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Review

Regulation of *Drosophila*-virus interaction

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ABSTRACT

Drosophila melanogaster is a useful model system for deciphering mammalian biological processes including development, innate immunity and cancer. Most genetic studies conducted in *Drosophila* have focused on the immune response against microbial infection and the results obtained have been extrapolated to other organisms. During the last decade the issue of the antiviral response attracted a great deal of interest. In this review we highlight recent discoveries in the role of RNA interference pathway in antiviral response in *Drosophila* with a focus on the role of miRNAs as both host defense elements and helpers of viral replication.

Contents

1. Introduction

The *Drosophila* immune response relies solely on the innate immunity. Hemocytes (blood cells) participate in phagocytosis, encapsulation, coagulation and melanization of intruders in the hemolymph (Lemaitre and Hoffmann, 2007). On the other hand, the fat-body, which is the analog of mammalian liver, produces antimicrobial peptides (AMPs) that are exported to the hemolymph. Synthesis of AMPs is mainly regulated by two signaling pathways: Toll and Immune deficiency (Imd), similar to the mammalian Toll-like receptor/interleukin-1 and tumor necrosis factor-α pathways, respectively (Lazzaro, 2008).

The Toll pathway, which is a serine protease cascade, is triggered by fungal and most Gram-positive bacterial cell component such as glucan and lysine-type peptidoglycan, respectively. On the contrary, the Imd pathway involves a kinase cascade induced by DAP (diaminopimelic acid)-type peptidoglycan, which is a common component in most Gram-negative bacteria. Both Toll and Imd pathways culminate in the activation of NF-κB related transcription factors and the transcription of several genes such as AMPs. In addition to Imd, the Jun-N-terminal kinase (JNK) pathway also protects from Gram-negative bacteria (Bond and Foley, 2009). Together with the well characterized antibacterial response, the *Drosophila* JAK/STAT signaling pathway is responsible for the

Abbreviations: miRNA, microRNA; siRNA, small interference RNA; piRNA, piwi-interacting RNA; mRNA, messenger RNA; AMP, antimicrobial peptide; NF-κB, nuclear factor-kappa B; VSR, viral suppressor of RNA; IκB, inhibitor kappa B.

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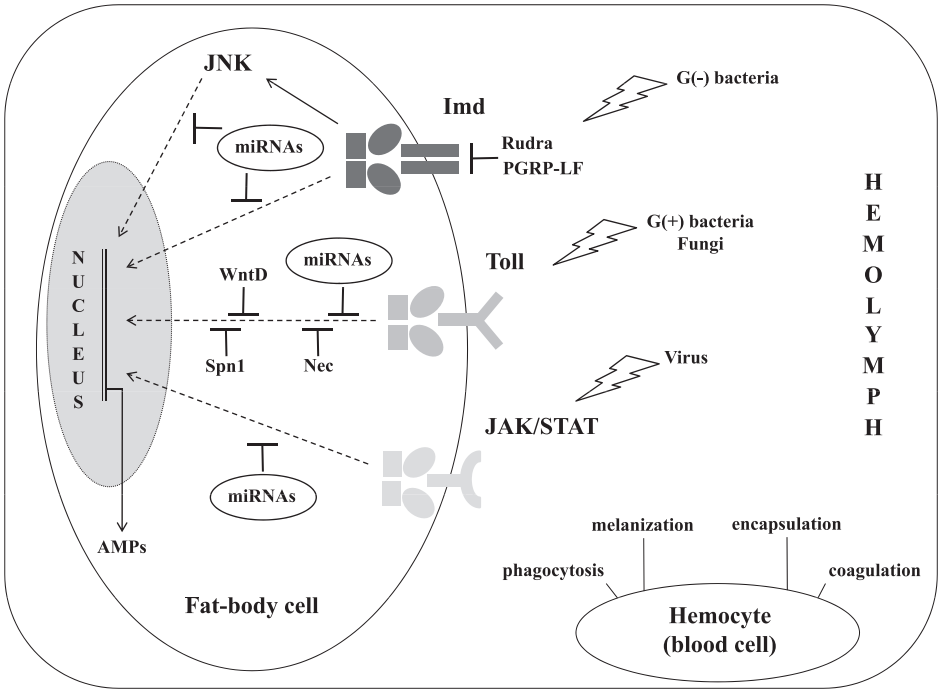

