Advances in the Immune Regulatory Role of Non-Coding RNAs (miRNAs and lncRNAs) in Insect-Pathogen Interactions

Ulrich Aymard Ekomi Moure1,2†, Tingshan Tan1†, Lin Sha1, Xiaoqin Lu1, Zhi Shao1, Guang Yang1*, Yi Wang1,3* and Hongjuan Cui2,4

1 Affiliated Hospital of Southwest University, the Ninth People’s Hospital of Chongqing, Chongqing, China, 2 Medical Research Institute, Southwest University, Chongqing, China, 3 Department of Gastrointestinal Surgery, the Ninth People’s Hospital of Chongqing, Chongqing, China, 4 State Key Laboratory of Silkworm Genome Biology, Key Laboratory of Sericultural Biology and Genetic Breeding, Ministry of Agriculture, Southwest University, Chongqing, China

Insects are by far the most abundant and diverse living organisms on earth and are frequently prone to microbial attacks. In order to counteract and overcome microbial invasions, insects have in an evolutionary way conserved and developed immune defense mechanisms such as Toll, immune deficiency (Imd), and JAK/STAT signaling pathways leading to the expression of antimicrobial peptides. These pathways have accessory immune effector mechanisms, such as phagocytosis, encapsulation, melanization, nodulation, RNA interference (RNAi), lysis, autophagy, and apoptosis. However, pathogens evolved strategies that circumvent host immune response following infections, which may have helped insects further sophisticate their immune response mechanisms. The involvement of ncRNAs in insect immunity is undeniable, and several excellent studies or reviews have investigated and described their roles in various insects. However, the functional analyses of ncRNAs in insects upon pathogen attacks are not exhaustive as novel ncRNAs are being increasingly discovered in those organisms. This article gives an overview of the main insect signaling pathways and effector mechanisms activated by pathogen invaders and summarizes the latest findings of the immune modulation role of both insect- and pathogen-encoded ncRNAs, especially miRNAs and lncRNAs during insect–pathogen crosstalk.

Keywords: insect immune pathways, insect–pathogen interaction, miRNAs and lncRNAs, mRNA targets, immune modulation

1 INTRODUCTION

Insects are often subjected to pathogen (bacteria, fungi, viruses, etc.) attacks, and their survival indicates advanced defense mechanisms (1). These organisms of interest endure physical barriers preventing intruders from entering their body cavity (hemocoeel) (2, 3). These intruders generally reach insect body parts through the degradation of the cuticle by enzymatic digestion or by ingestion (midgut) (4), which are followed by the induction of the host’s immune defense mechanisms via the
binding of their pathogen-associated molecular patterns (PAMPs) (5). Activation and coordination of insects’ innate immune defenses rely on evolutionary and highly conserved immune factors. This immunity is classified into humoral and cellular reactions (6). Humoral responses include the production of antimicrobial peptides (AMPs) (7). Studies showed that the Toll, immune deficiency (Imd), and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways are the most characterized in insects, and their activation results in the production of effector molecules such as AMPs with the potential to kill insect invaders (8–12) (Section 2; Figure 1). However, it was reported that these signaling pathways could be encountered by pathogens and lead to their replication and proliferation (13–15). Cellular reactions, meanwhile, rely on insect hemocytes (primary immune cell defenses) involved in phagocytosis, nodulation, encapsulation, autophagy, apoptosis, and so on (4, 16, 17). Given all this arsenal of immune defense described above, insects lack an adaptive immune response, unlike mammals, and rely only on innate immunity (18, 19). We may speculate that insect immune defense against pathogens lacks a memory immunity that allows rapid and robust responses to neutralize and kill pathogens quickly. Nonetheless, we are far from reaching the exhaustive portrayal of how these organisms that represent the Earth’s most abundant and diverse living organisms defend themselves.

Several factors such as insect gut microbiota, nutritional stress, and noncoding RNAs (ncRNAs) play an immune-modulatory role during insect–pathogen crosstalk (20–23). Noncoding RNAs (ncRNAs), also termed nonprotein-coding RNAs are RNA molecules that do not have the potentiality to encode proteins (24). In addition to ribosomal RNA (rRNA) and transfer RNA (tRNA), ncRNAs can be divided into two groups, small ncRNA

![Figure 1](image.png)

**FIGURE 1 |** Schematic illustration of the main signaling pathways (Toll, Imd, and JAK/STAT) in insects upon microbial attacks. See Section 2 for the description of the paths.
Aedes aegypti

miRNA candidates. In addition, by utilizing next-generation
in the abundance of miRNAs in the larvae. It highlighted the
and asymptomatic bacteriuria (ABU) caused signi-
fication pathways that mediate several different biological responses,
including immunity (50).

2.2 The Imd Signaling Pathway
This pathway is well characterized in Dipterans such as Drosophila; however, in Hemipterans like aphids and assassin
bugs, several components of that pathway are lacking. Imd
signaling components appear to be absent in Rhodnius prolixus
(Reedviidae) and Acyrthosiphon pisum (Aphididae) (51–53).
However, some study reported that the Toll and Imd pathways
are present in the hemipteran lineages like stinkbug Plautia stali,
but their functionality is blurred (54). The Imd pathway is
mainly responsive to Gram-negative bacteria and controls the
synthesis of several AMPs (55, 56).

During the infection, insect Imd transmembrane receptor
proteins (PGRPs) recognize pathogen diaminopimelic acid-
peptidoglycan (DAP-PGN). This recognition induces a cascade
reaction resulting in Relish (NF-κB family member) activation
(cytoplasm) and translocation (nucleus) for AMP production
(Diptericin) (38, 57). Like the Toll pathway, the Imd pathway is a
NF-κB-associated signaling pathway (8, 9). Although the Imd
and Toll pathways have independent functions and mediate the
specificity of innate immune responses towards different
microorganisms, some AMP genes can be activated by both
pathways. Interestingly, Tanji et al. (58) showed that a synergic
interaction occurs between the Toll and Imd signaling pathways,
downstream of the latter. Specifically, upon signal stimulation,
Dif, dorsal, and relish factors can independently bind to NF-κB1
or NF-κB2, and relish interacts after that with Dif or dorsal to
synergistically promote transcription. Alternatively, signal
stimulation may enhance the formation of relish/Dif or relish/
dorsal heterodimers and bind preferentially to NF-κB2 to
regulate transcription synergistically with interaction with NF-
κB1 (58).

2 INSECT MAIN SIGNALING PATHWAYS
TRIGGERED BY PATHOGENS

2.1 The Toll Signaling Pathway
This insects’ pathway is responsive to Gram-positive bacteria
and fungi and largely controls the expression of AMPs (28–
30). The Toll pathway is a NF-κB-associated signaling pathway
(8, 9). Stimulation of insects’ Toll pathway by appropriate
pathogens induces proteolytic cascades activated by secreted
recognition molecules, such as peptidoglycan receptor proteins
(PGRP-SA, PGRP-SD, GNPB1) (11, 31–34). These recognition
molecules are highly evolutionarily conserved from insects to
mammals. For instance, Drosophila, mosquito, and mammals
have families of 13, 7, and 4 PGRP genes, respectively (35). In
Drosophila where the molecular components of the Toll
signaling pathway and their functions in immune response
against pathogens are well characterized (36–38), the receptor
membrane Toll is activated and dimerized by the mature
proteolytic product Spätzle (39–42), which subsequently
causes the recruitment of three intracellular death domain-
containing proteins, MyD88, Tube, and Pelle (43–45). The IkB
homolog Cactus is then phosphorylated and degraded by the
proteasome, leading to the release of members of the nuclear
factor NF-κB family (Dif or dorsal) to translocate to the
nucleus (46–48), and activate genes encoding potent
antifungal and antibacterial peptides (AMPs), such as
Drosomycin (Dr) (11, 38, 49). The Toll-dorsal pathway in
Drosophila and the interleukin-1 receptor (IL-1R)-NF-κB
pathway in mammals are homologous signal transduction
pathways that mediate several different biological responses,
including immunity (50).

ncRNAs-Modulated Insect-Pathogen Interactions
2.3 The JAT/STAT Signaling Pathway

The JAK/STAT pathway was originally identified as a cytokine signaling pathway in mammals (59–62). This pathway provides an antiviral protection (27, 28, 63). Unlike the Toll and Imd pathways, little is known about the transcriptional cascade induced by the JAK/STAT pathway (14, 64). Souza-Neto et al. (65) showed that the JAK-STAT pathway is part of the A. aegypti mosquito’s antiviral defense and may act independently of the Toll pathway and the RNAi-mediated antiviral defenses.

The comparative genomic analysis of A. aegypti, Anopheles gambiae, and D. melanogaster genome sequence revealed orthologs for the core JAK/STAT pathway components (dome, Hop, and STAT) (66). Activation of this pathway in Drosophila starts when the virus-induced extracellular cytokine unpaired (Upd) binds to the cellular receptor dome. The latter recruits STAT, which then dimerizes and translocates to the nucleus and activates the transcription of AMP genes (nitric oxide synthase) after binding to the STAT-binding site (12, 65). Protein inhibitor of activated STAT (PIAS) and suppressor of cytokine signaling (SOCS), are two negative regulators of the JAK-STAT pathway in D. melanogaster (67). Orthologs of these two regulators (SUMO and SOCS) have been identified, confirming the proximity and evolutionary conservation of this pathway between mammals and insects (65).

