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The Fly Way of Antiviral Resistance and Disease Tolerance

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Abstract

Like humans, insects face the threat of viral infection. Despite having repercussions on human health and disease, knowledge gaps exist for how insects cope with viral pathogens. *Drosophila melanogaster* serves as an ideal insect model due to its genetic tractability. When encountering a pathogen, two major approaches to fight disease are resistance strategies and tolerance strategies. Disease resistance strategies promote the health of the infected host by reducing pathogen load. Multiple disease resistance mechanisms have been identified in *Drosophila*: RNA interference, Jak/STAT signaling, Toll signaling, IMD signaling, and autophagy. Disease tolerance mechanisms, in contrast, do not reduce pathogen load directly, but rather mitigate the stress and damage incurred by infection. The main benefit of tolerance mechanisms may therefore be to provide the host with time to engage antiviral resistance mechanisms that eliminate the threat. In this review, antiviral resistance mechanisms used by *Drosophila* will be described and compared to mammalian antiviral mechanisms. Disease tolerance will then be explained in a broader context as this is a burgeoning field of study.

1. INTRODUCTION

Arthropod-borne diseases are caused by pathogens that are able to thrive within an insect vector and a mammalian host. The study of these types of pathogens therefore requires a multi-host perspective of infection, yet modern biomedical research into such diseases has focused on the mammalian host. This type of approach runs the risk of scientists missing a vast swathe of the pathogen life cycle. In the context of arthropod-borne diseases, viruses are the most commonly studied infectious agent. For example, over the last two decades, research into Zika, Chikungunya, and Dengue fever viruses has increased substantially. Each of these viruses is transmitted to humans via the bite of mosquitoes. To decipher these complex interactions between pathogens and arthropods, researchers have increasingly turned to the fruit fly *Drosophila melanogaster* as a genetically tractable host. In this review article, we discuss the mechanisms and consequences of diverse antiviral defenses utilized in fruit flies.

We first focus on disease resistance mechanisms that prevent viral replication. A specific focus is given to RNA interference (RNAi), Jak/STAT signaling, IMD signaling, Toll signaling, and autophagy, which can lower viral replication rates in a pathogen-specific

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manner. A comparison to mammalian innate immune pathways will be done to highlight similarities and differences. Such comparative analyses provide an opportunity to determine gaps in our current knowledge and speculate on future discoveries.

After reviewing antiviral mechanisms, the remainder of this review will describe our current knowledge of disease tolerance mechanisms. Disease tolerance mechanisms are strategies an organism utilizes to maintain fitness during times of duress. While disease resistance mechanisms are focused upon reducing pathogen load to promote the health of the infected organism, disease tolerance mechanisms promote health without necessarily affecting the pathogen load. Disease tolerance mechanisms identified so far are quite limited. Thus, what is known in both plants and animals will be reviewed. Altogether, this review hopes to encompass the strategies taken by arthropods to counteract the deleterious effects of viral pathogens.

2. RNAi AS AN ANTIVIRAL DEFENSE MECHANISM IN INSECTS

RNAi is used as an antiviral defense strategy in arthropods, plants, fungi, and possibly mammals (Abubaker, Abdalla, Mahmud, & Wilkie, 2014). RNAi pathways exist in arthropods, and each has a distinct function. The microRNA (miRNA) pathway regulates endogenous gene expression. The piwi-interacting RNA (piRNA) pathway inhibits the activity of transposons embedded in the host genome. The small interfering RNA (siRNA) pathway detects viral nucleic acids and restricts viral replication. All three RNAi pathways are used to reduce gene expression at the transcript level, and they utilize similar mechanisms. The siRNA pathway will primarily be discussed since the siRNA pathway is the only one of the three currently thought to restrict viral infection (Petit et al., 2016; Zambon, Vakharia, & Wu, 2006).

The overall strategy to restrict viral replication by the siRNA pathway requires multiple steps (Fig. 1). Long, double-stranded RNA (dsRNA) derived from viral sequences must be recognized by Dicer 2 (Dcr2) (Lee et al., 2004). This RNA binding is mediated cooperatively with the accessory protein R2D2 (Liu, Jiang, Kalidas, Smith, & Liu, 2006). Upon RNA binding to the Dcr2/R2D2 complex, Dcr2 dices RNA to a 21-nucleotide length duplex with a 2-nucleotide overhang (Elbashir, Lendeckel, & Tuschl, 2001). Dcr2 then loads one RNA strand into Argonaute 2 (Ago2). Finally, Ago2 finds complementary RNA within the cytosol and sequesters or cleaves it to inhibit translation. Because RNAi is an important tool used in genetic screens, the molecular mechanisms of how translation inhibition is mediated have been a focus of research. However, questions remain to be answered, especially with regard to how viral infections are detected and restricted.

In *Drosophila*, the siRNA pathway resists infection by multiple classes of viruses. Of the viruses composed of an RNA genome, the siRNA pathway can inhibit infection by non-enveloped viruses and enveloped, single-stranded RNA viruses of both polarities (negative sense and positive sense) (Galiana-Arnoux, Dostert, Schneemann, Hoffmann, & Imler, 2006). Double-stranded RNA virus replication is also inhibited by the siRNA pathway (Zambon et al., 2006). In *Drosophila*, the DNA virus, invertebrate iridescent virus 6 (IIV-6), is restricted by the siRNA pathway (Bronkhorst et al., 2012). Depending on the type of

virus, different replication strategies are utilized. For example, the cellular compartments utilized for viral replication may be different depending on the type of virus. Despite these viral pathogens having various mechanisms for replication, the siRNA pathway can restrict them. The broad applicability of the siRNA pathway to many types of viruses might be facilitated by the generation of viral DNA, which can be transcribed to generate RNAs that are substrates for Dcr2. Endogenous reverse transcriptases encoded by retrotransposons can generate viral DNA (vDNA) from defective viral genomes (Goic et al., 2013; Poirier et al., 2018; Tassetto, Kunitomi, Tassetto, Kunitomi, & Andino, 2017). vDNA generation is Dcr2-dependent and utilizes a function distinct from its dicing activity (Poirier et al., 2018). The helicase domain of Dcr2 potentially serves as a pattern recognition receptor for viral transcripts that can be reverse transcribed into vDNA (Poirier et al., 2018). The exact mechanisms allowing for broad pathogen recognition and leading to reverse transcription of viral sequences into DNA have yet to be determined. Nonetheless, the long-lasting vDNA can serve as a template in the production of siRNA to restrict viral replication.

In addition to Dcr2, other proteins are necessary for dsRNA recognition, processing, and loading into Ago2 (Iwasaki et al., 2015). Loquacious was identified in vivo to serve a non-redundant role in facilitating the dicing of dsRNA to create a duplex of the correct length for Ago2 loading (Marques et al., 2009). R2D2 is essential in RNA binding and processing activities (Liu et al., 2006). The binding preference of R2D2 determines which strand of the dsRNA is the passenger strand and which is the guide strand that is loaded onto Ago2 (Tomari, Matranga, Haley, Martinez, & Zamore, 2004). R2D2 orients the dsRNA for loading into Ago2 depending upon the relative binding strength of each 5' end (Tomari et al., 2004). The dsRNA is then unwound, and the guide strand is loaded into Ago2 to create the RNAi silencing complex (RISC) (Tomari et al., 2004). A complex called C3PO, composed of Translin and Trax, facilitates the unwinding and subsequent removal of cleaved passenger strand RNA (Liu et al., 2009). RISC is then ready to silence viral mRNA transcripts.

After an RNA strand is loaded into Ago2 forming RISC, Ago2 searches for complementary RNA sequences to silence. Gene silencing can occur by one of two ways: slicing or obstruction of translation elongation of mRNA transcripts. Ago2 slicing activity requires that sequences are complementary to the siRNA, particularly the middle sequence region where slicing occurs (Elbashir, Lendeckel, et al., 2001; Elbashir, Martinez, Patkaniowska, Lendeckel, & Tuschl, 2001). The cleaved mRNA can no longer be used for translation and is quickly degraded due to a lack of a 5' cap or a 3' poly-A tail (Elbashir, Lendeckel, et al., 2001). If the mRNA cannot be sliced, RISC can still interfere with translation by obstructing translation elongation (Olsen & Ambros, 1999). These RISC-bound transcripts which can no longer be translated are then transported to P-bodies where they are degraded (Eulalio, Behm-Ansmant, Schweizer, & Izaurralde, 2007).

RNAi is a cell intrinsic mechanism of gene regulation, and the siRNA pathway specifically restricts the expression of viral genes inside the cell. However, the siRNA pathway uniquely functions in a cell extrinsic manner as well. Using a recombinant sindbis virus (SINV) expressing a GFP reporter gene, gene silencing was discovered to outpace the spread of virus, suggesting that RNAi targeting specific genes can be transferred from one cell to another (Saleh et al., 2009). Additional studies suggest that RISC can be transported

between cells via nanotube-like structures and that RNA binding proteins could mediate the endocytosis of the RNA (Karlikow et al., 2016; Saleh et al., 2006). Infected cells can also secrete siRNA via exosome-like vesicles to promote a systemic immune response (Tassetto et al., 2017). Thus, the intercellular spread of siRNA pathway components could prepare uninfected cells against potential viral infection.

The importance of the RNAi pathway in antiviral defense is highlighted by viral evasion mechanisms used to thwart the host. The B2 protein is encoded by flock house virus and efficiently inhibits RNAi activity by two mechanisms (Li, Li, & Ding, 2002). First, B2 protein can bind dsRNA, thereby inhibiting Dcr2 recognition (Chao et al., 2005; Lingel, Simon, Izaurralde, & Sattler, 2005). Second, B2 can directly interact with Dcr2 via its helicase domain in order to prevent recognition of viral RNA (Singh et al., 2009). In addition to B2, other insect viruses encode proteins that inhibit RNAi. *Drosophila* C virus also encodes an RNAi suppressor gene that binds to dsRNA (van Rij et al., 2006). Cricket paralysis virus encodes the 1a protein which antagonizes Ago2 function (Nayak et al., 2010). A more comprehensive discussion of viral strategies can be found elsewhere (Marques & Imler, 2016). Mechanisms to inhibit siRNA function demonstrate the importance of RNAi in resisting viral infection.

3. Jak/STAT-DEPENDENT SIGNAL TRANSDUCTION AND ANTIVIRAL IMMUNITY

The Jak/STAT signaling pathway was originally identified in efforts to understand Type I interferon signaling in mammals (Stark & Darnell, 2012). Type I interferons are signaling molecules that induce the transcription of genes whose products antagonize viral replication in numerous ways, thus creating an antiviral cellular state (Raftery & Stevenson, 2017). Signaling is initiated when extracellular interferon binds to the interferon receptor at the plasma membrane. In order for downstream signaling to occur, a class of cytoplasmic proteins called Janus kinases (Jaks) must become activated. Jaks are associated with signaling receptors via protein-protein interactions, and they operate in pairs to phosphorylate each other, thus becoming activated and poised to activate downstream signal transducer and activator of transcription (STAT) proteins via phosphorylation (Argetsinger et al., 1993; Muller et al., 1993). Activated STAT proteins serve as transcription factors, translocating to the nucleus to initiate gene transcription. In addition to playing a critical role in Type I interferon signaling, Jak and STAT proteins also mediate signal transduction downstream of Type II and Type III interferons and cytokines (Banerjee, Biehl, Gadina, Hasni, & Schwartz, 2017; Lin & Young, 2014).