Fig. 1. Immune response in *Drosophila*. The immune system of *Drosophila* is based only on the innate immunity. The humoral response is composed by different signaling pathways that culminate in the synthesis of AMPs in fat-body cells. Upon Gram-negative bacterial infection Imd and JNK cascades are activated, whereas Toll pathway is induced by fungal or Gram-positive bacterial challenge. In addition, the JAK/STAT pathway is required in the antiviral response. These signaling cascades are negatively regulated at distinct levels by diverse inhibitors. On the other hand, hemocytes are responsible for cellular response which consists of phagocytosis, melanization, encapsulation and coagulation processes.

antiviral response, which is triggered by cytokine binding to tyrosine kinase receptor, in collaboration with other mechanisms such as autophagy (Dostert et al., 2005; Shelly et al., 2009) (Fig. 1).

Misregulation of the immune response can cause cancer, chronic inflammatory disorders and developmental defects, therefore immune pathways have to be controlled at distinct levels (Han and Ulevitch, 2005). In *Drosophila*, two serpins (SERine Protease INhibitors), Necrotic and Spn1 (Levashina et al., 1999; Fullaondo et al., 2011), and the Wnt inhibitor of Dorsal (WntD) have been described as negative regulators of the Toll pathway. Serpins block the immune response targeting proteases in the cytoplasm, whereas WntD interferes with the nuclear translocation of transcription factors (Ganguly et al., 2005). The constitutive activation of the Imd pathway is inhibited by PGRP-LF receptor and Rudra (Pirk, poor Imd response upon knock-in and also known as Pims) by sequestering circulating peptidoglycan and by direct binding to PGRP-LC and PGRP-LE, respectively (Aggarwal et al., 2008; Maillet et al., 2008). Moreover, both Toll and Imd pathways, as well as JNK and JAK/STAT, have been recently proposed to be regulated by microRNAs (miRNAs) (Fullaondo and Lee, 2011) (Fig. 1).

Viruses are the most abundant pathogens on earth and an important cause of mortality worldwide; however, little is known about viral infection strategies and consequently about host antiviral immune responses in *Drosophila*. This review focuses on the interaction at molecular level between *Drosophila* and viruses highlighting the role of both viral and host miRNAs as key players in the regulation of the immune response.

2. *Drosophila* antiviral immune response

2.1. RNA interference pathways

The double-stranded RNA (dsRNA) present in the viral genome, replication complexes or resulting from transcription of DNA viruses, is the main molecular pattern recognized by host immune system. This dsRNA triggers the RNA interference (RNAi) pathway in plants and invertebrates, and the production of interferons in vertebrates (Li and Ding, 2005).

Three different RNAi pathways have been identified in *Drosophila*: the small interfering RNA (siRNA), the microRNA (miRNA) and the Piwi-interacting (piRNA) pathways. The siRNAs originate from the processing of long endogenous or exogenous dsRNA precursors such as those produced by inverted-repeat (IR) transgenes or viral RNAs respectively by the RNase III enzyme Dicer-2 (Dcr2), which is bound to r2d2 protein (Okamura et al., 2010). Then, the siRNAs are transferred to the RNA-induced silencing complex (RISC) containing Argonaute-2 (Ago2), which guides the cleavage of target mRNAs with perfect sequence complementarity (Ding and Voinnet, 2007).

The primary miRNAs (pri-miRNAs) folded into a hairpin structure are processed in the nucleus by the Microprocessor complex formed by RNase III enzyme Drosha and its partner Pasha. Next, the newly generated pre-miRNAs are transported to the cytoplasm by Exportin-5 and further processed by Dicer-1 (Dcr1). Then, the mature miRNAs are loaded into Argonaute-1 (Ago1)-dependent RISC complex and mediate silencing of gene expression by mRNA cleavage, when the sequence complementarity is perfect with the target, or by translational repression or RNA deadenylation, if complementarity is not perfect (Macfarlane and Murphy, 2010) (Fig. 2).

The piRNAs are produced from the cleavage of repetitive genetic element transcripts and then associate to Piwi clade Argonautes, Piwi and Aubergine. The piRNAs function in transposon silencing and genome maintenance during the germline development. Transposon elements are major structural elements of eukaryotic genomes and their mobilization can lead to genetic instability, such as deleterious mutations or alteration of gene expression. Because the regulation of the genetic information in the germline will be inherited, transposon silencing by piRNAs is critical (Khurana and Theurkauf, 2010).

