

## MIT Open Access Articles

### *Studying host-pathogen interactions and innate immunity in Caenorhabditis elegans*

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

**Citation:** Kim, D. "Studying Host-pathogen Interactions and Innate Immunity in Caenorhabditis Elegans." Disease Models and Mechanisms 1.4-5 (2008): 205–208. Copyright © 2008 Published by The Company of Biologists Ltd

**As Published:** <http://dx.doi.org/10.1242/dmm.000265>

**Publisher:** Company of Biologists

**Persistent URL:** <http://hdl.handle.net/1721.1/76794>

**Version:** Final published version: final published article, as it appeared in a journal, conference proceedings, or other formally published context

**Terms of use:** Creative Commons Attribution-Noncommercial-Share Alike 3.0



# Studying host-pathogen interactions and innate immunity in *Caenorhabditis elegans*

Dennis Kim<sup>1</sup>

The genetic analysis of mechanisms of pathogen resistance in the nematode *Caenorhabditis elegans* has revealed a role for evolutionarily conserved signaling pathways that are required for innate immunity in a wide range of organisms, from worms to mammals. *C. elegans* represents one of the more simple host organisms in which mechanisms of host defense can be dissected, and the use of *C. elegans* presents the researcher with a wide array of genetic and genomic tools to probe the host-pathogen interface. The study of host defense mechanisms in *C. elegans* continues to provide an ancient evolutionary perspective on innate immunity, which may generate insights into the conserved processes in phylogenetically diverse host organisms, including humans.

## **C. elegans AS A HOST ORGANISM FOR THE STUDY OF IMMUNITY: WHY AND HOW?**

Innate immunity serves as the first line of defense against infection, with roles in the initial recognition of pathogens, the development of a neutralizing response, and mobilization of the adaptive immune response (Akira et al., 2006). The innate immune system is crucial for normal host defense, but dysregulation of the innate immune system is implicated in a number of pathological states, such as in chronic inflammatory diseases. Studies of the regulation of antimicrobial peptides in the *Drosophila* and mouse immune responses to lipopolysaccharide revealed the functional conservation of Toll signaling in immunity between insects and mammals (Lemaitre et al., 1996; Poltorak et al., 1998). The convergence of these genetic analyses in evolutionarily diverse hosts suggested that the study of simple invertebrate systems could shed light on the ancient evolutionary origins and conserved signaling mechanisms of mammalian innate immunity (Hoffmann et al., 1999).

In recent years, studies involving a diversity of simple organisms, including zebrafish, fruitflies, mosquitoes, worms and *Dictyostelium* have addressed basic questions at the host-pathogen interface, including aspects of pathogen recognition,

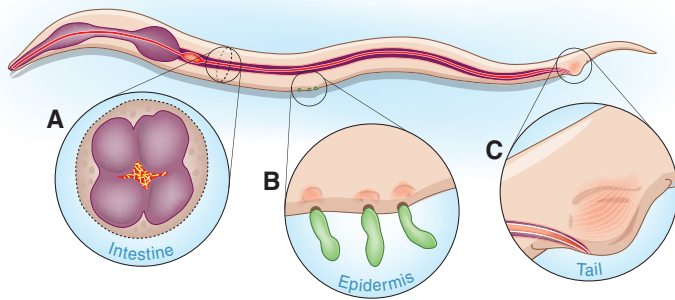
immune signaling and protective effector responses. What these approaches have in common is the analysis of a genetically tractable host that facilitates the identification and characterization of evolutionarily conserved mechanisms of host defense. Here, I discuss the use of the nematode *Caenorhabditis elegans* as a genetically tractable host organism in which to dissect mechanisms of innate immunity. Following the sequencing of the *C. elegans* genome (Sternberg, 2001), there has been a dramatic increase in the development of technologies that have facilitated the study of immunity in *C. elegans*.