3 INSECT IMMUNE EFFECTOR MECHANISMS

Pathogen neutralization and death are accomplished via insect immune effector mechanisms, known to be interconnected and to work synergistically (Figure 2). The primary immune cells are the hemocytes. Hemocytes are found in circulation (circulating hemocytes) and attached to tissues (sessile hemocytes), where they phagocytose, encapsulate and nodulate pathogens, and produce humoral immune factors. The fat body, the midgut, the salivary glands, and other tissues produce numerous humoral immune factors with, among other things, lytic and melanizing activity (Figure 3).

3.1 Phagocytosis

Small pathogens that become melanized, such as bacteria, are often phagocytosed (68, 69). Phagocytosis is an evolutionarily conserved cellular immune process used by vertebrates and invertebrate animals to neutralize and kill small pathogens. Due to its incredible speed, it takes seconds to hydrolyze foreign bodies following their internalization (69–71), and hundreds of bacteria can be phagocytosed at any time (72). The phagocytes, which can be circulating and sessile hemocytes (73) or granulocytes in the cases of Lepidoptera, Hemiptera, and other orders of insects, are able to recognize and engulf pathogens. The recognition process is mediated by pattern recognition receptors (PRRs) on the surface of the phagocyte, which bind to pathogen-associated molecular patterns (PAMPs) on the surface of the pathogen. Once the pathogen is internalized, the phagocytic vacuole fuses with lysosomes, and the contents of the lysosomes, including lipid, protein, and RNA degrading enzymes, are released to digest the engulfed pathogen. The digestion process is crucial for the elimination of the pathogen, as it prevents the pathogen from spreading to other cells or tissues.

**FIGURE 2 |** Schematic representation of insects’ immune effector mechanisms. (A) Insects use phagocytosis to neutralize and kill small pathogens. This process is mediated by phagocytes (hemocytes or granulocytes). (B) Encapsulation (cellular and melanotic) is a defense mechanism that insects used when pathogens are too large to be phagocytosed. Cellular encapsulation occurs without melanization, whereas melanotic humoral encapsulation is dependent on PO activity and can occur with or without the assistance of hemocytes. (C) Melanization is a process based on the conversion of PPO to PO, which leads to the formation of the melanotic capsule (melanotic enzymes) which mediates the killing of the foreign agent. (D) Nodulation is a process by which immune cells (granulocytes) adhere to each other to create layers that surround many bacteria or fungal spores. The granulocytes release their contents, which trap the bacteria in a floculent material. This step is often followed by melanization. (E) RNAi mechanism is based on the ribonuclease cleavage of viral dsRNA and is specifically used against viruses, and can be mediated by immune circulating cells (hemocytes, macrophages). (F) Lysis is a mechanism through which insects kill pathogens by disrupting their cellular membrane. The process involves the participation of peptides and proteins with antimicrobial activity, including lysozymes. (G) Autophagy provides protection to insects against pathogens through the degradation of cytoplasmic material (bacteria or viruses). (H) Apoptosis is a programmed cell death mediated by caspases. The killing of pathogen occurs through the formation of apoptotic bodies.
mosquitoes, and the plasmatocytes of fruit flies, identify the foreign body. The latter is then internalized into a membrane-delimited phagosome, which then fuses with a lysosome for enzymatic hydrolysis-mediated degradation (68, 69, 73, 74).

Although the intracellular patterns regulating phagocytosis remain poorly understood, phagocytosis initiates by binding of a cell-surface PRR or a humoral PRR on a PAMP. PRRs that have been empirically shown to be involved in phagocytosis include thioester-containing proteins, Nimrod proteins, DSCAM, β-integrins, and PGRPs (75–79). Different PRRs have different specificities. For example, D. melanogaster PGRP-LC mediates the phagocytosis of *Escherichia coli* (*E. coli*), but not *Staphylococcus aureus* (*S. aureus*) (79), and NimC1 mediates the phagocytosis of *S. aureus* and, to a lesser extent, *E. coli* (76).

### 3.2 Encapsulation

Encapsulation is a cellular immune response that insects use in response to pathogens that are too large to be phagocytosed. Two types of encapsulation are described in insects: cellular encapsulation, mainly described in *Lepidoptera*, and melanotic humoral encapsulation, more typical in some dipterans like *Drosophila* (80). Cellular encapsulation can occur without any sign of melanization. In contrast, melanotic encapsulation relies on PO activity and can occur with or without the assistance of hemocytes. In cellular encapsulation, granulocytes and plasmatocytes of *Lepidoptera*, plasmatocytes, and lamellocytes of *Drosophila* are the main hemocyte types involved in encapsulation (81, 82). In *Lepidoptera*, an inner layer of granulocytes and an outer one of plasmatocytes surrounded encapsulated objects (83, 84). Insects such as dipteran and lepidopteran larvae commonly employed this response to infection with the eggs of parasitoid wasps. In *Lepidoptera*, encapsulation starts when granulocytes attach to form a layer of cells, in a process dependent on the binding of integrins to specific sites defined by an Arg-Gly-Asp (RGD) sequence (85). This layer of cells surrounding the pathogen is in turn covered by several layers of plasmatocytes, which are then circled by the adhesion of additional granulocytes. A similar process occurs in *Drosophila*, except that the cells involved are plasmatocytes and lamellocytes (86). Depending on the pathogen and the insect, the capsule may become melanized.

### 3.3 Melanization

Melanization is an enzymatic process used by insects in several mechanisms, including immunity. The process involves coordinating pattern recognition receptors, serine proteases, serine protease inhibitors, and melanin production enzymes. When PRRs (β-1,3 glucan recognition proteins, C-type lectins, and Gram-negative binding proteins) identify PAMPs (87–89), the serine protease cascade is induced and leads to the conversion of the pro-phenoloxidase (PPO) to phenoloxidase (PO) (90, 91), which finally culminates in the formation of melanotic capsules. The killing of the foreign agent is mediated by two factors: the pathogen’s surrounding by the proteinaceous capsule and the oxidative stress damage or starvation (92–94). In addition, melanization also assists in the clearing of dead or
dying pathogens (95, 96). Many of the enzymes and PRRs that drive the melanization response are produced by hemocytes, with oenocytoids (crystal cells) being the primary producers of PPO (68, 97). Melanization is another essential facet of the mosquito immune defense against fungi infection. It plays a crucial role in encapsulating and retarding invasive Bauveria bassiana (B. bassiana) growth and dissemination in mosquitoes (98).

3.4 Nodulation

Although the molecular patterns underlying this immune process are still poorly understood, nodulation relies on eicosanoid-based signaling and the extracellular matrix-like protein, Noduler (99, 100). This process starts with the adherence of granulocytes to each other to create layers that surround many bacteria or fungal spores. The granulocytes release their contents, which trap the bacteria in a flocculent material. Plasmatocytes then aggregate around the surface of the nodule. This step is often followed by melanization (101, 102).

3.5 RNAi

RNA interference (RNAi) is an RNA-based mechanism for gene silencing. RNAi could be used to manage insect pests (103, 104). Nevertheless, one of the natural functions of RNAi is to protect the organism from viral infection (105–108).

The small interfering RNA (siRNA) pathway is the primary RNAi path that participates in the insect response against the virus. The process involves the ribonuclease cleavage of viral double-stranded RNA (dsRNA) by dicer-2 (Dcr2), forming a complex with its cofactor R2D2. This cleavage culminates in the production of viral-derived siRNAs (~21 nucleotides), and these siRNAs are loaded into pre-RNA induced silencing complexes (RISC) that include Argonaute-2 (Ago2). The siRNA in a RISC complex is unwound, one strand is discarded, and the other binds complementary viral RNA, which triggers its destruction by Ago2. The binding of viral RNA by Dcr2 also activates the transcription of Vago, which is a cysteine-rich polypeptide that negatively controls virus replication (109). In mosquitoes, the transcriptional activation of Vago is mediated by an NF-kB transcription factor associated with the Imd pathway (Rel2), and in turn, Vago activates the JAK/STAT pathway (110). The PIWI-associated RNA pathway (piRNA) also acts in the RNAi-mediated antiviral defense of mosquitoes but in a manner independent of dicer (105, 106).

3.6 Lysis

Pathogen killing via lysis refers to death due to the immune-based disruption of the cellular membrane. Some of the earliest studied insect factors that induce pathogen death via lysis are AMPs (~2 and 20 kDa). These peptides and proteins were initially identified for their antimicrobial activity in vitro (111–113). Most AMPs can be grouped into four categories (114–116). The defensin and defensin-like peptides (defensin, drosomycin, hloritcin, sacapin, and others) are rich in cysteines and contain cysteine disulfide bonds that have activity against Gram-positive bacteria. Still, others combat Gram-negative bacteria and fungi. The cecropin and cecropin-like peptides (moricins) are α-helical peptides that have activity against Gram-positive and Gram-negative bacteria and fungi. The attacins and gloverins are glycine-rich peptides and are mainly active against Gram-positive bacteria, whereas the gloverins, have activity against Gram-positive bacteria, Gram-negative bacteria, or fungi. The lebecins (lebecin, drosocin, metchnikowin, and others) are proline-rich peptides and are active against Gram-positive bacteria, Gram-negative bacteria, and some fungi. The production of antimicrobial peptides is governed by immune signaling pathways, as seen above with Toll, Imd, and JAK/STAT pathways.