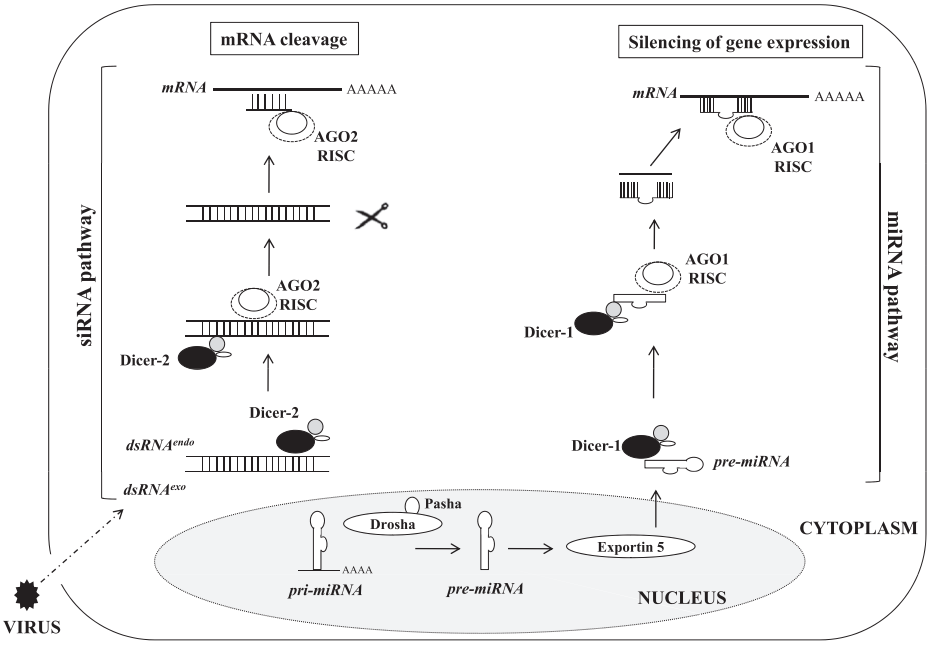

Fig. 2. RNA interference pathways in *Drosophila*. The biogenesis of siRNAs, which are derived from exogenous or endogenous sources of dsRNA located in the cytoplasm, requires several steps: (i) cleavage of dsRNA into siRNAs by Dicer-2, (ii) assembly of siRNAs into Ago2-dependent RISC and (iii) cleavage of target mRNAs. On the other hand, the synthesis of miRNA consist of: (i) formation of a primary transcript folded into a hairpin structure in the nucleus that is specifically cut near the stem-loop by Drosha/Pasha, (ii) transport of the newly generated pre-miRNA by Exportin-5 to the cytoplasm and its further processing by Dicer-1 and (iii) cleavage of target mRNAs mediated by Ago1/RISC complex.

2.2. Antiviral miRNAs

The siRNA pathway plays an essential role in the *Drosophila* antiviral response. Mutant flies for the genes *Dcr2*, *r2d2* and *Ago2* show increased susceptibility to infection of several RNA viruses such as *Drosophila* X virus (DXV), *Drosophila* C virus (DCV) and Flock house virus (FHV). Increased lethality of these deficient flies correlates with the augmented viral load, confirming the requirement of siRNA pathway in the antiviral response (Huszar and Imler, 2008).

On the contrary, the miRNA pathway was thought not to be involved in the antiviral immune response because (i) miRNAs are widely conserved across species and viral host ranges are very restricted; (ii) viruses evolve more rapidly than their hosts due to their short life cycles and high mutation rate and (iii) miRNAs could not have evolved to counter recently evolved new viruses (Umbach and Cullen, 2009). However, the enormous number of miRNAs identified in each animal species and the broad spectrum of target genes for each miRNA open the possibility of the existence of host antiviral miRNAs. Recently it has been demonstrated that miRNA-dependent silencing suppresses RNA virus infection in *Drosophila*. Flies deficient for *Ars2* (Arsenite-resistance protein 2) and the nuclear *cap binding complex* (CBC) genes are vulnerable to viral infection (Sabin et al., 2009). Ars2 and CBC form a complex that physically interacts with the Microprocessor. There are two models that may explain the function of Ars2-CBC complex. The first is a bridging model in which the complex recruits the pri-miRNA and the Microprocessor, promoting the generation of the pre-miRNA. The second model proposes that Ars2 functions as a cofactor for the enzymatic activity of Drosha and Dcr2. In both models Ars2-CBC complex regulate the biogenesis of siRNAs and miRNAs, a function conserved in mammals and plants (Gregory et al., 2008; Gruber et al., 2009).