In contrast to mammals which have four Jaks and seven STATs, *Drosophila* has one Jak and one STAT, hopscotch and STAT92E, respectively (Fig. 2) (Binari & Perrimon, 1994; Hou, Melnick, & Perrimon, 1996; Yan, Small, Desplan, Dearolf, & Darnell, 1996). Only one receptor, domeless, has been identified to activate hopscotch and STAT92E (Brown, Hu, & Hombria, 2001; Chen et al., 2002). The Jak/STAT pathway in *Drosophila* is important for development and tissue homeostasis, particularly in regulating cell proliferation (Zeidler, Bach, & Perrimon, 2000). Like mammalian Jak/STAT signaling, the *Drosophila* counterpart

is involved in hematopoiesis (Hanratty & Dearolf, 1993; Harrison, Binari, Nahreini, Gilman, & Perrimon, 1995; Luo, Hanratty, & Dearolf, 1995). Additionally, it is involved in cellular proliferation in the fly gut, which undergoes a high rate of cellular turnover (Jiang et al., 2009). While interferons do not exist in flies, three cytokine-like molecules have been identified to activate the receptor domeless: unpaired, unpaired2 and unpaired3 (upd, upd2, and upd3, respectively) (Agaisse, Petersen, Boutros, Mathey-Prevot, & Perrimon, 2003; Harrison, McCoon, Binari, Gilman, & Perrimon, 1998). The differences between these signaling molecules are still unclear, but it has been proposed that they have differential binding affinities for domeless and differential binding for the extracellular matrix (Wright, Vogt, Smythe, & Zeidler, 2011). A fourth potential ligand, vago, induces Jak/STAT signaling in mosquitoes (Paradkar, Trinidad, Voysey, Duchemin, & Walker, 2012). Vago mediates antiviral activity in *Drosophila*, but it has yet to be confirmed to induce Jak/STAT signaling in flies as well (Deddouche et al., 2008).

The antiviral function of the Jak/STAT pathway in insects is an area of active investigation. *Drosophila* C virus infection induces Jak/STAT signaling, and loss of function mutations in Jak result in decreased viability of infected flies (Dostert et al., 2005). Similarly, flies with mutations in the Jak/STAT pathway are also more susceptible to infection by cricket paralysis virus, *Drosophila* X virus, and IIV-6 (Kemp et al., 2013; West & Silverman, 2018). While Jak/STAT signaling is required to maintain viability during viral infection, work with *Drosophila* C virus and IIV-6 suggests that Jak/STAT signaling works in concert with additional signaling pathways (Dostert et al., 2005; West & Silverman, 2018). The antiviral role of Jak/STAT signaling is intriguing because different viruses are variably sensitive to this pathway. Flock house virus, SINV, and vesicular stomatitis virus (VSV) are unaffected by the Jak/STAT pathway (Kemp et al., 2013). In comparison to the broadly antiviral pathway of RNAi, Jak/STAT signaling is selectively beneficial. The reasoning for this selectivity is an interesting avenue for future work.

4. ANTIVIRAL ACTIVITIES OF THE TOLL RECEPTOR

The Toll pathway was originally discovered in a forward-genetic screen for genes involved in dorsal-ventral patterning of the *Drosophila* embryo (Anderson, Bokla, & Nüsslein-Volhard, 1985; Anderson, Jürgens, & Nüsslein-Volhard, 1985; Nüsslein-Volhard, 1980). Later, Toll pathway mutants were discovered to be susceptible to fungal and bacterial infections (Lemaitre, Nicolas, Michaut, Reichhart, & Hoffmann, 1996). In *Drosophila*, the Toll receptor is activated by the extracellular ligand, spätzle (Anderson & Nüsslein-Volhard, 1984), which is cleaved into its active form by proteases (Morisato & Anderson, 1994; Schneider, Jin, Morisato, & Anderson, 1994; Stein & Nüsslein-Volhard, 1992). The proteases are activated by peptidoglycan recognition proteins (PGRPs) that recognize microbe-derived molecules (Bischoff et al., 2004; Garver, Wu, & Wu, 2006; Michel, Reichhart, Hoffmann, & Royet, 2001). Downstream of the Toll receptor, protein-protein interactions mediate signaling. *Drosophila* MyD88 (dMyD88) is localized to the plasma membrane via protein-lipid interactions and binds to the cytoplasmic tail of the Toll receptor which recruits tube and pelle (Horng & Medzhitov, 2001; Marek & Kagan, 2012; Sun, Bristow, Qu, & Wasserman, 2002; Sun, Towb, Chiem, Foster, & Wasserman, 2004). Pelle is a serine/threonine kinase that mediates the phosphorylation and degradation of cactus (Hecht

& Anderson, 1993; Shelton & Wasserman, 1993). Cactus inhibits the NF- κ B transcription factors, dorsal and dorsal-related immunity factor (DIF) (Kidd, 1992). DIF and dorsal mediate the anti-microbial immune response and activate genes that define dorsal-ventral patterning of the embryo, respectively (Anderson, Bokla, et al., 1985; Lemaitre et al., 1996). Thus, the two transcription factors differentiate the functions of Toll signaling.

Toll signaling is involved in some antiviral responses (Fig. 3). *Drosophila* X virus infection induces the production of antimicrobial peptides (Zambon, Nandakumar, Vakharia, & Wu, 2005). Further analysis demonstrated that a loss of function mutant in DIF and a constitutively active mutant of the Toll receptor resulted in poorer survival outcomes compared to wild type animals (Zambon et al., 2005). Both loss of function and constitutive signaling of the Toll pathway resulted in decreased viability of infected animals. Thus, in the context of *Drosophila* X virus infection, the role of Toll signaling is complicated and requires further study. Toll signaling also seems to be involved in viral infection via the oral route. When flies were fed *Drosophila* C virus, Toll pathway mutants succumbed to infection faster than wild type animals (Ferreira et al., 2014). Similar survival defects were also observed with flock house virus, cricket paralysis virus, and Nora virus oral infections (Ferreira et al., 2014). While an increasing number of viral infections appear to be modulated by the Toll pathway in flies, many questions remain. For example, it is unclear how the Toll pathway is activated during these infections, as the upstream receptors that stimulate the spätzle cleavage cascade are classically viewed as sensors of bacterial and fungal infections. Commensal bacteria that occupy the fly intestine may therefore contribute to Toll activities during viral infections.

Homologs of the Toll receptor exist, numbered 2 through 9. These additional Toll receptors have been studied for their potential involvement in immune defense. Toll-7 had been implicated in inducing autophagy downstream of VSV and Rift Valley Fever virus (Moy et al., 2013; Nakamoto et al., 2012), although not all studies support this conclusion (Lamiable, Arnold, et al., 2016). Additionally, Toll-2 gene silencing resulted in increased VSV infectivity of S2 cells compared to control knockdowns (Nakamoto et al., 2012). To elucidate potential immune functions, the Toll receptors have been genetically modified by replacing their ectodomain with that of a constitutively active Toll mutant ectodomain. Only Toll-5 was observed to express the antimicrobial peptide drosomycin (Tauszig, Jouanguy, Hoffmann, & Imler, 2000). In an independent study, Toll-3, Toll-5 and Toll-9 induced antimicrobial peptides (Ooi, Yagi, Hu, & Ip, 2002). Although a second paper confirmed an immunological role for Toll-9 (Bettencourt, Tanji, Yagi, & Ip, 2004), a third study suggested that no defects were present in Toll-9 mutant flies (Narbonne-Reveau, Charroux, & Royet, 2011). Toll-2 mutants are defective in activating the transcription factor DIF for expression of the antimicrobial peptide attacin (Williams, Rodriguez, Kimbrell, & Eldon, 1997). In trachea, Toll-8 had been found to negatively regulate antimicrobial peptide production (Akhrouayri, Turc, Royet, & Charroux, 2011).

In addition to their potential roles in immune defense, the Toll receptors regulate development and mediate cell adhesion. Toll-2 has been implicated in the development of legs, salivary glands, fat body, and follicle cells (Eldon et al., 1994; Kleve, Siler, Syed, & Eldon, 2006; Kolesnikov & Beckendorf, 2007; Ligoxygakis, Bulet, & Reichhart, 2002).

Toll-8 is involved in neuronal glycosylation and leg development, and it antagonizes the function of decapentaplegic during wing formation (Kim, Chung, Yoon, Choi, & Yim, 2006; Seppo, Matani, Sharrow, & Tiemeyer, 2003; Yagi, Nishida, & Ip, 2010). Toll-6 and Toll-7 have been found to cooperate in the wiring of the olfactory system (Ward, Hong, Favaloro, & Luo, 2015). Only Toll-4 has no identified function. Given that Toll signaling has dual roles in development and immunity, the other Toll receptor family members might have undiscovered functions. Even Toll, Toll-2, and Toll-7, which have already been implicated in antiviral immunity, require further study to understand their mechanisms of action.

5. IMD-MEDIATED ANTIVIRAL DEFENSES

The IMD pathway is another *Drosophila* immune defense mechanism. IMD signaling confers protection against bacterial pathogens by activating the transcription of antimicrobial peptides (Lemaitre et al., 1995). In addition to its role during bacterial infection, the IMD pathway also affects the health of animals at steady state. In the *Drosophila* gut, the IMD pathway maintains homeostasis of the barrier epithelium with its associated commensal bacteria (Bosco-Drayon et al., 2012; Ryu et al., 2008). The IMD pathway also maintains the health of epithelia within the trachea, the respiratory tract of insects (Tzou et al., 2000). In the nervous system, some IMD pathway components such as DREDD and Relish have been implicated in mediating neurodegeneration (Cao, Chtarbanova, Petersen, & Ganetzky, 2013; Chinchore, Gerber, & Dolph, 2012; Petersen, Katzenberger, & Wassarman, 2013). Hyperactive signaling of the IMD pathway associated with aging can lead to shortened lifespan, similar to chronic inflammation in mammals (Landis et al., 2004; Pletcher et al., 2002; Valtonen, Kleino, Ramet, & Rantala, 2010; Zerofsky, Harel, Silverman, & Tatar, 2005). Thus, the IMD pathway is important during both healthy and diseased states.

Sensing of bacteria triggers a signaling cascade resulting in the activation of Relish, an NF- κ B-family transcription factor. To initiate the IMD signaling pathway, the receptors PGRP-LE and PGRP-LC detect bacteria-derived peptidoglycans (Kaneko et al., 2006; Lim et al., 2006; Stenbak et al., 2004; Takehana et al., 2002, 2004; Werner et al., 2000). PGRP-LC has multiple splice variants, resulting in transmembrane proteins that all have the same cytoplasmic and transmembrane domains but different PGRP ectodomains (Werner, Borge-Renberg, Mellroth, Steiner, & Hultmark, 2003). Unlike PGRP-LC, PGRP-LE is not anchored to the plasma membrane (Kaneko et al., 2006; Werner et al., 2000). Intracellular bacteria such as *Listeria monocytogenes* can be detected by PGRP-LE, but the detection of extracellular bacteria by PGRP-LE can also occur via unclear mechanisms (Kaneko et al., 2006; Yano et al., 2008). Recognition of bacterial ligands by PGRP-LC or PGRP-LE leads to the activation of IMD by forming amyloid fibrils between the receptors and IMD (Kleino et al., 2017). Homotypic death domain interactions between IMD and dFADD lead to dFADD activation (Naitza et al., 2002). dFADD then recruits and activates the caspase DREDD (Hu & Yang, 2000). Activated DREDD has proteolytic activity that cleaves IMD and the inhibitory domain of Relish (Erturk-Hasdemir et al., 2009; Leulier, Rodriguez, Khush, Abrams, & Lemaitre, 2000; Paquette et al., 2010; Stoven et al., 2003; Stöven, Ando, Kadalayil, Engström, & Hultmark, 2000). The cleaved IMD protein proceeds to activate the mitogen activated protein kinase (MAPK), Jun kinase (JNK), via dTAK1. Cleaved Relish,

which is released from its inhibitory domain, translocates to the nucleus to induce gene expression (Silverman et al., 2003).

