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The *Drosophila melanogaster* host model

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The deleterious and sometimes fatal outcomes of bacterial infectious diseases are the net result of the interactions between the pathogen and the host, and the genetically tractable fruit fly, *Drosophila melanogaster*, has emerged as a valuable tool for modeling the pathogen–host interactions of a wide variety of bacteria. These studies have revealed that there is a remarkable conservation of bacterial pathogenesis and host defence mechanisms between higher host organisms and *Drosophila*. This review presents an in-depth discussion of the *Drosophila* immune response, the *Drosophila* killing model, and the use of the model to examine bacterial–host interactions. The recent introduction of the *Drosophila* model into the oral microbiology field is discussed, specifically the use of the model to examine *Porphyromonas gingivalis*–host interactions, and finally the potential uses of this powerful model system to further elucidate oral bacterial-host interactions are addressed.

Keywords: *Drosophila melanogaster*; *Porphyromonas gingivalis*; *Pathogen-host interactions*; *Periodontitis*

Introduction

The use of invertebrate animal models has provided tremendous insight into the pathogen–host interactions of many human pathogens, and has revealed that many aspects of these interactions in higher host organisms are conserved in invertebrates. One of these animal models, the genetically tractable fruit fly, *Drosophila melanogaster* (*Drosophila*), has been well established as a model for studying the host–pathogen interactions of bacterial (1–9), fungal (10–17) and viral pathogens, and prior to the sequencing of the mosquito genome and the subsequent development of genetic tools, the malaria parasite (23–26); the model has also been used to probe the host defense response to infection.

This article will first provide an overview of the *Drosophila* model and address the advantages and drawbacks of the model for studying pathogen–host interactions. Next, the *Drosophila* immune response will be described in detail, and the various ways that the model has been used to study bacterial–host interactions will be addressed. Finally, the studies that used the *Drosophila* model to investigate *Porphyromonas gingivalis*–host interactions will be reviewed, followed by a discussion of the potential contributions that can be made using this model, toward a better understanding of oral bacteria–host interactions.

D. melanogaster as a model for studying host–pathogen interactions

Numerous studies have revealed that there is significant homology between the *Drosophila* immune response and the mammalian innate immune response (discussed in depth below). The absence of an adaptive immune response permits the study of the interactions between pathogens and the host's innate immune response in isolation. *Drosophila* are affordable to breed, are easy to handle, have a short generation time (10–14 days depending on the ambient temperature), have a clear endpoint (death), and can be used in quantities large enough to permit statistical analysis of the data. The *Drosophila* genome is fully sequenced (27–29), and numerous well-developed genetic tools are available for the manipulation and analysis of *Drosophila* responses [reviewed in (30, 31)]. Transposon mutagenesis has been used to successfully create loss-of-function mutants of at least 53% of *Drosophila* genes, with the ultimate goal of inactivating all genes (32, 33). Mutations are usually linked to visually identifiable markers, e.g. eye color or wing morphology, to allow for easy identification of mutant animals. Technologies for transgenic expression of genes in *Drosophila* are well developed (34–37) and have been enhanced by the development of expression systems like upstream activation sequence (UAS)/GAL4

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(yeast transcription activator) (38, 39) that allow for temporally and spatially regulated expression; linking transgenes with reporter genes like *lacZ* (β galactosidase) or *gfp* (green fluorescent protein) allows gene expression to be monitored. Mutant and transgenic *Drosophila* lines are readily available at stock centers and have been extensively used to probe the interactions between pathogens and the *Drosophila* host. *Drosophila* loss-of-function immune response gene mutants have been used to examine the roles of the genes in the response to infection with various pathogens [(11, 16, 19, 40–43) and Table 2], and transgenic *Drosophila* have been used to monitor the activation of immune response pathways upon infection (44, 45) and to examine the effects of transgenically expressed pathogen proteins on the host (21, 26, 46). Microarray and proteomic platforms as well as RNA interference (RNAi) lines and libraries have been developed and used to perform genome-wide analyses of *Drosophila* responses, in whole animals (41, 47–56) and in the well-established *Drosophila* cell culture lines, Schneider-2 (S2, embryonic-derived phagocytic cell) and malignant blood neoplasm (mbn-2) (56–59). As an estimated 50% of *Drosophila* genes have mammalian homologs (60), results from these and other studies are relevant to mammals. A comprehensive collection of *Drosophila* information can be found online at FlyBase (http://www.flybase.net) (61), including but not limited to gene annotation information, stock availability, images, references, and investigator contact information. Other databases containing *Drosophila* information include Berkeley Drosophila Genome Project (BDGP), Drosophila Interactions Database (DroID), and FlyView.

It is important to note that as *Drosophila* are not natural hosts for most of the human pathogens studied with this model and lack homologs for many mammalian immune response features, e.g. adaptive immunity, care should be taken when translating findings to mammals. *Drosophila* are usually reared at room temperature (25°C), and they can be incubated at temperatures of up to 30°C during infection experiments without affecting survival, although males become sterile at this temperature. Above 30°C, *Drosophila* physiological processes begin to deteriorate, and they are rapidly killed at 37°C (62), which is the optimal growth temperature for most human pathogens. In addition, some pathogen virulence genes are selectively expressed at 37°C, which could result in the absence of a virulence phenotype in the *Drosophila* model. Although the *Drosophila* hemolymph (blood) is not involved in respiration, it receives oxygen via the trachea, which likely makes it an inhospitable environment for obligate anaerobes. The *Drosophila* model may be more suitable for studying aerobic and facultatively anaerobic microbes; however, the model has been successfully used to examine the interactions between the obligately anaerobic oral bacterium *P. gingivalis* and the host. Even though *P. gingivalis* did not multiply in the flies, the bacterium viably persisted in them for up to 60 h after infection, likely aided by its high degree of aerotolerance (63).

In summary, the availability of a large number of well-developed tools for manipulating *Drosophila* genetics and analyzing *Drosophila* immune responses make it a powerful model for studying pathogen–host interactions, and numerous studies have convincingly demonstrated that strong correlation exists between microbial pathogenesis in mammals and in *Drosophila*.

The *Drosophila* immune system

Drosophila rely solely on an innate immune system to combat infecting microbes, and like mammals, they detect the presence of invading microbes using pattern recognition receptors, which recognize conserved microbial motifs and activate a response that is specific for the type of invading microbe [see (64) for a recent review].

Humoral immune response

The *Drosophila* humoral response is mediated by three signaling pathways: the Imd (immune deficiency), Toll, and JAK/STAT (janus kinase/signal transducer and activator of transcription) pathways (Fig. 1). The hallmark of this response is the transient synthesis and release of antimicrobial peptides (AMPs) by the fat body – the major immune responsive tissue in *Drosophila* and the functional equivalent of the mammalian liver – directly into the hemolymph, where they accumulate to their effective concentrations. The Toll and Imd pathways are major regulators of *Drosophila* immune response genes and have been very well studied [see (65) for a recent in depth review].

The Imd pathway

The Imd signaling pathway (Fig. 1) is homologous to the mammalian tumor necrosis factor receptor 1 signaling pathway, though they differ at the level of detection/activation. This pathway regulates both systemic and local (in response to natural infection) AMP expression, by fat body cells and gut epithelial cells, respectively, in response to primarily Gram-negative bacterial infections [(66–68) also see Table 2].

Activation and signal transduction. The pathway is activated by the direct detection of monomeric or polymeric meso-diaminopimelic (DAP)-type (Gram-negative type) peptidoglycan (PPG) by the transmembrane receptor, peptidoglycan recognition protein-LC (PGRP-LC) (69, 70). Binding of PPG by PGRP-LC leads to the intracellular recruitment of the adaptor molecule Imd (71–73) (receptor interacting protein, RIP, homolog), and the subsequent recruitment of *Drosophila* Fas-associated death domain protein

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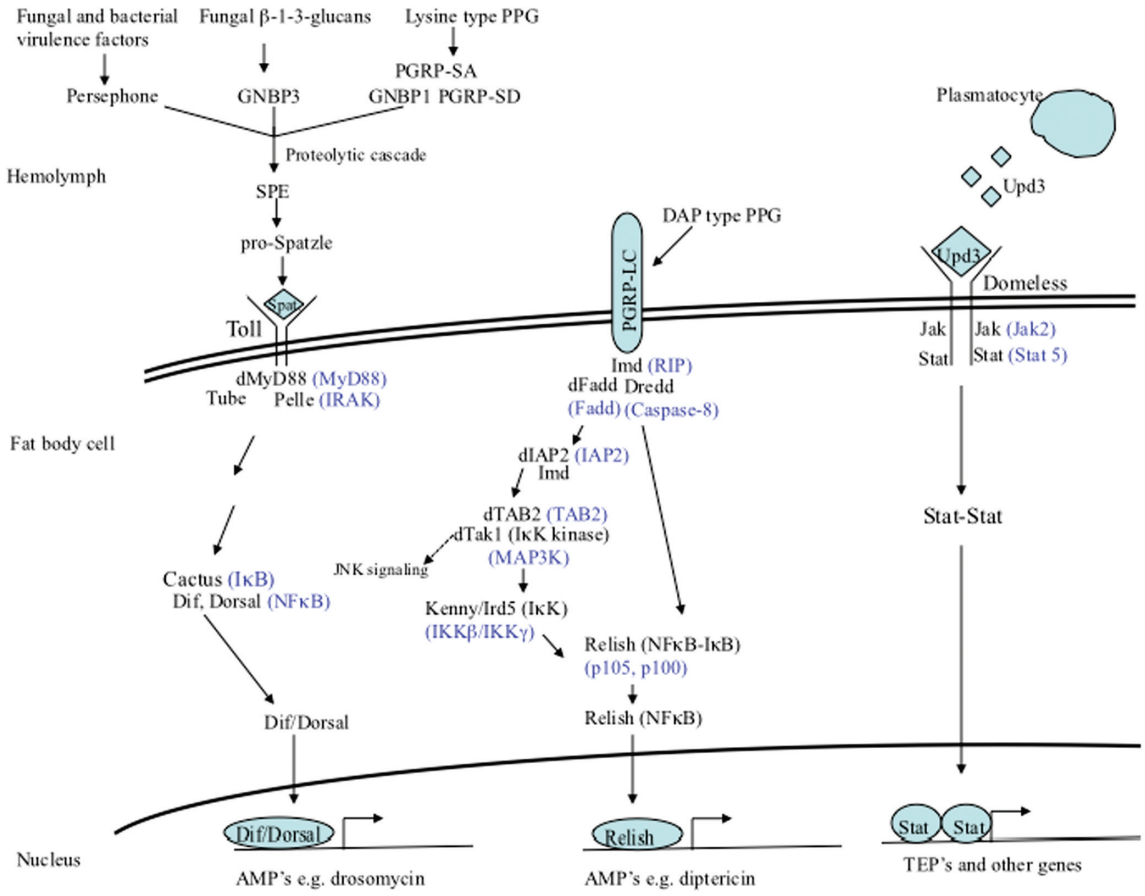

Fig. 1. Drosophila signaling pathways that regulate humoral responses. The Toll pathway regulates the expression of genes in response to the detection of Gram-positive type PPG or fungal β-1-3-glucans or activation by some fungal and bacterial proteases. The Imd pathway regulates the expression of genes in response to the detection of Gram-negative type PPG. The JAK/STAT pathway regulates the expression of genes in response to Upd3 signaling by plasmatocytes. Mammalian homologs of pathway components are shown in blue. Negative regulators of the pathways are not shown.