Lysoyzymes are another family of proteins with lytic activity. Lysoyzymes hydrolyze the β-1,4-glycosidic linkage between N-acetylMuramic acid and N-acetylglucosamine of the peptidoglycan present in bacteria’s cell wall. Lysoyzymes are usually present in low, constitutive levels and are transcriptionally upregulated following infection. Although lysoyzymes are classically active in the lytic antibacterial response (117, 118), some also have antiplasmodium and antifungal activity (119, 120) and can activate the PO-based melanization pathway (121, 122).

3.7 Autophagy

Autophagy is an evolutionarily conserved mechanism involved in the degradation of cytoplasmic material through the lysosomal degradation pathway. It plays crucial roles in cellular homeostasis, adaptive response to nutrient deprivation, energy homeostasis, and survival during starvation (123, 124). In immunity, autophagy provides insect protective immune responses against both viral and bacterial attacks. Evidence showed that the Toll pathway induces autophagy during an antiviral response. It is excluded in the activation of autophagy during an antibacterial response and therefore needs further investigation (125). In Drosophila, the activation of autophagy by vesicular stomatitis virus (VSV) is mediated by Toll-7 recognition of VSV-G, a glycoprotein on the virion surface. This PAMP-PRR interaction leads to autophagy initiation by acting on the (PI3K)-Akt-signaling pathway (126, 127). The connection of a Toll receptor to antiviral autophagy in flies suggests an evolutionarily conserved requirement for PRR in triggering autophagy between insects and mammals. Autophagy may, in some cases, promote virus infection as shown for the Sindbis virus, where the activation of the (PI3K)-Akt-signaling pathway enhances the infection of insect cells (128). Lysteria monocytogenes invades and replicates in hemocytes and is recognized by PGRP-LE (a PRR which senses a DAP-type PGN), eliciting an autophagic response that prevents intracellular growth of the bacterium and promotes host survival (125).

3.8 Apoptosis

Apoptosis is a form of programmed cell death. The caspase, Dronc, and the adaptor protein, Ark, form a complex at the molecular level. Dronc activates effector caspases such as Drice and Dcp1, and these caspases cleave proteins in a manner that eventually leads to programmed cell death (129). In Lepidoptera, apoptosis is involved in the response against baculoviruses (130). In mosquitoes, this process appears to be involved in the
response against West Nile Virus and Sindbis virus (131, 132), and in the fruit fly, apoptosis protects against Drosophila C virus in a manner that is dependent on the phagocytosis of virus-infected apoptotic cells (133).

4 ROLE OF ncRNAs IN INSECT IMMUNITY UPON PATHOGENIC INVASIONS

Using high-throughput sequencing techniques and advanced bioinformatics tools, researchers have done great work in discovering and identifying novel insect ncRNAs and their regulated transcripts, respectively (134, 135). The involvement of ncRNAs in insects’ immunity is evidenced, and changes in expression levels of these insect elements generally occur to be relatively altered upon microbial attacks. Below, we go beyond the altered differential expression of these insect elements and highlight their immune targets and influence on insect immune responses to pathogen invasions. Inversely, we will also consider the regulation of insect immunity by pathogen-encoded ncRNAs. Tables 1 and 2 enclose both conserved and novel insect- and pathogen-derived miRNAs and lncRNAs and their targets in insect-pathogen crosstalk.

4.1 Role of miRNAs in Insect Immunity During Microbial Invasions

miRNAs play a crucial role in host–pathogen interactions. The dynamic miRNA–mRNA is essential for immune response to pathogen attacks, including regulating critical insect signaling pathways to promote or inhibit innate immune responses and maintain homeostasis. For instance, differentially expressed miRNAs and mRNAs represented extensively dynamic changes during Drosophila with Micrococcus luteus (M. luteus) infection and enriched diverse signaling pathways, including Toll and Imd as other signaling pathways (139). Figure 4 summarizes the immune regulatory role of insect miRNAs when invaded by pathogens.

4.1.1 Upon Fungal Invasion

The expression levels of miRNAs in the insect–pathogen crosstalk used to be altered. Detection and characterization of miRNA cellular targets would therefore provide more insights to understand the immune modulatory role of these miRNAs. Insect immune mechanisms may deal directly with the pathogens by eliminating them from the host organism or disarm them by abolishing the synthesis of toxins and virulence factors that promote the invasion and destructive action of the invader within the host. Insect-encoded miRNAs seem to preferentially deal with fungal pathogens by disarming them through the suppression of invaders’ virulence factors. This silencing interferes with some translation factors associated with 5’-cap to 3’-tail structures of mRNAs (140). Recently, a study revealed mosquitoes increase the expression levels of both let-7 and miR-100 miRNAs when the fungus B. bassiana penetrates inside their hemocoel. Both miRNAs translocate into the fungal hyphae to silence the virulence-related genes sec2p and C6TF, encoding a Rab guanine nucleotide exchange factor and Zn(II) +Cys4 transcription factors, respectively (23). Suggestions state that extracellular vesicles (EVs) may transport those insect miRNAs to the fungal pathogen. To our knowledge, this is the first report describing the cross-kingdom miRNA transfer from arthropod hosts to their pathogen cells. Globally, the above evidence uncovers an insect defense mechanism whereby...

| Insect     | miRNA          | Target gene             | Expression level | Host target | Ref. |
|------------|----------------|-------------------------|------------------|-------------|------|
| G. mellonella | gme-new-135-3p | TNF-8-like              | –                | G. mellonella | (136) |
|            | gme-miR-274-3p | TNF-8-like              | –                | –           |      |
|            | gme-miR-8-5p  | TNF-8-like              | –                | –           |      |
|            | gme-new-161-3p| Poly(A)-pol             | –                | –           |      |
|            | gme-new-160-5p| zfl-UTAF-like           | Up               | –           |      |
|            | gme-new-135-5p| Invertebrate-type lysozyme | –              | –           |      |
|            | gme-miR-263a-5p| Invertebrate-type lysozyme | –              | –           |      |
|            | gme-new-70-3p | Linear gramicidin synthase subunit D | – | – |      |
|            | gme-new-147-3p| Long-chain-fatty-acid CoA ligase 5 | – | – |      |
|            | gme-new-54-3p | Ras guanine-nucleotide exchange factor | – | – |      |
|            | gme-new-40-3p | phosphatidylinositol 3-kinase | Up | – |      |
|            | gme-new-138-3p| AMP-dependent synthetase/ligase | Up | – |      |
|            | gme-new-72-3p | Atlastin                | –                | –           |      |
|            | gme-new-4-5p  | Long-chain-fatty-acid-CoA ligase 4 | – | – |      |
|            | gme-new-135-3p| Long-chain-fatty-acid-CoA ligase ACSBG2 | – | – |      |
| Drosophila | miR-959       | Tube                    | Down             | Drosophila | (137) |
|            | miR-960       | Tube                    | Down             | –           |      |
|            | miR-961       | Dorsal                  | Down             | –           |      |
|            | miR-962       | Dorsal                  | Down             | –           |      |
|            | let-7         | Toll                    | Down             | B. bassiana | (23) |
|            | miR-100       | sec2p                   | Down             | –           |      |
| A. aegypti | miR-2b        | URM                     | Down             | A. aegypti | (26) |
TABLE 2 | Novel and conserved insect-derived lncRNAs and their immune targets.

| LncRNA    | Target gene | Expression level | Host target | Ref.  |
|-----------|-------------|------------------|-------------|-------|
| L. striatellus | MSTRG15394  | PI               | Up          | L. striatellus | (138) |
|           | MSTRG31066  | PI               | Up          |       |
|           | MSTRG31416  | PI               | Up          |       |
|           | MSTRG3494   | CREB-A           | Up          |       |
|           | MSTRG12659  | CREB-A           | Up          |       |
|           | MSTRG21101  | CREB-A           | Up          |       |
|           | MSTRG32119  | CREB-A           | Up          |       |
|           | MSTRG33257  | CREB-A           | Up          |       |

infection leads to the upregulation of specific miRNAs that are transferred to the invading fungus and suppress fungal virulence genes, in this way, bestowing antifungal protection. This study opens avenues to improve fungal virulence by expression of host miRNA sponges.

4.1.2 Upon Bacterial Invasion

Another crucial role played by miRNAs is to restore insects’ immune homeostasis by negatively regulating immune signaling pathways. This role is achieved by an individual or synergistic action of miRNAs, which repress expression levels of hosts’ immune signaling key components. Di and Toll are critical transcription and transmembrane factors, respectively, whereas the tube is an indispensable effector molecule in the Toll pathway. The synergistic regulatory mechanism of miR-959-962 cluster in the Drosophila immune response to M. luteus infection could negatively regulate the Toll pathway in combination via directly targeting the 3’UTR of the tube, dl, or Toll miRNAs, leading to a reduced survival rate of flies by inhibiting the expression of AMPs at the late stage of the infection (137).