Although the IMD pathway has been mainly studied for its antibacterial properties, this pathway may also serve as an antiviral defense (Fig. 4). Flies with genetic mutations in *relish*, *PGRP-LC*, *dTak1*, *ird5*, and *kenny*, all of which belong in the IMD pathway, were more susceptible to cricket paralysis virus infection (Costa, Jan, Sarnow, & Schneider, 2009). The IMD pathway also restricts SINV replication (Avadhanula, Weasner, Hardy, Kumar, & Hardy, 2009). In a subsequent study, dipterin B, an antimicrobial peptide induced by the IMD pathway, protected against SINV infection (Huang, Kingsolver, Avadhanula, & Hardy, 2013). Whether additional genes regulated by the IMD pathway also conferred resistance was unclear, and the mechanism of how dipterin B inhibited infection remains to be examined. A conflicting report did not find *kenny* and IMD mutant flies to have decreased survival compared to wild type flies during SINV infection (Lamiable, Kellenberger, et al., 2016). Contrary to the previous work, a knockout of *diedel*, a negative regulator of the IMD pathway, resulted in decreased survival of SINV infected flies (Lamiable, Kellenberger, et al., 2016). This finding would suggest that activating the IMD pathway during SINV infection results in poorer outcomes for the flies. However, a group of insect DNA viruses were found to encode genes homologous to *diedel*, a negative regulator of the IMD pathway (Lamiable, Kellenberger, et al., 2016). The existence of viral mimics would suggest that the pathway to which the host gene belongs is important in regulating viral infection. Although the IMD pathway seems to have an effect on viral infections, the consequences of IMD signaling are not entirely clear and may be virus-specific.

6. AUTOPHAGY AND ANTIVIRAL DEFENSE

Autophagy is a cellular process whereby cytoplasmic contents can be captured and degraded in lysosomes (Zirin & Perrimon, 2010). A phagophore, also known as an isolation membrane, forms, elongates and engulfs the cargo to be degraded. In the process, the characteristic double membrane of autophagosomes forms. Autophagy has been studied in model organisms such as yeast, flies, and mice, and the genes and processes involved are highly conserved (Reggiori & Klionsky, 2002). The use of multiple species to study autophagy has resulted in an effort to standardize the nomenclature (Klionsky et al., 2011, 2003).

Many proteins are involved in autophagy. Initiation of autophagy involves a complex of proteins including Atg1 and Atg13 which become activated by upstream signals such as metabolic shifts (Kamada et al., 2000; Melendez et al., 2003; Scott, Schuldiner, & Neufeld, 2004). The activation of the Atg1 complex leads to the activation of another complex of proteins including Vps34, which produces phosphatidylinositol-3-phosphate (Simonsen & Tooze, 2009). This step results in the nucleation of the phagophore which in turn recruits more proteins such as Atg18 (Berry & Baehrecke, 2007; Pace et al., 2002). Once the phagophore has been created, it must elongate in order to engulf its intended cargo. This elongation step is mediated by two conjugation systems. In the first conjugation system Atg7 and Atg10 mediate covalent binding of Atg5 to Atg12, and Atg5 and Atg12 form a complex with Atg16 (Fujita et al., 2008; Kuma, Mizushima, Ishihara, & Ohsumi, 2002; Mizushima et

al., 1998; Shintani et al., 1999). The second conjugation system links Atg8 to phosphatidylethanolamine (PE) via an amide bond (Ichimura et al., 2000). The conjugation of Atg8 to PE involves Atg4, Atg7, and Atg3 which bind and transfer Atg8 akin to the ubiquitin ligation system (Kirisako et al., 2000). Once the phagophore completely envelops its cargo, the autophagosome fuses with endosomes and then lysosomes to degrade the contents.

In *Drosophila*, autophagy was demonstrated to be an antiviral defense (Fig. 5). A genomic screen in the *Drosophila* S2 cell line revealed that VSV infection was poorly restricted in cells where autophagy genes were knocked down (Shelly, Lukinova, Bambina, Berman, & Cherry, 2009). When flies were infected, knockdown or mutation of autophagy genes resulted in worse survival outcomes compared to control flies (Shelly et al., 2009). In a subsequent study, Toll-7 was implicated in the detection of VSV glycoprotein, resulting in the induction of autophagy (Nakamoto et al., 2012). Although Toll-7 is homologous to Toll, downstream mediators of Toll signaling (dMyD88 and DIF) were not involved in restricting VSV infection, suggesting that Toll-7 induced autophagy noncanonically (Nakamoto et al., 2012). In addition to VSV, Rift Valley Fever virus infection is sensitive to autophagy as an antiviral defense (Moy et al., 2013). In the case of Rift Valley Fever virus, Toll-7 was also responsible for virus recognition and induction of autophagy (Moy et al., 2013). However, another group found no evidence for Toll-7 restricting VSV infection (Lamiable, Arnold, et al., 2016). However, this study did confirm that autophagy is an antiviral defense mechanism (Lamiable, Arnold, et al., 2016). Interestingly, flock house virus was less virulent when Atg7 was mutated, suggesting that some viruses can use the autophagy pathway to their advantage (Lamiable, Arnold, et al., 2016).

Recent work has also found autophagy to be involved in controlling Zika virus infection in flies (Liu et al., 2018). Although the mechanism for how Zika virus induces autophagy is unclear, *Drosophila* STING (dSTING) and the IMD pathway NF- κ B protein, Relish, are involved. The findings corroborate data published on *Listeria* infection of flies which suggests that dSTING activates IMD signaling to mount an antibacterial response (Martin, Hiroyasu, Guzman, Roberts, & Goodman, 2018). Martin et al. suggest that bacterial-derived cyclic dinucleotides activate dSTING, which also occurs with mammalian STING (Burdette et al., 2011). In the mammalian innate immune system, cyclic dinucleotides can be synthesized by the host when cytoplasmic DNA is sensed by the cyclic guanosine monophosphate-adenosine monophosphate synthase enzyme (cGAS) (Sun, Wu, Du, Chen, & Chen, 2013; Wu et al., 2013). However, the fly ortholog of cGAS lacks a critical DNA binding domain which would theoretically allow viral DNA sensing (Wu et al., 2014). How Zika virus is sensed to initiate STING-dependent autophagy remains to be determined. Many compelling questions about the mechanisms for inducing antiviral autophagy remain to be answered.

7. SIMILARITIES BETWEEN THE DROSOPHILA IMMUNE SYSTEM AND THE MAMMALIAN INNATE IMMUNE SYSTEM

As described above, *Drosophila* has numerous ways of fighting pathogens. Some of these disease resistance mechanisms are evolutionarily conserved across species. Mammals such as humans and mice share some of these disease resistance mechanisms in common with flies. Comparisons across species offer the opportunity to determine the most important features and strategies of the immune system. Also intriguing are the disease resistance mechanisms that have not been conserved between flies and mammals.

For example, the *Drosophila* Toll pathway shares similarities with its mammalian counterpart, the Toll-like receptors (TLRs). Broadly, both are used for the detection of microbes and result in the activation of NF- κ B proteins to initiate a transcriptional immune response. TLRs are expressed on the plasma membrane or endosomal compartments, directly detecting pathogen associated molecular patterns (PAMPs). In contrast, only Toll has been well characterized for its immune function, and detection of PAMPs by *Drosophila* Toll is indirect. An upstream receptor detects PAMPs and subsequently promotes the cleavage of the pro-form of spatzle by the spatzle processing enzyme in order to activate the Toll receptor. Thus, the *Drosophila* Toll receptor signaling pathway involves upstream mediators which are not involved in mammalian TLR signaling.

Downstream of TLRs are adaptor proteins that recruit kinases and additional factors, forming a supramolecular organizational center (SMOC) that initiates inflammatory and defense-associated gene expression (Kagan, Magupalli, & Wu, 2014). *Drosophila* Toll also requires adaptors, dMyD88 and tube, and a kinase, pelle. The mammalian and insect systems are similar in that they require protein-protein interactions mediated by protein domains such as the TIR and death domains. Interestingly, the functionality of the adaptor molecules seems to be conserved across species. TIRAP is a mammalian adaptor molecule localized to the plasma membrane and endosomes via lipid interaction, while MyD88 is cytoplasmic (Bonham et al., 2014; Kagan & Medzhitov, 2006). In the *Drosophila* system, dMyD88 is localized to the plasma membrane and tube is cytoplasmic (Marek & Kagan, 2012). Although the composition of structural domains between proteins in the mammalian and insect systems differs, the localization of the adaptor molecules and their recruitment of downstream kinases are conserved.

TLR signaling differs from *Drosophila* Toll signaling in that MAPKs are activated in mammals (Brown, Wang, Hajishengallis, & Martin, 2011; Wang et al., 2001). No MAPK pathway has been identified to be initiated downstream of *Drosophila* Toll signaling. However, MAPKs are indeed part of the response to pathogens in flies. In the IMD pathway, dTAK1 mediates signaling to JNK (Silverman et al., 2003). Another MAPK pathway, ERK, is involved in defense against VSV infection via the oral route (Xu et al., 2013). Thus, MAPK signaling is involved in the immune response in both insects and mammals but the signaling pathways might not function identically.

While TLRs survey the extracellular environment for potential pathogens, PRRs also exist inside the cytoplasm. One such family is the nucleotide-binding, leucine-rich repeat (NLR)

containing protein family. Some NLRs are involved in recognizing PAMPs and other danger signals such as potassium efflux (Muñoz-Planillo et al., 2013). Upon activation, PAMP-detecting NLRs oligomerize, forming specialized SMOCs called inflammasomes that ultimately lead to caspase activation. Caspases cleave proteins such as the cytokine pro-IL-1 β which is then secreted from the cell cytoplasm to the extracellular space to initiate an inflammatory response in the tissue. Although caspases exist in *Drosophila* and are involved in apoptotic cell death, NLRs do not exist in *Drosophila*. It is interesting to note that PGRP receptors upstream of the Toll receptor are responsible for direct detection of PAMPs. Their activation also leads to a protein cleavage event (spatzle) which then allows receptor binding (Toll) and downstream signaling. The cascade of events leading to spatzle cleavage is reminiscent of the activation of IL-1 β in mammals. Although spatzle activation occurs in the extracellular space and not in the cytoplasm, the two pathways are reminiscent of each other. While SMOC formation is speculative for the Toll pathway, PGRPs do oligomerize to form amyloid fibrils in the Imd pathway (Kleino et al., 2017).

Another family of mammalian cytoplasmic receptors is the RIG-I-like receptor (RLR) family. The RLR family includes RIG-I, MDA5, and LGP2 receptors. RIG-I and MDA5 recognize viral RNA, and LGP2 is a proposed regulator of signaling (Rothenfusser et al., 2005; Satoh et al., 2010; Venkataraman et al., 2007). Upon recognition of viral RNA, RLRs bind to the adaptor protein MAVS which is localized on mitochondria and peroxisomes (Dixit et al., 2010; Seth, Sun, Ea, & Chen, 2005). MAVS then recruits downstream signaling mediators which ultimately lead to the activation of the transcription factors IRF3, IRF7, and NF- κ B (Kawai et al., 2005). Activation of the RLR signaling pathway induces a transcriptional response that produces Type I and III interferons. In *Drosophila*, no orthologs of MAVS, IRF3, or interferons have been identified. While the RLR pathway is absent in flies, one group has drawn a comparison between Dcr2 and the RLR family. They each have closely related DExD/H-box helicase domains (Deddouche et al., 2008). Their common phylogenetic clade also includes drh1 which is a dicer protein that mediates antiviral immunity in *Caenorhabditis elegans* (Deddouche et al., 2008; Guo, Zhang, Wang, Ding, & Lu, 2013; Lu, Yigit, Li, & Ding, 2009). All these related proteins are involved in antiviral immunity in their respective species. Thus, the antiviral siRNA pathway in *Drosophila* might have some relationship to the mammalian RLR family.