(dFADD, FADD homolog) and the caspsase-8 homolog DREDD to the Imd/PGRP-LC complex (74). *Drosophila* inhibitor of apoptosis-2 (dIAP2) (75) and transforming-growth-factor-β activating kinase (dTAK1, IκB-kinase-kinase) (68) are also recruited, and dTAK1 becomes activated by TAK1-binding protein 2 (TAB2). dTAK1 phosphorylates the IκB-kinase complex (IKKβ/Ird5 and IKKγ/Kenny) (76), which in turn phosphorylates the dual domain nuclear factor kappa B (NFκB) family protein Relish (77–79). Relish is cleaved, possibly by DREDD, which separates the inhibitory ankyrin domain from the Rel-homology domain (80). The phosphorylated Rel domain then translocates into the nucleus where it activates the transcription of Imd regulated genes (81) (discussed below).

Downregulation. To prevent the deleterious effects of an unchecked inflammatory response, the Imd pathway is regulated on multiple levels. Several PGRPs function as *N*-acetylmuramoyl-l-alanine amidases that scavenge and degrade PPG, converting it into non-immunostimulatory fragments. These include PGRP-SC1, -SC2 (82), -SB1 (83), and –LB, which is itself upregulated by the pathway creating a negative feedback loop (84). PGRP-LF is a membrane-bound receptor that sequesters DAP-type PPG and prevents it from binding to PGRP-LC and activating Imd signaling (85). Two proteins Caspar (Fas-associated factor 1 homolog) and Dnr1 block DREDD mediated cleavage of Relish (86, 87). Pirk/Rudra/PIMS (PGRP-LC-interacting inhibitor of Imd signaling) functions in a negative feedback loop to disrupt Imd/PGRP-LC binding and inhibit Imd signaling (88–90), and the Ras/MAP kinase [mitogen-activated protein kinase (MAPK)] pathway negatively regulates Imd pathway activation by modulating the expression of Pirk/Rudra/PIMS (91). Finally, a ubiquitin-proteasome complex represses Imd signaling by degrading one of the pathway components, likely Relish (92).

The Toll pathway

The Toll signaling pathway (Fig. 1) is homologous to the mammalian Toll/IL-1 receptor-signaling pathway,

although unlike the mammalian receptors *Drosophila* Toll does not directly recognize bacterial components. The pathway regulates systemic AMP expression by the cells of the fat body, primarily in response to fungal and Gram-positive bacterial infections [(93–97), also see Table 2].

Activation and signal transduction. The pathway is activated in response to the detection of lysine-type (Gram-positive type) PPG (LPPG) by circulating recognition molecules PGRP-SA (95) and Gram-negative binding protein 1 (GNBP1) (98), which function in a complex (99), and PGRP-SD (100). The Toll pathway is also activated in response to the detection of fungal cell wall β-1-3-glucans by GNBP3 or by the activation of the *Drosophila* protease Persephone by fungal- and bacterial-secreted virulence factors (101–104). A common proteolytic cascade is activated downstream of the recognition molecules (105) that culminates in the activation of Spatzle processing enzyme (SPE), which cleaves and activates the cytokine Spatzle (106). Activated Spatzle binds to the cell transmembrane receptor, Toll, and induces the receptor's dimerization, which recruits the adaptors, *Drosophila* myeloid differentiation factor 88 (dMyD88, homolog of mammalian MyD88), Tube (serine-threonine kinase), and Pelle (homolog of mammalian IL-1R associated kinase) intracellularly (96, 107). Subsequent to Pelle activation Cactus (inhibitor of kappa B, IκB) becomes phosphorylated and is then degraded, freeing the (NFκB) family proteins Dif and Dorsal to translocate into the nucleus and activate the expression of Toll-regulated genes (108–111). A functional redundancy exists between Dif and Dorsal in the control of immune response gene expression (as measured by drosomycin induction) in *Drosophila* larvae (112), but not in adults where Dif alone is sufficient for the induction of defensin and drosomycin (113), and the presence of either protein is sufficient for the induction of cecropin (94). Also, deformed epidermal autoregulatory growth factor 1 (Deaf1) is required downstream of Dorsal and Dif to activate genes (e.g. drosomycin) in response to fungal infections (114).

Downregulation. Like the Imd pathway, the Toll pathway is negatively regulated on multiple levels. Toll pathway activation induces the expression of Cactus (NFκB inhibitor) (115) and WntD (Wnt inhibitor of Dorsal) (116), creating negative feedback loops. *Drosophila* Ubc9 (dUbc9) also inhibits Toll pathway signaling at the level of Dif and Dorsal (117). Finally, a serine protease inhibitor (Spn1) acts upstream of SPE to downregulate Toll signaling in response to fungal infections, in a negative feedback manner (118).

Toll- and Imd-regulated genes

The Toll and Imd pathways regulate the expression of a subset of *Drosophila* genes that are induced upon septic injury (52, 53, 56). There is clearly some functional overlap and synergy in gene regulation between the pathways, as revealed by microarray- (53) and RNAi-analyses (119), the detection of Dif-Relish heterodimers in fly extracts (120), and the observation that *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Ehrlichia chafeensis* activate both signaling pathways (4, 121–123). The most well characterized *Drosophila* immune effectors regulated by the pathways are the AMPs, of which there are seven classes. Cecropin (antibacterial) (124) and Defensin (antibacterial) (125) form ion channels in the cytoplasmic membrane (126, 127), diptericin (antibacterial) (128) and drosomycin (antifungal) (129, 130) act on the cytoplasmic membrane causing lysis (131, 132), Attacin (antibacterial) (133) interferes with outer membrane synthesis (133), Drosocin (antibacterial) (134) interferes with the activities of DnaK (135), and the mode of action of Metchnikowin (antibacterial and –fungal) (136) has not yet been determined. The Toll and Imd signaling pathways together regulate most of the AMPs, but diptericin and drosomysin are primarily regulated by Imd and Toll, respectively, and are, therefore, used to monitor pathway activation (53, 66, 137). Microarray analysis showed that 283 of 400 previously identified (52) *Drosophila* immune response genes are regulated by either one or both pathways. In addition to the AMPs and pathway regulators, the proteins encoded by these genes are involved in iron metabolism, opsonization, melanization, iron sequestration, coagulation, reactive oxygen species (ROS) production, Jun kinase (JNK) pathway signaling, stress response, and many proteases (53).

JAK/STAT pathway

The *Drosophila* JAK/STAT signaling pathway (Fig. 1) comprises the same components as the mammalian pathway, although they differ in the number of JAKs (one in *Drosophila*, four in mammals) and STATs (one in *Drosophila*, seven in mammals) they possess.

Activation and signal transduction. In response to septic injury, *Drosophila* plasmatocytes (macrophage like cells) secrete the cytokine, unpaired-3 (Upd3) (138, 139), which binds to the fat body cell transmembrane receptor, Domeless (homolog of mammalian class 1 cytokine receptor) (140, 141). Upd3 binding induces Domeless dimerization and the intracellular recruitment and phosphorylation of two Hopscotch (JAK) molecules, followed by two STAT92E (homolog of mammalian STAT5) molecules. Activated STAT92E molecules dimerize and translocate into the nucleus where they activate the expression of target genes (139).

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Regulated genes. The JAK/STAT pathway regulates numerous physiological processes [reviewed in (142, 143) including immune responses; however, the contribution of this pathway to immune regulation has not been as well studied as the Toll and Imd pathways. In response to septic injury, the JAK/STAT pathway induces the expression of *CG11501*, which remains functionally uncharacterized (56) and, in combination with several other pathways (144), induces the expression of Turandots, which are stress-response proteins currently found only in *Drosophila* (145). The pathway is involved in the immune response in the *Drosophila* gut, where it helps maintain epithelial cell homeostasis, by regulating stem cell proliferation (51, 146). Activation of the JAK/STAT pathway in response to an intestinal *Serratia marcescens* infection negatively impacts *Drosophila* survival (51).

Downregulation. The JAK/STAT pathway is regulated on multiple levels like Imd and Toll. *Drosophila* protein inhibitor of activated STATs (dPIAS) inhibits STAT92E-dependent transcription by physically interacting with and blocking its DNA binding ability (147). Suppressor of cytokine signaling 36E (SOCS36E, mammalian soc5 homolog) expression is induced by JAK/STAT signaling and inhibits the pathway in a negative feedback loop (148, 149); a truncated form of STAT92E, which is transcribed from an alternate promoter, acts as a dominant negative regulator of JAK/STAT signaling (150); eye transformer, a receptor that is structurally related to Domeless, negatively regulates JAK/STAT signaling by interfering with STAT92E phosphorylation and activation (151).

Although the *Drosophila* antiviral immune response is not well characterized, a role for the Imd, Toll, and JAK-STAT pathways has been demonstrated (156–159).

Cellular immune response

The *Drosophila* cellular immune response is mediated by three sets of hemocytes: plasmatocytes, crystal cells, and lamellocytes, and while all three are found in larvae, only plasmatocytes are found in adults (160).

Plasmatocytes

These cells are similar to mammalian macrophages in that they express scavenger receptors (70, 161–165), secrete cytokines (139, 166), and are responsible for the phagocytosis of microbes (45, 167–170) and apoptotic cells (167, 171). *Drosophila* plasmatocytes also contribute to hemolymph coagulation by secreting the major clotting factor hemolectin (172), promote tolerance to intracellular bacterial infection (see below) (173), and utilize autophagy to control some intracellular pathogens (see below).