The same study further demonstrated that miR-960 might modulate antibacterial defense only at the late 12 h stage upon infection. At the same time, miR-959 may constantly repress the Dr expression at 6 and 12 h, respectively. Meanwhile, miR-961 may contribute more than miR-962 to repress antibacterial defense. Furthermore, using in silico screening strategy combined with the Gal80T-Gal4 driver system, miR-958 was identified as a candidate miRNA that could potentially regulate the Toll pathway signaling, both in vitro and in vivo, by negatively targeting Dif and Toll. The latter is specifically and significantly inhibited by miR-958 at its site 3, as the Toll 3’UTR harbors four miR-958-binding sites (141). Similarly, Drosophila miR-317 negatively regulated Drosophila Toll signaling response via suppressing only the Dif-Rc of Dif four isoforms (142). However, the same authors found in a previous study that miR-317 regulates the Drosophila Toll pathway via targeting the other three Dif isoforms (Dif-Ra/b/d) (141). A set of studies showed the involvement of miR-317 in Drosophila reproductive responses and larval ovary morphogenesis (143–145). Remarkably, flies transiently overexpressing miR-317 have poor survival. In contrast, the knockout miR-317 flies (miR-317 KO/+ ) have better survival than the control group, respectively, during Gram-positive bacterial infection (142), implying a new insight of miRNA involved in the crosstalk between Drosophila immune and survival. Moreover, four members of the Drosophila miR-310, namely miR-310, miR-311, miR-312, and miR-313, negatively regulated the Toll-mediated immune response by repressing the expression of Drs and directly cotargeting the 3’UTR of Drs in Drosophila upon Gram-positive bacterial infection (146). In summary, insect-encoded miRNAs can act individually or collectively to inhibit AMP expression and impair antibacterial defenses for the purpose of immune homeostasis. These mechanisms, in most cases, involve the regulation of the Toll pathway components and allow not only the identification of a new miRNAs but also enrich the repertoire of Toll-related immune-modulating miRNAs in insects.

While some insects enjoy the conservation of genes encoding immune effectors, other from the hemipteran species such as pea aphid Acyrthosiphon pisum showed decreased immune responses as they lack the genes coding for AMP, IMD, PGRPs, and other immune-related molecules (53). Fortunately, the conserved Jun N-terminal kinase (JNK) pathway has been suggested in the pea aphid immune defense. In their investigation on how this pathway regulates the pea aphid immune defense upon bacterial invasion, Ma et al. (147) found that miRNA-184a/b targeted the JNK-3’UTR and repressed its expression, hence resulting in more bacteria in the aphids and increased aphids’ mortality after infection. The expression of miRNA-184a and miRNA-184b remarkably dropped after M. luteus and Pseudomonas aeruginosa infection, with the lowest expression observed at 24 h postinfection, revealing a negative correlation with JNK expression (147). Interestingly, PO, reactive oxygen species, and phagocytosis are under the control of the JNK pathway, suggesting miRNA-184 indirectly controls these antibacterial immune response mechanisms in the pea aphid. Finally, the regulation of the JNK pathway by miRNA-184 is likely a universal mechanism in animals, as prediction using the RNAhybrid program showed that JNK is a potential target of miRNA-184 in insects, zebrafish, frogs, mice, and humans (147).

UPEC strains provoke symptomatic urinary tract infections in humans, whereas commensal-like E. coli strains in the urinary bladder cause long-term asymptomatic bacteriuria (ABU). G. mellonella is a surrogate insect model host used to study human pathogens, including UPEC (148). miRNA sequencing in G. mellonella larvae infected with UPEC strain CFT073 or ABU strain 83972 showed significant changes in the expression levels of G. mellonella miRNAs, respectively, suggesting that insect immune response-mediated miRNAs can distinguish between pathogenic and commensal E. coli invasions (136).
4.1.3 Upon Viral Invasion

Host miRNAs have an essential role in defense against viral attacks, hence influencing the course of the infection. For example, during chikungunya virus (CHIKV) infection of *A. aegypti*, upregulated *A. aegypti* miR-2b binds to the ubiquitin-related modifier (Urm) 3'UTR, decreasing its translation. This finally leads to decreasing CHIKV replication within *A. aegypti* (26).

In contrast, in some cases, insect miRNAs can promote viral replication by reducing the expression of virus-induced host genes. That is the case for the insulin-related peptide-binding protein 2 (IBP2) which is known to be significantly upregulated in viruses- infected B. mori (149) but has been negatively regulated by miR-278-3p in vitro and vivo, leading to *BmCPV* replication. However, the definite mechanism of miR-278-3p and IPB2 on *BmCPV* replication has been ambiguous, requiring further investigation in the future (150).

4.2 Role of IncRNAs in Insect Immunity Upon Microbial Invasions

The functional exploration of IncRNAs in insects is by far exhaustive, mainly upon microbial attacks. Figure 5 gives an overview of how insects use IncRNAs to defend against fungal, bacterial, or viral attacks.

4.2.1 Upon Fungal Invasion

Few reports investigated interactions between insects and fungi, mainly insect IncRNA response to fungal stress. The western honeybee *Apis mellifera* (*A. mellifera*) is domestically used worldwide for honey production and crop pollination. The spore-forming and obligate intracellular fungal pathogen, *Nosema ceranae* (*N. ceranae*), can infect a variety of insects, including honeybees (151). The expression of *A. mellifera* IncRNAs was drastically altered by *N. ceranae* infection, revealing 4,749 conserved IncRNAs and 1,604 novel IncRNAs. Some differentially expressed IncRNAs regulated gene expression in *cis* and *trans* manners or served as precursors of miRNAs, or competitive endogenous RNAs (ceRNAs) acting as miRNA sponges, leading to the activation of the host key signaling pathways and infection control (152). One of the essential suggestions was that through their sponge potential, *A. mellifera* differential expressed IncRNAs might suppress *N. ceranae* via crosstalk with miR-25-x, miR-30-x, and miR-30-y. However, the binding of these IncRNAs and miRNAs is limited.
to bioinformatic prediction, and further experimental study is required.

**4.2.2 Upon Bacterial Invasion**

LncRNAs can promote or impair insect immune responses, especially the Toll immune response upon bacterial attacks. *Dif* and *Dorsal* genes are two crucial components of the Toll immune signaling in insects. These two effectors can activate the transcription of AMPs for pathogen eradication. The LncRNA CR46018 was approximatively tenfold overexpressed after infection of *Drosophila* with *M. luteus*. In addition, RNA-seq analysis of LncRNA CR46018-overexpressing *Drosophila* after infection with *M. luteus* revealed that upregulated genes were mainly enriched in Toll and Imd signaling pathways, supported by bioinformatics predictions and RNA-immunoprecipitation experiments which showed that CR46018 interacted with the transcription factors Dif and dorsal to enhance the Toll pathway. All this indicates LncRNA-CR46018 as a positive regulator of the Toll signaling pathway and essential for *Drosophila* survival. Moreover, LncRNA-CR46018 could up- and downregulate genes involved in the phagosome pathway and metabolism-related regulation, respectively. The latter pathway seems to be a promising target of insect-derived LncRNAs during insect-pathogen interactions. For example, a previous report unveiled that the *Drosophila* LncRNA CR44404 (lincRNA-IBIN) links immunity and metabolism in *Drosophila* upon infection with *M. luteus* (28). However, how insect-derived LncRNAs regulate this pathway to support insect immune response mechanisms needs further investigation.

Inversely, LncRNAs could negatively regulate insect immune responses by suppressing insect immune effectors to avoid abusive immune stimulation at a late stage of the infection. A recent study reports that at the later stage (24 h) of infection of *Drosophila* with *M. luteus*, the LncRNA-CR11538 inhibited the transcription of AMPs via decoying Dif/dorsal away from AMP promoter, thereby negatively modulating the Toll signaling pathway.
pathway and inhibiting their transcriptions to prevent abusive immune activation in Drosophila with M. luteus infection (153).

4.2.3 Upon Viral Invasion

The RNAi pathway is an essential antiviral response in insects. However, several studies suggested that the RNAi pathway is ineffective in preventing viral replication (154, 155). An obvious viral strategy to bypass insect RNAi defense mechanisms is to encode RNAi suppressors. Several RNAi cancellers have been described in plant and insect RNA viruses (156, 157). Meanwhile, no RNAi suppressors have been associated with arboviruses until the recent investigation of Zhang et al. (158). During infection of Drosophila with the Drosophila C virus (DCV), the antiviral lncRNA VINR was accumulated in the nucleus because of DCV’s viral RNAi suppressors for their inhibition. LncRNA VINR acted by binding to cactin, preventing its degradation by ubiquitin-proteasome and promoting a noncanonical antiviral and AMP defense leading to a reduced viral replication (159). This suggests a counter counter-defense strategy activated by an lncRNA in response to the viral suppression of the primary antiviral RNAi immunity in Drosophila.

