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MINIREVIEW

Worms and Flies as Genetically Tractable Animal Models To Study Host-Pathogen Interactions

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Flies and worms have been chosen as model genetic organisms based on a number of traits that strike an excellent compromise between complexity and genetic tractability. Although an antibacterial defense system in flies was reported in the early 1970s (11), *Drosophila melanogaster* actually emerged as a model animal to study innate immunity 20 years later, when the first parallels between mammalian and *Drosophila* innate immunity were discovered after a NF-κB/Rel protein was found to translocate to the nucleus in response to microbial infection (39, 53, 76). In addition, in seminal studies Lemaitre et al. demonstrated that *Toll* and *imd* genes control the expression of antimicrobial defense in *D. melanogaster* and showed that mutations in the Toll signaling pathway dramatically reduce survival after fungal infection (52, 54). *Caenorhabditis elegans* emerged a few years later when Tan et al. reported in 1999 that the nematode can be used to model *Pseudomonas aeruginosa* pathogenesis in mammals (82). Since then, these organisms have been extensively used to study host-pathogen interactions (reviewed in references 1, 5, 14, 35, 50, 69, and 78).

As in vertebrates, physical barriers and antimicrobial substances protect *D. melanogaster* and *C. elegans* from microbial attacks, but successful microorganisms are capable of overcoming the first line of defense by causing infections that in many cases result in the death of the infected animal. Although the interactions between *D. melanogaster* and *C. elegans* and a wide variety of microorganisms are somehow artificial, since these animals have not been described to be the natural hosts, they appear to have evolved mechanisms to respond to different microorganisms with some degree of specificity (18, 21, 22, 38, 40, 55, 59).

In the case of *D. melanogaster*, growing evidence shows that certain microorganisms are capable of penetrating the exoskeleton or the intestinal epithelium to cause an infection following the so-called "physiological" or "natural" route of infection (85). The physiological infection consists of either (i) feeding *D. melanogaster* larvae or adult flies with the microorganism of interest distributed in the food or (ii) spraying fungal spores or microorganisms directly onto the fly exoskeleton. Various microorganisms, however, are unable to break the first line of defense and need to be inoculated. This is accomplished by (i) pricking the dorsal part of the fly thorax (or abdomen) body cavity of the insect with a sharp needle that has been dipped into a microbial suspension or (ii) microinjecting a precise dose of microbes directly into the body cavity. The disadvantages of these methods are that the mechanical manipulation itself appears to affect the host defense response to some extent and that there seems to be significant differences in the *Drosophila* defense response depending on the route of inoculation (7).

In contrast, all the *C. elegans* pathogens described so far seem to use a relatively more physiological route of infection. Typically, *C. elegans* animals are propagated in the laboratory on petri dishes containing a lawn of a slow-growing strain of *Escherichia coli* OP50. The nematodes, which feed almost constantly during their adult life cycle, use muscle contractions to pump food into the pharynx where the pharyngeal grinder uses specialized cuticular structures to effectively disrupt most bacteria. Thus, essentially no intact *E. coli* cells can be found in the intestinal lumen. However, when *C. elegans* is fed certain human pathogens, the nematodes die and, in many cases, intact microorganisms can be found within the intestine (4, 28, 41, 48, 71, 82). A specific *C. elegans* pathogen, *Microbacterium nematophilum*, has also been isolated. These bacteria adhere to the anal region of the nematodes and induce localized swelling of the underlying hypodermal tissue. Although the *C. elegans-M. nematophilum* interaction is not lethal to the worm, it has been suggested to be pathogenic due to the morphological changes induced by *M. nematophilum* and lack of obvious benefits for the host (34).

Studies on the *D. melanogaster* and *C. elegans* genomes have yielded new insights into the mechanisms of a variety of human diseases including Alzheimer's disease, stroke, cancer, retinitis pigmentosa, diabetes, and kidney diseases (33, 77). Here, we will discuss seminal genetic and functional genomic studies performed with *D. melanogaster* and *C. elegans* that have served to identify and characterize a variety of conserved innate immunity-related genes and virulence factors.

IDENTIFICATION OF INNATE IMMUNITY PATHWAYS

***Drosophila melanogaster*.** The *D. melanogaster* immune response against microorganisms lacks adaptive components and relies solely on innate defenses. This, together with its genetic

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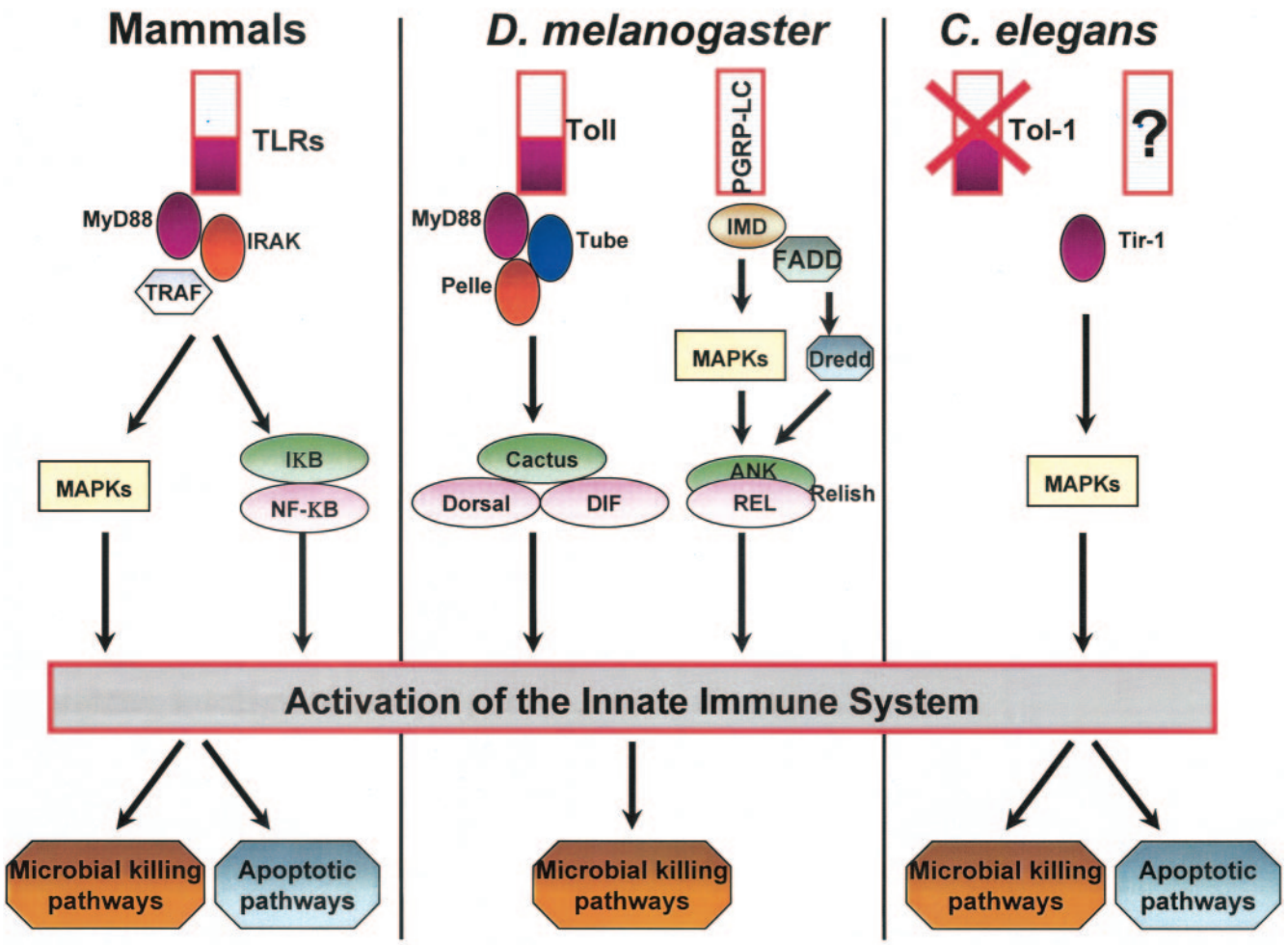