The standard propagation of *C. elegans* in the laboratory involves growing the nematodes on lawns of attenuated *Escherichia coli* strains that allow the worms to survive for 3 weeks. Interest in the development of alternative hosts to study mechanisms of bacterial pathogenesis has led a number of groups to develop experimental host-pathogen systems in which the *C. elegans* host feeds on pathogenic bacteria or fungi (Couillault and Ewbank, 2002; Couillault et al., 2004; Darby et al., 1999; Garsin et al., 2001; Mahajan-Miklos et al., 1999; Mylonakis et al., 2002; Tan et al., 1999a). A number of initial studies have used the human opportunistic pathogen *Pseudomonas aeruginosa*, which appears to

be able to infect an evolutionarily diverse range of hosts (Rahme et al., 1995). *P. aeruginosa* infection leads to death of the worm within 2-3 days; the *C. elegans* killing process is dependent on a number of bacterial virulence factors that are also required for pathogenesis in mammalian hosts (Tan et al., 1999a; Tan et al., 1999b). Under specific experimental conditions, *P. aeruginosa* can also kill *C. elegans* through the secretion of toxins (Darby et al., 1999; Mahajan-Miklos et al., 1999). Of note, even *Salmonella* species, which unlike *P. aeruginosa* are generally thought to have a narrow, highly specific host tropism, have been shown to infect and kill *C. elegans* (Aballay et al., 2000; Labrousse et al., 2000).

The initial bias in the development of experimental host-pathogen systems involving bacterial or fungal infection of *C. elegans* has been to use microbial pathogens of humans, in part because of interest in using the *C. elegans* host to dissect virulence mechanisms in the human pathogens. In addition, at least two infections that appear to occur naturally in *C. elegans* have been characterized in detail. First, *Microbacterium nematophilum* adheres to the cuticle of *C. elegans* and induces a protective, local anal swelling response that results in compromised defecation function (Hodgkin et al., 2000). Second, *Drechmeria coniospora* is a member of the agriculturally important nematophilous fungi that capture *C. elegans* and kill the worms through conidia that penetrate the outer cuticle and epidermal layers of *C. elegans* (Couillault et al., 2004). The different modes of infection of *C. elegans* are depicted in Figure 1. Little is known about the natural ecology and microbial flora that *C. elegans* encounters in the wild; although, as a soil nematode, *C. elegans* undoubtedly encounters innumerable microorganisms, some of which are pathogenic. Such information will help to illuminate studies of *C. elegans* immunity and provide insights into the evolution of specific host defense mechanisms in *C. elegans*.

<sup>1</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA (e-mail: dhkim@mit.edu)



**Fig. 1. Different modes of infection of *C. elegans* elicit immune responses.** Three distinct modes of infection of *C. elegans* have been described that elicit conserved innate immune signaling pathways. (A) Intestinal infection with a wide range of pathogens involves proliferation of bacteria in the intestinal lumen. (B) The nematophagous fungus *Drechmeria* initiates infection by attachment to the cuticle with subsequent penetration of the epidermis. (C) The attachment of *M. nematophilum* to the tail region of *C. elegans* and subsequent rectal invasion induces a protective swelling response at the site of infection.

### CONSERVED PATHWAYS OF IMMUNE SIGNALING IN *C. elegans*

Studies of immunity in *C. elegans* have focused on three principal approaches: (1) forward genetic screening for mutants with altered pathogen susceptibility, (2) reverse genetic approaches assaying the role of conserved genes that are known to function in innate immunity in other organisms, and (3) analysis of gene expression that is either induced by pathogenic infection, or under the regulation of immune signaling pathways.

A genetic screen for mutants with enhanced susceptibility to *P. aeruginosa* killing identified a requirement for a conserved p38 mitogen-activated protein kinase (MAPK) pathway in *C. elegans* immunity (Kim et al., 2002). The *C. elegans* p38 MAPK pathway is homologous to the mammalian apoptosis signal-regulating kinase 1 (ASK1)–MAPK kinase 3/6 (MKK3/6)–p38 MAPK pathway, which is required for mammalian innate immune responses. Prior biochemical and cell biology studies established a pivotal role for the p38 MAPK pathway in innate immune responses to infection and inflammatory cytokines, whereas genetic analysis of p38 MAPK signaling was hampered by embryonic lethality of mice that were homozygous for a p38 deletion (Dong et al., 2002). Recent analysis of ASK1-null mice has demonstrated a crucial role for ASK1-dependent signaling in response to lipopolysaccharide (Matsuzawa et al., 2005).