LncRNAs regulate gene expression via cis- or trans-acting regulation (160, 161). In a comprehensive study of lncRNAs associated with Rice black-streaked dwarf virus (RBSDV) infection in Laodelphax striatellus midgut, all predicted and differentially expressed mRNA targets were regulated in a trans manner by 176 differentially expressed lncRNAs. In addition, although the KEGG pathway analysis revealed significantly enriched pathways such as purine metabolism, valine, leucine, and isoleucine degradation, fatty acid elongation, and so on as the most significantly enriched pathways of those trans-regulated genes, the fact remains that the Human papillomavirus infection pathway (which is pivotal for viral infection) was enriched considerably during RBSDV infection. It, therefore, might be involved in RBSDV infection of L. striatellus midgut (138). The eight differentially expressed lncRNAs and the two coexpressed targets in the Human papillomavirus pathway predicted by KEGG analysis and confirmed by RT-qPCR can be found in Table 1. Moreover, a protease inhibitor (PI), one of the lncRNAs’ targets, plays a vital role in antivirus and preventing carcinogenesis (162, 163). Interestingly, lncRNA MSTRG15394 and its target PI (15-fold) were significantly expressed, and scrutinizing their involvement in RBSDV infection of L. striatellus midgut showed that knockdown of MSTRG15394 or PI drastically increased the expression patterns of RBSDV replication-related genes, S5-1, S6, and S9-1, suggesting that MSTRG15394 and PI could inhibit the accumulation and proliferation of RBSDV in L. striatellus midgut (138). Moreover, upon B. mori cypovirus (BmCPV) infection of silkworm larvae, the expression of mRNA targets was mainly affected via trans-regulation by BmCPV-induced IncRNAs (164). Interestingly and remarkably, analysis of the differentially expressed lncRNAs and mRNAs network reveals that these differentially expressed lncRNAs simultaneously targeted some genes involved in relevant mechanisms such as apoptosis (PDCD6), autophagy (ATG3), and immune response (IPB2). Genes such as PDCD6, ATG3, IPB2, MFB1, and VPS52 could be trans-targeted by the most significantly expressed lncRNA MSTRG.20486.1 (164).

5 PATHOGEN-ENCODED ncRNAs MODULATING INSECT IMMUNITY

Pathogens can encounter insect immune responses and create a suitable environment for their replication. Insect pathologists have employed several strategies to escape host immune responses, including pathogen-encoded miRNAs and lncRNAs. The pathogen-encoded ncRNAs targeting insect-derived genes are listed in Table 3.

5.1 Pathogen-Encoded miRNAs Modulating Insect Immunity

Pathogen-derived miRNAs or miRNA-like-RNAs (miRNAs) are necessary components for host–pathogen crosstalk, promoting pathogen proliferation and replication in most cases. These miRNAs can modulate both pathogen and insect host immune genes. The following concentrates on the miRNA modulation of insect key genes. Figure 6 highlights the manipulation of insect immunity by pathogen-encoded miRNAs.

5.1.1 Fungus-Encoded miRNAs

miRNAs play crucial roles during fungal invasion (169). However, few fungal-derived miRNAs have been reported. Still, their active investigation remains challenging.

Delivering cell-entering effector molecules is a well-known employed strategy of plant pathogenic fungi to suppress host immunity (170, 171). No description has been documented for such effector molecules for pathogenic insect fungi until the brilliant discovery of Cui et al. (158). The pathogenic fungal

| Pathogen | miRNA | Target | Expression level | Host target | Ref. |
|----------|-------|--------|------------------|-------------|------|
| B. bassiana | bba-miR1 | Spz4 | Down | A. stephensi | (158) |
| B. bassiana | bba-miR1 | CLIPB9 | Up | A. stephensi | (159) |
| N. bombycis | Nb-miR8 | BmPKc16 | Down | B. mori | (166) |
| BmCPV | BmCPV-miR1 | BmRan | Down | B. mori | (166) |
| BmCPV | BmCPV-miR3 | BmRan | Down | B. mori | (166) |
| BmCPV | BmCPV-miR-10 | BmCSDE1 | Down | B. mori | (167) |
| BmCPV | BmCPV-miR-10 | Bmpapaf-1 | Down | B. mori | (167) |
| BmCPV | BmCPV-miR-1 | BmIAP | Down | B. mori | (168) |
B. bassiana deployed a miRNA-like RNA (bba-miR1) acting as a cell-entering effector to suppress mosquito immune response. The authors found that during the early stages of infection, B. bassiana bba-miR1 expression increased remarkably and translocated into the mosquito cells to mitigate mosquito immune responses by suppressing the expression of the critical activator gene Spz4. During this early invasion in the integument, the bba-miR1 is not accessible to circulating hemocytes, the site of CLIPB9 gene expression. In contrast, during the late stage of infection (hemocoel invasion), B. bassiana strategically decreased the expression level of bba-miR1 and avoided induction of CLIPB9 and activation of melanization. Quantification of bba-miR1 expression level during B. bassiana infection of Anopheles stephensi (A. stephensi) showed that bba-miR1 was induced by ~30-fold at 36 h postinfection and then declined to deficient levels as the fungus enters the host’s hemocoel at about 60 h after infection (158). Moreover, Nosema bombycis (N. bombycis) proliferation within B. mori is mediated by the increased expression of its miRNA-like RNA, Nb-miR8, which negatively regulates the B. mori peroxisome metabolic pathway via BmPXE16 gene expression, ultimately inhibiting the latter expression (165).

5.1.2 Virus-Encoded miRNAs
Studies have shown that one miRNA can target multiple genes and several miRNAs can also regulate a target gene. Generally, miRNA mainly binds to the 3’UTR of mRNA to repress the target gene translation, and cooperativity between two or more miRNA-binding sites can enhance the repression of the mRNA translation (172–174). In the case of viral infections, virus-encoded miRNAs act individually or synergistically to negatively regulate the expression of insect key genes (Ran, Apaf-1, etc.) by targeting their 3’UTR regions. The latter downregulation reduces host miRNAs’ production, leading to viral replication and proliferation (purple). However, the figure does not show the bacterial-derived miRNAs due to missing data on their role in insect-bacterium interaction.
miR-3, that co-operatively targeted the 3’UTR of B. mori GTP-binding nuclear protein Ran (BmRan) by lowering its expression level. Ran is a GTP-binding nuclear protein of 25 kDa, which plays a role of transporting small RNAs, including pre-miRNAs, from the nucleus to the cytoplasm (176), and its repression level led to a significant reduction in nuclear export of pre-miRNA. Similarly, BmCPV-miR-10 downregulated the B. mori cold shock domain E1 protein (BmCSDE1) mRNA expression level after binding to its 3’UTR in the infected larvae. BmCPV-miR-10 also inhibited the expressional changes of B. mori apoptotic protease activating factor 1 (BmApafl-1) during in vivo infection, creating suitable conditions for virus replication and proliferation (167). BmCPV-miR-1 inhibited cell apoptosis in the infected silkworm via increasing B. mori inhibitor of apoptosis protein (BmIAP) expression, promoting the virus replication (168). Obviously, the roles of virus-encoded miRNAs in the crosstalk seem to be largely explored compared with miRNA-encoded fungi or bacteria. An interesting insight in investigating this element-encoded virus is that they have the possibility to simultaneously regulate both insect innate and cellular immune responses, and further demonstrate that there is a dynamic evolution in the insect–pathogen crosstalk implicating ncRNAs, where insects evolutionally sophisticate their defense mechanisms while pathogens simultaneously elaborate strategies to encompass these immune obstacles for their own benefit.

### 5.2 Pathogen-Encoded IncRNAs Modulating Insect Immunity

Although new technologies and bioinformatics tools emerge for the detection of ncRNAs, most IncRNAs’ expression is low, probably limiting their identification and functional characterization (177). Several studies identified pathogen-encoded IncRNAs, their differential expression levels, and how the latter regulates pathogen’s genes (178). However, studies describing how these pathogen-encoded IncRNAs regulate insect immune responses during insect–pathogen interactions are considerably missing. For example, no virus-encoded IncRNAs were generated during silkworm larvae-BmCPV interaction, while 41 B. mori IncRNAs among 1845 were identified (164). We are tempted to speculate that pathogen-encoded IncRNAs may act similarly to miRNAs derived-pathogens, where their differential expression negatively regulates insect key genes and creates a favorable environment for pathogen replication and proliferation. However, the above suggestion needs further experimental shreds of evidence. This area could be an exciting and hotspot topic for researchers to investigate in the future.

### 6 CONCLUSIONS AND PERSPECTIVES

ncRNAs are the emerging and fate-determining players of insect–pathogen interactions. The long history of host and pathogen coevolution suggests that the pathogen keeps on evolving its arsenals to succeed in infection, whereas the host continues investing in defense mechanisms. Therefore, portraying the landscape of ncRNAs in insect–pathogen crosstalk at the immunological level is of great scientific worth. In short, we briefly reviewed the main signaling pathways and accessory immune mechanisms engaged in insect defense mechanisms and the recent progress on the involvement of ncRNAs, especially miRNAs and IncRNAs, in promoting or suppressing insect immune responses upon pathogen invasion. In addition, how pathogen-encoded miRNAs and IncRNAs regulate insect immunity was a part of the scope of this study.

The Toll, Imd, and JAK/STAT pathways are evolutionarily conserved pathways playing a manifest role in insect immune defense mechanisms against pathogens. They protect a wide range of pathogens (bacteria, fungi, parasites, etc.). In addition, with the assistance of immune effector mechanisms, this protection might be strengthened. However, looking deep inside, although the conferred protection, several factors exclude the idea of total protection. The fact that these pathways are not present in all insects might influence and change the ability of insects to defend themselves upon pathogens invasions. Moreover, the inexistence of adaptive immunity, which confers immune memory, allowing organisms to mount a more rapid and potent immune response when it is re-exposed to the same pathogen or a highly related one, might probably be a situation that affects insect immune defense intensity and efficacy. However, it has been hypothesized that immune priming occurs because the insect is inherently able to activate immune responses more rapidly during a second exposure or because pathogens or pathogen components are retained within the insect, which maintains the animal in a heightened state of immune alertness (179).