FIG. 1. Comparison of the *D. melanogaster* innate immunity pathway with homolog pathways in *C. elegans* and mammals. In mammals and *D. melanogaster*, Toll-like receptors are crucial in the recognition of microbial pathogens prior to the elicitation of innate immune responses. The *C. elegans* Tol-1 receptor is apparently not involved in this process. The intracellular TIR domain of Toll interacts with a similar domain contained in the MyD88 conserved protein. In mammals, this leads to the activation of both MAPKs and NF-κB that ultimately activates the innate immune system. Similarly, Toll activation triggers innate immunity in *D. melanogaster* through the activation of the NF-κB-like transcription factors Dorsal and DIF. Upon the recognition of microbial pathogens, a *C. elegans* receptor(s) yet to be identified activates innate immunity through a Tir-1/MAPK signaling pathway. As in mammals, *C. elegans* defense responses involve the activation of microbial killing pathways and apoptotic pathways. Although caspases are required for the activation of innate immunity in *D. melanogaster*, an apoptotic defense response has not yet been reported.

tractability, makes *D. melanogaster* an excellent animal model to study innate responses without the intervention of adaptive responses. As in many other metazoans, however, innate responses in *D. melanogaster* involve both cellular and humoral components. The cellular response, which has not been studied as much as the humoral response, comprises three cell lineages (extensively reviewed in reference 61). Plasmatocytes are professional phagocytes dedicated to the elimination of invading microorganisms by engulfment. Lamellocytes correspond to a cell type that differentiates and forms a multilayer capsule around parasites. Encapsulation, together with melanization supported by the crystal cells, results in the elimination of the invading parasites. The humoral response involves the secretion of antimicrobial peptides that are synthesized by the fat body and secreted into the hemolymph. As described in the examples provided in the following paragraphs, innate immunity pathways involved in pathogen recognition and expression of antimicrobial substances have been very well dissected in *D. melanogaster*.

Before the first genetic screen to identify innate immunity-related genes was performed (86), various *D. melanogaster* mutants were used to define the Toll and IMD pathways as key regulators of antimicrobial defense in flies (52, 54). Subsequent studies demonstrated striking similarities between these pathways, which regulate the expression of most of the defense-related genes in response to fungal and bacterial infection through NF-κB-like transcription factors, and their vertebrate counterparts (reviewed in references 14, 36, and 50).

In *D. melanogaster*, the Toll pathway has been described as a key defense response against fungi and gram-positive bacteria (Fig. 1). Toll is a transmembrane receptor which is part of a leucine-rich repeat subgroup of proteins that also contain

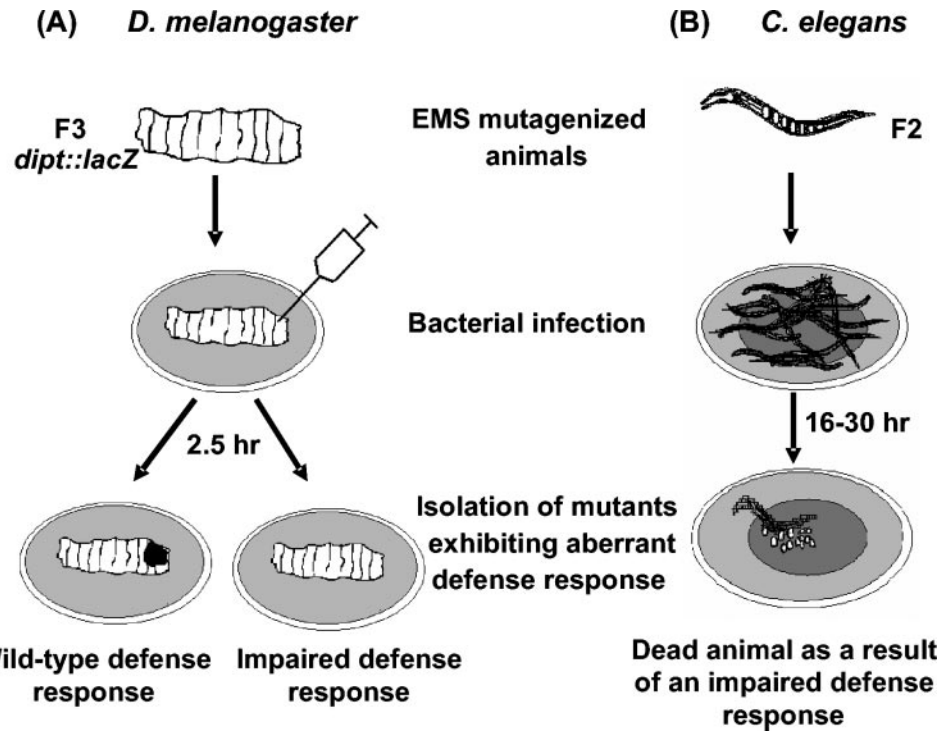

FIG. 2. Genetic analysis identified innate immunity pathways required for proper defense responses in flies and nematodes. (A) *D. melanogaster* F_3 male mutagenized larvae carrying the transgene *dipt*::*lacZ* were inoculated with a diluted culture of *E. coli.* After 2.5 h, the larvae were inspected for melanization at the wound site and then the β-galactosidase activity was evaluated to isolate mutants exhibiting an impaired defense response. (B) An EMS-mutagenized F_2 population of worms was transferred to agar plates seeded with *P. aeruginosa* to identify *C. elegans* mutants impaired in defense response. Because wild-type animals infected with *P. aeruginosa* typically start to die at approximately 34 h, dead mutant animals were isolated during a period of 16 to 30 h. Because *C. elegans* eggs are not infected by bacterial pathogens, the candidate mutants were recovered by transferring individual dead worms containing their brood to plates seeded with nonpathogenic *E. coli.*

Toll-IL-IR (TIR) domains (reviewed in references 9, 42, 74, and 80). Following an infection, the Toll pathway is activated by a serine protease cascade that leads to the processing of a Spaetzle. The physical interaction between Spaetzle and Toll initiates an intracellular cascade that involves the adaptor proteins dMyD88 and Tube and the threonine-serine kinase Pelle. This leads to the degradation of Cactus and nuclear translocation of NF-κB-like transcription factors Dorsal and Dif, which ultimately regulate the expression of antimicrobial peptides. The Imd pathway seems to be particularly important against gram-negative bacteria. As in the case of the Toll signaling pathway, this pathway regulates the expression of antimicrobial peptides through the Rel family transcription factor Relish (Fig. 1). Two well-defined cascades have been involved in the activation of Relish (14, 36, 50). A mitogen-activated protein kinase (MAPK) signaling pathway involving TAK1 and IKK has been implicated in the phosphorylation of Relish, and a pathway involving the caspase Dredd has been shown to subsequently proteolytically activate Relish. Recently, the MAPK Jun N-terminal protein kinase has been demonstrated to be both down-regulated by Relish and involved in transient expression of innate immunity-related genes (72).