Phagocytosis. Phagocytosis involves several receptors many of which have mammalian homologs [see (174) for a recent in-depth review of *Drosophila* phagocytosis]. These include EGF repeat containing proteins Nimrod (162), Eater (161), and Draper (121), the scavenger receptors Croquemort (CD36 family), Peste (CD36 family) (165, 175), and Scavenger receptor CI (dSR-CI) (163), PGRP-LC (70), and Down syndrome cell adhesion molecule (Dscam, homolog of mammalian DSCAM) (164). Thus far, convincing *in vivo* evidence for the role of receptors in phagocytosis has only been obtained for Eater (161) and Nimrod (162). Phagocytosis may also be enhanced by the thiolester proteins (TEPs), which are members of the complement C3 (C3)/α2-macroglobulin (α2M) superfamily. The *Drosophila* genome encodes four Teps (I–IV) that are expressed at basal levels by fat body cells and larval hemocytes but strongly upregulated after a bacterial challenge (I, II, and IV in larvae and II and IV in adults); a fifth gene Tep V is believed to be a pseudogene (176). *In vitro* RNAi treatment of *Drosophila* S2 cells revealed that Teps II and III enhance the phagocytosis of *Escherichia coli* and *S. aureus*, respectively, likely by functioning as opsonins, while a sixth protein Tep VI (also known as macroglobulin complement related, Mcr) binds to and enhances the phagocytosis of *Candida albicans* (177). It has been suggested that Tep II may function as an α2M by virtue of its five splice isoforms that only differ in a domain that is similar to the bait region of α2Ms (176); however, this function has not been demonstrated. Although an *in vivo* role for the Teps (II and IV) in combating *P. gingivalis* infection has recently been demonstrated (178), Teps I–IV have been found to be dispensable for *Drosophila* survival after infection with a variety of other bacterial and fungal pathogens (179). RNAi studies have demonstrated that phagocytosis involves actin rearrangement (7, 177, 180), and phagocytosed microbes are taken up into a phagosome (57) and destroyed by mechanisms that are not yet clearly understood.

Autophagy. Autophagy is an evolutionarily conserved process by which cells envelope their cytoplasmic contents into double-membrane vesicles and, subsequently, deliver them to the lysosome for degradation. It is involved in the control of intracellular pathogen infections in mammals and was first reported as a *Drosophila* immune response by Yano et al., who observed that *in vivo* RNAi targeting an essential autophagy factor (Atg5) rendered *Drosophila* more susceptible to infection by the intracellular pathogen *L. monocytogenes* (181). They subsequently observed *L. monocytogenes* containing autophagosomes in *Drosophila* hemocytes using electron microscopy and identified the intracellular form of PGRP-LE (which detects DAP-type PPG) as the receptor responsible for the induction of autophagy. Autophagy is a part of the *Drosophila* antiviral immune response; however, the initiating receptor remains unidentified (182). Autophagy can also promote infection

by pathogens such as the intracellular fungus *Cryptococcus neoformans*, which co-opts the autophagic system to maintain its lifestyle within *Drosophila* plasmatocytes (180).

Lamellocytes and crystal cells

Only present in *Drosophila* larvae, these cells are involved in encapsulation (lamellocytes) and melanization (crystal cells), immune responses that have no mammalian counterparts. Parasites that are too large to be phagocytosed, e.g. wasp eggs, are surrounded by lamellocytes and encapsulated, walling them off from the rest of the hemocoel (body cavity) (183, 184). The encapsulated particle is then destroyed by the local production of ROSs (185, 186), RNSs (187), and melanization, which is the synthesis and deposition of toxic melanin (188, 189). Melanization also occurs at the site of a cuticle breach and in *Drosophila* adults.

Coagulation

Coagulation is an immediate reaction that occurs at the site of a wound and prevents hemolymph loss, promotes wound healing, and acts as a secondary barrier to infection by immobilizing microbes (190, 191). Studies on hemolymph clots from *Drosophila* larvae revealed that the most abundant protein in the clot is hemolectin (homolog of mammalian clotting factor von Willebrand factor), which is secreted by plasmatocytes (172, 190, 192). Once the primary clot fibers have been formed, they are cross linked to each other and to other clot proteins like Fondue (193), by the enzyme transglutaminase (homolog of mammalian factor XIIIa) (194). Transglutaminase also accumulates on the surface of microbes and targets them to the clot, a role also observed for factor XIIIa in mammals (195). *Drosophila* clotting factor mutants have coagulation defects and are more sensitive to microbial infection (172, 190, 195).

Additional immune response features

MAPK pathways

Two MAPK pathways, the p38 MAPK pathway and the JNK pathway, contribute to the *Drosophila* immune response and are homologous to the mammalian pathways.

p38 MAPK pathway. p38 signaling in *Drosophila* has been shown to be involved in mediating stress responses (196–198) and immunity (40, 173, 199). Two MAPKs, p38a and p38b, are encoded by the *Drosophila* genome (196, 199), and they are activated in response to infection by the upstream MAPK-kinase (MKK) Licorne (homolog of mammalian MKK3) (199), which itself is activated by the MKK-kinase MEKK1 (homolog of mammalian MEKK4) (196, 198–200). Once activated, p38 can then regulate the activity of transcription factors, such as ATF2 and d-Jun to control gene expression (199, 201). p38 null *Drosophila* are more susceptible than wildtype animals to killing by bacterial (40, and Igboin et al., unpublished) and fungal pathogens (40). Also overexpression of p38b conferred increased survival to *Drosophila* infected with intracellular bacteria (e.g. *Salmonella typhimurium*) without a corresponding decrease in bacterial load, and this was due to a p38b-dependent sequestering of the bacteria within plasmatocyte phagosomes (173) (see below for a discussion of tolerance). p38 signaling is negatively regulated by Alphabet, a phosphatase that antagonizes MEKK1 phosphorylation (202).

JNK pathway. In *Drosophila*, immune activation of JNK pathway signaling can occur via the binding of the cytokine Eiger (mammalian tumor necrosis factor homolog) to its receptor Wengen (mammalian TNF receptor homolog) (203–205), also via the Imd pathway at the level of dTak1 (76), and mediates apoptosis, wound healing, morphogenesis, and immunity (206–208). Downstream of Eiger/Wengen binding, one or more JNK kinase kinases (JNKKK) are activated by currently poorly understood mechanisms. The JNKKK dTak1, a shared component with the Imd pathway (76), activates two JNKKs, Hemipterous (mammalian MKK7 homolog) and Mkk4 (mammalian MKK4 homolog) (209), which in turn activate Basket (mammalian JNK homolog) (199, 209, 210). Activated JNK can then turn on the expression of transcription factors like AP1 (211) and other target genes. JNK signaling regulates a subset of immune response genes induced after septic injury (56) and also regulates AMP gene expression by the Imd pathway, downstream of Relish (212, 213). It has been reported that *Drosophila* JNK signaling in response to infection can either be beneficial or detrimental, as evidenced by the observation that Eiger null mutants are more susceptible than wildtype animals to killing by some pathogens, e.g. *P. gingivalis* and *S. aureus* (178, 214), but are more resistant than wildtype animals to killing by some intracellular pathogens, e.g. *S. typhimurium* (1, 214). It has also been reported that Eiger null mutants are no more susceptible to Gram-positive cocci, including *S. aureus*, infections than wildtype animals (215). Several negative regulators of JNK signaling have been identified, including Alphabet, a phosphatase that antagonizes dTAK1 phosphorylation (202), Pva (receptor tyrosine kinase) and Peroxiredoxin (redox sensing enzyme), which antagonize dTAK1 phosphorylation of JNK (216, 217), Puc, a phosphatase that antagonizes JNK activity (218), and Relish, whose activation results in proteosomal degradation of dTAK1 (219).

Tolerance

Tolerance is a process by which plants and animals endure a microbial infection and is one of the more

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recently described immune responses of *Drosophila*. Tolerance differs from resistance, in which the host reduces the fitness of the pathogen, thereby reducing the pathogen load. Tolerance in *Drosophila* has only been observed in response to bacterial infections, and the hallmarks of this response are increased fly survival without a corresponding decrease in the bacterial load (1, 173, 220, 221) or an increase in bacterial load without a corresponding decrease in fly survival (222). As examples of the first scenario, *S. typhimurium*-infected *eiger* null *Drosophila* survived 3 days longer than *wt* animals even though their bacterial loads were similar (1), and *Drosophila* overexpressing p38b survived *S. typhimurium*, *L. monocytogenes*, and *Legionella pneumophilia* infections longer than wildtype animals without a reduction in bacterial load (173). The mechanism of tolerance to *S. typhimurium* was shown to be via the sequestration of the bacteria within *Drosophila* hemocytes. As an example of the second scenario, wildtype and CG3066 (signaling protease involved in melanization) null *Drosophila* were killed at the same rate by *Burkholderia cepacia*, even though up to 25 times more bacteria were present in the mutant animals (222).

Reactive intermediate production

As mentioned previously, ROSs, e.g. superoxide anion, and RNSs, e.g. nitric oxide, are produced in *Drosophila* larvae during the melanotic encapsulation of particles that are too large to be phagocytosed (185, 186, 223). They also play a role in the immune response in the *Drosophila* gut where they are induced in response to natural infection (i.e. ingestion) (224, 225).

Drosophila dual oxidase (dDuox) is present on gut epithelial cells and contains a nicotinamide-adenine-dinucleotide-phosphate (NADPH)-oxidase domain and a peroxidase domain. The NADPH oxidase domain generates superoxide anion, which dismutates to hydrogen peroxide (H_2O_2), and the peroxidase domain uses the H_2O_2 to produce hypochlorous acid (HOCl) (224). Excessive ROS production is prevented by immune responsive catalase, which converts the H_2O_2 to water and oxygen (226).

NO participates in signaling in the gut of *Drosophila* larvae, which upregulate nitric oxide synthase expression upon natural infection with some Gram-negative bacteria. NO contributes to the induction of the AMP, diptericin, in the gut and hemocytes of naturally infected *Drosophila* larvae, and pharmacological inhibition of NOS has been shown to impair the ability of *Drosophila* larvae to survive septic and natural infections (225). The availability of a NO synthase null *Drosophila* mutant should shed additional light on the role of NO in the immune response.

Iron sequestration

The *Drosophila* genome encodes conserved iron-binding proteins transferrin (227) and ferritin (228), which can limit iron availability to invading microbes. Transferrin, ferritin, and iron transporters are upregulated in response to microbial challenges, which suggests a role for iron sequestration in controlling microbial infections in *Drosophila* (49, 50, 52, 227, 229).

RNA interference (RNAi)

RNAi involves the sequence-specific degradation of mRNA and is involved in the *Drosophila* antiviral immune response (19, 22, 230, 231). The role of RNAi in the *Drosophila* immune response was first reported by Li et al., who observed the accumulation of Flockhouse virus (FHV)-specific small interfering RNAs (siRNAs) in infected S2 cells (232). Galiana-Arnoux et al. (233), Zambon et al. (234), and van Rij et al. (230) demonstrated an *in vivo* role for RNAi in combating viral infections. *Drosophila* also possess dsRNA uptake machinery (235, 236), which is important for the systemic RNAi response to extracellularly released (by controlled export or from lysed host cells) viral RNA (237).