Insect immunity is well known to be modulated by several factors, including microbiota and ncRNAs. The latter, mainly miRNAs and IncRNAs, hugely reinforce insect host immune defense mechanisms during their crosstalk with pathogens. Their positive and negative modulation of insects’ immune responses passes through their ability to target insect- or pathogen-encoded miRNA targets and influence the course of the infection for the benefit of insects. These elements can be viewed as alternative insects’ defense mechanisms such as those mentioned above (immune effector mechanisms). They can be put on the stage as primary insect immune defense mechanisms, when principal pathways are inefficient to secure insects’ integrity from being invaded. The RNAi immune effector mechanism, for example, is well documented to provide a robust protection to insects against viral attacks. However, pathogen VSRs were recently proved to overcome this path (180). In this present case, the insect’s survival was accountable to the immune modulation ability of ncRNAs. Therefore, it is hoped that these noncoding elements would be exploited as a mainstream player to achieve food security or avoid economic waste in the industry by tackling different insect invaders.
the pathogen. Interestingly, in opposition to pathogen-derived miRNAs, there is a deficiency of studies describing the modulation of insect immunity by pathogen-derived lncRNAs in order to facilitate pathogen replication and proliferation. Such topics should retain researchers’ attention, as exploring this area will undoubtedly bring new insights into the role of these elements in insect–pathogen crosstalk. In addition, it seems like ncRNAs support insect immunity against pathogens by regulating insect main and evolutionarily conserved signaling pathways (Toll, Imd). Simultaneously, their action is less observed in insect accessory immune mechanisms. The regulation of these insect accessory defense mechanisms by ncRNAs has not been extensively explored. Few studies investigated the link between ncRNAs and insect accessory defense mechanisms. miRNAs of Snellenius manilae bracovirus (SmBV-miR-199b5p and SmBV-miR-2989) were found to regulate innate (domeless and toll-7) and cellular (encapsulation and phagocytosis activity of the hemocytes) immune responses of its host Spodoptera litura (181). Plutella xylostella miR-8 is downregulated following parasitization by Diadegma semiclausum, leading to significant declines in Serpin 27 transcript levels (182). The serine protease inhibitor Serpin 27 regulates activation of the Toll pathway and PPO involved in the melanization response in insects. Taken together, we are tempted to speculate that there is no direct role of ncRNAs on insect accessory immunity. Nonetheless, to a lesser extent, and based on evidence, ncRNAs undoubtedly modulate insect immune effector mechanisms indirectly by targeting insect main signaling pathway components. The Toll ligand Spätzle 3 controlled the melanization process in the stripe pattern formation of caterpillars (183).

It is now known that ncRNAs have a drastic immunomodulation influence in insect–pathogen crosstalk. All the changes, such as the differentially expressed miRNAs, lncRNAs, the co/target transcripts, and the diverse signaling pathways activated during insect–pathogen crosstalk, are proof of their crucial role. Proper understanding of insect–pathogen interaction is essential to deciphering the immune molecular patterns employed by insects against various pathogens. This understanding will positively impact the economy based on important insects, such as D. melanogaster and B. mori. Here are some perspectives: (1) improvement of methods and technologies used for ncRNA investigations; (2) promotion of more in-depth studies instead of preliminary ones; and (3) the link between pathogen-encoded lncRNAs and insect immunity represents an attracting topic to investigate and may hide tons of critical information, which will undoubtedly give new insights into the immunological landscape of insect–pathogen interaction.

**REFERENCES**

1. Satyavathi V, Ghosh R, Subramanian S. Long Non-Coding RNAs Regulating Immunity in Insects. Non-coding RNA. (2017) 3(1):14. doi: 10.3390/ncrna3001014
2. Lundgren JG, Jurat-Fuentes JL. The Physiology and Ecology of Host Defense Against Microbial Invaders. *Insect Pathol* (2012) 2nd edition):460–80. doi: 10.1016/B978-0-12-384984-7.00013-0
3. Siva-Jothy MT, Moret Y, Rolff J. Insect Immunity: An Evolutionary Ecology Perspective. In: SJ Simpson, editor. *Advances in Insect Physiology*, vol. 32 . Academic Press (2005). p. 1–48.
4. Hillyer JF. Insect Immunology and Hematopoiesis. *Dev Comp Immunol* (2016) 58:102–18. doi: 10.1016/j.dci.2015.12.006
5. Kingsolver MB, Huang Z, Hardy RW. Insect Antiviral Innate Immunity: Pathways, Effectors, and Connections. *J Mol Biol* (2013) 425(24):4921–36. doi: 10.1016/j.jmb.2013.10.006
6. Zhang W, Tettamanti G, Bassal T, Heryanto C, Eleftherianos I, Mohamed A. *Insect Antiviral Innate Immunity: Regulators and Signalling in Insect Antimicrobial Innate Immunity: Functional Molecules and Cellular Pathways. Cell signalling* (2021) 83:110003. doi: 10.1016/j.cellsig.2021.110003
7. Mylonakis E, PodsiadloWS, Muhammed M, Vilcinskas A. Diversity, Evolution and Medical Applications of Insect Antimicrobial Peptides. *Philos Trans R Soc Lond Ser B Biol Sci* (2016) 371(1695):20150290. doi: 10.1098/rstb.2015.0290
8. Lemaitre B, Hoffmann J. The Host Defense of Drosophila Melanogaster. *Annu Rev Immunol* (2007) 25:697–743. doi: 10.1146/annurev.immunol.25.022106.141615
9. Hultmark D. Drosophila Immunity: Paths and Patterns. *Curr Opin Immunol* (2005) 15(1):12–9. doi: 10.1016/S0952-7915(02)00005-5
10. Ferrandon D, Imler J-L, Hetru C, Hoffmann JA. The Drosophila Systemic Immune Response: Sensing and Signalling During Bacterial and Fungal Infections. *Nat Rev Immunol* (2007) 7(11):862–74. doi: 10.1038/nri2194
11. Valanne S, Wang JH, Rämö M. The Drosophila Toll Signalling Pathway. *J Immunol* (Baltimore Md 1950) (2011) 186(2):649–56. doi: 10.4049/jimmunol.1002302
12. Myllymäki H, Rämet M. JAK/STAT Pathway in Drosophila Immunity. *Scandinavian J Immunol* (2014) 79(6):377–85. doi: 10.1111/sji.12170
13. Vallet-Gely I, Lemaitre B, Boccard F. Bacterial strategies to Overcome Insect Defence. *Nat Rev Microbial* (2008) 6(4):302–13.
14. Dostert C, Jouanguy E, Irving P, Troyler L, Galiana-Arnoux D, Hetru C, et al. The Jak-STAT Signalling Pathway is Required But Not Sufficient for the Antiviral Response of Drosophila. *Nat Immunol* (2005) 6(9):946–53. doi: 10.1038/nl1237
15. Palmer WH, Joosten J, Overheul G, Jansen PW, Vermeulen M, Obbard DJ, et al. Induction and Suppression of NF-kB Signalling by a DNA Virus of Drosophila. *bioRxiv* (2018) 358176.
16. Strand MR. The Insect Cellular Immune Response. *Insect Sci* (2008) 15(1):1–14. doi: 10.1111/j.1744-7917.2008.00183.x

**AUTHOR CONTRIBUTIONS**

UAEM and TT reviewed the literature, designed the figures, and wrote the manuscript. LS, GY, and XL designed the tables and reviewed the manuscript. ZS, YW, and HC reviewed and revised the text. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

**FUNDING**

This work was supported by the National Natural Science Foundation of China (31802142), the Doctoral Start-up Fund of Southwest University (SWU120019, SWU020023), the Fundamental Research Funds for the Central Universities (XDJK2019C089), and the China Postdoctoral Science Foundation (2019T120801 and 2017M620408).

Frontiers in Immunology | www.frontiersin.org April 2022 Volume 13 Article 856457
17. Browne N, Heelan M, Kavanagh K. An Analysis of the Structural and Functional Similarities of Insect Hemocytomes and Mammalian Phagocytes. *Virulence* (2013) 4(7):597–603. doi: 10.4161/viru.25906

18. Kounatidis I, Ligysygysik P, Drosophila as a Model System to Unravel the Layers of Innate Immunity to Infection. *Open Biol* (2012) 2(5):120075. doi: 10.1098/rsob.120075

19. Hoffmann JA. The Immune Response of Drosophila. *Nature* (2003) 426 (6962):33–8. doi: 10.1038/nature02021

20. Bai L, Wang L, Vega-Rodriguez J, Wang G, Wang S. A Gut Symbiotic Bacterium Serratia Marcescens Renders Mosquito Resistance to Plasmodium Infection Through Activation of Mosquito Immune Responses. *Front Microbiol* (2019) 10:1580. doi: 10.3389/fmicb.2019.01580

21. Castelli L, Branchiccela B, Garrido M, Invernizzi C, Porrini M, Romero H, et al. Impact of Nutritional Stress on Honeybee Gut Microbiota, Immunity, and Nosema Ceranae Infection. *Microbiol Ecol* (2020) 80(4):90–19. doi: 10.1007/s00248-020-01535-8

22. Ahmad P, Bensaoud C, Mekki I, Rehman MU, Kotsyfakis M. Long Non-Coding RNAs and Their Association With Wolbachia and Vector-Host-Pathogen Triad. *Front Microbiol* (2021) 12:603. doi: 10.3390/fmicb.2021.0603