For one of the first genetic screens, Wu and Anderson used a reporter strain expressing β-galactosidase under the promoter of the *diptericin* gene, which encodes an antibacterial peptide (86). The rationale for using the *diptericin* promoter was that the Toll signaling pathway was not involved in the activation of diptericin and that only the *imd* gene was known to be required for induction of *diptercin* expression (17, 52, 54). The commonly used chemical mutagen ethyl methane sulfonate (EMS), which randomly induces point mutations, was used to mutagenize homozygous males carrying the *dipt*::*lacZ* transgene on chromosome 3. Homozygous flies for both the *dipt*::*lacZ* transgene and the mutagenized chromosome 3, obtained after a series of crosses, were assayed for their immune responses (Fig. 2).

The immune response of 3,627 lines was evaluated by monitoring the induction of the *dipt*::*lacZ* reporter gene after the inoculation of a diluted culture of *E. coli*. Wu and Anderson identified 57 lines containing mutations in more than 40 different genes that were deficient in *dipt*::*lacZ* expression. Mutations in six of these genes were named *ird* (*i*mmune *r*esponse *d*eficient) and found to affect the nuclear import of the NF-κB-like protein Dif, which translocates to the nucleus in response to infection (39, 53, 76). The receptor that activates the expression of *diptericin* was not identified in this screen, but welcome information on the events upstream of the activation of antimicrobial peptide expression was obtained. Four years later, three independent studies identified the peptidoglycan recognition protein LC (PGRP-LC) as a crucial receptor involved in the detection of gram-negative bacteria and subsequent activation of antibacterial peptide biosynthesis through the *imd* gene (16, 30, 75). The discovery of additional members

of the PGRP family of pattern recognition molecules and members of the family of gram-negative binding proteins, as well as their role in the recognition of gram-negative and gram-positive bacteria and fungi, has recently been reviewed (14, 50).

***Caenorhabditis elegans*.** As *D. melanogaster*, the nematode *C. elegans* seems to rely only on innate immunity to deal with microbial infections. Although several markers of conserved innate immune responses have been recently described for *C. elegans*, phagocytosis, an important component of innate immunity, does not appear to play a role in microorganism clearance. The hermaphrodite animal has six phagocytic cells in the pseudocoelom, but they are not mobile and do not seem to be involved in the engulfment of microorganisms. The relatively simple innate immune system of *C. elegans* and the number of traits that facilitate genetic and genomic analysis using this organism, including a hermaphroditic lifestyle and short 2- to 3-week lifespan, have nurtured rapid advances into the understanding of *C. elegans* innate immunity during recent years.

Programmed cell death (PCD) is the first marker of a conserved innate immune response observed in evolutionarily disparate species that was identified in *C. elegans*. Interestingly, it was found that *Salmonella enterica* but not *P. aeruginosa* elicits programmed cell death in the *C. elegans* germ line cells. Using a set of *C. elegans* mutants in which PCD is blocked, it was shown that *S. enterica*-elicited germ line cell death is dependent on the well-characterized CED-9/CED-4/CED-3 pathway, which is homologous to the BCL2/APAF-1/CASPASE pathway in mammalian cells. Moreover, *ced-3* and *ced-4* mutants were found to be hypersensitive to *S. enterica*-mediated killing, suggesting that PCD (or the CED9/CED4/CED3 signal transduction pathway) may be involved in a *C. elegans* defense response to pathogen attack (2). In addition, taking advantage of both host and pathogen mutants, it was shown that *S. enterica* lipopolysaccharide acts as a pathogen-associated molecular pattern that triggers programmed cell death in *C. elegans*. Similar to mammals, the pathogen-induced CED-3 pathway in *C. elegans* appears to lie downstream of a PMK-1/P38 MAPK signaling pathway (3). Since *S. enterica* persistently colonizes the *C. elegans* intestinal lumen, these results suggest that *S. enterica* infection triggers somatic signals that induce the CED-3 pathway in the germ line. Induction of the CED-3 pathway may serve a protective role when *C. elegans* encounters an adverse environmental stimulus, such as the attack of a potentially pathogenic bacterium, maintaining homeostasis by eliminating the excess germ line cells or sick cells potentially detrimental to the organism. In contrast to somatic cells, germ cells do not have a fixed lineage or population of cells. The CED-3 pathways could also operate in the *C. elegans* intestine, which is in direct contact with potential bacterial pathogens, to trigger a somatic defense response independent of cell death.

Another genetic approach focused on the characterization of the Toll signaling pathway in *C. elegans*. Although *C. elegans* and *D. melanogaster* have been placed in sister phyla, *C. elegans* does not appear to have an intact Toll signaling pathway. The nematode genes encoding proteins homologous to several components of the Toll signaling pathway, Toll/TOL-1, dTraf/TRF-1, Pelle/PIK-1, and Cactus/IKB-1, were identified, and the corresponding deletion mutants were generated. However, none of these mutants exhibited enhanced susceptibility to several pathogens compared to a nonpathogenic *E. coli* control (73).

The Ausubel laboratory performed a pioneering genetic analysis of *C. elegans* to identify innate immunity genes required for proper defense response against a bacterial infection (45). An EMS-mutagenized F_2 generation was screened to isolate mutants exhibiting an *e*nhanced *s*usceptibility to *p*athogens (Esp) phenotype according the scheme shown in Fig. 2. The pathogen used in this study was *P. aeruginosa* strain PA14, which was previously shown to kill *C. elegans* by two mechanisms. *P. aeruginosa* grown on nematode growth medium accumulates within the lumen of the *C. elegans* intestine, killing worms relatively slowly over the course of 2 to 3 days (called "slow killing") (81). In contrast, PA14 grown on rich and high-osmolarity media kills worms quickly by excreting low-molecular-weight toxins ("fast killing") (58, 81). Using the slow-killing conditions, 14,000 lines were screened and 10 mutants were isolated. Mutations in two of these animals, *esp-8* and *esp-2*, were found to correspond to the *nsy-1* and *sek-1* genes, respectively (45). NSY-1 and SEK-1 are components of a conserved PMK-1/p38 MAPK signaling pathway previously shown to mediate asymmetric cell fate decisions during neuronal development (83). Further studies revealed that TOL-1 is not the *C. elegans* receptor sensing the stimulatory signal for PMK-1 activation (3). The PMK-1/p38 MAPK pathway does not appear to play a role against the natural *C. elegans* pathogen *M. nematophilum*, which, as indicated above, causes a persistent infection that correlates with a swollen tail. However, the extracellular signal-regulated kinase MAPK signaling pathway has been shown to mediate both tail swelling and a protective response against *M. nematophilum* attack (68).

The Aroian laboratory also performed an elegant genetic study to understand the mechanism of the *Bacillus thuringiensis* toxin Cry5B. First, it was demonstrated that Cry5B damages the *C. elegans* intestine, reduces the brood size, and eventually kills the nematodes. Second, an EMS-mutagenized F_2 population was used to isolate 10 recessive mutants resistant to the toxin's effects that ultimately defined five *B. thuringiensis re*sistance (*bre*) genes (60). In subsequent studies, the *bre-5* gene was cloned and its product was characterized. It was hypothesized that the putative galactosyltransferase BRE-5 is involved in the formation of a carbohydrate structure required at the gut surface for proper toxin binding. Consistent with this hypothesis, the study of mosaic animals revealed that the presence of BRE-5 in the intestine is necessary for Cry5B-mediated toxicity (32). BRE-5 is part of a larger family of proteins involved in glycosylation that function in the intestine and are required for the interaction of Cry5B toxin with the host cells (31).