In summary, three distinct pathways, Imd, Toll, and JAK/STAT, mediate the *Drosophila* humoral response, and the main humoral effectors are the AMPs, which are synthesized and secreted by the fat body. Two MAPK signaling pathways, JNK and p38, also regulate *Drosophila* immune responses, and all five signaling pathways are homologous to the pathways in mammals. The cellular immune response in *Drosophila* adults is mediated by plasmatocytes, macrophage-like cells that detect the presence of invading microbes via scavenger receptors, and phagocytose and subsequently destroy said microbes. Phagocytosis may be aided by the Teps, C3/α2M family proteins that function as opsonins. Additional *Drosophila* immune defense strategies include the production of reactive intermediates (oxygen and nitrogen), RNAi (to combat viral pathogens), the sequestration of iron, autophagy (to combat intracellular pathogens), and endurance of infections.

Drosophila infection models

Drosophila cultured cell lines (S2 and mbn-2), embryos, larvae, and adults (male and female) have been used to study host–pathogen interactions; however, adults and cultured cells are the most widely used. *Drosophila* embryos, larvae, and adults offer the advantage of whole animal models to examine pathogen–host interactions, and in all three models, the readout for pathogen virulence is fly mortality. A *Drosophila* embryo model was developed to study *Photorhabdus asymbiotica* infection, as it offered the added advantage of allowing the interaction between the bacterium and actively migrating *Drosophila* hemocytes to be followed *in vivo*, and in real

time, using confocal microscopy (238). *Drosophila* larvae and adults have been used to identify and characterize pathogen and host factors that promote or inhibit infection by pathogens like *P. gingivalis* (63, 178), *Pseudomonas fluorescens* (3, 239), *P. aeruginosa* (40, 122, 240), *Vibrio cholera* (241–243), *S. aureus* (244, 245), *Klebsiella pneumonia* (246), *Streptococcus agalactiae* (247), West Nile virus (19), *Aspergillus fumigatus* (13, 17), and *Rhizopus oryzae* (41). *Drosophila* adults have also been used to test the efficacy of antifungal (248–251) and antibacterial drugs (252–254) and other antibacterial therapies (255, 256). It is important to take into account the immunological differences between the models when choosing one. For example, *Drosophila* larvae possess all three types of hemocytes but adults possess only plasmatocytes. Also, larval hemocytes do not actively migrate to a site of infection but rather arrive there by direct capture from the open circulatory system, in which they are passively pumped around (257).

Drosophila S2- and mbn2-cell lines are used as models for plasmatocytes because they are immunocompetent, phagocytic, and express many plasmatocyte-specific scavenger receptors. They are very useful for studying the phagocytosis, trafficking, proliferation, and cell-to-cell spread of pathogens (2, 121, 177, 180, 258) and for large scale screening by RNAi to identify host factors that interact with pathogens (20, 51, 259–262).

Routes of infection

Three routes of infection have been used to introduce pathogens into *Drosophila*: injection/septic injury, ingestion/natural infection, and rolling (in fungal spores). The injection method introduces the pathogen directly into the *Drosophila* hemocoel and involves either pricking the body cavity (usually the thorax in adults) with a needle dipped into a concentrated culture or the injection of a precise dose of the pathogen using a nanoinjector. Ingestion is also referred to as natural infection and involves introducing the animals into vials containing filter disks soaked with media containing the bacterium of interest or vials containing a pregrown lawn of fungal spores. Some ingestion models require that the animals be starved prior to their introduction into food containing vials, which drives subsequently ingested food to the crop. The rolling method of infection has only been used with fungal pathogens and involves rolling the flies over a lawn of spores growing on a plate. The rolling and injection methods of infection require anesthetization, which is usually done with carbon dioxide, and the injection and rolling methods require subsequent transfer of the animals into vials with food. After infection, the animals are incubated at 25–30°C (embryos are covered with oil), and their survival is monitored. It is important to note that the route of infection affects the interaction of the pathogen with the host and can give different results. For example, the *alb1* gene is involved in *A. fumigatus* virulence when the fungus is ingested by or introduced onto the cuticle of *Drosophila* but not when the fungus is injected into the animals (17).

Transgenic expression (non-infection based)

In situations where the *Drosophila* model is not permissive for infection, it has been possible to introduce pathogen virulence factors into the flies by transgenically expressing the virulence factors in the animals. Pathogen genes can be expressed ubiquitously (26) or in specific fly tissues such as the fat body (21), wings (263), and eyes (264) by placing the transgene under the control of tissue-specific promoters. This has facilitated the identification of host cell targets of *Plasmodium* sporozite proteins (26), anthrax lethal factor and edema factor (263, 265), pertussis toxin (266), HIV-1 viral protein U (21), and Epstein-Barr virus immediate-early protein BZLF1 (264) (Table 1).

Drosophila–pathogen interactions

Drosophila as a model for analyzing bacterial virulence

Numerous studies have shown that bacterial virulence factors that are important for the successful infection of mammals are also involved in infecting *Drosophila* (1, 63, 170, 238, 240, 246, 247, 252, 254, 263, 267). Bacterial virulence factors that promote or inhibit infection in *Drosophila*, many of which are also involved in mammalian infection, are listed in Table 1. These include *F. novicida* oxidative stress transcriptional regulator, OxyR (268), *L. monocytogenes* actin polymerization protein, actA (4), and *V. cholera* toxin (252). *P. aeruginosa* has been extensively studied using the *Drosophila* model [see (64) for a recent review], and in the interest of brevity, this section of the review will address the use of the model to study *Francisella tularensis* virulence.

F. tularensis is a facultative intracellular pathogen that is the etiological agent of tularemia, a disease that affects humans and small vertebrates. Three subspecies, *F. holartica*, *F. tularensis*, and *F. novicida*, cause disease in humans and are vectored by blood-sucking insects. *F. tularensis* is able to infect and propagate in *Drosophila* mbn2 (170) and S2 cells (269) *in vitro*, killing the cells in the process. The bacterium also infects and kills adult flies in a dose-dependent manner (170, 269). Injection of GFP-expressing *F. tularensis* enabled the systemic spread of the bacteria and their localization to *Drosophila* hemocytes (about 55% of the bacteria were intracellular at the time of death) to be observed. Similar to infections in human cells, the bacteria are taken up into phagosomes in *Drosophila* S2 cells, which do not fuse with lysosomes, and from which the bacteria can escape within 30–60 min postinfection (269). The intracellular growth locus (*igl*)

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Table 1. Human bacterial pathogen genes that are involved in the infection of *Drosophila*

Pathogen	*Drosophila* model (mode of infection)†	Pathogen genes involved in infection: Promote infection	Pathogen genes involved in infection: Inhibit infection	References
Bacillus anthracis	Adult (TE)	*lef* (lethal factor), *cya* (edema factor)		(263)
Bordetella pertussis	Adult (TE)	*ptx* (pertussis toxin)		(266)
Burkholderia cenocepacia	Adult (SI)	*BCAL2831* (Two component regulatory system component) *bscN* (Type III secretion system ATP-binding protein) *cepI* (Two component regulatory system component) *hldA* (LPS biosynthesis) *htrA* (stress response protease) *zmpA* (zinc metalloprotease) *zmpB* (zinc metalloprotease)	*cepR* (Two component regulatory system component)	(267)
Francisella novicida	Adult (SI)	*FTN_0649* (FAD-dependent 4Fe-4S ferrodoxin) *FTN_0869* (putative transglutaminase) *FTN_0889* (putative transcriptional regulator) *glpD* (anaerobic glycerol-3-phosphate dehydrogenase) *nadC* (nicotinate-nucleotide pyrophosphorylase) *OxyR* (oxidative stress transcriptional regulator) *pmrA* response regulator *udp* (uridine phosphorylase) *uvrA, uvrB, recB, ssb, mutM, ruvC* (DNA repair) DNA repair: 9 Protein repair: 1 Transporter: 3 Other: 43		(268)*
	Adult (SI)	FPI genes: 14 Other: 29	*FTN_0119* *pilA, pilB, pilM, fimT* (Type IV pilus) *pckA* (Phosphoenolpyruvate carboxykinase)	(271)*
	Adult (SI)	*iglB, iglD* (FPI proteins) *mglA* (Virulence gene transcriptional regulator)		(269)
	Adult (SI)	Cell division: 1 DNA modification: 9 FPI: 3 Hypothetical: 24 Intergenic: 5 Metabolic: 41		(58)*

Table 1 (*Continued*)

Pathogen	*Drosophila* model (mode of infection)[†]	Pathogen genes involved in infection: Promote infection	Pathogen genes involved in infection: Inhibit infection	References
		Other: 13 Transcription/translation: 2 Transferases: 8 Transport: 28 Type IV Pili: 1 Unknown: 33		
Francisella tularensis (LVS)	Adult (SI)	*iglB, iglC, iglD* (FPI proteins)		(170)
		mglA (Virulence gene transcriptional regulator)		
Klebsiella pneumoniae	Adult (SI)	*TrpC (*tryptophan biosynthesis) *WaaQ* (capsule and LPS biosynthesis)		(246)
Listeria monocytogenes	Adult (SI)	*actA* (Actin polymerization)		(277)
		hly (Listeriolysin, hemolysin)		
Photorhabdus asymbiotica	Embryos (SI)	*mcf1* (toxin)		(238)
Porphyromonas gingivalis	Adult (SI)	capsular polysaccharide locus		(63)
		fimA (fimbriae) *kgp* (lysine specific protease) *mfa* (minor fimbriae) *rgpA rgpB* (arginine specific proteases)		
Pseudomonas aeruginosa	Adult (SI)	*kerV (hypothetical methyltransferase)*		(241)
	Adult (SI)	*nrdD, nrdJ* (Ribonucleotide reductases)		(301)
	Adult (F)	*lasI;rhlI* (Quorum sensing proteins)	*qteE* (Quorum sensing regulator)	(302)
	Adult (SI)	*toxA* (Exotoxin A)		(122)
	Adult (SI)	*iscR* (Transcriptional regulator)		(303)
	Adult (SI)	*katA* (Catalase A)		(303)–(305)
	Adult (SI)	*hcnC* (Cyanide)		(279)
	Adult (F)	*exoS* (Exotoxin)		(274)
	Adult (SI)	*OxyR* (Oxidative stress transcriptional activator)		(306)
	Adult (F)	*relA* (Nucleotide synthesis, stringent response)		(307)

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Table 1 (*Continued*)