The relationship between Dcr2 and RLR family proteins has been one aspect of a larger mystery. While RNAi has been established as an antiviral mechanism in plants, fungi, and arthropods, the relative importance of RNAi as an antiviral defense in mammals has been debated (Cullen, Cherry, & tenOever, 2013). One group found that in the absence of Drosha in mammalian cells, SINV titers increased (Shapiro et al., 2014). This effect was independent of Dicer and of Type I interferons (Shapiro et al., 2014). The absence of a role for Dicer would suggest a different mechanism from how RNAi antiviral defense is mediated in *Drosophila* and other species. Another study found that mice lacking Dcr1 were more susceptible to VSV infection than wild type mice (Otsuka et al., 2007). The researchers found that the susceptibility was due to a loss of miR24 and miR93 expression, which targeted VSV L transcripts, and that Dcr1 did not produce small RNAs from viral transcripts (Otsuka et al., 2007). In the case of VSV infection, the protection conferred by Dcr1 seems more happenstance instead of being a targeted mechanism directed against the

virus. Several studies using embryonic cells do support the idea of RNAi as an antiviral response in mammals. Early studies found that long dsRNA could be substrates for producing siRNA in embryonic cells lines (Billy, Brondani, Zhang, Muller, & Filipowicz, 2001; Paddison, Caudy, & Hannon, 2001). Later studies also supported the idea that embryonic cells could utilize the RNAi pathway as an antiviral defense. Those studies additionally demonstrated that virus-encoded B2 protein could suppress host production of siRNA (Li, Lu, Han, Fan, & Ding, 2013; Maillard et al., 2013). However, studies have not extended these findings to prove any significant relevance to differentiated cells. Indeed, others have noted that RNAi seems to function in cell types that have attenuated Type I interferon responses (Pare & Sullivan, 2014). In agreement with this hypothesis, another publication showed that RISC is modified via poly-ADP-ribosylation shortly after infection, inhibiting its silencing function in somatic cells (Seo et al., 2013). In fact, interferon-stimulated gene induction seemed to be acutely regulated by RNAi, suggesting an antagonistic relationship between the two (Seo et al., 2013). In support of the idea that RNAi and interferon signaling antagonize each other, a more recent publication took a different approach of introducing *Drosophila* siRNA genes into mammalian cells. Expression of *Drosophila* Dcr2 in HEK293 cells resulted in the production of viral RNAs of 21-nucleotide length, but viral replication was not suppressed (Girardi et al., 2015). Instead, *Drosophila* Dcr2 expression resulted in the disruption of interferon signaling (Girardi et al., 2015). Thus, if the RNAi pathway is an antiviral defense mechanism in mammals, it seems to be most relevant in cases where the interferon signaling pathway is attenuated.

While RNAi has been a point of contention, comparative analyses of DNA sensing between *Drosophila* and mammals have only begun. Direct DNA recognition in *Drosophila* has yet to be demonstrated. However, DNA virus infection is restricted by RNAi in *Drosophila* (Bronkhorst et al., 2012). As discussed, RNAi largely does not seem to operate in mammals as an antiviral defense. In contrast, detection of pathogen-derived DNA in the cytoplasm of mammalian cells is mediated by the cGAS/STING pathway (Chow, Franz, & Kagan, 2015). DNA in the cytoplasm is directly sensed by cGAS, resulting in the production of the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) (Sun et al., 2013; Wu et al., 2013). Binding of cGAMP to STING, localized to the ER, results in a signaling cascade that activates IRF3. Like in RLR signaling, Type I interferons are produced, leading to the induction of interferon-stimulated genes and the promotion of an antiviral state (Sun et al., 2013). Again, Type I interferons do not exist in *Drosophila*, although ligands do exist to induce Jak/STAT signaling. Additionally, the ortholog of cGAS in flies lacks a functional domain for DNA recognition (Wu et al., 2014). It is tantalizing to speculate that accessory proteins might act in concert with the cGAS ortholog to facilitate DNA recognition, especially since vDNA is produced by endogenous reverse transcriptases. Regardless of cGAS function, recent data suggest that the dSTING gene is immunostimulatory. dSTING has been found to induce autophagy during Zika virus infection (Liu et al., 2018). Autophagy-induction is an attribute common between mammalian and dSTING (Watson et al., 2015). In the context of bacterial infection using *L. monocytogenes*, dSTING utilizes IMD pathway components to activate the NF- κ B protein Relish (Martin et al., 2018). In downstream signaling mediators, dSTING is different from its mammalian counterpart because mammalian STING preferentially activates IRF3 instead of NF- κ B. Within

endosomal spaces, some mammalian cells have the capacity to sense DNA using TLR9 (Latz et al., 2004). Despite multiple Toll receptors in flies, none have been found to mediate DNA sensing.

Overall, the main area of overlap between the *Drosophila* immune system and the mammalian innate immune system has been the Toll and TLR pathways. The defined mediators of Toll signaling function similar to TLR signaling, but further comparisons between the two pathways hinge upon further study of the *Drosophila* system. The same is true for other innate immune pathways because the mammalian system is better defined. We have an increasing knowledge of RLRs, CLRs, NLRs, and the cGAS/STING pathway, but our knowledge of other innate immune systems is lagging. By understanding the immune strategies utilized by fruit flies, the field can move beyond the question of what immune defenses exist. Having the ability to compare different immune systems will allow us to start tackling the questions of what immune defenses are most effective and why.

8. DISEASE TOLERANCE

While host-pathogen interactions have mainly focused upon disease resistance mechanisms, as described above, alternative strategies exist for a host to mitigate an infection. Instead of applying mechanisms to restrict the growth of a pathogen, an infected host can employ disease tolerance mechanisms. Disease tolerance is the idea of altering homeostatic conditions to improve the health of the host without necessarily reducing the pathogen load. To differentiate disease resistance and disease tolerance one must recognize that the health of the organism is not always directly dependent upon pathogen load. Instead, the health of an organism is dependent upon its ability to mitigate the effects of damage and stress.

The idea of disease tolerance is a budding field that originally stemmed from studies of plant biology (Raberg, Graham, & Read, 2009). In one of the early studies to note disease tolerance, several varieties of winter wheat were compared for their yield, physical characteristics, and chemical composition when grown in the face of infection by the fungus *Puccinia triticina*. One particular variety of wheat, Fulhard, was noted to have comparable levels of infection as other varieties but still had the best grain yield (Caldwell, Kraybill, Sullivan, & Compton, 1934). The observation was confirmed by a separate group that also subjected the Fulhard wheat, along with other varieties of wheat, to *P. triticina* infection (Salmon & Laude, 1932). What was keenly noted by these researchers was that some varieties had reduced fungal infection and resultant healthier crops (disease resistance). Meanwhile, the Fulhard variety was struck with the highest pathogen load but still managed to yield the most grain (disease tolerance). Although the exact mechanism for disease tolerance was not identified, the hallmarks of disease tolerance were identified, namely the modulation of health without a dependency on changing pathogen load.

Some noteworthy principles about disease tolerance have arisen from plant studies. Plants have been noted for disease tolerance strategies such as increasing photosynthesis, increasing growth, and taking up more nutrients (Redondo-Gomez, 2013). These strategies help to offset the damage caused by a pathogen by increasing nutrient production and availability. Thus, disease tolerance is focused upon mitigating the effects of tissue damage.

Another important lesson about disease tolerance taken from plant studies is that stresses that result in damage can come from biotic and abiotic sources (Redondo-Gomez, 2013). Infection by pathogens such as fungi would fall in the category of biotic stress. Also within the category of biotic stresses would be herbivores, such as insects, that might consume parts of the plant. Interestingly, disease tolerance can be broadened in scope to include mechanisms that deal with abiotic stresses. Abiotic stresses would include environmental stresses such as high/low temperatures, salinity, heavy metal exposure, ultraviolet light damage, and drought. By noting that pathogenic organisms are only one category to induce disease tolerance mechanisms, one can appreciate that disease tolerance focuses on responding to damage caused by a stress, regardless of whether it is biotic or abiotic.

From plant biology, the concept of disease tolerance has spread into studies in the animal kingdom. Disease tolerance was first noted in the study of *Plasmodium chabaudi* infection of mice (Råberg, Sim, & Read, 2007). Several inbred strains of mice were infected with *P. chabaudi*, and red blood cell count and animal weight were used as indicators of organismal health (Råberg et al., 2007). Pathogen load was measured and correlated to red blood cell count and animal weight. Depending on the mouse strain, the same pathogen load resulted in differing effects on red blood cell count and weight, suggesting that disease tolerance mechanisms varied from one mouse strain to the other (Råberg et al., 2007).

Two other early studies confirming disease tolerance in animals utilized *Drosophila*. In one, a forward-genetic screen was performed to find genes important for survival during *L. monocytogenes* infection (Ayres, Freitag, & Schneider, 2008). When mutated, one category of identified genes resulted in poor survival without changes in bacterial growth, indicating that disease tolerance pathways had been impaired (Ayres et al., 2008). In the second study, infection by *Wolbachia* bacteria altered survival outcomes of flies additionally infected by virus (Teixeira, Ferreira, & Ashburner, 2008). In the case of flock house virus infection, elimination of *Wolbachia* resulted in poorer survival, while viral titers were minimally affected. IIV-6 had the opposite result. When *Wolbachia* was removed from flies by tetracycline treatment, IIV-6 infected flies had improved survival (Teixeira et al., 2008). While the effects of *Wolbachia* on flies can be complicated, the lack of significant change in viral titers would suggest that *Wolbachia* alters disease tolerance pathways that are relevant to virus infection.

Wolbachia affecting disease tolerance such that the host is more susceptible to an additional pathogen is not a unique situation. A similar effect has been observed in mice co-infected with *Legionella pneumophila* and influenza virus. While an infection of either pathogen can be administered to mice at a sublethal dose, the respective sublethal doses become lethal in combination (Jamieson et al., 2013). Coinfection did not affect pathogen load in comparison to single infections, suggesting that mortality was a result of a deficiency in disease tolerance. Indeed, Jamieson and colleagues found that administration of amphiregulin, an epithelial growth signal, rescued coinfecting mice from mortality, suggesting that increased tissue regrowth is a disease tolerance mechanism (Jamieson et al., 2013). As a proof of concept, Jamieson and colleagues demonstrated that two biotic stressors can be used in combination to reveal disease tolerance mechanisms.

Compounding stressors need not always be biotic to affect host health. Flies have a natural ability to maintain viability in high carbon dioxide environments, perhaps a trait necessary in its larval stages. In soft rotting fruits where fly eggs develop into larvae, oxygen can become low and carbon dioxide levels can increase. Flies have mechanisms to tolerate high carbon dioxide levels that acidify their nervous systems, as observed in the lab when anesthetizing animals. During VSV infection, the expression of viral glyco-protein in the nervous system renders flies acutely sensitive to an acidifying neuronal environment (Chow, Marka, Bartos, Marka, & Kagan, 2017). The acidic environment produced by high carbon dioxide levels induces viral glycoproteins to fuse neurons and glia, paralyzing flies. Thus, the ability to cope with the abiotic stress of high carbon dioxide levels can be lost when combined with a biotic stress.

Damage to the host does not necessarily need to be induced by multiple stressors. In some situations, such as *P. chabaudi* infection, damage can occur directly from one pathogen. *P. chabaudi* lyses red blood cells as part of its life cycle. Seixas and colleagues demonstrated that heme released from damaged red blood cells sensitizes hepatocytes to TNF-mediated cell death, resulting in liver failure and death (Seixas et al., 2009). The group was able to provide mechanistic insight as to how heme-oxygenase-1, produced by hepatocytes, can serve as a disease tolerance mechanism by catabolizing free heme. Infected heme-oxygenase-1 mutant mice suffered from increased mortality without any effects on pathogen load (Seixas et al., 2009). Thus, hemeoxygenase-1 was important for reducing circulating heme liberated by *P. chabaudi* infection. Disease tolerance mechanisms can target damage caused directly by a pathogen.