FUNCTIONAL GENOMICS TO UNDERSTAND DEFENSE RESPONSES

Expression profiling analyses. Microarray studies have been used not only to assess *D. melanogaster* and *C. elegans* innate immune response to microbial challenge (21, 40, 59) but also to dissect the pathways involved in this response. For example, to study the contribution of the Toll and Imd pathways in defense response, De Gregorio et al. compared the expression profile of *D. melanogaster* mutants in the Toll and Imd pathways

infected with *Escherichia coli* or *Micrococcus luteus* to the expression profile of uninfected and *Beauveria bassiana*-infected (physiological infection) wild-type flies (22). Most of the genes regulated by microbial infection were found to be regulated by the Toll and Imd pathways. However, since the authors found that the expression profile of some of the pathogen-responsive genes were unaffected in mutant flies in both pathways, they concluded that other undefined pathways regulate a subset of immune-responsive genes. Boutros et al. showed that *D. melanogaster* genes induced by microbial challenges are regulated by the Toll and Imd pathways and by the Jun N-terminal protein kinase and JAK/STAT pathways (12). In addition, they demonstrated that the expression of some of these genes is altered by the mechanical manipulation itself and that there is a connection between the pathways that control tissue repair and innate immunity. In a recent whole-genome analysis of innate immunity pathways transcriptionally regulated by *P. aeruginosa* infection, Apidianakis et al. studied not only the injury effects but also the effects of a *P. aeruginosa* avirulent strain-induced response. Thus, virulence-related responses specifically elicited by virulent *P. aeruginosa* were identified (6). Future studies could further dissect the link between the pathways that control tissue repair and innate immunity and lead to the identification of pathogen-specific pathways involved in innate immunity.

In the case of *C. elegans*, a microarray approach was also employed to identify effectors of the innate immune system important in defense response against the fungus *Drechmeria coniospora* (18). Various fungus-inducible genes were identified, but only *nlp-29* and a related gene were also found to be strongly induced by both *D. coniospora* and *Serratia marcescens* infection. The *D. coniospora*-responsive gene *nlp31* was found to encode a 75-amino-acid protein that has antifungal activity comparable to that of drosomycin, a potent *D. melanogaster* antifungal peptide. Consistent with previous findings that indicate that the TOL-1 signaling pathway is not required for proper defense response in *C. elegans* (3, 73), the expression of fungus-induced antimicrobial peptide NLP-31 was found to be independent of TOL-1. However, the expression of both NLP-29 and NLP-31 was found to be regulated by TIR-1. Independently, Liberati et al. also reported the involvement of TIR-1 in *C. elegans* innate immunity (56).

Recently published expression profiling analyses also illustrate that stress response, innate immunity, and lifespan are governed by interacting and intersecting pathways in *D. melanogaster* and *C. elegans*. To evaluate the role of oxidative damage in aging in *D. melanogaster*, Landis et al. compared the expression profiles of young, old, and oxygen-stressed flies, defined as flies grown on a 100% oxygen atmosphere for 7 days. Both aging and oxidative stress induce a set of common genes, including innate immunity-related genes (49). Similarly, it has been found with *C. elegans* that induction of various innate immunity-related genes correlates with an increased lifespan. Several of the induced genes in the long-lived mutant *daf-2* were found to shorten the lifespan of the animals when inhibited using RNA interference (RNAi) (65). In addition, other investigators have shown that long-lived *daf-2* and *age-1* mutants are resistant to *Enterococcus faecalis*, *Staphylococcus aureus*, and *P. aeruginosa* (29). In the context of lifespan regulation, it was shown that *daf-16*, which encodes a forkhead transcription factor (57, 70), translocates into the nucleus and modulates transcription when *daf-2* signaling is abrogated (51). Thus, strong *daf-16* alleles suppress the long-lived phenotype of *daf-2* mutants. Garsin et al. showed that a *daf-16*;*daf-2* double mutant exhibits wild-type susceptibility to microorganisms, indicating that *daf-16* alleles suppress the enhanced resistance to microorganisms of *daf-2* mutants. Interestingly, although *daf-16* alleles suppress the increased resistance to the microorganism phenotype of *daf-2* animals and *daf-16* mutants show a short lifespan, *daf-16* mutants present susceptibility to microorganisms comparable to that of wild-type animals (29). The molecular mechanisms that modulate aging and immune response were recently reviewed (47).

Expression profiling analysis also pinpointed the PMK-1/p38 MAPK as a key component of defense response against the Cry5B toxin and helped identify two of its downstream targets, *ttm-1* and *ttm-2* (38). Although the effect of the PMK-1/p38 MAPK pathway was not as strong as in the case of defense response against Cry5B toxin, the pathway was also shown to be required for proper response to cadmium-induced stress, providing yet another example that pathways involved in innate immunity and stress responses are interconnected.

RNA interference. Since the development of the RNAi technology, several systematic genome-wide screens have been performed (reviewed in reference 15). However, although RNAi has been used in *D. melanogaster* to study phagocytosis, which led to the identification of PGRP-LC (75), only one laboratory so far has taken advantage of this technology to perform a systematic genome-wide study of *D. melanogaster* innate immunity (23) and there are no reports of genome-wide RNAi-based screens to dissect *C. elegans* innate immunity.

Foley and O'Farrell generated an RNAi library containing 7,216 double-stranded RNAs corresponding to the majority of the phylogenetically conserved genes in the *D. melanogaster* genome (23). This library was used in a tissue culture system to genetically dissect the Imd pathway. An S2 blood cell line containing the *dipt*::*lacZ* reporter transgene was generated and used to identify genes whose inhibition by RNAi resulted in an altered expression of the reporter gene under standard laboratory conditions or when the cells were challenged with lipopolysaccharide. RNAi was also used to carry out epistasis analysis, allowing assignment of a large set of candidate genes into pathways and regulatory hierarchies. Although RNAi mimics loss-of-function mutations instead of null mutations, which are desired to avoid ambiguities in interpreting epistasis data, the investigators succeeded at discovering that Defense repressor 1 (Dnr1) inhibits Dipt::LacZ expression by blocking Dredd signaling. It was also shown that Dnr1 is up-regulated by Dredd in a feedback loop (23). RNAi is a powerful technology that has brought light to various biological processes, including innate immunity. Genome-wide studies of *D. melanogaster* and *C. elegans* should help elucidate the differences and similarities of innate immunity in vertebrates and invertebrates.

GENETIC ANALYSES TO IDENTIFY VIRULENCE FACTORS

The use of genetic techniques to identify microbial virulence factors involved in mammalian pathogenesis is often complicated by the tediousness, expense, and ethical considerations

of using large numbers of vertebrate animals to identify mutants exhibiting reduced virulence. *D. melanogaster* and *C. elegans* have been adopted as an alternative to vertebrate models for the study of microbial pathogenesis. A broad range of human pathogens has been shown to infect and kill these organisms, and a variety of virulence factors required for full pathogenicity in mammalian systems has also been shown to be required for virulence in flies and worms. As discussed earlier, several human pathogens are unable to penetrate *D. melanogaster* to cause an infection and need to be artificially inoculated. Although this represents a limitation for large-scale genetic analyses of pathogen virulence factors, *D. melanogaster* has successfully been used, for example, to identify novel *P. aeruginosa* virulence factors (20). About 1,500 independent transposon insertion mutants were screened to identify virulence-related genes by isolating mutants exhibiting reduced virulence in inoculated flies. The molecular analysis of 33 candidate strains mapped the mutations to the *pilGHIJKL chpAB CDE* gene cluster. Although these genes are known to be required for twitching and motility, it was demonstrated that lack of twitching and motility itself is not responsible for the reduced virulence of the strains, which is consistent with the involvement of the *pil chp* genes in the regulation of the expression of additional virulence factors (20).