Because the blockade of the miRNA pathway favors viral infection (Sabin et al., 2009), one can expect that specific miRNAs with antiviral function exist in *Drosophila*. In mammals, several cellular miRNAs have been reported to play a role in antiviral response *in vitro*. For example miR-24 and miR-93 down-regulate vesicular stomatitis virus (VSV) protein synthesis in mice and other miRNAs, including miR-28, miR-125b, miR-150, miR-223 and miR-382, suppress human immunodeficiency virus-1 (HIV-1) replication. Yet, the function of these miRNAs has to be proven *in vivo* (Umbach and Cullen, 2009). In this context *Drosophila* represents a fantastic model to investigate the existence of antiviral host miRNAs and could consequently facilitate the understanding of human antiviral response.

2.3. Signaling cascades regulating antiviral response

Drosophila immune responses to various bacteria and fungi are well characterized at molecular level (Ferrandon et al., 2007). However, the signaling cascades regulating the antiviral immunity remain unclear. The JAK/STAT pathway is known to contribute to the response against DCV and FHV infection. It is thought that DCV induces the activation of an unidentified cytokine, probably a member of Unpaired (Upd) ligand family, which in turn triggers Domeless receptor and Jak kinase hopscotch leading to the induction of STAT transcription factor and then expression of specific genes including *vir-1* (virus-induced RNA 1) (Dostert et al., 2005). FHV infection induces the expression of *Turandot M* gene through the activation of JAK/STAT pathway (Dostert et al., 2005).

Previously, it was suggested that Toll and Imd pathways have no role in the *Drosophila* antiviral response. Yet, recently it has been shown that the Toll cascade controls the survival of DXV-infected flies (Zambon et al., 2005) and that the Imd pathway is required in the defense against both cricket paralysis virus (CrPV) and alphavirus Sindbis virus (SINV) (Avadhanula et al., 2009; Costa et al., 2009).

Indeed, the importance of NF-κB signaling pathways in the antiviral immune response has been strengthened by the identification of viral proteins mimicking IκB inhibitors (described in *Viral suppressors of RNAs* section).

Additionally, autophagy, a cell-intrinsic mechanism that degrades cytoplamic contents, was identified to play a direct antiviral role against vesicular stomatitis virus (VSV) in *Drosophila*. Upon viral infection, autophagy is activated via the attenuation of the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway and decreases viral replication in a cell-autonomous manner. Flies depleted of Atg 18, which is a component of the PI3K-Akt signaling pathway, showed high susceptibility against VSV infection but not DCV infection (Shelly et al., 2009).

3. Viral strategies: escape from *Drosophila* immune response

3.1. Viral suppressors of RNAs

The inhibition of the antiviral RNAi pathways is a crucial requirement for effective propagation of virus within the host (Fig. 3). Not surprisingly, viruses have evolved distinct strategies to block antiviral RNAi response such as viral suppressors of RNAs (VSRs).

VSRs are very diverse in sequence and structure across viral kingdoms, but operate through a few evolutionarily conserved strategies: (i) binding to host dsRNAs to inhibit their processing by Dicer proteins; (ii) sequestering siRNA duplexes to prevent their loading into Ago complexes or (iii) interacting directly with Dicer or Ago proteins to impair their antiviral activities (Li and Ding, 2005). Two VSRs have been identified in *Drosophila* viruses, B2 from Flock house virus (FHV) and 1A from *Drosophila* C virus (DCV). These proteins directly interact with dsRNA preventing recognition and cleavage by Dcr2. In addition, FHV-B2 is also able to bind siRNAs. Because of the high affinity binding between these proteins and dsRNAs, point mutations affecting this interaction suppress VSR activity (van Rij et al., 2006; Aliyari et al., 2008).

3.2. Viral miRNAs

miRNAs are particularly useful for viruses due to (i) their small size which is ideal for the tight space constraints typical of viral genomes, (ii) their capacity to evolve rapidly comparing to regulatory proteins, (iii) their ability to knock-down specific genes to establish favorable conditions for viral replication and (iv) their lack of immunogenicity.