Pathogen	*Drosophila* model (mode of infection)[†]	Pathogen genes involved in infection: Promote infection	Pathogen genes involved in infection: Inhibit infection	References
	Adult (F)	*relA;spoT* (Nucleotide synthesis, stringent response)		(308)
	Adult (SI)	*chpA, fimV, orf406, pilGHI, pilJ, pilK, pilL, orf2982* (Twitching motility)		(309)
	Adult (SI)	*dsbA (*Disulfide isomerase)		(8), (122), (240)
	Adult (F)		*qscR*	(5)
	Adult (SI)	*muxA* (efflux pump) *pilA* (Type IV pilus)		(310)
	Adult (SI)	*exsA* (Transcription activator, Type III secretory system) *exsD* (Type III secretory apparatus component) *pilV* (Type IV pili, twitching motility)		(8)
	Adult (SI)	*33C7, 44B1* (Unknown) *gacA, mvfR* (Two component regulatory system regulators, quorum sensing) *mtrR, pstP* (Transcriptional activators) *phzB* (Phenazine biosynthesis) *plcS* (Phospholipase C) *pqsB* (Hydroxy-alkylquinoline synthesis, quorum sensing)		(122)
	Adult (SI)	*PA3001* (Oxidoreductase) *PA4489, PA5441* (Hypothetical, unknown function) *pgk* (Phosphoglycerate kinase) *pgm* (Phosphoglycerate mutase) *pilI, cca* (Twitching motility) *pyrF* (Orotidine decarboxylase)		(311)
	Adult (SI)	*fabF1 (*Beta-ketoacyl-acyl carrier protein synthase II) *flhB* (Flagellar biosynthesis) *opdO* (Outer membrane porin) *PA0272, prpR, hudR* (transcription factors) *PA0369, PA2077* (Hypotherical proteins) *PA14_35740* (Transposase) *PA2002* (Fatty acid transporter) *pilF* (Type IV pilus assembly protein) *pvdI* (Peptide synthesis) *wspF (*Methyl esterase)		(240)

Table 1 (*Continued*)

Pathogen	*Drosophila* model (mode of infection)[†]	Pathogen genes involved in infection: Promote infection	Pathogen genes involved in infection: Inhibit infection	References
Pseudomonas fluorescens	larvae (F)	*A4589* (Unknown function)		(3)
		gmd (LPS biosynthesis)		
		gacA (Two component regulatory system protein)		(239)
Salmonella typhimurium	Adult (SI)	*orgA* (Salmonella pathogenicity island 1 component)		(1)
		phoP (two component regulatory system sensor)		
		slrP (type III secretion effector)		
		spiC, sseA, sseB, sseC, sseD (SPI2 translocation machinery)		
		ssrA (Salmonella pathogenicity island 2 component)		
Serratia marcescens	Adult (SI)	*wzm* (LPS biosynthesis, O antigen)		(312)
Streptococcus agalactiae	Adult (SI)	*bca* (alpha C protein, adhesin)		(247)
		clyE (cytolisin)		
		cpsE (capsular polysaccharide)		
		dltA (D-alanyl-lipoteichoic acid)		
Staphylococcus aureus	Adult (SI)	*perR* (Transcription regulator, oxidative stress and iron storage)		(254)
		pheP (Amino acid permease)		
	Adult (SI)	*atl* (amidase–glucosaminidase, peptidoglycan degradation)		(245)
		dltA (D-alanine ligase)		
		mprF (lysylphosphatidylglycerol synthesis)		
		SA0614 (Two component regulatory system component)		
		SA0615 (Two component regulatory system component)		
		ypfP (Glycolipid synthesis)		
	Adult (SI)		*itaS* (lipoteichoic acid synthesis)	(121)
Vibrio cholera	Adult (F)	*ctxB* (Cholera toxin)		(241)
		kerV (hypothetical methyltransferase)		(241), (252)
Yersinia pseudotuberculosis	Adult (F)	*kerV* (hypothetical methyltransferase)		(241)

*Studies where genome-wide screens were performed, and resulted in the identification of >20 genes. Genes that were further characterized are listed. If genes were functionally or otherwise classified, and the number of genes in each group was reported, we included the numbers in this table.

[†]SI: septic injury, TE: transgenic expression (of pathogen virulence factors), F: feeding, R: rolling (in spores).

Abbreviations: LPS: lipopolysaccharide FPI: Francisella pathogenicity island LVS: Live vaccine strain

and macrophage growth locus (*mgl*) are involved in *F. tularensis* virulence in mice (270), and when several genes from these loci were mutated and the null mutants tested in *Drosophila*, they were found to be less virulent than the wildtype parental strain (170, 269).

Two hundred and forty-nine *F. novicida* transposon insertion mutants were individually evaluated in a *Drosophila* killing model, and 49 genes that were required for normal virulence in the flies were identified (Table 1) (271). The majority of the identified genes were novel *F. novicida* virulence factors; 43 mutants had attenuated killing, and the mutated loci in these strains included most of the *Francisella* pathogenicity island (FPI) genes; six mutants were hypervirulent in the model and included mutants in pilus assembly proteins, an outer membrane protein and a kinase. Most of the attenuated strains also had low proliferation abilities in mammalian macrophages (murine J774 cells), and many of them also demonstrated reduced cytotoxicity; the hypervirulent mutants were significantly more cytotoxic to the murine macrophages than the wildtype strains. The strong correlation between *F. novicida* mutant phenotypes in *Drosophila* and in mouse macrophages suggested that the survival of *F. novicida*-infected flies is a good indicator of the bacterium's pathogenesis in mammals.

A *F. novicida* transposon mutant library was screened to identify genes that were essential for growth and survival in *Drosophila*, and 149 negatively selected genes (56 confirmed, Table 1) were identified (268). Sixty of the genes had previously been identified as virulence factors in murine models. Seven of the negatively selected genes encoded proteins that are important for the bacterium's ability to resist oxidative damage, and the mutants showed increased sensitivity to reactive-oxygen producing agents in subsequent *in vitro* experiments. Screening the library in wt and Imd pathway-null *Drosophila* identified a subset of seven *F. novicida* genes that are required to resist *Drosophila* Imd-regulated defenses (genes that were negatively selected in wt flies but rescued in the mutant flies). Five of the seven mutants showed increased sensitivity to AMPs, modeled by polymyxin B, which are the main Imd-regulated immune effectors. The two mutants that showed no phenotype in the AMP killing assay were attenuated due to other Imd-dependent mechanisms, as similar levels of AMPs were induced in response to infection with these mutants as with the wt strain. Five genes, *recB*, *pilA*, *pilB*, *pyrF*, and *manB*, identified in this study as being essential for *F. novicida* growth and survival within the fly, were also identified by Ahlund et al. as being involved in *F. tularensis* virulence in *Drosophila* (271).

Another *F. novicida* transposon mutant library (3,050 alleles representing 1,448 genes) was first screened in cultured S2 cells to identify genes required for the intracellular proliferation of the bacterium, and then, a subset of the mutants was tested for their lethality in adult flies (Table 1) (58). Three hundred and ninety-four genes that when mutated resulted in a significant reduction in intracellular growth of the bacterium were identified, and 80 of the 168 subsequently tested mutants exhibited reduced lethality and proliferation in the adult animals. The mutated loci encoded FPI proteins and proteins involved in metabolism, type IV pili biogenesis, transport, and DNA modification. One hundred and thirty-five genes were also required for *F. novicida* replication in human macrophages. Genes that were newly identified as playing a role in *F. novicida* pathogenesis may not be identifiable by currently available mammalian models, or could be specifically required for the insect phase of the bacterium's life cycle. Four genes, *minD*, *iglC*, *iglD*, and FTN_0109, identified in this study as being impaired in growth and/or lethality in *Drosophila*, were also identified by Ahlund et al. as being involved in *F. tularensis* virulence in *Drosophila* (271). Seven genes, *ruvC*, *glpD*, *mdaB*, *ilvE*, *kdpC*, *pilQ*, and FTN_1014, identified in this study, were also identified by Moule et al. as being essential for *F. novicida* growth and survival within the fly (268).

The studies described above and referenced in Table 1 show that bacterial pathogens utilize many of the same virulence mechanisms to infect mammals and *Drosophila*, and that the *Drosophila* model is useful for identifying novel virulence factors.

Drosophila as a model for analyzing the host response to bacterial infection

The host response activated upon a bacterial infection can fight and resolve the infection or contribute to the pathology caused by the infection, and the vast array of *Drosophila* mutant and transgenic lines, and microarray, RNAi, and proteomics platforms has facilitated in-depth analyses of the host response to infection by a variety of pathogens. The results of studies in which several different *Drosophila* tools (immune response gene mutants, transgenic expression, whole genome microarray, and RNAi) were exploited to examine the host response to infection are discussed in this section of the review. *Drosophila* genes that are involved in the response to human bacterial pathogens are shown in Table 2.

Loss-of-function immune response gene mutants

A large collection of loss-of-function *Drosophila* mutants have been generated by numerous investigators in the field and are readily available for use to identify *Drosophila* immune response components that play a role during infection. The use of knockout mutants of pathway components demonstrated that the well-characterized Toll- and Imd-signaling pathways are involved in the response to a variety of bacterial infections (4, 45, 49, 122, 170, 272–275). For example, *Drosophila Dif*

Table 2. *Drosophila* components that are involved in the response to bacterial infection