An experimental advantage of using *C. elegans* as a host is that thousands of microbial clones from a mutagenized library can be individually screened for avirulent mutants on separate petri plates seeded with many animals. This has led to numerous genetic studies that have identified many microbial virulence factors required for full virulence in *C. elegans* and in mammalian systems.

Bacterial pathogens kill the nematode by different mechanisms that involve diffusible low-molecular-weight toxins, finely tuned host-specific strategies to establish pathogenic relationships, and biofilm formation (recently reviewed in reference 79). Thus, a large number of *C. elegans*-based screens have been used to identify novel bacterial virulence factors by isolating insertion mutants exhibiting reduced virulence in nematodes. These studies involved gram-negative bacteria *P. aeruginosa* (26, 58, 82), *Burkholderia pseudomallei* (27), *Burkholderia cenocepacia* (37), *S. marcescens* (46), *S. enterica* (84), and *Yersinia pseudotuberculosis* (43) and gram-positive bacteria *S. aureus* (8), *E. faecalis* (28), and *Enterococcus faecium* (64). Overall, these genetic studies demonstrate that there is a remarkable overlap among bacterial virulence factors required for human and nematode pathogenesis.

Although it was not known whether *C. elegans* could feed on yeasts, a recent report shows that *C. elegans* can use nonpathogenic fungi, including *Cryptococcus laurentii* and *Cryptococcus kuetzingii*, as a sole source of food, producing brood sizes similar to those achieved with growth on *E. coli*. It was found that nematodes grown on *C. laurentii* have a lifespan similar to that observed for worms fed *E. coli*, while animals fed *C. kuetzingii* exhibited a longer lifespan (66). However, the human pathogenic yeast *Cryptococcus neoformans* was found to kill *C. elegans*, and the *C. neoformans* polysaccharide capsule and several *C. neoformans* genes previously shown to be involved in mammalian virulence were also found to play a role in *C. elegans* killing. As in bacterial pathogens, the exact mechanism of killing of *C. elegans* by *C. neoformans* is not yet clear. Recently, the first screen of fungal pathogens was completed and seven mutants exhibiting reduced virulence in *C. elegans* were isolated (67). Genetic analysis of one strain revealed an insertion in a gene homologous to *Saccharomyces cerevisiae KIN1*, which encodes a serine/threonine protein kinase. *C. neoformans kin1* mutants exhibit significant defects in virulence in murine inhalation and hematogenous infection models and also increased binding to alveolar and peritoneal macrophages.

USE OF TRANSGENIC ANIMALS TO STUDY THE MECHANISMS OF VIRULENCE FACTORS

Virulence factors such as bacterial toxins, which may be excreted directly into the medium or released only on bacterial lysis, and effector proteins, which are injected into the cytosol of host cells, specifically interfere with host cellular processes to promote pathogen survival in the host. Several approaches have been used to study the mechanisms by which these virulence factors contribute to bacterial pathogenesis, including the direct expression of virulence factors in mammalian cells. Although these methods proved insightful, they mainly provide clues about gross morphological changes at the cellular level and lack genetic tractability. To test the hypothesis that *C. elegans* can be used to study the molecular mechanisms of toxins, the well-characterized pertussis toxin (PTX) was expressed in the neurons and muscles of the nematode (19). The rationale to express PTX in neurons and muscles was that its putative target, a G(o/i)alpha protein, is primarily expressed in these cell types. The phenotype conferred by PTX expression was remarkably similar to that observed in nematodes carrying a loss-of-function mutation in the gene *goa-1*, which encodes the G(o/i)alpha protein presumably targeted by PTX. In addition, PTX expression suppressed the phenotype imposed by the expression of a constitutively active form of GOA-1 in *C. elegans* (19).

A recent *C. elegans*-based screen identified a set of *S. enterica* virulence-related genes, including genes related to the type three secretion system (TTSS) encoded in *Salmonella* pathogenicity island 1 (SPI-1). Since the SPI-1 TTSS-exported effector protein SptP was found to contribute to *S. enterica*-mediated killing of *C. elegans*, it was studied whether ectopic expression of SptP in *C. elegans* intestinal cells would affect *C. elegans* innate immunity (84). The SptP carboxyl-terminal domain has tyrosine phosphatase activity in vitro and displays amino acid sequence similarity to the *Yersinia* spp. tyrosine phosphatase YopH (10, 44, 62). The amino-terminal domain of SptP has GTPase-activating protein activity for Cdc42 and Rac and is similar to the bacterial cytotoxins YopE and ExoS (24, 25, 63). Intestinal expression of SptP rendered transgenic animals more susceptible to *S. enterica* infection, presumably by down-regulating the PMK-1/p38 MAPK signaling pathway (84) (Fig. 3). Further studies will be needed to study which SptP catalytic domain is required for this process or whether both domains are necessary. *S. enterica* TTSS-exported effector proteins also appear to contribute to *S. enterica*-mediated killing of *D. melanogaster*. It is intriguing that although *spi-1* and *spi-2* mutants are less virulent than wild-type bacteria, they replicate better in flies. It seems that the lack of manipulation of cellular pathways by *S. enterica* effector proteins is beneficial for both the host and the pathogen (13). It would be interesting

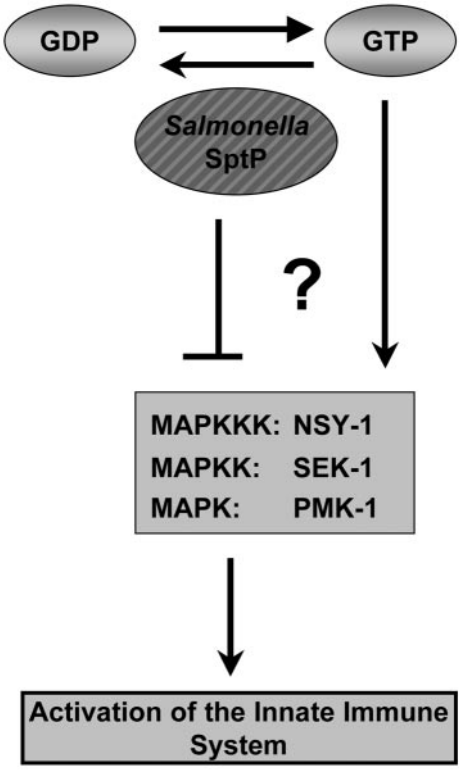

FIG. 3. Expression of bacterial virulence factors in *C. elegans* cells diminishes innate immunity. A genetic analysis indicates that *S. enterica* TTSS-related genes are required for full virulence in *C. elegans.* To confirm that effector proteins secreted through SPI-1 TTSS affect *C. elegans* innate immunity, SptP was directly expressed in the intestinal cells of nematodes. The intestinal expression of SptP diminishes *C. elegans* innate immunity apparently by preventing the activation of MAPK PMK-1. It remains unknown whether the SptP GTPase activating protein activity, the tyrosine phosphatase activity, or both are required for the downregulation of MAPK PMK-1.

to study the effects of ectopic expression of effector proteins in *D. melanogaster*. Future modifier genetic screens using transgenic animals expressing virulence factors to identify suppressors and enhancers of the phenotype exhibited by the animals should aid in the molecular characterization of host-pathogen interactions.