23. Xiao T, Towb P, Wasserman SA, Sprang SR. Three-Dimensional Structure of the Spätzle Protein De

24. De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaître B. The Toll and IMD Pathways are the Major Regulators of the Immune Response in Drosophila. *EMBO J* (2002) 21(11):2568–79. doi: 10.1093/emboj/21.11.2568

25. Lemaître B, Reichhart JM, Hoffmann JA. Drosophila Host Defense: Differential Induction of Antimicrobial Peptide Genes After Infection by Various Classes of Microorganisms. *Proc Natl Acad Sci USA* (1997) 94(26):14614–9. doi: 10.1073/pnas.94.26.14614

26. Weber AN, Tauszig-Delamasure S, Hoffmann JA, Lelièvre E, Gascan H, Ray KP, et al. Binding of the Drosophila Cytokine Spätzle to Toll is Direct and Establishes Signaling, *Nat Immuno* (2003) 4(8):794–800. doi: 10.1038/nij

27. Hu X, Yagi Y, Tanji T, Zhou S, Ip YT. Multimerization and Interaction of Toll and Spätzle in Drosophila. *Proc Natl Acad Sci USA* (2004) 101(25):9369–74. doi: 10.1073/pnas.0307062101

28. Wang L, Weber AN, Atilano ML, Filipe SR, Gay NJ, Ligoxygakis P. Sensing and Nosema Ceranae Infection. *Microbial Ecol* (2013) 4(7):597. doi: 10.1007/s00248-012-01538-1

29. Maarouf M, Chen B, Chen Y, Wang X, Rai KR, Zhao Z, et al. Identification of a Dorsal-Related Gene That Mediates an Immune Response in Drosophila. *Cell* (1993) 75(4):753–63. doi: 10.1016/0092-8674(93)90495-C

30. Michel T, Reichhart JM, Hoffmann JA. IFN-α/β and IFN-γ in Drosophila as a Model System to Unravel the Layers of Innate Immunity to Infection. *Proc Natl Acad Sci USA* (2001) 98(22):12590–5. doi: 10.1073/pnas.221458698

31. Michel T, Reichhart JM, Hoffmann JA. IFN-α/β and IFN-γ in Drosophila as a Model System to Unravel the Layers of Innate Immunity to Infection. *Proc Natl Acad Sci USA* (2001) 98(22):12590–5. doi: 10.1073/pnas.221458698

32. De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaître B. The Toll and IMD Pathways are the Major Regulators of the Immune Response in Drosophila. *EMBO J* (2002) 21(11):2568–79. doi: 10.1093/emboj/21.11.2568

33. Lemaître B, Reichhart JM, Hoffmann JA. Drosophila Host Defense: Differential Induction of Antimicrobial Peptide Genes After Infection by Various Classes of Microorganisms. *Proc Natl Acad Sci USA* (1997) 94(26):14614–9. doi: 10.1073/pnas.94.26.14614

34. Weber AN, Tauszig-Delamasure S, Hoffmann JA, Lelièvre E, Gascan H, Ray KP, et al. Binding of the Drosophila Cytokine Spätzle to Toll is Direct and Establishes Signaling, *Nat Immuno* (2003) 4(8):794–800. doi: 10.1038/nij

35. Hu X, Yagi Y, Tanji T, Zhou S, Ip YT. Multimerization and Interaction of Toll and Spätzle in Drosophila. *Proc Natl Acad Sci USA* (2004) 101(25):9369–74. doi: 10.1073/pnas.0307062101

36. Tauszig-Delamasure S, Bilak H, Capovilla M, Hoffmann JA, Imler JL. Drosophila MyD88 is Required for the Response to Fungal and Gram-Positive Bacterial Infections. *Nat Immuno* (2002) 3(1):91–7. doi: 10.1038/ni747

37. Horròg T, Medzhitov R. Drosophila MyD88 is an Adapter in the Toll Signaling Pathway. *Proc Natl Acad Sci USA* (2001) 98(22):12654–8. doi: 10.1073/pnas.231471798

38. Xiao T, Towb P, Wasserman SA, Sprang SR. Three-Dimensional Structure of a Complex Between the Death Domains of Pelle and Tube. *Cell* (1999) 99(5):545–55. doi: 10.1016/S0092-8674(00)81542-1

39. Weber AN, Tauszig-Delamasure S, Hoffmann JA, Lelièvre E, Gascan H, Gonzalez Crespo S, et al. Dif, a Dorsal-Related Gene That Mediates an Immune Response in Drosophila. *Cell* (1993) 75(4):753–63. doi: 10.1016/0092-8674(93)90495-C

40. Michel T, Meister M, Govind S, Georgell P, Stewart R, Reichhart JM, et al. Functional Analysis and Regulation of Nuclear Import of Dorsal During the Immune Response in Drosophila. *EMBO J* (1995) 14(3):536–45. doi: 10.1002/j.1460-2075.1995.tb07029.x

41. Wu LP, Anderson KV. Regulated Nuclear Import of Rel Proteins in the Drosophila Immune Response. *Nature* (1998) 392(6671):93–7. doi: 10.1038/32195

42. Hetru C, Hoffmann JA. NF-kappaB in the Immune Response of Drosophila. *Cold Spring Harbor Perspect Biol* (2009) 1(6):a000232. doi: 10.1101/cshperspect.a000232

43. Belvin MP, Anderson KV. A Conserved Signaling Pathway: The Drosophila Toll-Dorsal Pathway. *Annu Rev Cell Dev Biol* (2016) 12:393–416. doi: 10.1146/annurev.cellbio.12.1.393

44. Ribeiro JM, Genta FA, Sorgine MH, Logullo R, Mesquita RD, Paiva-Silva GO, et al. An Insight Into the Transcriptome of the Digestive Tract of the Bloodsucking Bug, Rhodnius Prolixus. *Plos neglected Trop Dis* (2014) 8(1):e2594. doi: 10.1371/journal.pntd.0002594

45. Mesquita RD, Vionette-Amaral RJ, Lowenberger C, Rivera-Pomar C, Monteiro FA, Minx P, et al. Genome of Rhodnius Prolixus, an Insect Vector of Chagas Disease, Reveals Unique Adaptations to Hematophagy and Parasite Infection. *Proc Natl Acad Sci United States America* (2015) 112(48):14936–41. doi: 10.1073/pnas.1506226112

46. Gerardo NM, Altincicek B, Anselme C, Atamian H, Barribeau SM, de Vos I, et al. Immunity and Other Defenses in Pea Aphids, *Cell*

47. De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaître B. The Toll and IMD Pathways are the Major Regulators of the Immune Response in Drosophila. *EMBO J* (2002) 21(11):2568–79. doi: 10.1093/emboj/21.11.2568

48. Tzou P, Reichhart JM, Lemaître B. Constitutive Expression of a Single Antimicrobial Peptide can Restore Wild-Type Resistance to Infection in Immunodeficient Drosophila Mutants. *Proc Natl Acad Sci USA* (2002) 99(4):2152–7. doi: 10.1073/pnas.042411999
66. Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS, Bartholomay LC, et al. Evolutionary Dynamics of Immune-Related Genes and Pathways Independently of its Toll-Pathway Activation Function. Cell Mol Immunol 2009 50(5):1244–54. doi: 10.1002/jem.20904164

67. Hoffman JA, Reichhart JM. Drosophila Innate Immunity: An Evolutionary Perspective. Nat Immunol (2002) 3(2):121–6. doi: 10.1038/nri8202-121

68. Hillyer JF, Schmidt SL, Christensen BM. Rapid Phagocytosis and the Mosquito Aedes Aegypti. Science (2003) 306(5698):1738–43. doi: 10.1126/science.1103862

69. Hillyer JF, Strand MR. Granular Cells are Required for Encapsulation of Nematodes in Two Lepidopteran Insects. Appl Entomol Zoology (2020) 55(3):337–44. doi: 10.1007/s1335-020-00687-6

70. Hillyer JF, Strand MR. Mosquito Hemocyte-Mediated Immune Responses. Int J Insect Morphol Embryol (2013) 42(1):207–31. doi: 10.1016/j.ijim.2012.09.002

71. Hillyer JF, Schmidt SL, Christensen BM. HMGB1 and Its Drosophila Homolog: Allotopic Expression during Immune Response. Trends Immunol (2010) 31(10):427–30. doi: 10.1016/j.it.2009.10.013

72. Hillyer JF, Ishibashi J, Ragan EJ, Jiang H, Kanost MR. Functions of Manduca Hyperphagocytic Haemocytes in Manduca Sexta. J Insect Physiol (2009) 55:90–9. doi: 10.1016/j.jinsphys.2008.02.009

73. Hillyer JF, Strand MR. Mosquito Hemocyte-Mediated Immune Responses. Cell Mol Immunol 2009 50(5):1244–54. doi: 10.1002/jem.20904164

74. Honti V, Coeiras G, Kurucz E, Markus R, Ando I. The Cell-Mediated Immunity of Drosophila Melanogaster: Hemocyte Lineages, Immune Compartmentalization, Microanatomy and Regulation. Dev Comp Immunol (2014) 42(1):37–56. doi: 10.1016/j.devimm.2013.06.005

75. Hoffmann JA, Reichhart JM. Drosophila Innate Immunity: An Evolutionary Perspective. Nat Immunol (2002) 3(2):121–6. doi: 10.1038/nri8202-121