Disease tolerance mechanisms can also target damage caused by the host immune response (immunopathology). A prime example of disease tolerance targeting immunopathology is the situation of sepsis. During sepsis, an overabundance of inflammatory cytokines is produced, leading to multiple organ failure and death. Using mice as an experimental model, one can induce septic shock by injecting a large amount of lipopolysaccharide (LPS) into mice. However, a tolerogenic effect can be induced if the mice are first injected with a sublethal dose of LPS (Dobrovolskaia & Vogel, 2002). In one mechanistic study, LPS tolerance was traced to epigenetic changes that inhibited expression of inflammatory genes (Foster, Hargreaves, & Medzhitov, 2007). In this example of disease tolerance, the stress (a lethal dosage of LPS) is not changed in dosage, and in contrast to a live pathogen, LPS is not directly cytopathic. Epigenetic changes induced by a sublethal LPS dose prevent a cytokine storm and subsequent death. The example of LPS tolerance demonstrates that disease tolerance mechanisms can target damage that is self-inflicted.

Disease tolerance mechanisms are not always separate from disease resistance mechanisms. Sometimes, a cellular pathway can serve as either, depending on the context. Autophagy is a disease resistance mechanism, reducing pathogen load for the benefit of host survival (Levine, Mizushima, & Virgin, 2011). In some instances, the pathogen is observed to be destroyed by autophagy (Gomes & Dikic, 2014). However, autophagy can also serve as a disease tolerance mechanism. In *Staphylococcus aureus* infection of mice, α -toxin produced by the bacterium can bind to the protein ADAM10, inducing integrin cleavage that leads to destruction of epithelial and endothelial barrier integrity (Inoshima et al., 2011; Powers,

Kim, Wang, & Wardenburg, 2012). A hypomorph mutation in ATG16L, resulting in reduced autophagic activity, led to an accumulation of ADAM10 and increased mortality when mice were infected with *S. aureus* expressing α -toxin (Maurer et al., 2015). By reducing ADAM10 protein expression, autophagy minimized endothelial barrier destruction and maintained survival of the host (Maurer et al., 2015). Thus, depending on the context, a cellular process can serve as a disease resistance mechanism or a disease tolerance mechanism.

So far, the examples of disease tolerance mechanisms that have been described above seem very specific to the pathogen/stress. However, this may be due to the fact that only a handful of disease tolerance mechanisms in animals have been identified to date. Disease tolerance mechanisms might be more broadly applicable to multiple pathogens. One potential example comes from studies of food supplementation or restriction of infected animals. Depending upon the infection, food restriction versus supplementation can have profound effects upon host survival. In flies infected with *Salmonella typhimurium*, food restriction improved survival of infected flies without an effect on bacterial load, suggesting activation of a disease tolerance mechanism (Ayres & Schneider, 2009). Interestingly, food restriction during *L. monocytogenes* infection resulted in worse survival outcomes for flies because pathogen resistance weakened, again demonstrating that disease tolerance and resistance can be affected by the same stimulus depending on context (Ayres & Schneider, 2009). In the case of mice however, forced consumption of food, or simply just glucose supplementation, during *L. monocytogenes* infection resulted in poorer survival outcomes (Wang et al., 2016). The decreased survival was not due to changes in bacterial load or increased inflammatory cytokine production, and the result could be replicated simply using an LPS sepsis model as well (Wang et al., 2016). In contrast to bacterial infection, influenza challenge or poly(I:C) injection (simulating a viral infection) resulted in improved survival outcomes when mice were force fed (Wang et al., 2016). Viral titers were unaffected by dietary supplementation. The dichotomous outcomes of food intake during bacterial versus viral infection in mice suggest that disease tolerance mechanisms can apply to multiple pathogens, albeit with different outcomes. However, multiple underlying molecular mechanisms of dietary restriction/supplementation might exist, and that is important to note. This possibility is suggested by the differing results observed in mice and flies.

Disease tolerance is slowly gaining recognition as an important feature of host-pathogen interactions, but the biggest challenge thus far has been the identification of disease tolerance mechanisms. Genetic tools such as CRISPR/Cas9 are improving the ability to manipulate model organisms such as mice and flies. However, host-pathogen interactions can be complex. Mammalian organisms such as mice have the innate and adaptive arms of the immune system that function coordinately, utilizing cross-talk among many cell types. Pathogens often have strategies to evade the immune system and manipulate the host to their advantage. For disease tolerance models utilizing two pathogens, the complexity is increased. Future work identifying disease tolerance mechanisms could greatly benefit from simple host-pathogen models with easy-to-manipulate genetics.

9. CONCLUSION

In flies, a number of antiviral pathways have been identified. RNAi, Toll, IMD, Jak/STAT, and autophagy restrict viral replication. However, a major gap in our knowledge remains in how exactly viruses are being sensed. Currently, no unifying themes exist. Without this knowledge, we are unable to manipulate these systems to our advantage. Other than RNAi, it is unclear how these pathways mediate their antiviral effects downstream. Do Toll, IMD, or Jak/STAT signaling activate genes to suppress viral replication? Perhaps especially potent antiviral gene products could be utilized in novel ways. With regards to disease tolerance, elucidating tolerance mechanisms in more contexts will provide the opportunity to determine if some mechanisms can be broadly applicable. At which point, we will be able to assess the feasibility of modulating these pathways in arthropods or humans to our benefit.

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REFERENCES

- Abubaker S, Abdalla S, Mahmud S, & Wilkie B (2014). Antiviral innate immune response of RNA interference. *Journal of Infection in Developing Countries*, 8, 804–810. [PubMed: 25022288]
- Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, & Perrimon N (2003). Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Developmental Cell*, 5, 441–450. [PubMed: 12967563]
- Akhouayri I, Turc C, Royet J, & Charroux B (2011). Toll-8/Tollo negatively regulates antimicrobial response in the *Drosophila* respiratory epithelium. *PLoS Pathogens*, 7, e1002319. [PubMed: 22022271]
- Anderson KV, Bokla L, & Nüsslein-Volhard C (1985). Establishment of dorsal-ventral polarity in the *Drosophila* embryo: The induction of polarity by the Toll gene product. *Cell*, 42, 791–798. [PubMed: 3931919]
- Anderson KV, Jürgens G, & Nüsslein-Volhard C (1985). Establishment of dorsalventral polarity in the *Drosophila* embryo: Genetic studies on the role of the Toll gene product. *Cell*, 42, 779–789. [PubMed: 3931918]
- Anderson KV, & Nüsslein-Volhard C (1984). Information for the dorsal-ventral pattern of the *Drosophila* embryo is stored as maternal mRNA. *Nature*, 311, 223–227. [PubMed: 6434989]
- Argetsinger LS, Campbell GS, Yang X, Witthuhn BA, Silvennoinen O, Ihle JN, et al. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell*, 74, 237–244. [PubMed: 8343952]
- Avadhanula V, Weasner BP, Hardy GG, Kumar JP, & Hardy RW (2009). A novel system for the launch of alphavirus RNA synthesis reveals a role for the Imd pathway in arthropod antiviral response. *PLoS Pathogens*, 5, e1000582. [PubMed: 19763182]
- Ayres JS, Freitag N, & Schneider DS (2008). Identification of *Drosophila* mutants altering defense of and endurance to *Listeria monocytogenes* infection. *Genetics*, 178, 1807–1815. [PubMed: 18245331]
- Ayres JS, & Schneider DS (2009). The role of anorexia in resistance and tolerance to infections in *Drosophila*. *PLoS Biology*, 7, e1000150. [PubMed: 19597539]
- Banerjee S, Biehl A, Gadina M, Hasni S, & Schwartz DM (2017). JAK–STAT signaling as a target for inflammatory and autoimmune diseases: Current and future prospects. *Drugs*, 77, 521–546. [PubMed: 28255960]

- Berry DL, & Baehrecke EH (2007). Growth arrest and autophagy are required for salivary gland cell degradation in *Drosophila*. *Cell*, 131, 1137–1148. [PubMed: 18083103]
- Bettencourt R, Tanji T, Yagi Y, & Ip YT (2004). Toll and Toll-9 in *Drosophila* innate immune response. *Journal of Endotoxin Research*, 10, 261–268. [PubMed: 15373972]
- Billy E, Brondani V, Zhang H, Muller U, & Filipowicz W (2001). Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 14428–14433. [PubMed: 11724966]
- Binari R, & Perrimon N (1994). Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in *Drosophila*. *Genes & Development*, 8, 300–312. [PubMed: 8314084]
- Bischoff V, Vignal C, Boneca IG, Michel T, Hoffmann JA, & Royet J (2004). Function of the *drosophila* pattern-recognition receptor PGRP-SD in the detection of Gram-positive bacteria. *Nature Immunology*, 5, 1175–1180. [PubMed: 15448690]
- Bonham KS, Orzalli MH, Hayashi K, Wolf AI, Glanemann C, Weninger W, et al. (2014). A promiscuous lipid-binding protein diversifies the subcellular sites of toll-like receptor signal transduction. *Cell*, 156, 705–716. [PubMed: 24529375]
- Bosco-Drayon V, Poidevin M, Boneca IG, Narbonne-Reveau K, Royet J, & Charroux B (2012). Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota. *Cell Host & Microbe*, 12, 153–165. [PubMed: 22901536]
- Bronkhorst AW, van Cleef KWR, Vodovar N, Ince IA, Blanc H, Vlak JM, et al. (2012). The DNA virus Invertebrate iridescent virus 6 is a target of the *Drosophila* RNAi machinery. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E3604–E3613. [PubMed: 23151511]
- Brown S, Hu N, & Hombria JC (2001). Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene domeless. *Current Biology*, 11, 1700–1705. [PubMed: 11696329]
- Brown J, Wang H, Hajishengallis GN, & Martin M (2011). TLR-signaling networks: An integration of adaptor molecules, kinases, and cross-talk. *Journal of Dental Research*, 90, 417–427. [PubMed: 20940366]
- Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, Hyodo M, et al. (2011). STING is a direct innate immune sensor of cyclic di-GMP. *Nature*, 478, 515–518. [PubMed: 21947006]
- Caldwell RM, Kraybill HR, Sullivan JT, & Compton LE (1934). Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. *Journal of Agricultural Research*, 48, 1049–1071.
- Cao Y, Chtarbanova S, Petersen AJ, & Ganetzky B (2013). Dnr1 mutations cause neurodegeneration in *Drosophila* by activating the innate immune response in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E1752–E1760. [PubMed: 23613578]
- Chao JA, Lee JH, Chapados BR, Debler EW, Schneemann A, & Williamson JR (2005). Dual modes of RNA-silencing suppression by Flock House virus protein B2. *Nature Structural & Molecular Biology*, 12, 952–957.
- Chen H-W, Chen X, Oh S-W, Marinissen MJ, Gutkind JS, & Hou SX (2002). mom identifies a receptor for the *Drosophila* JAK/STAT signal transduction pathway and encodes a protein distantly related to the mammalian cytokine receptor family. *Genes & Development*, 16, 388–398. [PubMed: 11825879]
- Chinchore Y, Gerber GF, & Dolph PJ (2012). Alternative pathway of cell death in *Drosophila* mediated by NF- κ B transcription factor Relish. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E605–E612. [PubMed: 22328149]
- Chow J, Franz KM, & Kagan JC (2015). PRRs are watching you: Localization of innate sensing and signaling regulators. *Virology*, 479–480, 104–109.
- Chow J, Marka Z, Bartos I, Marka S, & Kagan JC (2017). Environmental stress causes lethal neuro-trauma during asymptomatic viral infections. *Cell Host & Microbe*, 22, 48–60. [PubMed: 28704652]
- Costa A, Jan E, Sarnow P, & Schneider D (2009). The Imd pathway is involved in antiviral immune responses in *Drosophila*. *PLoS One*, 4, e7436. [PubMed: 19829691]