CONCLUDING REMARKS

In addition to *D. melanogaster* and *C. elegans*, a variety of alternative model hosts have been used to study microbial pathogenesis and defense response. Each alternative model system has advantages and disadvantages, which highlights the need to use many models to understand the mechanisms by which pathogens manipulate the innate immune system to cause disease. In the long run, an in-depth understanding of how pathogenic mechanisms and host defense responses interact in evolutionarily diverse hosts should contribute to the understanding of key aspects of the pathogenic process that may help in the design of novel preventive and therapeutic approaches.

There are practical limitations associated with the use of *D. melanogaster* and *C. elegans* as alternative hosts to model mammalian host-pathogen interactions. For example, *D. melanogaster* and *C. elegans* cannot survive at 37°C and the administration of exact inocula or antimicrobial substances is technically demanding in these systems. In addition, there are many differences in the innate immune systems of metazoans. For example, one remarkable difference between flies and mammals corresponds to the pathogen recognition mechanism by Toll receptors. While Toll receptors are critical in the recognition of fungal pathogens and gram-positive bacteria in flies, the mammalian counterparts are key in the recognition of gram-negative bacteria. Differences in the role of Toll receptors in the pathogen recognition process are not only found between invertebrates and vertebrates. Although *C. elegans* and *D. melanogaster* are two invertebrates that correspond to relatively related phyla, the single Tol-1 receptor does not appear to be involved in the recognition of pathogen-associated molecular patterns. In addition, NF-κB-like molecules or other transcription factors that control the expression of immunity effectors remain unknown.

It is logical to conclude that the use of *D. melanogaster* and *C. elegans* to study host-pathogen interactions may identify interactions specific to pathogens and these invertebrates in addition to interactions that can potentially be translated to mammalian systems. In some cases, these interactions may not have direct relevance to human health but will prove important to understand the pathogenic mechanisms in nonvertebrate hosts that could eventually be translated to improve human health. Despite these disadvantages, the results described here indicate that the highly sophisticated *D. melanogaster* and *C. elegans* genetic systems can be used to collect new information relevant to bacterial and fungal pathogenesis in mammals. There are differences not only between vertebrate and invertebrate innate immunity but also in the innate immune systems of *D. melanogaster* and *C. elegans*. Future work should help answer the key question of to what extent findings in *D. melanogaster* and *C. elegans* can be translated to mammalian innate immunity.

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REFERENCES

1. **Aballay, A., and F. M. Ausubel.** 2002. *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. Curr. Opin. Microbiol. **5:**97–101.
2. **Aballay, A., and F. M. Ausubel.** 2001. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. Proc. Natl. Acad. Sci. USA **98:**2735–2739.
3. **Aballay, A., E. Drenkard, L. R. Hilbun, and F. M. Ausubel.** 2003. *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. Curr. Biol. **13:**47–52.
4. **Aballay, A., P. Yorgey, and F. M. Ausubel.** 2000. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans.* Curr. Biol. **10:**1539–1542.
5. **Alegado, R. A., M. C. Campbell, W. C. Chen, S. S. Slutz, and M. W. Tan.** 2003. Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. Cell. Microbiol. **5:**435–444.
6. **Apidianakis, Y., M. N. Mindrinos, W. Xiao, G. W. Lau, R. L. Baldini, R. W. Davis, and L. G. Rahme.** 2005. Profiling early infection responses: *Pseudomonas aeruginosa* eludes host defenses by suppressing antimicrobial peptide gene expression. Proc. Natl. Acad. Sci. USA **102:**2573–2578.