Viral miRNAs can inhibit both viral and host transcripts. Those miRNAs that regulate viral gene expression help maintaining a persistent infection by lowering antigenicity of viral proteins or preventing viral replication. On the other hand, most host mRNAs targeted by viral miRNAs play a role in either regulation of apoptosis or modulation of host antiviral response (Grundhoff and Sullivan, 2011).

Viral miRNAs were first discovered in the herpesvirus and to date most of the viral miRNAs found are from mammalian viruses (Pfeffer et al., 2004). The first miRNA from an insect virus was found in *Heliothis virescens* ascovirus (HvAV), a pathogen of prevalent lepidopteran pests. The HvAV-miR-1 is transcribed from the *major capsid protein* (MCP) gene. Although MCP is constitutively expressed, HvAV-miR-1 is specifically transcribed late in viral infection and coincides with a remarkable reduction of the expression of HvAV DNA polymerase I. This result suggests that HvAV-miR-1 is involved in the regulation of viral replication. Indeed, several human viruses express miRNAs that tightly control virus replication, preventing the rapid decline of the host and thus favoring the amplification of viral progeny (Hussain et al., 2008). Recently four miRNAs have been characterized from the *Bombyx mori*-specific baculovirus, a natural pathogen of the silkmoth that inflicts high mortality. The computationally predicted viral and host targets of these miRNAs play a key role in pathogen-host interaction by modulating viral replication as well as those involved in host immune defense machinery (Singh et al., 2010). To date no virus-encoded miRNAs have been identified in *Drosophila*.

3.3. Inhibitor κB-like proteins

Among the counterstrategies viruses have developed to circumvent host immune responses, those mimicking host inhibitor κB (IκB) proteins are of much interest. In resting cells NF-κB transcription factors exist in an inactive state and form complexes with IκBs through ankyrin-repeat domains present and highly

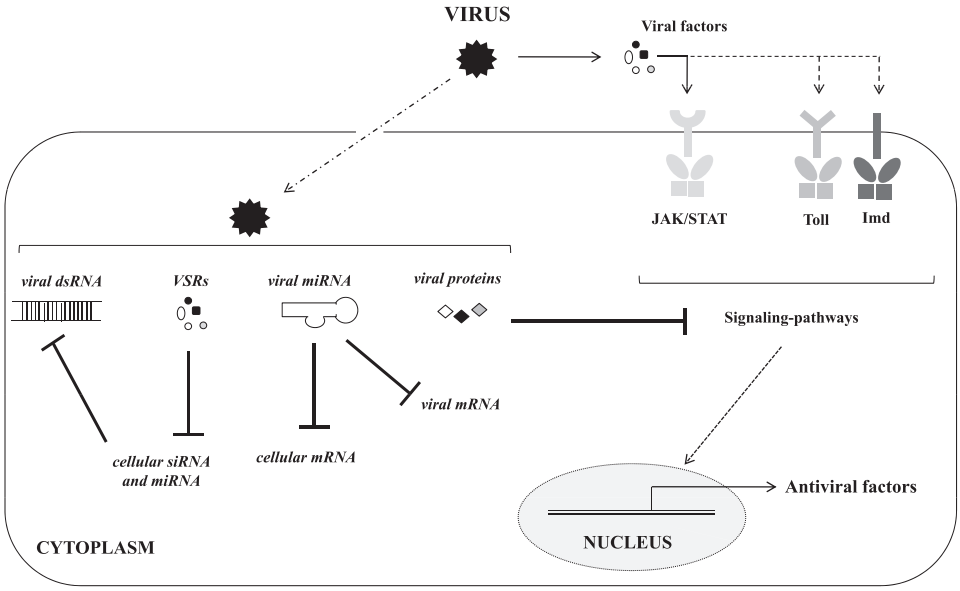

Fig. 3. Host-pathogen interactions. Several strategies are used by viruses to favor their replication within the host. VSRs are responsible for inhibiting through distinct mechanisms host siRNA and miRNA pathways, which in turn are key players in antiviral response. In order to maintain a persistent infection and to avoid the induction of the antiviral response, viruses also resort to their miRNAs to target either viral or host mRNAs. Once in the cytoplasm, viruses can also secrete distinct proteins such as IκB-like proteins that block NF-κB pathways such as Toll, Imd and JAK/STAT. These cascades are initiated by viral challenge and induce the transcription of distinct factors to eliminate the infected viruses.

conserved in the inhibitors. Upon immune challenge, IκBs are phosphorylated and then degraded, leading to the nuclear translocation of the transcription factors (Hetru and Hoffmann, 2009).