	Drosophila component	*Drosophila* model*	Bacteria‡																	References
			Listeria monocytogenes	*Staphylococcus aureus*	*Enterobacter cloacae*	*Enterococcus faecalis*	*Ehrlichia chafeensis*	*Francisella tularensis* LVS	*Porphyromonas gingivalis*	*Francisella novicida*	*Burkolderia cenocepacia*	*Klebsiella pneumoniae*	*Photorhabdus luminescens*	*Legionella pneumophilia*	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Salmonella typhimurium*	*Streptococcus pneumoniae*	*Vibrio cholera*	*Streptococcus agalactiae*	*Photorhabdus asymbiotica*	
Imd pathway components	*Dredd* (Death-related ced-3)	Adult (SI)					■														45
		Adult (F)																	■		242
		Adult (SI)											■								272
		Adult (F)														■					9
	FADD (Fadd)	Adult (F)														■					9
		Adult (F)																	■		242
	Iap2 (Inhibitor of apoptosis 2)	Adult (SI)			■																273
	imd (Immune deficiency)	Adult (SI)								■											268
		Adult (F)														■					9
		Adult (SI)															■				1
		Adult (SI)	■																		4
		Adult (SI)		■																	121
		Adult (SI)						■													170
	ird5 (immune response deficient 5)	Adult (SI)						■													170
	key (Kenny)	Adult (SI)							■												178†
		Adult (F)														■					9
		Adult (F)														■					51
		Adult (F)																	■		242
	PGRP-LC (peptidoglycan recognition protein-LC)	Adult (SI)			■																69
		Adult (SI)	■																		181
	PGRP-LE (peptidoglycan recognition protein-LE)	Adult (SI)																			181
	pirk (Poor Imd response upon knock-in)	Adult (SI)			■																89
	Rel (Relish)	Adult (SI)					■														45
		Adult (F)																	■		242
		Adult (F)														■					9
		Adult (SI)						■													170
		Adult (SI)							■												268
		Adult (SI)													■						40
		Adult (SI)			■																69
	Tak1 (TGF-β activated kinase 1)	Adult (F)													■						276
		Adult (SI)			■							■									276
Toll pathway components	*cact* (Cactus)	Adult (SI)													■						122
	Dif (Dorsal-related immunity factor)	Adult (SI)													■						122
		Adult (SI)	■																		4
		Adult (F)														■					9
	dl (Dorsal)	Adult (SI)													■						122
	GNBP1 (Gram-negative binding protein, Toll pathway component)	Adult (SI)		■		■															313
	MyD88 (MyD88)	Adult (SI)				■															96
	pll (Pelle)	Adult (SI)				■															45
	SPE (Spatzle processing enzyme)	Adult (SI)				■															106
	spz (Spatzle)	Adult (SI)													■						122
		Adult (SI)		■																	244
	Tl (Toll)	Adult (SI)					■														45
		Adult (SI)		■																	121, 40, 245
		Adult (SI)													■						122
	wntD (Wnt inhibitor of Dorsal)	Adult (SI)	■																		116
JAK/STAT signaling pathway compone	*dome* (Domeless)	Adult (F)														■					51
	PIAS (Pias)	Adult (F)														■					51
	Pp1α-96A (JAK-STAT pathway negative regulators)	Adult (F)														■					51
	upd3 (Unpaired 3)	Adult (F)														■					51
Effectors	*Ars2*	Adult (SI)																	■		242
Antimicrobial peptides	*AttA* (Attacin)	Adult (SI)													■						280
Thiol-ester proteins		Adult (SI)																	■		243
Clotting proteins	*CecA (* Cecropin A)	Adult (SI)													■						280
Melanization proteins	*CG3066* (Serine protease 7)	Adult (SI)								■											268
RNAi		Adult (SI)	■	■													■	■			222
	Def (Defensin)	Adult (SI)													■						280
	Drc (Drosocin)	Adult (SI)													■						280

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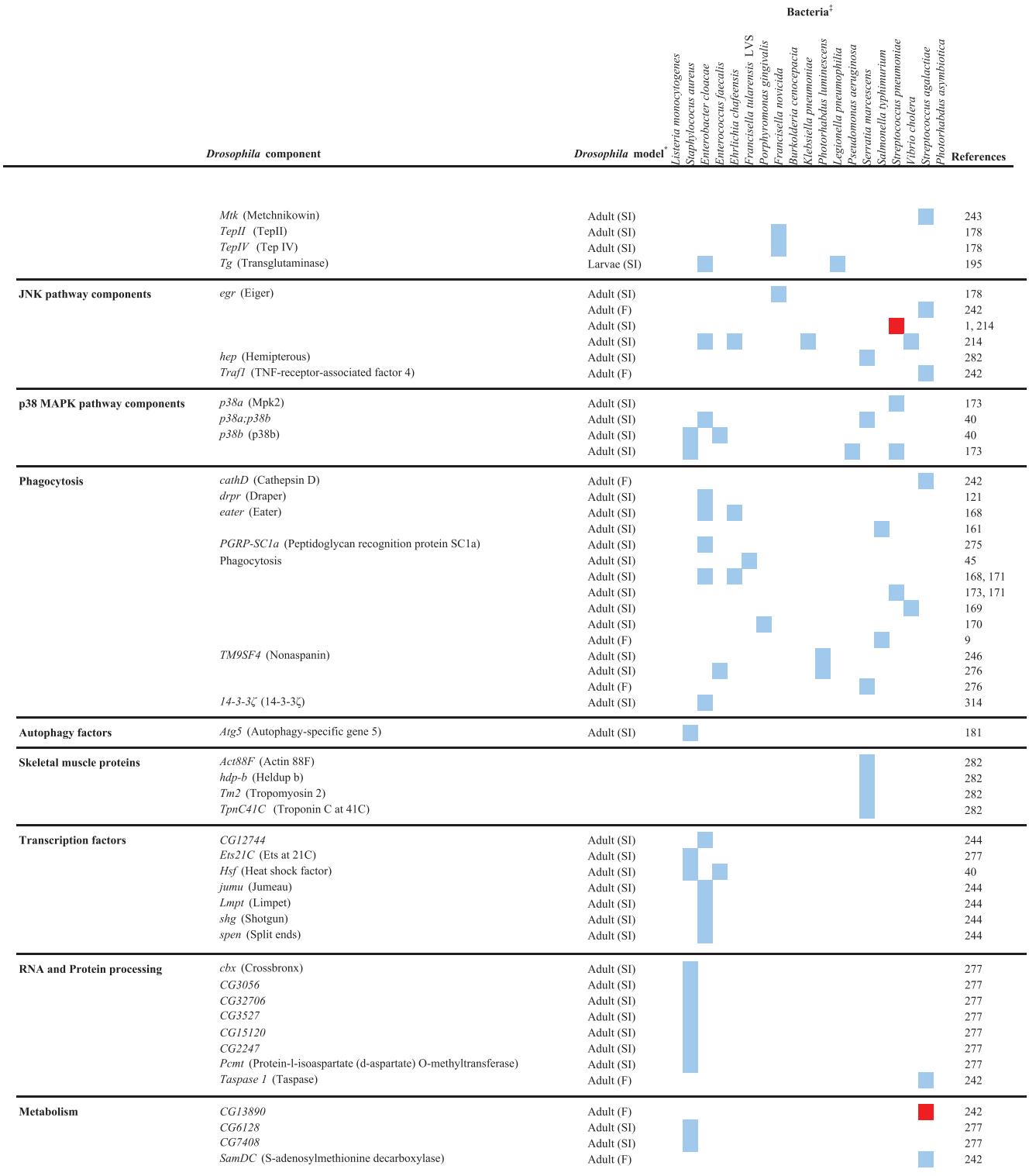
Table 2 (*Continued*)



Drosophila component		*Drosophila* model*	References
	Mtk (Metchnikowin)	Adult (SI)	243
	TepII (TepII)	Adult (SI)	178
	TepIV (Tep IV)	Adult (SI)	178
	Tg (Transglutaminase)	Larvae (SI)	195
JNK pathway components	*egr* (Eiger)	Adult (SI)	178
		Adult (F)	242
		Adult (SI)	1, 214
		Adult (SI)	214
	hep (Hemipterous)	Adult (SI)	282
	Traf1 (TNF-receptor-associated factor 4)	Adult (F)	242
p38 MAPK pathway components	*p38a* (Mpk2)	Adult (SI)	173
	p38a;p38b	Adult (SI)	40
	p38b (p38b)	Adult (SI)	40
		Adult (SI)	173
Phagocytosis	*cathD* (Cathepsin D)	Adult (F)	242
	drpr (Draper)	Adult (SI)	121
	eater (Eater)	Adult (SI)	168
		Adult (SI)	161
	PGRP-SC1a (Peptidoglycan recognition protein SC1a)	Adult (SI)	275
	Phagocytosis	Adult (SI)	45
		Adult (SI)	168, 171
		Adult (SI)	173, 171
		Adult (SI)	169
		Adult (SI)	170
		Adult (F)	9
	TM9SF4 (Nonaspanin)	Adult (SI)	246
		Adult (SI)	276
		Adult (F)	276
	14-3-3ζ (14-3-3ζ)	Adult (SI)	314
Autophagy factors	*Atg5* (Autophagy-specific gene 5)	Adult (SI)	181
Skeletal muscle proteins	*Act88F* (Actin 88F)		282
	hdp-b (Heldup b)		282
	Tm2 (Tropomyosin 2)		282
	TpnC41C (Troponin C at 41C)		282
Transcription factors	*CG12744*	Adult (SI)	244
	Ets21C (Ets at 21C)	Adult (SI)	277
	Hsf (Heat shock factor)	Adult (SI)	40
	jumu (Jumeau)	Adult (SI)	244
	Lmpt (Limpet)	Adult (SI)	244
	shg (Shotgun)	Adult (SI)	244
	spen (Split ends)	Adult (SI)	244
RNA and Protein processing	*cbx* (Crossbronx)	Adult (SI)	277
	CG3056	Adult (SI)	277
	CG32706	Adult (SI)	277
	CG3527	Adult (SI)	277
	CG15120	Adult (SI)	277
	CG2247	Adult (SI)	277
	Pcmt (Protein-l-isoaspartate (d-aspartate) O-methyltransferase)	Adult (SI)	277
	Taspase 1 (Taspase)	Adult (F)	242
Metabolism	*CG13890*	Adult (F)	242
	CG6128	Adult (SI)	277
	CG7408	Adult (SI)	277
	SamDC (S-adenosylmethionine decarboxylase)	Adult (F)	242

Table 2 (*Continued*)

	Drosophila component	*Drosophila* model*	Bacteria‡																References
			Listeria monocytogenes	*Staphylococcus aureus*	*Enterobacter cloacae*	*Enterococcus faecalis*	*Ehrlichia chafeensis*	*Francisella tularensis* LVS	*Porphyromonas gingivalis*	*Francisella novicida*	*Burkolderia cenocepacia*	*Klebsiella pneumoniae*	*Photorhabdus luminescens*	*Legionella pneumophilia*	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Salmonella typhimurium*	*Streptococcus pneumoniae*	*Vibrio cholera*	*Streptococcus agalactiae*	*Photorhabdus asymbiotica*	
Calcium Binding	*CBP* (Calcium binding protein)	Adult (F)																				242
	NUCB1 (NucB1)	Adult (F)																				242
GTPases	*Rac1* (Rac1)	Embryos (SI)																				238
	Rac2 (Rac2)	Adult (SI)																				274
		Adult (F)																				274
Electron transport and redox maintana	*Cyp6a20* (Cyp6a20)	Adult (F)																				242
	Cyp6a13 (Cyp6a13)	Adult (SI)																				277
	Prx5 (Peroxiredoxin 5)	Adult (SI)																				217
	Trx-2 (Thioredoxin 2)	Adult (SI)																				244
Miscellanous	*BobA* (Brother of Bearded A)	Adult (SI)																				277
	ced-6 (Ced6)	Adult (SI)																				121
	CG11489	Adult (SI)																				277
	Gr28b (Gustatory receptor 28b)	Adult (SI)																				277
	Gsa60A (Stimulatory G protein alpha subunit)	Adult (SI)																				252
	Gst2 (Glutathione S transferase S1)	Adult (SI)																				282
	Hsp27 (Heat shock protein)	Adult (SI)																				40
	insc (Inscuteable)	Adult (F)																				242
	rut (Rutabaga)	Adult (SI)																				252
	SK (Small conductance calcium-activated potassium channel)	Adult (SI)																				252
	ttv;Ext2 (Tout-velu;Ext2)	Adult (SI)																				247
	Wwox (WW domain containing oxidoreductase)	Adult (F)																				242
Unknown function	*CG11293*	Adult (SI)																				277
	CG12004	Adult (SI)																				244
	CG13564	Adult (SI)																				277
	CG14722	Adult (F)																				242
	Der-2 (Derlin-2)	Adult (SI)																				277
	edin (Elevated during infection)	Adult (SI)																				281
	Fit1 (Fermitin 1)	Adult (F)																				242
	tyf (Twenty-four)	Adult (SI)																				277

