKUN replication, reduced miR-2940 leads to lowered *ftsh* levels and decreased viral titers suggesting a host antiviral mechanism. A similar decline in miR-2940 was observed in A. albopictus cells infected with Chikungunya virus, indicating that suppression of this miRNA may be a common response to viral infection.

In *Aedes aegypti*, two immune-related genes *cactus* and *REL1* were identified as targets of the blood-meal–induced miRNA aae-miR-375. Interestingly, aae-miR-375 enhances the expression of *cactus*, a suppressor of the Toll pathway, while simultaneously reducing the levels of *REL1*, a transcription factor that drives AMP production. This combined increase in *cactus* and decrease in *REL1* was shown to facilitate DENV replication because AMPs negatively impact the virus. Thus, the suppression of AMP activity after blood feeding may inadvertently benefit viral fitness. Moreover, immune-related genes are known to evolve more rapidly than non-immune genes due to constant selective pressure from pathogens. However, an analysis of 50 genes from the Drosophila Toll and Imd pathways revealed a strong negative correlation between a gene's evolutionary rate and the number of miRNAs regulating it. This indicates that immune genes under extensive miRNA regulation tend to evolve more slowly, likely due to tighter functional constraints highlighting a potential role for miRNAs in shaping the evolutionary trajectory of insect immune genes.

Conclusions

Over the past ten years, significant progress has been made in understanding how miRNAs are produced and how they function within different aspects of insect biology. Much of this work has emphasized developmental processes, particularly in *Drosophila melanogaster*. More recently, however, attention has shifted toward the involvement of miRNAs in insect–pathogen interactions. As a starting point, most studies have examined how infections alter host miRNA expression profiles, leading to the identification of many miRNAs whose abundance changes upon pathogen challenge. The next important step will be to validate the functional roles of these infection-responsive miRNAs through experimental studies, clarifying their contributions to immunity and host–microbe interactions. In parallel, several virus-derived miRNAs have been discovered, some of which directly influence viral replication or modulate host genes and miRNAs to enhance viral success. Looking forward, applying miRNA research beyond basic biology offers exciting opportunities

such as reducing crop losses caused by insect pests, protecting beneficial insects, and limiting the spread of vector-borne diseases. However, practical applications may face regulatory hurdles, especially when genetic modification of insects or plants is required. Despite these challenges, early laboratory and cell-based studies have produced promising results. Given the rapid pace of advancement in miRNA research, further significant developments in this field are expected in the near future.

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