- Cullen BR, Cherry S, & tenOever BR (2013). Is RNA interference a physiologically relevant innate antiviral immune response in mammals? *Cell Host & Microbe*, 14, 374–378. [PubMed: 24139396]
- Deddouche S, Matt N, Budd A, Mueller S, Kemp C, Galiana-Arnoux D, et al. (2008). The DExD/H-box helicase Dicer-2 mediates the induction of antiviral activity in drosophila. *Nature Immunology*, 9, 1425–1432. [PubMed: 18953338]
- Dixit E, Boulant S, Zhang Y, Lee ASY, Odendall C, Shum B, et al. (2010). Peroxisomes are signaling platforms for antiviral innate immunity. *Cell*, 141, 668–681. [PubMed: 20451243]
- Dobrovolskaia MA, & Vogel SN (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes and Infection*, 4, 903–914. [PubMed: 12106783]
- Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, Hetru C, et al. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nature Immunology*, 6, 946–953. [PubMed: 16086017]
- Elbashir SM, Lendeckel W, & Tuschl T (2001). RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes & Development*, 15, 188–200. [PubMed: 11157775]
- Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, & Tuschl T (2001). Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *The EMBO Journal*, 20, 6877–6888. [PubMed: 11726523]
- Eldon E, Kooyer S, D'Evelyn D, Duman M, Lawinger P, Botas J, et al. (1994). The *Drosophila* 18 wheeler is required for morphogenesis and has striking similarities to Toll. *Development*, 899, 885–899.
- Erturk-Hasdemir D, Broemer M, Leulier F, Lane WS, Paquette N, Hwang D, et al. (2009). Two roles for the *Drosophila* IKK complex in the activation of Relish and the induction of antimicrobial peptide genes. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 9779–9784. [PubMed: 19497884]
- Eulalio A, Behm-Ansmant I, Schweizer D, & Izaurralde E (2007). P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Molecular and Cellular Biology*, 27, 3970–3981. [PubMed: 17403906]
- Ferreira G, Naylor H, Esteves SS, Pais S, Martins NE, & Teixeira L (2014). The Toll-dorsal pathway is required for resistance to viral oral infection in *Drosophila*. *PLoS Pathogens*, 10, e1004507. [PubMed: 25473839]
- Foster SL, Hargreaves DC, & Medzhitov R (2007). Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature*, 447, 972–978. [PubMed: 17538624]
- Fujita N, Itoh T, Omori H, Fukuda M, Noda T, & Yoshimori T (2008). The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Molecular Biology of the Cell*, 19, 2092–2100. [PubMed: 18321988]
- Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, & Imler J-L (2006). Essential function in vivo for Dicer-2 in host defense against RNA viruses in *Drosophila*. *Nature Immunology*, 7, 590–597. [PubMed: 16554838]
- Garver LS, Wu J, & Wu LP (2006). The peptidoglycan recognition protein PGRP-SC1a is essential for Toll signaling and phagocytosis of *Staphylococcus aureus* in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 660–665. [PubMed: 16407137]
- Girardi E, Lefèvre M, Chane-Woon-Ming B, Paro S, Claydon B, Imler J-L, et al. (2015). Cross-species comparative analysis of Dicer proteins during Sindbis virus infection. *Scientific Reports*, 5, 10693. [PubMed: 26024431]
- Goic B, Vodovar N, Mondotte JA, Monot C, Frangeul L, Blanc H, et al. (2013). RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model *Drosophila*. *Nature Immunology*, 14, 396–403. [PubMed: 23435119]
- Gomes LC, & Dikic I (2014). Autophagy in antimicrobial immunity. *Molecular Cell*, 54, 224–233. [PubMed: 24766886]
- Guo X, Zhang R, Wang J, Ding S, & Lu R (2013). Homologous RIG-I-like helicase proteins direct RNAi-mediated antiviral immunity in *C. elegans* by distinct mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 16085–16090. [PubMed: 24043766]

- Hanratty WP, & Dearolf CR (1993). The *Drosophila* Tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. *Molecular & General Genetics*, 238, 33–37. [PubMed: 8479437]
- Harrison DA, Binari R, Nahreini TS, Gilman M, & Perrimon N (1995). Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *The EMBO Journal*, 14, 2857–2865. [PubMed: 7796812]
- Harrison DA, McCoon PE, Binari R, Gilman M, & Perrimon N (1998). *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes & Development*, 12, 3252–3263. [PubMed: 9784499]
- Hecht PM, & Anderson KV (1993). Genetic characterization of tube and pelle, genes required for signaling between Toll and dorsal in the specification of the dorsal-ventral pattern of the *Drosophila* embryo. *Genetics*, 135, 405–417. [PubMed: 8244004]
- Hornig T, & Medzhitov R (2001). *Drosophila* MyD88 is an adapter in the Toll signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 12654–12658. [PubMed: 11606776]
- Hou XS, Melnick MB, & Perrimon N (1996). Marelle acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell*, 84, 411–419. [PubMed: 8608595]
- Hu S, & Yang X (2000). dFADD, a novel death domain-containing adapter protein for the *Drosophila* caspase DREDD. *The Journal of Biological Chemistry*, 275, 30761–30764. [PubMed: 10934188]
- Huang Z, Kingsolver MB, Avadhanula V, & Hardy RW (2013). An antiviral role for antimicrobial peptides during the arthropod response to alphavirus replication. *Journal of Virology*, 87, 4272–4280. [PubMed: 23365449]
- Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, et al. (2000). A ubiquitin-like system mediates protein lipidation. *Nature*, 408, 488–492. [PubMed: 11100732]
- Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, et al. (2011). A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. *Nature Medicine*, 17, 1310–1314.
- Iwasaki S, Sasaki HM, Sakaguchi Y, Suzuki T, Tadakuma H, & Tomari Y (2015). Defining fundamental steps in the assembly of the *Drosophila* RNAi enzyme complex. *Nature*, 521, 533–536. [PubMed: 25822791]
- Jamieson AM, Pasman L, Yu S, Gamradt P, Homer RJ, Decker T, et al. (2013). Role of tissue protection in lethal respiratory viral-bacterial coinfection. *Science*, 340, 1230–1234. 80. [PubMed: 23618765]
- Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, & Edgar BA (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell*, 137, 1343–1355. [PubMed: 19563763]
- Kagan JC, Magupalli VG, & Wu H (2014). SMOCs: Supramolecular organizing centres that control innate immunity. *Nature Reviews. Immunology*, 14, 821–826.
- Kagan JC, & Medzhitov R (2006). Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling. *Cell*, 125, 943–955. [PubMed: 16751103]
- Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, & Ohsumi Y (2000). Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *The Journal of Cell Biology*, 150, 1507–1513. [PubMed: 10995454]
- Kaneko T, Yano T, Aggarwal K, Lim J-H, Ueda K, Oshima Y, et al. (2006). PGRP-LC and PGRP-LE have essential yet distinct functions in the *drosophila* immune response to monomeric DAP-type peptidoglycan. *Nature Immunology*, 7, 715–723. [PubMed: 16767093]
- Karlikow M, Goic B, Mongelli V, Salles A, Schmitt C, Bonne I, et al. (2016). *Drosophila* cells use nanotube-like structures to transfer dsRNA and RNAi machinery between cells. *Scientific Reports*, 6, 1–9. [PubMed: 28442746]
- Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, et al. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nature Immunology*, 6, 981–988. [PubMed: 16127453]

- Kemp C, Mueller S, Goto A, Barbier V, Paro S, Bonnay F, et al. (2013). Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in *Drosophila*. *Journal of Immunology*, 190, 650–658.
- Kidd S (1992). Characterization of the *Drosophila* cactus locus and analysis of interactions between cactus and dorsal Proteins. *Cell*, 71, 623–635. [PubMed: 1423619]
- Kim S, Chung S, Yoon J, Choi K, & Yim J (2006). Ectopic expression of Tollo/Toll-8 antagonizes Dpp signaling and induces cell sorting in the *Drosophila* wing. *Genesis*, 44, 541–549. [PubMed: 17078066]
- Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, et al. (2000). The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *The Journal of Cell Biology*, 151, 263–275. [PubMed: 11038174]
- Kleino A, Ramia NF, Bozkurt G, Shen Y, Nailwal H, Huang J, et al. (2017). Peptidoglycan-sensing receptors trigger the formation of functional amyloids of the adaptor protein imd to initiate *Drosophila* NF- κ B signaling. *Immunity*, 47, 635–647.e6. [PubMed: 29045898]
- Kleve CD, Siler DA, Syed SK, & Eldon ED (2006). Expression of 18-wheeler in the follicle cell epithelium affects cell migration and egg morphology in *Drosophila*. *Developmental Dynamics*, 235, 1953–1961. [PubMed: 16607637]
- Klionsky DJ, Baehrecke EH, Brumell JH, Chu CT, Codogno P, Cuervo AM, et al. (2011). A comprehensive glossary of autophagy-related molecules and processes (2nd edition). *Autophagy*, 7, 1273–1294. [PubMed: 21997368]
- Klionsky DJ, Cregg JM, Dunn WA, Emr SD, Sakai Y, Sandoval IV, et al. (2003). A unified nomenclature for yeast autophagy-related genes. *Developmental Cell*, 5, 539–545. [PubMed: 14536056]
- Kolesnikov T, & Beckendorf SK (2007). 18 wheeler regulates apical constriction of salivary gland cells via the Rho-GTPase-signaling pathway. *Developmental Biology*, 307, 53–61. [PubMed: 17512518]
- Kuma A, Mizushima N, Ishihara N, & Ohsumi Y (2002). Formation of the ~350-kDa Apg12-Apg5-Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. *The Journal of Biological Chemistry*, 277, 18619–18625. [PubMed: 11897782]
- Lamiabie O, Arnold J, Joao da Silva de Faria I, Olmo RP, Bergami F, Meignin C, et al. (2016). Analysis of the contribution of hemocytes and autophagy to percent survival percent survival. *Journal of Virology*, 90, 5415–5426. [PubMed: 27009948]
- Lamiabie O, Kellenberger C, Kemp C, Troxler L, Pelte N, Boutros M, et al. (2016). Cytokine Dieldel and a viral homologue suppress the IMD pathway in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 698–703. [PubMed: 26739560]
- Landis GN, Abdueva D, Skvortsov D, Yang J, Rabin BE, Carrick J, et al. (2004). Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 7663–7668. [PubMed: 15136717]
- Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, et al. (2004). TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nature Immunology*, 5, 190–198. [PubMed: 14716310]
- Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, et al. (2004). Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell*, 117, 69–81. [PubMed: 15066283]
- Lemaitre B, Kromer-metzger E, Michaut L, Nicolas E, Meister M, Georgel P, et al. (1995). A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. *Proceedings of the National Academy of Sciences of the United States of America*, 92, 9465–9469. [PubMed: 7568155]
- Lemaitre B, Nicolas E, Michaut L, Reichhart JM, & Hoffmann JA (1996). The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell*, 86, 973–983. [PubMed: 8808632]