7. **Apidianakis, Y., L. G. Rahme, J. Heitman, F. M. Ausubel, S. B. Calderwood, and E. Mylonakis.** 2004. Challenge of *Drosophila melanogaster* with *Cryptococcus neoformans* and role of the innate immune response. Eukaryot. Cell **3:**413–419.
8. **Bae, T., A. K. Banger, A. Wallace, E. M. Glass, F. Aslund, O. Schneewind, and D. M. Missiakas.** 2004. *Staphylococcus aureus* virulence genes identified by *bursa aurealis* mutagenesis and nematode killing. Proc. Natl. Acad. Sci. USA **101:**12312–12317.
9. **Barton, G. M., and R. Medzhitov.** 2003. Toll-like receptor signaling pathways. Science **300:**1524–1525.
10. **Bolin, I., and H. Wolf-Watz.** 1988. The plasmid-encoded Yop2b protein of *Yersinia pseudotuberculosis* is a virulence determinant regulated by calcium and temperature at the level of transcription. Mol. Microbiol. **2:**237–245.
11. **Boman, H. G., I. Nilsson, and B. Rasmuson.** 1972. Inducible antibacterial defense system in *Drosophila.* Nature **237:**232–235.
12. **Boutros, M., H. Agaisse, and N. Perrimon.** 2002. Sequential activation of signaling pathways during innate immune responses in *Drosophila.* Dev. Cell **3:**711–722.
13. **Brandt, S. M., M. S. Dionne, R. S. Khush, L. N. Pham, T. J. Vigdal, and D. S. Schneider.** 2004. Secreted bacterial effectors and host-produced Eiger/TNF drive death in a *Salmonella*-infected fruit fly. PLoS Biol. **2:**e418.
14. **Brennan, C. A., and K. V. Anderson.** 2004. *Drosophila*: the genetics of innate immune recognition and response. Annu. Rev. Immunol. **22:**457–483.
15. **Carpenter, A. E., and D. M. Sabatini.** 2004. Systematic genome-wide screens of gene function. Nat. Rev. Genet. **5:**11–22.
16. **Choe, K. M., T. Werner, S. Stoven, D. Hultmark, and K. V. Anderson.** 2002. Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in *Drosophila.* Science **296:** 359–362.
17. **Corbo, J. C., and M. Levine.**1996. Characterization of an immunodeficiency mutant in *Drosophila.* Mech. Dev. **55:**211–220.
18. **Couillault, C., N. Pujol, J. Reboul, L. Sabatier, J. F. Guichou, Y. Kohara, and J. J. Ewbank.** 2004. TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. Nat. Immunol. **5:**488–494.
19. **Darby, C., and S. Falkow.** 2001. Mimicry of a G protein mutation by pertussis toxin expression in transgenic *Caenorhabditis elegans.* Infect. Immun. **69:** 6271–6275.
20. **D'Argenio, D. A., L. A. Gallagher, C. A. Berg, and C. Manoil.** 2001. *Drosophila* as a model host for *Pseudomonas aeruginosa* infection. J. Bacteriol. **183:**1466–1471.
21. **De Gregorio, E., P. T. Spellman, G. M. Rubin, and B. Lemaitre.** 2001. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. Proc. Natl. Acad. Sci. USA **98:**12590–12595.
22. **De Gregorio, E., P. T. Spellman, P. Tzou, G. M. Rubin, and B. Lemaitre.** 2002. The Toll and Imd pathways are the major regulators of the immune response in *Drosophila.* EMBO J. **21:**2568–2579.
23. **Foley, E., and P. H. O'Farrell.** 2004. Functional dissection of an innate immune response by a genome-wide RNAi screen. PLoS Biol. **2:**E203.
24. **Forsberg, A., and H. Wolf-Watz.** 1990. Genetic analysis of the *yopE* region of *Yersinia* spp.: identification of a novel conserved locus, *yerA*, regulating *yopE* expression. J. Bacteriol. **172:**1547–1555.
25. **Fu, Y., and J. E. Galan.** 1999. A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. Nature **401:**293–297.
26. **Gallagher, L. A., and C. Manoil.** 2001. *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. J. Bacteriol. **183:**6207–6214.
27. **Gan, Y. H., K. L. Chua, H. H. Chua, B. Liu, C. S. Hii, H. L. Chong, and P. Tan.** 2002. Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. Mol. Microbiol. **44:**1185–1197.
28. **Garsin, D. A., C. D. Sifri, E. Mylonakis, X. Qin, K. V. Singh, B. E. Murray, S. B. Calderwood, and F. M. Ausubel.** 2001. A simple model host for identifying gram-positive virulence factors. Proc. Natl. Acad. Sci. USA **98:**10892–10897.
29. **Garsin, D. A., J. M. Villanueva, J. Begun, D. H. Kim, C. D. Sifri, S. B. Calderwood, G. Ruvkun, and F. M. Ausubel.** 2003. Long-lived *C. elegans* daf-2 mutants are resistant to bacterial pathogens. Science **300:**1921.
30. **Gottar, M., V. Gobert, T. Michel, M. Belvin, G. Duyk, J. A. Hoffmann, D. Ferrandon, and J. Royet.** 2002. The *Drosophila* immune response against gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature **416:**640–644.
31. **Griffitts, J. S., D. L. Huffman, J. L. Whitacre, B. D. Barrows, L. D. Marroquin, R. Muller, J. R. Brown, T. Hennet, J. D. Esko, and R. V. Aroian.** 2003. Resistance to a bacterial toxin is mediated by removal of a conserved glycosylation pathway required for toxin-host interactions. J. Biol. Chem. **278:** 45594–45602.
32. **Griffitts, J. S., J. L. Whitacre, D. E. Stevens, and R. V. Aroian.** 2001. Bt toxin resistance from loss of a putative carbohydrate-modifying enzyme. Science **293:**860–864.
33. **Hariharan, I. K., and D. A. Haber.** 2003. Yeast, flies, worms, and fish in the study of human disease. N. Engl. J. Med. **348:**2457–2463.
34. **Hodgkin, J., P. E. Kuwabara, and B. Corneliussen.** 2000. A novel bacterial pathogen, Microbacterium nematophilum, induces morphological change in the nematode *C. elegans.* Curr. Biol. **10:**1615–1618.
35. **Hoffmann, J. A.** 2003. The immune response of *Drosophila.* Nature **426:**33–38.
36. **Hoffmann, J. A., and J. M. Reichhart.** 2002. *Drosophila* innate immunity: an evolutionary perspective. Nat. Immunol. **3:**121–126.
37. **Huber, B., F. Feldmann, M. Kothe, P. Vandamme, J. Wopperer, K. Riedel, and L. Eberl.** 2004. Identification of a novel virulence factor in *Burkholderia cenocepacia* H111 required for efficient slow killing of *Caenorhabditis elegans.* Infect. Immun. **72:**7220–7230.
38. **Huffman, D. L., L. Abrami, R. Sasik, J. Corbeil, F. G. van der Goot, and R. V. Aroian.** 2004. Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. Proc. Natl. Acad. Sci. USA **101:**10995–11000.
39. **Ip, Y. T., M. Reach, Y. Engstrom, L. Kadalayil, H. Cai, S. Gonzalez-Crespo, K. Tatei, and M. Levine.** 1993. Dif, a dorsal-related gene that mediates an immune response in *Drosophila.* Cell **75:**753–763.
40. **Irving, P., L. Troxler, T. S. Heuer, M. Belvin, C. Kopczynski, J. M. Reichhart, J. A. Hoffmann, and C. Hetru.** 2001. A genome-wide analysis of immune responses in *Drosophila.* Proc. Natl. Acad. Sci. USA **98:**15119–15124.
41. **Jansen, W. T., M. Bolm, R. Balling, G. S. Chhatwal, and R. Schnabel.** 2002. Hydrogen peroxide-mediated killing of *Caenorhabditis elegans* by *Streptococcus pyogenes.* Infect. Immun. **70:**5202–5207.
42. **Janssens, S., and R. Beyaert.** 2003. Role of Toll-like receptors in pathogen recognition. Clin. Microbiol. Rev. **16:**637–646.
43. **Joshua, G. W., A. V. Karlyshev, M. P. Smith, K. E. Isherwood, R. W. Titball, and B. W. Wren.** 2003. A *Caenorhabditis elegans* model of *Yersinia* infection: biofilm formation on a biotic surface. Microbiology **149:**3221–3229.
44. **Kaniga, K., J. Uralil, J. B. Bliska, and J. E. Galan.** 1996. A secreted protein tyrosine phosphatase with modular effector domains in the bacterial pathogen *Salmonella typhimurium.* Mol. Microbiol. **21:**633–641.
45. **Kim, D. H., R. Feinbaum, G. Alloing, F. E. Emerson, D. A. Garsin, H. Inoue, M. Tanaka-Hino, N. Hisamoto, K. Matsumoto, M. W. Tan, and F. M. Ausubel.** 2002. A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. Science **297:**623–626.
46. **Kurz, C. L., S. Chauvet, E. Andres, M. Aurouze, I. Vallet, G. P. Michel, M. Uh, J. Celli, A. Filloux, S. De Bentzmann, I. Steinmetz, J. A. Hoffmann, B. B. Finlay, J. P. Gorvel, D. Ferrandon, and J. J. Ewbank.** 2003. Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by in vivo screening. EMBO J. **22:**1451–1460.
47. **Kurz, C. L., and M. W. Tan.**2004. Regulation of aging and innate immunity in *C. elegans.* Aging Cell **3:**185–193.
48. **Labrousse, A., S. Chauvet, C. Couillault, C. L. Kurz, and J. J. Ewbank.** 2000. *Caenorhabditis elegans* is a model host for *Salmonella typhimurium.* Curr. Biol. **10:**1543–1545.
49. **Landis, G. N., D. Abdueva, D. Skvortsov, J. Yang, B. E. Rabin, J. Carrick, S. Tavare, and J. Tower.** 2004. Similar gene expression patterns characterize aging and oxidative stress in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **101:**7663–7668.
50. **Leclerc, V., and J. M. Reichhart.**2004. The immune response of *Drosophila melanogaster.* Immunol. Rev. **198:**59–71.
51. **Lee, R. Y., J. Hench, and G. Ruvkun.** 2001. Regulation of *C. elegans* DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. Curr. Biol. **11:**1950–1957.
52. **Lemaitre, B., E. Kromer-Metzger, L. Michaut, E. Nicolas, M. Meister, P. Georgel, J. M. Reichhart, and J. A. Hoffmann.** 1995. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. Proc. Natl. Acad. Sci. USA **92:**9465–9469.
53. **Lemaitre, B., M. Meister, S. Govind, P. Georgel, R. Steward, J. M. Reichhart, and J. A. Hoffmann.** 1995. Functional analysis and regulation of nuclear import of dorsal during the immune response in *Drosophila.* EMBO J. **14:**536–545.
54. **Lemaitre, B., E. Nicolas, L. Michaut, J. M. Reichhart, and J. A. Hoffmann.** 1996. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell **86:**973–983.
55. **Lemaitre, B., J. M. Reichhart, and J. A. Hoffmann.** 1997. *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc. Natl. Acad. Sci. USA **94:**14614–14619.
56. **Liberati, N. T., K. A. Fitzgerald, D. H. Kim, R. Feinbaum, D. T. Golenbock, and F. M. Ausubel.** 2004. Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. Proc. Natl. Acad. Sci. USA **101:**6593–6598.
57. **Lin, K., J. B. Dorman, A. Rodan, and C. Kenyon.** 1997. *daf-16*: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans.* Science **278:**1319–1322.
58. **Mahajan-Miklos, S., M. W. Tan, L. G. Rahme, and F. M. Ausubel.** 1999. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa-Caenorhabditis elegans* pathogenesis model. Cell **96:**47–56.
59. **Mallo, G. V., C. L. Kurz, C. Couillault, N. Pujol, S. Granjeaud, Y. Kohara, and J. J. Ewbank.**2002. Inducible antibacterial defense system in *C. elegans.* Curr. Biol. **12:**1209–1214.