The p38 MAPK pathway is required for immunity in *C. elegans* against not only *P.*

*aeruginosa*, but also other intestinal pathogens including *Salmonella*, Gram-positive pathogens such as *Enterococcus faecalis* and *Staphylococcus aureus*, and pathogenic fungi such as *Cryptococcus neoformans* (Aballay et al., 2003; Kim et al., 2002; Mylonakis et al., 2002; Sifri et al., 2003). In addition, based on the analysis of *C. elegans* resistance to *E. coli* which have been engineered to express the Bt toxin (Huffman et al., 2004), a toxin of important agricultural interest expressed by *Bacillus thuringiensis*, the p38 MAPK pathway has been implicated in defense against the action of pore-forming toxins. The requirement for p38 MAPK in *C. elegans* resistance to intestinal pathogens, and the corresponding homologous function of p38 MAPK signaling in mammalian innate immunity, demonstrates that the study of *C. elegans* immunity should shed light on evolutionarily conserved mechanisms of mammalian innate immunity.

Two genes in *C. elegans* encode proteins with Toll/interleukin-1 receptor (TIR) domains and have been the subject of reverse genetic analysis. The study of *tol-1*, which encodes a homolog of the Toll-like receptor (TLR), has been complicated by the lethality of a *tol-1* null allele. Analysis of a *tol-1* allele that is viable, even though the cytoplasmic domain is predicted to be deleted, revealed no enhanced susceptibility of worms to the lethality of *P. aeruginosa* or *Serratia marcescens*, but mutant animals failed to avoid a lawn of *S. marcescens* (Pujol et al., 2001). A recent study suggests that *C. elegans* with the viable *tol-1* mutant allele

showed increased mortality following *Salmonella* infection (Tenor and Aballay, 2008). Interestingly, no orthologs of the crucial downstream TLR signaling component MyD88, or the downstream transcription factor NF- $\kappa$ B, are predicted to be encoded by the *C. elegans* genome (Pujol et al., 2001).

The other gene encoding a TIR domain protein in *C. elegans* is *tir-1*, which encodes an ortholog of the human TIR domain protein SARM, also referred to as MyD88-5 (Mink et al., 2001). SARM is one of five members of the TIR adaptor protein family that includes MyD88, MAL, TRIF and TRAM (O'Neill and Bowie, 2007). TIR-1 functions upstream of the ASK1-dependent PMK-1 p38 MAPK pathway in pathogen defense (Liberati et al., 2004). In addition, TIR-1 is required for immunity to intestinal pathogens, as well as the epidermal expression of the antifungal peptide NLP-29 in response to the nematophagous fungus *Drechmeria coniospora* (Couillault et al., 2004). A role for TIR-1 in neuronal development has also been demonstrated in AWC chemosensory neurons, where TIR-1 was found to function in ASK1-dependent activation of a MAPK that is distinct from PMK-1 (Chuang and Bargmann, 2005).

Subsequently, studies of the role of SARM in mammals have provided conflicting results regarding its role in innate immunity. Overexpression of SARM resulted in inhibition of TRIF-mediated TLR signaling, and siRNA knockdown of SARM was reported to attenuate TLR4-mediated responses (Carty et al., 2006). However, the study of macrophages derived from a SARM knockout mouse revealed no deficits in immunity (Kim et al., 2007). Furthermore, this study revealed that expression of SARM occurred predominantly in neurons and that SARM has a role in mediating neuronal cell death in response to oxygen and glucose deprivation (Kim et al., 2007). Although studies of SARM/TIR-1 in *C. elegans* indicate an ancient role in innate immunity that may predate the evolution of TLR-mediated immunity (Kim and Ausubel, 2005), whether or not TLR-independent immunity has been conserved in mammals requires further investigation.