- Leulier F, Rodriguez A, Khush RS, Abrams JM, & Lemaitre B (2000). The *Drosophila* caspase Dredd is required to resist gram-negative bacterial infection. *EMBO Reports*, 1, 353–358. [PubMed: 11269502]
- Levine B, Mizushima N, & Virgin HW (2011). Autophagy in immunity and inflammation. *Nature*, 469, 323–335. [PubMed: 21248839]
- Li H, Li WX, & Ding SW (2002). Induction and suppression of RNA silencing by an animal virus. *Science*, 296, 1319–1321. [PubMed: 12016316]
- Li Y, Lu J, Han Y, Fan X, & Ding S-W (2013). RNA interference functions as an antiviral immunity mechanism in mammals. *Science*, 342, 231–234. [PubMed: 24115437]
- Ligoxygakis P, Bulet P, & Reichhart J (2002). Critical evaluation of the role of the Toll-like receptor 18-Wheeler in the host defense of *Drosophila*. *EMBO Reports*, 3, 666–673. [PubMed: 12101100]
- Lim J-H, Kim M-S, Kim H-E, Yano T, Oshima Y, Aggarwal K, et al. (2006). Structural basis for preferential recognition of diaminopimelic acid-type peptidoglycan by a subset of peptidoglycan recognition proteins. *The Journal of Biological Chemistry*, 281, 8286–8295. [PubMed: 16428381]
- Lin F, & Young HA (2014). Interferons: Success in anti-viral immunotherapy. *Cytokine & Growth Factor Reviews*, 25, 369–376. [PubMed: 25156421]
- Lingel A, Simon B, Izaurralde E, & Sattler M (2005). The structure of the flock house virus B2 protein, a viral suppressor of RNA interference, shows a novel mode of double-stranded RNA recognition. *EMBO Reports*, 6, 1149–1155. [PubMed: 16270100]
- Liu Y, Gordesky-Gold B, Leney-Greene M, Weinbren NL, Tudor M, & Cherry S (2018). Inflammation-induced, STING-dependent autophagy restricts zika virus infection in the *Drosophila* brain. *Cell Host Microbe*, 24, 57–68.e3. [PubMed: 29934091]
- Liu X, Jiang F, Kalidas S, Smith D, & Liu Q (2006). Dicer-2 and R2D2 coordinately bind siRNA to promote assembly of the siRISC complexes. *RNA*, 12, 1514–1520. [PubMed: 16775303]
- Liu Y, Ye X, Jiang F, Liang C, Chen D, Peng J, et al. (2009). C3PO, an endoribonuclease that promotes RNAi by facilitating RISC activation. *Science*, 325, 750–753. [PubMed: 19661431]
- Lu R, Yigit E, Li W-X, & Ding S-W (2009). An RIG-I-like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in *Caenorhabditis elegans*. *PLoS Pathogens*, 5, e1000286. [PubMed: 19197349]
- Luo H, Hanratty WP, & Dearolf CR (1995). An amino acid substitution in the *Drosophila* hopTum-I Jak kinase causes leukemia-like hematopoietic defects. *The EMBO Journal*, 14, 1412–1420. [PubMed: 7729418]
- Maillard PV, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW, et al. (2013). Antiviral RNA interference in mammalian cells. *Science*, 342, 235–238. [PubMed: 24115438]
- Marek LR, & Kagan JC (2012). Phosphoinositide binding by the Toll adaptor dMyD88 controls antibacterial responses in *Drosophila*. *Immunity*, 88, 1–11.
- Marques JT, & Imler J-L (2016). The diversity of insect antiviral immunity: Insights from viruses. *Current Opinion in Microbiology*, 32, 71–76. [PubMed: 27232381]
- Marques JT, Kim K, Wu P-H, Alleyne TM, Jafari N, & Carthew RW (2009). Loqs and R2D2 act sequentially in the siRNA pathway in *Drosophila*. *Nature Structural & Molecular Biology*, 17, 24–30.
- Martin M, Hiroyasu A, Guzman RM, Roberts SA, & Goodman AG (2018). Analysis of *Drosophila* STING reveals an evolutionarily conserved antimicrobial function. *Cell Reports*, 23, 3537–3550.e6. [PubMed: 29924997]
- Maurer K, Reyes-Robles T, Alonzo FI, Durbin J, Torres VJ, & Cadwell K (2015). Autophagy mediates tolerance to *Staphylococcus aureus* alpha-toxin. *Cell Host & Microbe*, 17, 429–440. [PubMed: 25816775]
- Melendez A, Tallóczy Z, Seaman M, Eskelinen E-L, Hall DH, & Levine B (2003). Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science*, 301, 1387–1391. [PubMed: 12958363]
- Michel T, Reichhart JM, Hoffmann JA, & Royet J (2001). *Drosophila* Toll is activated by Gram-positive bacteria through a circulating peptidoglycan recognition protein. *Nature*, 414, 756–759. [PubMed: 11742401]

- Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, et al. (1998). A protein conjugation system essential for autophagy. *Nature*, 395, 395–398. [PubMed: 9759731]
- Morisato D, & Anderson KV (1994). The spatzle gene encodes a component of the extracellular signaling pathway establishing the dorsal-ventral pattern of the *Drosophila* embryo. *Cell*, 76, 677–688. [PubMed: 8124709]
- Moy RH, Gold B, Molleston JM, Schad V, Yanger K, Salzano M-V, et al. (2013). Antiviral autophagy restricts rift valley fever virus infection and is conserved from flies to mammals. *Immunity*, 40, 51–65. [PubMed: 24374193]
- Muller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, et al. (1993). The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. *Nature*, 366, 129–135. [PubMed: 8232552]
- Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith BL, Rajendiran TM, & Núñez G (2013). K⁺ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity*, 38, 1142–1153. [PubMed: 23809161]
- Naitza S, Rosse C, Kappler C, Georgel P, Belvin M, Gubb D, et al. (2002). The *Drosophila* immune defense against gram-negative infection requires the death protein dFADD. *Immunity*, 17, 575–581. [PubMed: 12433364]
- Nakamoto M, Moy RHH, Xu J, Bambina S, Yasunaga A, Shelly SSS, et al. (2012). Virus recognition by Toll-7 activates antiviral autophagy in *Drosophila*. *Immunity*, 36, 658–667. [PubMed: 22464169]
- Narbonne-Reveau K, Charroux B, & Royet J (2011). Lack of an antibacterial response defect in *Drosophila* Toll-9 mutant. *PLoS One*, 6, e17470. [PubMed: 21386906]
- Nayak A, Berry B, Tassetto M, Kunitomi M, Acevedo A, Deng C, et al. (2010). Cricket paralysis virus antagonizes Argonaute 2 to modulate antiviral defense in *Drosophila*. *Nature Structural & Molecular Biology*, 17, 547–554.
- Nusslein-Volhard C (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature*, 287, 795–801. [PubMed: 6776413]
- Olsen PH, & Ambros V (1999). The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Developmental Biology*, 216, 671–680. [PubMed: 10642801]
- Ooi JY, Yagi Y, Hu X, & Ip YT (2002). The *Drosophila* Toll-9 activates a constitutive antimicrobial defense. *EMBO Reports*, 3, 82–87. [PubMed: 11751574]
- Otsuka M, Jing Q, Georgel P, New L, Chen J, Mols J, et al. (2007). Hypersusceptibility to vesicular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR24 and miR93 expression. *Immunity*, 27, 123–134. [PubMed: 17613256]
- Pace KE, Lebestky T, Hummel T, Arnoux P, Kwan K, & Baum LG (2002). Characterization of a novel *Drosophila melanogaster* galectin. *The Journal of Biological Chemistry*, 277, 13091–13098. [PubMed: 11809773]
- Paddison PJ, Caudy AA, & Hannon GJ (2001). Stable suppression of gene expression by RNAi in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 1443–1448.
- Paquette N, Broemer M, Aggarwal K, Chen L, Husson M, Ertürk-Hasdemir D, et al. (2010). Caspase-mediated cleavage, IAP binding, and ubiquitination: Linking three mechanisms crucial for *Drosophila* NF-kappaB signaling. *Molecular Cell*, 37, 172–182. [PubMed: 20122400]
- Paradkar PN, Trinidad L, Voysey R, Duchemin J-B, & Walker PJ (2012). Secreted Vago restricts West Nile virus infection in *Culex* mosquito cells by activating the Jak-STAT pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 18915–18920. [PubMed: 23027947]
- Pare JM, & Sullivan CS (2014). Distinct antiviral responses in pluripotent versus differentiated cells. *PLoS Pathogens*, 10, e1003865. [PubMed: 24516379]
- Petersen AJ, Katzenberger RJ, & Wassarman DA (2013). The innate immune response transcription factor relish is necessary for neurodegeneration in a *Drosophila* model of ataxia-telangiectasia. *Genetics*, 194, 133–142. [PubMed: 23502677]

- Petit M, Mongelli V, Frangeul L, Blanc H, Jiggins F, & Saleh M-C (2016). piRNA pathway is not required for antiviral defense in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E4218–E4227. [PubMed: 27357659]
- Pletcher SD, Macdonald SJ, Marguerie R, Certa U, Stearns SC, Goldstein DB, et al. (2002). Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. *Current Biology*, 12, 712–723. [PubMed: 12007414]
- Poirier EZ, Goic B, Tome-Poderti L, Frangeul L, Boussier J, Gausson V, et al. (2018). Dicer-2-dependent generation of viral DNA from defective genomes of RNA viruses modulates antiviral immunity in insects. *Cell Host & Microbe*, 23, 353–365. [PubMed: 29503180]
- Powers ME, Kim HK, Wang Y, & Wardenburg JB (2012). ADAM10 mediates vascular injury induced by *Staphylococcus aureus* alpha-hemolysin. *The Journal of Infectious Diseases*, 206, 352–356. [PubMed: 22474035]
- Raberg L, Graham AL, & Read AF (2009). Decomposing health: Tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B*, 364, 37–49.
- Råberg L, Sim D, & Read AF (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science*, 318, 812–814. [PubMed: 17975068]
- Raftery N, & Stevenson NJ (2017). Advances in anti-viral immune defence: Revealing the importance of the IFN JAK/STAT pathway. *Cellular and Molecular Life Sciences*, 74, 2525–2535. [PubMed: 28432378]
- Redondo-Gomez S (2013). Abiotic and biotic stress tolerance in plants In Rout GR & Das AB (Eds.), *Molecular stress physiology of plants* (pp. 1–20). India: Springer.
- Reggiori F, & Klionsky DJ (2002). Autophagy in the eukaryotic Cell. *Eukaryotic Cell*, 1, 11–21. [PubMed: 12455967]
- Rothenfusser S, Goutagny N, Diperna G, Gong M, Monks BG, Schoenemeyer A, et al. (2005). The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *Journal of Immunology*, 175, 5260–5268.
- Ryu J-H, Kim S-H, Lee H-Y, Bai JY, Nam Y-D, Bae J-W, et al. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science*, 319, 777–782. [PubMed: 18218863]
- Saleh M-C, Tassetto M, van Rij RP, Goic B, Gausson V, Berry B, et al. (2009). Antiviral immunity in *Drosophila* requires systemic RNA interference spread. *Nature*, 458, 346–350. [PubMed: 19204732]
- Saleh M-C, Van Rij RP, Hekele A, Gillis A, Foley E, Farrell PHO, et al. (2006). The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. *Nature Cell Biology*, 8, 793–802. [PubMed: 16862146]
- Salmon SC, & Laude HH (1932). Twenty years of testing varieties and strains of winter wheat at the Kansas Agricultural Experimental Station. Topeka: Kansas State Printing Plant, Walker BP, State Printer.
- Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, et al. (2010). LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 1512–1517. [PubMed: 20080593]
- Schneider DS, Jin Y, Morisato D, & Anderson KV (1994). A processed form of the Spätzle protein defines dorsal-ventral polarity in the *Drosophila* embryo. *Development*, 120, 1243–1250. [PubMed: 8026333]
- Scott RC, Schuldiner O, & Neufeld TP (2004). Role and regulation of starvation-induced autophagy in the *Drosophila* fat body. *Developmental Cell*, 7, 167–178. [PubMed: 15296714]
- Seixas E, Gozzelino R, Chora A, Ferreira A, Silva G, Larsen R, et al. (2009). Heme oxygenase-1 affords protection against noncerebral forms of severe malaria. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 15837–15842. [PubMed: 19706490]
- Seo GJ, Kincaid RP, Phanaksri T, Burke JM, Pare JM, Cox JE, et al. (2013). Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host & Microbe*, 14, 435–445. [PubMed: 24075860]