*SI: septic injury, F: feeding

† Unencapsulated Pg strains only

‡ Genes shaded blue fight bacterial infection (knockout enhances fly mortality), genes shaded red enhance bacterial infection (knockout enhances fly survival)

Abbreviations:
LVS: Live vaccine strain
JNK: c-Jun N-terminal kinase
MAPK: Mitogen-activated protein kinase
JAK/STAT:Janus kinase/Signal transducer and activator of transcription

(NFκB)-null mutants were more susceptible to killing than *wt* animals by *L. monocytogenes*, demonstrating a role for the Toll pathway in the defense against the bacterium (4). Imd pathway mutants were more susceptible than wildtype animals to killing by *F. tularensis* LVS (170), and both Imd and Toll mutants were more susceptible to killing by *Erlichia chaffeensis* (45). Once a *Drosophila*–pathogen model has been established, mutants that are deficient in specific host processes can be used to examine the interaction between those processes and the microbe. For example, *Phg1* (nonaspanin)-null *Drosophila* were found to be highly susceptible to Gram-negative (*P. aeruginosa*, *E. cloacae*, and *K. pneumoniae*) bacterial infections, and it was demonstrated that this enhanced susceptibility was due to the inefficient phagocytic abilities of the fly hemocytes (276).

A large library of 1,231 transposon insertion mutants representing 8% of the *Drosophila* genome was screened to identify host genes required to survive a *L. monocytogenes* septic injury infection (277). Eighteen *Drosophila* mutants with increased susceptibility to killing by the bacterium were identified (Table 2), and the mutated loci included those involved in ubiquitination, RNA processing, and transcription activation. A comparison of the growth rates of *L. monocytogenes* in sensitive mutants and wildtype animals identified two classes of mutants: those in which bacterial growth was elevated relative to wildtype and those in which the bacterial levels remained unchanged relative to wildtype. This suggested that the first class of mutants is immunocompromised, i.e. deficient in genes required to resist the *L.monocytogenes* infection, while the second class of mutants has reduced endurance-characterized by lowered survival without a corresponding increase in bacterial proliferation-to the bacterial infection. Surprisingly, no overlap was observed between the *Drosophila* genes identified in

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this study and genes identified in two previous RNAi screens, likely because the use of S2 cells for the RNAi screens limited the observations to the processes involved in the interaction between *L.monocytogenes* and just one type of cell, whereas the whole animal model allowed for a much wider variety of processes to be examined.

An even larger library of 6,200 *Drosophila* transposon mutants was screened for altered susceptibility to a *V. cholera* natural infection, and 16 mutants were identified that contained disruptions in genes encoding proteins with homology to conserved domain proteins or mammalian proteins of know function (242) (Table 2). Seven of the *Drosophila* mutants demonstrated enhanced resistance, while nine demonstrated lowered resistance relative to the wildtype strain. Interestingly, the Imd pathway was found to contribute to pathology under normal circumstances (i.e. pathway mutants were more resistant to infection). It was determined that the mechanism of Imd involvement in causing pathology was due in part to an enhancement of cholera toxin activity, as imd pathway mutants were more resistant than wildtype animals to killing by a cholera toxin null *V. cholera* mutant. A significantly higher number of apoptotic intestinal epithelial cells was observed in infected imd pathway mutants than in wildtype animals, which suggested that under normal circumstances programmed cell death- a defense mechanism against intracellular pathogens in eukaryotes- is repressed by the Imd pathway in response to *V. cholera* infection.

Transgenic Drosophila

As discussed above (Routes of infection), transgenic *Drosophila* expressing pathogen virulence factors have been used to identify host targets. Host genes that are absent from *Drosophila* have also been transgenically expressed in the flies to study their interactions with pathogens. For example, the paraoxonase (PON) family of enzymes can degrade the *P. aeruginosa* quorum sensing (QS) signaling molecule N-3-oxododecanoyl homoserine lactone, and although they are conserved in mammals and *Caenorhabditis elegans*, the enzymes are absent from *Drosophila*. A QS deficient *P. aeruginosa* strain, synthetic acyl-homoserine lactones (AHLs), and *Drosophila* overexpressing human PON1 were used to examine the effect of the enzyme on the signaling molecule and on *P. aeruginosa* virulence *in vivo* (278). *Drosophila* that were infected with the *P. aeruginosa* QS-deficient mutant and ingested a control sucrose solution survived significantly better that flies that ingested synthetic AHLs, showing that QS is important for *P. aeruginosa* virulence *in vivo*. When human PON1 was overexpressed in the *Drosophila*, the animals were protected from the lethality of a wildtype *P. aeruginosa* infection. When AHLs were administered to *P. aeruginosa* QS mutant-infected *Drosophila*, PON1 overexpressing *Drosophila* but not wildtype animals were protected from killing, which showed that the basis of the observed protection was the inactivation of the AHLs by PON1. Therefore, the manipulation of PON expression could be explored as a treatment for some infectious diseases. In another study, it was observed that *Drosophila* overexpressing rhodanase, a bovine enzyme that detoxifies cyanide, survived longer than wildtype animals when infected with cyanogenic *P. aeruginosa* strains (279).

Whole genome microarray analysis

Whole-genome transcriptional profiling of whole animals has been used to assess the *Drosophila* global immune response to various infections (280, 281). The transcriptional profiles of *Drosophila* septically infected with virulent (PA14) and avirulent (CF5) strains of *P. aeruginosa* were compared, in order to identify defence-specific genes (expression altered after CF5 infection) and pathogenesis-specific genes (expression altered after PA14 infection) (280). Based on these criteria, 213 defence-specific genes (133 upregulated, 80 downregulated) and 28 pathogenesis-specific genes (16 upregulated, 12 downregulated) were identified. Interestingly, while infection with strain CF5 significantly upregulated AMP gene expression, infection with strain PA14 did not. Microarray analysis of AMP transcript levels following infection with a PA14 avirulent isogenic mutant revealed similar levels of expression as CF5, suggesting that the ability to reduce *Drosophila* AMP expression is a virulence trait of PA14 and likely other virulent *P. aeruginosa* strains.

Although transcriptional profiling provides a large amount of data, a subset of genes can be selected for more in depth examination. For example, a follow-up study examined the defense-specific genes that were non-immunity related, in a bid to understand the susceptibility to infection that occurs following trauma (282). It was observed that skeletal muscle genes (SMGs) were upregulated upon CF5 infection but not upon PA14 infection, suggesting that PA14 may suppress SMG induction in response to infection. It was determined that the JNK pathway is involved in the regulation of SMGs, as the transcriptional profile of a JNK pathway hypomorph infected with CF5 showed almost no induction of SMGs. Also, bacterial counts were higher in the thoraces of the JNK hypomorph than the wildtype strain but similar in the abdomens, suggesting that the JNK pathway protects against *P. aeruginosa* infection by promoting local tissue reconstruction after an infection. Interestingly, this role for the JNK pathway in the local defense against *P. aeruginosa* in tissue was conserved in a mouse open wound trauma model.

RNAi analysis

RNAi has been used extensively to examine the *Drosophila* host response to microbial infection in recent years

due to the establishment of RNAi lines (47), dsRNA libraries representing the majority of *Drosophila* genes (283, 284), and well-developed, relatively easy methodology for use with bacterial (259), fungal (59), and viral pathogens (285).

The generation of over 22,000 transgenic *Drosophila* RNAi lines representing 12,800 genes allow for the knock down of host genes in specific tissues of the whole animal (47). Thirteen thousand and fifty-three RNAi lines representing 78% of the genome were screened to identify host factors that affected *Drosophila* survival after an ingestion infection with *S. marcescens* (51). Eight hundred and eighty-five lines with altered survival were identified, of which 790 were susceptible (RNAi of the genes increased susceptibility to killing by *S. marcescens*), and 95 were resistant (RNAi of the genes decreased susceptibility to killing by *S. marcescens*). Subsequent RNAi screening of the genes in the *Drosophila* gut epithelium identified 166 genes that affected *Drosophila* survival, most notably JAK/STAT signaling pathway components. Further study of the role of JAK/STAT signaling revealed that the pathway negatively regulates *Drosophila* survival in response to a *S. marcescens* infection, due to a disruption of intestinal cell homeostasis.

Drosophila S2 cells readily take up dsRNA added to their culture medium, and use it to silence genes (286), and the *Drosophila* RNAi Screening Center developed an RNAi library representing 13,900 *Drosophila* genes (284). This library has been used to perform genome-wide screens in S2 cells that aimed to identify host factors involved in infection by several pathogens (20, 165, 260–262). A smaller targeted RNAi library representing 7,216 *Drosophila* genes that are conserved in metazoans (283) has been used to look specifically at host genes that may be relevant during human infection (177, 287, 288).

Drosophila as a model for examining polymicrobe–host interactions

Some human infectious diseases like periodontitis and cystic fibrosis are caused or exacerbated (in the case of CF) by polymicrobial communities; however, there is a dearth of *in vivo* models for studying the interactions among these microbes and between the microbes and the host. The polymicrobial community that colonizes the cyctic fibrosis airway results in persistent inflammation that ultimately destroys the lung, and *P. aeruginosa* is one of the primary CF pathogens. A *Drosophila* natural infection model was developed to examine the interaction between *P. aeruginosa* and other oropharyngeal (OF) species (including *Streptococci*, *Neisseria*, and *Actinomyces*) isolated from cystic fibrosis patients (289). A comparison of the survival of *Drosophila* infected with *P. aeruginosa* alone, each of the 40 OF species alone, and a combination of *P. aeruginosa* and the OF species identified three classes of microbes. The virulent class consisted of species that alone are able to kill *Drosophila* and enhanced the killing of *Drosophila* in combination with *P. aeruginosa*. The synergistic class represented species that alone are not pathogenic to *Drosophila* but, in combination with *P. aeruginosa*, significantly enhanced *Drosophila* killing. The avirulent group represented species that alone are not pathogenic to *Drosophila* and do not enhance the killing of *Drosophila* in combination with *P. aeruginosa*. A novel luminescence assay was used to monitor the expression of individual *P. aeruginosa* virulence genes in individual flies in real-time, during a coinfection with representatives of the synergistic class of OF species. *P. aeruginosa* virulence gene expression was altered in the presence of these OF species with half of the genes being upregulated, including several QS genes that are responsive to the interspecies signaling molecule autoinducer-2. This suggested that interspecies communication is important for modulating *P. aeruginosa* gene expression in a mixed microbial infection.