60. **Marroquin, L. D., D. Elyassnia, J. S. Griffitts, J. S. Feitelson, and R. V. Aroian.** 2000. *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans.* Genetics **155:** 1693–1699.
61. **Meister, M., and M. Lagueux.** 2003. *Drosophila* blood cells. Cell. Microbiol. **5:**573–580.
62. **Michiels, T., and G. Cornelis.** 1988. Nucleotide sequence and transcription analysis of yop51 from *Yersinia enterocolitica* W22703. Microb. Pathog. **5:**449–459.
63. **Michiels, T., P. Wattiau, R. Brasseur, J. M. Ruysschaert, and G. Cornelis.** 1990. Secretion of Yop proteins by *Yersiniae*. Infect. Immun. **58:**2840–2849.
64. **Moy, T. I., E. Mylonakis, S. B. Calderwood, and F. M. Ausubel.** 2004. Cytotoxicity of hydrogen peroxide produced by *Enterococcus faecium.* Infect. Immun. **72:**4512–4520.
65. **Murphy, C. T., S. A. McCarroll, C. I. Bargmann, A. Fraser, R. S. Kamath, J. Ahringer, H. Li, and C. Kenyon.** 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans.* Nature **424:**277–283.
66. **Mylonakis, E., F. M. Ausubel, J. R. Perfect, J. Heitman, and S. B. Calderwood.** 2002. Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a model of yeast pathogenesis. Proc. Natl. Acad. Sci. USA **99:**15675–15680.
67. **Mylonakis, E., A. Idnurm, R. Moreno, J. El Khoury, J. B. Rottman, F. M. Ausubel, J. Heitman, and S. B. Calderwood.** 2004. *Cryptococcus neoformans* Kin1 protein kinase homologue, identified through a *Caenorhabditis elegans* screen, promotes virulence in mammals. Mol. Microbiol. **54:**407–419.
68. **Nicholas, H. R., and J. Hodgkin.** 2004. The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in *C. elegans.* Curr. Biol. **14:**1256–1261.
69. **Nicholas, H. R., and J. Hodgkin.** 2004. Responses to infection and possible recognition strategies in the innate immune system of *Caenorhabditis elegans.* Mol. Immunol. **41:**479–493.
70. **Ogg, S., S. Paradis, S. Gottlieb, G. I. Patterson, L. Lee, H. A. Tissenbaum, and G. Ruvkun.** 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans.* Nature **389:** 994–999.
71. **O'Quinn, A. L., E. M. Wiegand, and J. A. Jeddeloh.** 2001. *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin-mediated paralysis. Cell. Microbiol. **3:**381–393.
72. **Park, J. M., H. Brady, M. G. Ruocco, H. Sun, D. Williams, S. J. Lee, T. Kato, Jr., N. Richards, K. Chan, F. Mercurio, M. Karin, and S. A. Wasserman.** 2004. Targeting of TAK1 by the NF-kappa B protein Relish regulates the JNK-mediated immune response in *Drosophila.* Genes Dev. **18:**584–594.
73. **Pujol, N., E. M. Link, L. X. Liu, C. L. Kurz, G. Alloing, M. Tan, K. P. Ray, R. Solari, C. D. Johnson, and J. J. Ewbank.** 2001. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans.* Curr. Biol. **11:**809–821.
74. **Qureshi, S. T., and R. Medzhitov.** 2003. Toll-like receptors and their role in experimental models of microbial infection. Genes Immun. **4:**87–94.
75. **Ramet, M., P. Manfruelli, A. Pearson, B. Mathey-Prevot, and R. A. Ezekowitz.** 2002. Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for E. coli. Nature **416:**644–648.
76. **Reichhart, J. M., P. Georgel, M. Meister, B. Lemaitre, C. Kappler, and J. A. Hoffmann.** 1993. Expression and nuclear translocation of the rel/NF-kappa B-related morphogen dorsal during the immune response of *Drosophila.* C. R. Acad. Sci. Ser. III **316:**1218–1224.
77. **Rubin, G. M., M. D. Yandell, J. R. Wortman, G. L. Gabor Miklos, C. R. Nelson, I. K. Hariharan, M. E. Fortini, P. W. Li, R. Apweiler, W. Fleischmann, J. M. Cherry, S. Henikoff, M. P. Skupski, S. Misra, M. Ashburner, E. Birney, M. S. Boguski, T. Brody, P. Brokstein, S. E. Celniker, S. A. Chervitz, D. Coates, A. Cravchik, A. Gabrielian, R. F. Galle, W. M. Gelbart, R. A. George, L. S. Goldstein, F. Gong, P. Guan, N. L. Harris, B. A. Hay, R. A. Hoskins, J. Li, Z. Li, R. O. Hynes, S. J. Jones, P. M. Kuehl, B. Lemaitre, J. T. Littleton, D. K. Morrison, C. Mungall, P. H. O'Farrell, O. K. Pickeral, C. Shue, L. B. Vosshall, J. Zhang, Q. Zhao, X. H. Zheng, and S. Lewis.** 2000. Comparative genomics of the eukaryotes. Science **287:**2204–2215.
78. **Schulenburg, H., C. L. Kurz, and J. J. Ewbank.** 2004. Evolution of the innate immune system: the worm perspective. Immunol. Rev. **198:**36–58.
79. **Sifri, C. D., J. Begun, and F. M. Ausubel.** 2005. The worm has turned—microbial virulence modeled in *Caenorhabditis elegans.* Trends Microbiol. **13:**119–127.
80. **Takeda, K., and S. Akira.** 2003. Toll receptors and pathogen resistance. Cell. Microbiol. **5:**143–153.
81. **Tan, M. W., S. Mahajan-Miklos, and F. M. Ausubel.** 1999. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. Proc. Natl. Acad. Sci. USA **96:**715–720.
82. **Tan, M. W., L. G. Rahme, J. A. Sternberg, R. G. Tompkins, and F. M. Ausubel.** 1999. *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. Proc. Natl. Acad. Sci. USA **96:**2408–2413.
83. **Tanaka-Hino, M., A. Sagasti, N. Hisamoto, M. Kawasaki, S. Nakano, J. Ninomiya-Tsuji, C. I. Bargmann, and K. Matsumoto.** 2002. SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in *Caenorhabditis elegans.* EMBO Rep. **3:**56–62.
84. **Tenor, J. L., B. A. McCormick, F. M. Ausubel, and A. Aballay.** 2004. *Caenorhabditis elegans*-based screen identifies *Salmonella* virulence factors required for conserved host-pathogen interactions. Curr. Biol. **14:**1018–1024.
85. **Tzou, P., E. De Gregorio, and B. Lemaitre.** 2002. How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions. Curr. Opin. Microbiol. **5:**102–110.
86. **Wu, L. P., and K. V. Anderson.** 1998. Regulated nuclear import of Rel proteins in the *Drosophila* immune response. Nature **392:**93–97.

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