The *Microplitis demolitor* virus encodes a family of genes with homology to IκB proteins from insects and mammals. In *Drosophila,* two proteins of this family, H4 and N5, are able to suppress the expression of AMPs by blocking the translocation of NF-κBs to the nucleus. H4 and N5 bind to Dif and Relish, transcription factors of Toll and Imd signaling pathways respectively. Because H4 and N5 are insensitive to host signaling factors that regulate phosphorylation, degradation, or cleavage after immune challenge, they could function as irreversible inhibitors of NF-κBs (Thoetkiattikul et al., 2005).

4. Conclusions

Despite recent progress in understanding the fly antiviral immunity, numerous unanswered questions remain. The antiviral role of miRNA and siRNA pathways has changed our perception of host-virus interactions; however, the molecular basis of this interplay is still far from complete. Consequently, more detailed studies of host antiviral response and viral infection strategies are crucial, particularly the development of *in vivo* models (Fig. 3). Today most antiviral drugs designed in the laboratories operate directly against virally encoded proteins, making the implementation of a host-oriented pharmacological approach necessary. For this purpose *Drosophila* represents a fantastic model to facilitate further genetic and molecular dissection of the antiviral response and to help elucidating the human immune response.

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References

Aggarwal, K., Rus, F., Vriesema-Magnuson, C., Erturk-Hasdemir, D., Paquette, N., Silverman, N., 2008. Rudra interrupts receptor signaling complexes to negatively regulate the IMD pathway. PLoS Pathog 4, e1000120.

Aliyari, R., Wu, Q., Li, H.W., Wang, X.H., Li, F., Green, L.D., Han, C.S., Li, W.X., Ding, S.W., 2008. Mechanism of induction and suppression of antiviral immunity directed by virus-derived small RNAs in Drosophila. Cell Host Microbe 4, 387–397.

Avadhanula, V., Weasner, B.P., Hardy, G.G., Kumar, J.P., Hardy, R.W., 2009. A novel system for the launch of alphavirus RNA synthesis reveals a role for the Imd pathway in arthropod antiviral response. PLoS Pathog 5, e1000582.

Bond, D., Foley, E., 2009. A quantitative RNAi screen for JNK modifiers identifies Pvr as a novel regulator of Drosophila immune signaling. PLoS Pathog 5, e1000655.

Costa, A., Jan, E., Sarnow, P., Schneider, D., 2009. The Imd pathway is involved in antiviral immune responses in Drosophila. PLoS One 4, e7436.

Ding, S.W., Voinnet, O., 2007. Antiviral immunity directed by small RNAs. Cell 130, 413–426.

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J.A., Imler, J.L., 2005. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nat. Immunol. 6, 946–953.

Ferrandon, D., Imler, J.L., Hetru, C., Hoffmann, J.A., 2007. The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. Nat. Rev. Immunol. 7, 862–874.

Fullaondo, A., Garcia-Sanchez, S., Sanz-Parra, A., Recio, E., Lee, S.Y., Gubb, D. (2011). Spn1 regulates the GNBP3-dependent Toll-signalling pathway in Drosophila melanogaster. Mol. Cell. Biol.

Fullaondo, A., Lee, S.Y. (2011). Identification of putative miRNA involved in Drosophila melanogaster immune response. Dev. Comp. Immunol.

Ganguly, A., Jiang, J., Ip, Y.T., 2005. Drosophila WntD is a target and an inhibitor of the Dorsal/Twist/Snail network in the gastrulating embryo. Development 132, 3419–3429.

Gregory, B.D., O'Malley, R.C., Lister, R., Urich, M.A., Tonti-Filippini, J., Chen, H., Millar, A.H., Ecker, J.R., 2008. A link between RNA metabolism and silencing affecting Arabidopsis development. Dev. Cell 14, 854–866.