Using AMP (diptericin, cecropin, and drosomycin) expression as a readout for the immune response, three different host responses to a coinfection with *P. aeruginosa* and another OF species were observed: increased AMP expression as a result of the additive effect of both species, a suppression of AMP expression, and a synergistic activation of AMP expression to levels greater than would be achieved by the additive effect of both species. It was hypothesized that the hyperactivation of the immune response seen in the third instance could be detrimental to the animals. The results of this study demonstrate the complexity of polymicrobial- and polymicrobe–host-interactions and the power of the *Drosophila* model for deciphering these interactions.

Drosophila as a model for drug therapy and antibacterial screening

Drosophila provides the benefit of a whole animal context for drug and antibacterial testing, although the model is currently not as powerful as *C. elegans* for which high-throughput techniques have been developed (290). *Drosophila* can be used as an *in vivo* screen for antibiotics against bacteria like *S. aureus* (254) and *V. cholera* (252). *Drosophila* fed tetracycline or methicillin – which are used clinically to treat *S. aureus* infections – resisted a *S. aureus* infection that killed all flies fed a control sucrose solution (254). *V. cholera* infection in a *Drosophila* natural infection model mimics human cholera, and when the potassium ion channel blocker, clotrimazole, was co-administered with the bacterium the flies were resistant to the otherwise lethal infection (252).

Alternate antibacterial treatments such as phage therapy have been tested using *Drosophila* and have shown some promise. The lytic bacteriophage *Caudovirales* strains MPK1 and MPK6 were isolated based on their

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abilities to form plaques on lawns of *P. aeruginosa* (256). These phage also killed *P. aeruginosa* grown in broth culture, reducing the levels of the bacterium from 10^7 to 10^2 colony-forming units within 2 h. The efficacy of the phage against a *P. aeruginosa* infection *in vivo* was determined using a *Drosophila* septic injury model, and it was observed that when the phage were fed to the *Drosophila*, *P. aeruginosa* proliferation was inhibited and fly survival was enhanced. The phages were also protective against a *P. aeruginosa* infection in a mouse intraperitoneal model of infection.

D. melanogaster in oral microbiology

P. gingivalis

P. gingivalis is the first oral microbe that has been studied using the *D. melanogaster* model. More importantly, it is the first obligately anaerobic bacterium to be successfully studied using the model (63), despite the inhospitable oxygenated environment of the *Drosophila* hemolymph.

Using a septic injury route of infection, *P. gingivalis* was pathogenic to *Drosophila*, in a dose-dependent manner, with an intermediate level of pathogenicity between the non-pathogenic *E. coil* DH5-alpha and the highly pathogenic *P. aeruginosa* PA01 (Fig. 2). A comparison of clinically prevalent heteroduplex type strains of *P. gingivalis* revealed that all of the strains were virulent to some degree in *Drosophila* and that the highly disease-associated type strain, W83, was also the most pathogenic in the flies. *P. gingivalis* colony-forming unit levels did not increase in the *Drosophila*; however, the bacterium was able to persist in the flies up to 60 h postinfection. The relatively low temperature at which the infected flies were incubated (30°C), in addition to the oxygen rich environment of the hemolymph likely contribute to the inability of *P. gingivalis* to multiply in the host. However, *P. gingivalis* is aerotolerant and can survive exposure to air for up to 5 h without any loss in viability, which likely accounted for the bacterium's ability to persist in the *Drosophila* postinfection. *P. gingivalis* killing of *Drosophila* was not due to overt destruction of the fly tissues or a high bacterial burden as was observed with other pathogens, rather the observation that both live and heat-killed *P. gingivalis* effectively killed *Drosophila* suggested that the pathology may be due primarily to the host's own exaggerated immune response. Futher experiments are warranted to determine the exact cause of death of *P. gingivalis*-infected *Drosophila*, for example, *in vivo* RNAi could be used to dampen *Drosophila* immune responses and assess whether fly survival of *P. gingivalis* infection is enhanced as a result.

Five *P. gingivalis* virulence gene mutants (arginine gingipains, lysine gingipain, major fimbriae, minor fimbriae, and capsule) that have been shown to be attenuated in rodent models of infection were also attenuated in the *Drosophila* model, demonstrating that *P. gingivalis* uses similar mechanisms of virulence in *Drosophila* and in mammals and that the bacterium's killing of *Drosophila* is multifactorial. It was hypothesized that as *P. gingivalis* spreads systemically (FITC-labeled bacteria were observed systemically using confocal microscopy), bacterial surface-associated components induce a systemic hyperactivation of the *Drosophila* immune response, which is not only bad for the bacterium but also harmful to the host.

To examine the *Drosophila* immune response to *P. gingivalis* infection (178), six immune response components were selected, and null mutants of these components were screened for altered susceptibility to *P. gingvalis* induced killing. *Drosophila* thiol-ester proteins, Tep II, Tep IV, and the JNK pathway ligand, Eiger (TNF homolog) were involved in the immune response against *P. gingivalis* infection, while the scavenger receptors Eater and Croquemort were dispensable for the response to *P. gingivalis* infection. Interestingly, the Imd pathway was initially found to be dispensable for the immune response against *P. gingivalis*, and because the strain that was used to infect the *Drosophila* (strain W83) is encapsulated, it was reasoned that the capsule may nullify any Imd pathway effects. This was supported by the observation that an unencapsulated *P. gingivalis* strain, strain 381, was significantly more pathogenic in Imd pathway-null flies than in wildtype flies. In a subsequent experiment, *P. gingivalis* strain W50UK, which is highly similar if not identical to W83, behaved like strain W83, while its isogenic capsule-null mutant, GPC, behaved like strain 381, when used to infect wildtype and Imd pathway-null flies, confirming that the bacterial capsule was responsible for the observed nullification of the Imd pathway.

To determine whether the *P. gingivalis* capsule prevented the activation of the Imd pathway by shielding the bacterium's peptidoglycan from detection by PGRP-LC, a *dipt-LacZ* reporter *Drosophila* strain was used to monitor activation of the pathway in response to W50UK and GPC infections. Beta galactosidase expression was detected in both cases, which demonstrated that the capsule of strain W50UK did not shield the bacterium's PPG from detection. To determine whether the *P. gingivalis* capsule could protect the bacterium from killing by Imd pathway regulated AMPs, the survival of strains W50UK and GPC after exposure to cecropin A and drosocin was compared. Strain GPC was significantly more susceptible to killing by the AMPs than W50UK, which showed that the *P. gingivalis* capsule mediates resistance to killing by *Drosophila* Imd-regulated AMPs. The results are also relevant to humans as the capsule also protected W50UK from killing by human beta-defensin-3. This was the first report

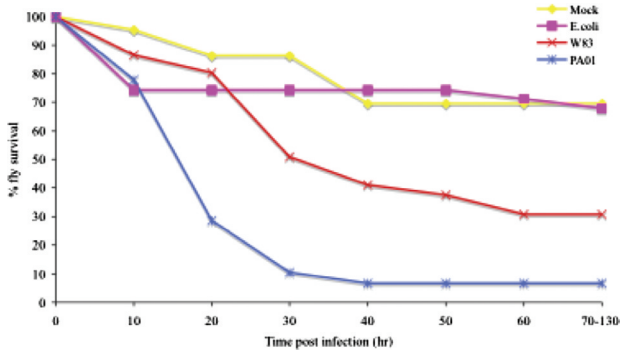

Fig. 2. Survival curves of adult, female *Drosophila* infected with *P. gingivalis* and other bacterial species. *E. coli* DH5α (pink curve), *P. aeruginosa* strain PA01 (blue curve), *P. gingivalis* strain W83 (red curve), and vehicular control (yellow curve) (63).

to demonstrate that the *P. gingivalis* capsule is protective against host AMPs.

The future of the Drosophila model in the oral microbiology arena

Although the use of the *Drosophila* model for the study of oral microbiology is in its infancy, a wealth of information about oral bacteria–host interactions can be gleaned using this model. The major diseases of the oral cavity (periodontitis and caries) are polymicrobial in nature, and many putative periodontal (291–293) and caries (294, 295) pathogens have been identified and are yet to be characterized in terms of their interactions with the host. The introduction of the high-throughput *Drosophila* model will facilitate the screening of these potential pathogens, and the further characterization of species that are found to be virulent, as is being done with *P. gingivalis*. The *Drosophila* model is useful for studying *P. gingivalis* because this bacterium is aerotolerant, whereas a lot of oral bacteria will be killed by exposure to even a small amount of air and are, thus, unlikely to have virulent phenotypes in this model. Additional oral species have been screened for virulence using the *Drosophila* model, and a range of virulence has been observed (unpublished).

Further characterization of oral bacteria that are found to be pathogenic in the *Drosophila* model should involve screening for pathogen virulence factors, as well as host components that play a role in the response to infection. A large number of *P. gingivalis* mutants with *in vitro* phenotypes such as non-pigmentation, (296–299), enhanced biofilm forming ability, and loss of capsule synthesis (300) have been generated by transposon mutagenesis; however, likely due to the impracticality of using rodent models for large-scale screening, very few of these mutants have been tested for changes in virulence *in vivo*. The *Drosophila* model offers a high-throughput option to perform large-scale screening of these mutants to look for changes in virulence, which can then be further characterized. Also, the small size of *Drosophila* will make it relatively easy to profile its systemic response to infection, say with *P. gingivalis*, in a whole animal context, and could lead to the identification of novel host components that interact with the bacterium. Interesting candidates could be followed up on using null *Drosophila* mutants and/or *in vivo* RNAi.

The mixed microbial nature of periodontitis and caries warrants the study of polymicrobe interactions as well as polymicrobe–host interactions, and the work by Sibley et al. (289) has laid the groundwork for the use of *Drosophila* to study these interactions *in vivo*. Studies are ongoing to examine the virulence of oral microbe mixtures comprised of *P. gingivalis* and other oral species, using *Drosophila*. The model could potentially be used to examine mixtures of microbes that are even more complex, possibly as complex as oral plaque.

Finally, *Drosophila* could potentially be used to test the efficacy of antimicrobials against oral bacteria in a whole-animal context; however, the model has not yet been developed for high-throughput drug testing.

It is important to keep in mind that *Drosophila* is not a natural host for oral microbes and that, due to anatomical and some host response – e.g. lack of an adaptive immune response – differences, the model is not directly comparable to humans. However, the large body of evidence obtained from studies in which the model was used to evaluate the host–microbe interplay has demonstrated that many mechanisms of microbial pathogenesis and the host response have been conserved between mammals and *Drosophila*. It is our belief that the addition of the *Drosophila* model to the repertoire of tools with which to study oral microbiology will facilitate infection research in this field considerably.

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