- Seppo A, Matani P, Sharrow M, & Tiemeyer M (2003). Induction of neuron-specific glycosylation by Tollo/Toll-8, a *Drosophila* Toll-like receptor expressed in non-neural cells. *Development*, 130, 1439–1448. [PubMed: 12588858]
- Seth RB, Sun L, Ea C, & Chen ZJ (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF- κ B and IRF3. *Cell*, 122, 669–682. [PubMed: 16125763]
- Shapiro JS, Schmid S, Aguado LC, Sabin LR, Yasunaga A, Shim JV, et al. (2014). Drosba as an interferon-independent antiviral factor. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 7108–7113. [PubMed: 24778219]
- 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. [PubMed: 19362021]
- Shelton CA, & Wasserman SA (1993). pelle encodes a protein kinase required to establish dorsoventral polarity in the *Drosophila* embryo. *Cell*, 72, 515–525. [PubMed: 8440018]
- Shintani T, Mizushima N, Ogawa Y, Matsuura A, Noda T, & Ohsumi Y (1999). Apg10p, a novel protein-conjugating enzyme essential for autophagy in yeast. *The EMBO Journal*, 18, 5234–5241. [PubMed: 10508157]
- Silverman N, Zhou R, Erlich RL, Hunter M, Bernstein E, Schneider D, et al. (2003). Immune activation of NF- κ B and JNK requires *Drosophila* TAK1. *The Journal of Biological Chemistry*, 278, 48928–48934. [PubMed: 14519762]
- Simonsen A, & Tooze SA (2009). Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *The Journal of Cell Biology*, 186, 773–782. [PubMed: 19797076]
- Singh G, Popli S, Hari Y, Malhotra P, Mukherjee S, & Bhatnagar RK (2009). Suppression of RNA silencing by Flock house virus B2 protein is mediated through its interaction with the PAZ domain of Dicer. *The FASEB Journal*, 23, 1845–1857. [PubMed: 19193719]
- Stark GR, & Darnell JE (2012). The JAK-STAT pathway at twenty. *Immunity*, 36, 503–514. [PubMed: 22520844]
- Stein D, & Nusslein-Volhard C (1992). Multiple extracellular activities in *Drosophila* egg perivitelline fluid are required for establishment of embryonic dorsal-ventral polarity. *Cell*, 68, 429–440. [PubMed: 1739964]
- Stenbak CR, Ryu J-H, Leulier F, Pili-Floury S, Parquet C, Herve M, et al. (2004). Peptidoglycan molecular requirements allowing detection by the *Drosophila* immune deficiency pathway. *Journal of Immunology*, 173, 7339–7348.
- Stöven S, Ando I, Kadalayil L, Engström Y, & Hultmark D (2000). Activation of the *Drosophila* NF- κ B factor Relish by rapid endoproteolytic cleavage. *EMBO Reports*, 1, 347–352. [PubMed: 11269501]
- Stoven S, Silverman N, Junell A, Hedengren-Olcott M, Erturk D, Engstrom Y, et al. (2003). Caspase-mediated processing of the *Drosophila* NF- κ B factor Relish. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 5991–5996. [PubMed: 12732719]
- Sun H, Bristow BN, Qu G, & Wasserman SA (2002). A heterotrimeric death domain complex in Toll signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 12871–12876. [PubMed: 12351681]
- Sun H, Towb P, Chiem DN, Foster BA, & Wasserman SA (2004). Regulated assembly of the Toll signaling complex drives *Drosophila* dorsoventral patterning. *The EMBO Journal*, 23, 100–110. [PubMed: 14685264]
- Sun L, Wu J, Du F, Chen X, & Chen ZJ (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*, 339, 786–791. [PubMed: 23258413]
- Takehana A, Katsuyama T, Yano T, Oshima Y, Takada H, Aigaki T, et al. (2002). Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in *Drosophila* larvae. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 13705–13710. [PubMed: 12359879]

- Takehana A, Yano T, Mita S, Kotani A, Oshima Y, & Kurata S (2004). Peptidoglycan recognition protein (PGRP)-LE and PGRP-LC act synergistically in *Drosophila* immunity. *The EMBO Journal*, 23, 4690–4700. [PubMed: 15538387]
- Tassetto M, Kunitomi M, Tassetto M, Kunitomi M, & Andino R (2017). Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in *Drosophila*. *Cell*, 169 314–325.e13. [PubMed: 28388413]
- Tauszig S, Jouanguy E, Hoffmann JA, & Imler J (2000). Toll-related receptors and the control of antimicrobial peptide expression in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 10520–10525. [PubMed: 10973475]
- Teixeira L, Ferreira A, & Ashburner M (2008). The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biology*, 6, e2.
- Tomari Y, Matranga C, Haley B, Martinez N, & Zamore PD (2004). A protein sensor for siRNA asymmetry. *Science*, 306, 1377–1380. [PubMed: 15550672]
- Tzou P, Ohresser S, Ferrandon D, Capovilla M, Reichhart JM, Lemaitre B, et al. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. *Immunity*, 13, 737–748. [PubMed: 11114385]
- Valtonen TM, Kleino A, Ramet M, & Rantala MJ (2010). Starvation reveals maintenance cost of humoral immunity. *Evolutionary Biology*, 37, 49–57.
- van Rij RP, Saleh M-C, Berry B, Foo C, Houk A, Antoniewski C, et al. (2006). The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes & Development*, 20, 2985–2995. [PubMed: 17079687]
- Venkataraman T, Valdes M, Elsby R, Kakuta S, Caceres G, Saijo S, et al. (2007). Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. *Journal of Immunology*, 178, 6444–6455.
- Wang C, Deng L, Hong M, Akkaraju GR, Inoue J-I, & Chen ZJ (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature*, 412, 346–351. [PubMed: 11460167]
- Wang A, Huen SC, Luan HH, Yu S, Zhang C, Gallezot J-D, et al. (2016). Opposing effects of fasting metabolism on tissue tolerance in bacterial and viral inflammation. *Cell*, 166, 1512–1525. [PubMed: 27610573]
- Ward A, Hong W, Favaloro V, & Luo L (2015). Toll receptors instruct axon and dendrite targeting and participate in synaptic partner matching in a *Drosophila* olfactory circuit. *Neuron*, 85, 1013–1028. [PubMed: 25741726]
- Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, Olivas J, et al. (2015). The cytosolic sensor cGAS detects *Mycobacterium tuberculosis* DNA to induce type I interferons and activate autophagy. *Cell Host & Microbe*, 17, 811–819. [PubMed: 26048136]
- Werner T, Borge-Renberg K, Mellroth P, Steiner H, & Hultmark D (2003). Functional diversity of the *Drosophila* PGRP-LC gene cluster in the response to lipopolysaccharide and peptidoglycan. *The Journal of Biological Chemistry*, 278, 26319–26322. [PubMed: 12777387]
- Werner T, Liu G, Kang D, Ekengren S, Steiner H, & Hultmark D (2000). A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 13772–13777. [PubMed: 11106397]
- West C, & Silverman N (2018). p38b and JAK-STAT signaling protect against invertebrate iridescent virus 6 infection in *Drosophila*. *PLoS Pathogens*, 14, e1007020. [PubMed: 29746571]
- Williams MJ, Rodriguez A, Kimbrell DA, & Eldon ED (1997). The 18-wheeler mutation reveals complex antibacterial gene regulation in *Drosophila* host defense. *The EMBO Journal*, 16, 6120–6130. [PubMed: 9321392]
- Wright VM, Vogt KL, Smythe E, & Zeidler MP (2011). Differential activities of the *Drosophila* JAK/STAT pathway ligands Upd, Upd2 and Upd3. *Cellular Signalling*, 23, 920–927. [PubMed: 21262354]
- Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. (2013). Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*, 339, 826–830. [PubMed: 23258412]

- Wu X, Wu F, Wang X, Wang L, Siedow JN, Zhang W, et al. (2014). Molecular evolutionary and structural analysis of the cytosolic DNA sensor cGAS and STING. *Nucleic Acids Research*, 42, 8243–8257. [PubMed: 24981511]
- Xu J, Hopkins K, Sabin L, Yasunaga A, Subramanian H, Lamborn I, et al. (2013). ERK signaling couples nutrient status to antiviral defense in the insect gut. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 15025–15030. [PubMed: 23980175]
- Yagi Y, Nishida Y, & Ip YT (2010). Functional analysis of Toll-related genes in *Drosophila*. *Development, Growth & Differentiation*, 52, 771–783.
- Yan R, Small S, Desplan C, Dearolf CR, & Darnell JE (1996). Identification of a Stat gene that functions in *Drosophila* development. *Cell*, 84, 421–430. [PubMed: 8608596]
- Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, et al. (2008). Autophagic control of listeria through intracellular innate immune recognition in *drosophila*. *Nature Immunology*, 9, 908–916. [PubMed: 18604211]
- Zambon RA, Nandakumar M, Vakharia VN, & Wu LP (2005). The Toll pathway is important for an antiviral response in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 7257–7262. [PubMed: 15878994]
- Zambon RA, Vakharia VN, & Wu LP (2006). RNAi is an antiviral immune response against a dsRNA virus in *Drosophila melanogaster*. *Cellular Microbiology*, 8, 880–889. [PubMed: 16611236]
- Zeidler MP, Bach EA, & Perrimon N (2000). The roles of the *Drosophila* JAK/STAT pathway. *Oncogene*, 19, 2598–2606. [PubMed: 10851058]
- Zerofsky M, Harel E, Silverman N, & Tatar M (2005). Aging of the innate immune response in *Drosophila melanogaster*. *Aging Cell*, 4, 103–108. [PubMed: 15771614]
- Zirin J, & Perrimon N (2010). *Drosophila* as a model system to study autophagy. *Seminars in Immunopathology*, 32, 363–372. [PubMed: 20798940]

Fig. 1.

The RNAi pathway restricts viral replication. Viral RNA is sensed by Dcr2, and a subset is reverse transcribed by endogenous retrotransposon reverse transcriptases into viral DNA. dsRNA is detected by Dcr2 and diced to a 21-nucleotide length so that one strand can be loaded into Ago2 for viral RNA silencing. Viral DNA and RNA and RISC can be transported out of infected cells to promote viral transcript silencing in a cell extrinsic manner.

Fig. 2. Jak/STAT signaling induces antiviral gene transcription. By currently unknown mechanisms, viral infection is detected within a cell. Unpaired proteins or vago signal for Jak/STAT activation. STAT92E serves as a transcription factor to transcribe antiviral response genes.

Fig. 3.

Toll signaling is antiviral in some instances such as Nora virus, flock house virus, and cricket paralysis virus infections. The mechanism for detecting viral infection is unclear, but spatze is likely cleaved to engage Toll receptor signaling. Although the Toll signaling pathway has been studied in the context of embryonic development and bacterial and fungal infections, published work has only begun to probe the roles of downstream mediators during viral infection. How Toll signaling promotes an antiviral response is unclear.

Fig. 4.

The IMD pathway is involved in antiviral signaling. Similar to Toll signaling during viral infection, how viruses are sensed by the IMD pathway is unclear. IMD signaling leads to the activation of the NF- κ B protein Relish. Nuclear translocation of Relish promotes the transcription of *dSTING* and potentially other antiviral genes.

Fig. 5.

Autophagy restricts viral replication. Toll-7 has been proposed to detect viruses such as VSV and Rift Valley Fever virus. While TRAF6 has been implicated in inducing antiviral autophagy, other canonical Toll signaling proteins have not been assigned to this signaling axis. Additionally, dSTING has been identified to induce autophagy during Zika virus infection. Upstream signaling for dSTING activation is unknown. Whether autophagosomes directly destroy viral products or has some other indirect role during infection is unclear.