Gruber, J.J., Zatechka, D.S., Sabin, L.R., Yong, J., Lum, J.J., Kong, M., Zong, W.X., Zhang, Z., Lau, C.K., Rawlings, J., Cherry, S., Ihle, J.N., Dreyfuss, G., Thompson, C.B., 2009. Ars2 links the nuclear cap-binding complex to RNA interference and cell proliferation. Cell 138, 328–339.

Grundhoff, A., Sullivan, C.S., 2011. Virus-encoded microRNAs. Virology 411, 325–343.

Han, J., Ulevitch, R.J., 2005. Limiting inflammatory responses during activation of innate immunity. Nat. Immunol. 6, 1198–1205.

Hetru, C., Hoffmann, J.A., 2009. NF-kappaB in the immune response of Drosophila. Cold Spring Harb Perspect Biol 1, a000232.

Hussain, M., Taft, R.J., Asgari, S., 2008. An insect virus-encoded microRNA regulates viral replication. J. Virol. 82, 9164–9170.

Huszar, T., Imler, J.L., 2008. Drosophila viruses and the study of antiviral host-defense. Adv. Virus Res. 72, 227–265.

Khurana, J.S., Theurkauf, W., 2010. PiRNAs, transposon silencing, and Drosophila germline development. J. Cell. Biol. 191, 905–913.

Lazzaro, B.P., 2008. Natural selection on the Drosophila antimicrobial immune system. Curr. Opin. Microbiol. 11, 284–289.

Lemaitre, B., Hoffmann, J., 2007. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. 25, 697–743.

Levashina, E.A., Langley, E., Green, C., Gubb, D., Ashburner, M., Hoffmann, J.A., Reichhart, J.M., 1999. Constitutive activation of toll-mediated antifungal defense in serpin-deficient Drosophila. Science 285, 1917–1919.

Li, H.W., Ding, S.W., 2005. Antiviral silencing in animals. FEBS Lett. 579, 5965–5973.

Macfarlane, L.A., Murphy, P.R., 2010. MicroRNA: Biogenesis, Function and Role in Cancer. Curr. Genomics 11, 537–561.

Maillet, F., Bischoff, V., Vignal, C., Hoffmann, J., Royet, J., 2008. The Drosophila peptidoglycan recognition protein PGRP-LF blocks PGRP-LC and IMD/JNK pathway activation. Cell Host Microbe 3, 293–303.

Okamura, K., Robine, N., Liu, Y., Liu, Q., Lai, E.C., 2010. R2D2 organizes small regulatory RNA pathways in Drosophila. Mol. Cell. Biol. 31, 884–896.

Pfeffer, S., Zavolan, M., Grasser, F.A., Chien, M., Russo, J.J., Ju, J., John, B., Enright, A.J., Marks, D., Sander, C., Tuschl, T., 2004. Identification of virus-encoded microRNAs. Science 304, 734–736.

Sabin, L.R., Zhou, R., Gruber, J.J., Lukinova, N., Bambina, S., Berman, A., Lau, C.K., Thompson, C.B., Cherry, S., 2009. Ars2 regulates both miRNA- and siRNA-dependent silencing and suppresses RNA virus infection in Drosophila. Cell 138, 340–351.

Shelly, S., Lukinova, N., Bambina, S., Berman, A., Cherry, S., 2009. Autophagy is an essential component of Drosophila immunity against vesicular stomatitis virus. Immunity 30, 588–598.

Singh, J., Singh, C.P., Bhavani, A., Nagaraju, J., 2010. Discovering microRNAs from Bombyx mori nucleopolyhedrosis virus. Virology 407, 120–128.

Thoetkiattikul, H., Beck, M.H., Strand, M.R., 2005. Inhibitor kappaB-like proteins from a polydnavirus inhibit NF-kappaB activation and suppress the insect immune response. Proc. Natl. Acad. Sci. USA 102, 11426–11431.

Umbach, J.L., Cullen, B.R., 2009. The role of RNAi and microRNAs in animal virus replication and antiviral immunity. Genes Dev. 23, 1151–1164.

Van Rij, R.P., Saleh, M.C., Berry, B., Foo, C., Houk, A., Antoniewski, C., Andino, R., 2006. The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in Drosophila melanogaster. Genes Dev. 20, 2985–2995.

Zambon, R.A., Nandakumar, M., Vakharia, V.N., Wu, L.P., 2005. The Toll pathway is important for an antiviral response in Drosophila. Proc. Natl. Acad. Sci. USA 102, 7257–7262.