76. Hillyer JF, Schmidt SL, Christensen BM. Hemocyte-Mediated Phagocytosis. J Immunol (Baltimore Md 1950) (2014) 192(8):3455–62. doi: 10.4049/jimmunol.1303309

77. Hillyer JF, Schmidt SL, Christensen BM. Hemocyte-Mediated Phagocytosis. J Immunol (Baltimore Md 1950) (2014) 192(8):3455–62. doi: 10.4049/jimmunol.1303309
94. Napi AJ, Christensen BM. Melanogenesis and Associated Cytotoxic Reactions: Applications to Insect Innate Immunity. Insect Biochem Mol Biol (2005) 35(5):443–59. doi:10.1016/j.ibmb.2005.01.014

95. Osta MA, Christophides GK, Kafatos FC. Effects of Mosquito Genes on Plasmodium Development. Sci (New York) (2004) 303(5666):2030–2. doi:10.1126/science.10911789

96. Volz J, Müller HM, Zdanowicz A, Kafatos FC, Osta MA. A Genetic Module Regulates the Melanization Response of Anopheles to Plasmodium. Cell Microbiol (2006) 8(9):1392–405. doi:10.1111/j.1462-882X.2006.00718.x

97. Ashida M, Ochiai M, Niki T. Immunolocalization of Prophenoloxidase Among Hemocytes of the Silkworm, Bombyx Mori. Tissue Cell (1988) 20(4):599–610. doi:10.1016/0040-8166(88)90061-4

98. Yassine H, Kamareddine L, Osta MA. The Mosquito Melanization Response Is Implicated in Defense Against the Entomopathogenic Fungus Beauveria Bassiana. PLoS Pathog (2012) 8(11):e1003029. doi:10.1371/journal.ppat.1003029

99. Carton Y, Frey F, Stanley DW, Vass E, Nappi AJ. Dexamethasone Inhibition of Cellular Immune Response of Drosophila Melanogaster Against a Parasitoid. J parasitology (2002) 88(2):405–7. doi:10.1645/2002-3395(2002)088[0405:DIOTCI]2.0.CO;2

100. Gandhe AS, John SH, Nagaraju J. Nodulator, a Novel Immune Up-Regulated Protein Mediates Nodulation Response in Insects. J Immunol (Baltimore Md 1950) (2007) 179(10):6943–51. doi:10.4049/jimmunol.179.10.6943

101. Ratcliffe NA, Green S. Studies on the In Vivo Cellular Reactions of Insects: An Ultrastructural Analysis of Nodule Formation in Galleria mellonella. Tissue Cell (1977) 9(1):73–85. doi:10.1016/0040-8166(77)90050-7

102. Satyavathi VV, Minz A, Nagaraju J. Nodulation: An Unexplored Cellular Defense Mechanism in Insects. Cell signalling (2014) 26(8):1753–63. doi:10.1016/j.cellsig.2014.02.024

103. Scott JT, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, et al. Towards the Elements of Successful Insect RNAi. Tissue Cell (2014) 13(3):166–74. doi:10.1016/j.jinsphys.2014.06.002

104. Yu N, Christiaens O, Liu J, Niu J, Cappelle K, Caccia S, et al. Delivery of dsRNA for RNAi in Insects: An Overview and Future Directions. Insect Sci (2013) 20(1):4–11. doi:10.1117/1.4747917.2012.01534a

105. Blair CD, Olson KE. Mosquito Immune Responses to Arbovirus Infection. Curr Opin Infect Sci (2014) 3:22–9. doi:10.1016/j.cois.2014.07.005

106. Bronkhorst AW, van Rij RP. The Long and Short of Antiviral Defense: Small RNA-Based Immunity in Insects. Curr Opin Virol (2014) 7:19–28. doi:10.1016/j.coviro.2014.03.010

107. Karlikow M, Goic B, Saleh MC. RNAi and Antiviral Defense in Drosophila: A Genome-Wide Analysis of Antimicrobial Effector Genes and Their Transcription Patterns in Manduca Sexta. Insect Biochem Mol Biol (2015) 62:23–37. doi:10.1016/j.ibmb.2015.01.015

108. Tettamanti G, Malagoli D, Marchesini E, Congiu T, De Guglielmo M, Ottaviani E. Oligomycin A Induces Autophagy in the IPLB-LdFB Insect Cell Line. Cell Tissue Res (2006) 326(1):179–86. doi:10.1007/s00441-006-0206-4

109. Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, et al. Autophagy is an Essential Component of Antimicrobial Responses in Drosophila. Immunity (2008) 9(8):809–16. doi:10.1016/j.immuni.2008.03.010

110. Kajla MK, Shi L, Li B, Luckhart S, Li J, Paskewitz SM. A New Role for an Old Antimicrobial: Lysozyme C-1 can Function to Protect Malaria Parasites in Anopheles Mosquitoes. PloS One (2011) 6(5):e19649. doi:10.1371/journal.pone.0019649

111. Imler JL. Overview of Drosophila Immunity: A Historical Perspective. Dev Comp Immunol (2014) 42(1):3–15. doi:10.1016/j.dci.2013.08.018

112. Yu HY, Chowdhury M, Huang YD, Yu XQ. Insect Antimicrobial Peptides and Their Applications. Appl Microbiol Biotechnol (2014) 98(13):5807–22. doi:10.1007/s00253-014-5792-6

113. Elmagy M, Bassal TT, Yousef HA, Dorrah MA, Mohamed AA, Duvic B. Isolation, Characterization, Kinetics, and Enzymatic and Nonenzymatic Microbical Activities of a Novel C-Type Lysozyme From Plasma of Schistocerca Gregaria (Orthoptera: Acrididae). J Insect Sci (2015) 15(1):57. doi:10.1093/jisesa/iev038

114. He Y, Cao X, Li K, Hu Y, Chen YR, Blissard G, et al. A Genome-Wide Analysis of Antimicrobial Effector Genes and Their Transcription Patterns in Manduca Sexta. Insect Biochem Mol Biol (2015) 62:23–37. doi:10.1016/j.ibmb.2015.01.015

115. Yang S, Zhang I, Wang S, Zhang X, Liu Y, Xi J. Identification and Profiling of miRNAs in Overwintering Lissornitrus oryzaeae via Next-Generation Sequencing.
172. Saetrom P, Heale BS, Sneve Ojr., Aagaard L, Alluin J, Rossi JJ. Distance Constraints Between microRNA Target Sites Dictate Efficacy and Cooperativity. Nucleic Acids Res (2007) 35(7):2333–42. doi: 10.1093/nar/gkm133

173. Fang Z, Rajewsky N. The Impact of miRNA Target Sites in Coding Sequences and in 3′UTRs. PLoS One (2011) 6(3):e18067. doi: 10.1371/journal.pone.0018067

174. Liu G, Zhang R, Xu J, Wu CI, Lu X. Functional Conservation of Both CDS- and 3′-UTR-Located microRNA Binding Sites Between Species. Mol Biol Evol (2015) 32(3):623–8. doi: 10.1093/molbev/msu323

175. Singh CP, Singh J, Nagaraju J. A Baculovirus-Encoded MicroRNA (miRNA) Suppresses its Host miRNA Biogenesis by Regulating the Exportin-5 Cofactor Ran. J Virol (2012) 86(15):7867–79. doi: 10.1128/JVI.00064-12

176. Bischoff FR, Ponstingl H. Catalysis of Guanine Nucleotide Exchange on Ran by the Mitotic Regulator RCC1. Nature (1991) 354(6348):80–2. doi: 10.1038/354080a0

177. Bergmann JH, Spector DL. Long Non-Coding RNAs: Modulators of Nuclear Structure and Function. Curr Opin Cell Biol (2014) 26:10–8. doi: 10.1016/j.ceb.2013.08.005

178. Guo R, Chen D, Xiaoj C, Hou C, Zheng Y, Fu Z, et al. First Identification of Long Non-Coding RNAs in Fungal Parasite Nosema Ceranae. Apidologie (2018) 49(5):660–70. doi: 10.1007/s13592-018-0593-z

179. Masri L, Cremer S. Individual and Social Immunisation in Insects. Trends Immunol (2014) 35(10):471–82. doi: 10.1016/j.it.2014.08.005

180. Betting V, Van Rij RP. Countering Counter-Defense to Antiviral RNAi. Trends Microbiol (2020) 28(8):660–2. doi: 10.1016/j.tim.2020.05.018

181. Tang CK, Tsai CH, Wu CP, Lin YH, Wei SC, Lu YH, et al. MicroRNAs From Snellenius Manilae Bracovirus Regulate Innate and Cellular Immune Responses of its Host Spodoptera Litura. Commun Biol (2021) 4(1):52. doi: 10.1038/s42003-020-01563-3

182. Elebadi K, Asgari S. Conserved microRNA miR-8 Blocks Activation of the Toll Pathway by Upregulating Serpin 27 Transcripts. RNA Biol (2013) 10(8):1356–64. doi: 10.4161/rna.25481

183. KonDo Y, Yoda S, Mizoguchi T, Ando T, Yamaguchi J, Yamamoto K, et al. Toll Ligand Spätzle3 Controls Melanization in the Stripe Pattern Formation in Caterpillars. Proc Natl Acad Sci United States America (2017) 114(31):8336–41. doi: 10.1073/pnas.1707896114

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Moure, Tan, Sha, Lu, Shao, Yang, Wang and Cui. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.