Additional conserved signaling pathways have been implicated in mediating pathogen resistance in *C. elegans*. Whereas the TIR-1–PMK-1 p38 MAPK pathway has been found to be required for resistance to intestinal pathogens, as well as to fungi that

kill through the cuticle, the protective swelling response to *M. nematophilum* requires a distinct MAPK signaling pathway, the ERK MAPK pathway (Nicholas and Hodgkin, 2004). In addition, the TGF- $\beta$  pathway has been shown to be involved in defense against *S. marcescens* (Mallo et al., 2002), and insulin signaling appears to contribute to pathogen resistance against a broad range of pathogens (Garsin et al., 2003).

### TRANSCRIPTIONAL RESPONSES TO INFECTION: ANCIENT EFFECTORS OF INNATE IMMUNITY

The availability of genomic resources and the tractability of *C. elegans* have led a number of groups to study the gene expression profile induced by pathogen infection (Mallo et al., 2002; O'Rourke et al., 2006; Shapira and Tan, 2008; Troemel et al., 2006; Wong et al., 2007). These studies suggest that pathogens induce common classes of genes, such as those associated with lysozymes and C-type lectin domain-containing proteins, to promote pathogen defense. Also induced, are a number of short peptides that are predicted to be secreted. These might represent antimicrobial peptides, although sequence conservation among antimicrobial peptides is limited. A few of these antimicrobial peptides have been expressed, purified and characterized in vitro (Couillault et al., 2004; Kato et al., 2002), but the primary focus of studies on candidate downstream effectors of *C. elegans* immunity has been microarray-based studies of genes induced by pathogen infection. These studies suggest that there are distinct classes of pathogen resistance genes that are either induced by pathogen infection or expressed constitutively at a basal level (O'Rourke et al., 2006; Shapira and Tan, 2008; Troemel et al., 2006; Wong et al., 2007). Based on microarray studies, a comparative analysis of pathogen-induced genes with genes that are regulated by the PMK-1 pathway revealed that approximately 15% of PMK-1-regulated genes are also induced by *P. aeruginosa* infection, further implicating the PMK-1 pathway in the *C. elegans* innate immune response to pathogens (Troemel et al., 2006). This study also suggested that there are additional, yet to be identified signaling pathways that control pathogen-induced genes upon infection.

### Characteristics of experimental systems involving infection of the *C. elegans* host

- *C. elegans* responds to infection with a simple innate immune system, key aspects of which have been shown to be conserved
- *C. elegans* does not appear to have specific immune cells and has no adaptive immunity; instead, cells adjacent to infection, such as the intestine or epidermis, serve a primary role in a 'mucosal' innate immunity
- A number of bacteria and fungi of medical relevance, as well as naturally occurring pathogens of *C. elegans*, have been used to infect *C. elegans* in the lab, often providing insights into general mechanisms of pathogen virulence, as well as host defense
- A comprehensive set of genetic and genomic resources greatly facilitates the systematic analysis of gene function in *C. elegans*
- Neuronally-mediated pathogen avoidance responses have been demonstrated in *C. elegans*, underscoring the whole organism physiology of responses to microbes, and can be studied by taking advantage of the simple anatomy and physiology of *C. elegans*

### PATHOGEN RECOGNITION AND *C. elegans* PHYSIOLOGY

Although most studies of the *C. elegans* immune response have focused on tissues that are directly adjacent to the site of infection, such as the intestine and epidermis, the study of *C. elegans* behavior in the presence of pathogens has revealed that *C. elegans* can not only 'smell' or sense pathogens (Pradel et al., 2007; Zhang et al., 2005), but that it also responds with aversive learning behaviors (Zhang et al., 2005). These studies may provide insights into ancient mechanisms by which the nervous system plays a role in pathogen defense. In addition, there is evidence that physiological processes such as reproduction (Miyata et al., 2008) and diverse cellular stress responses (Chavez et al., 2007; Kim et al., 2004; Mizuno et al., 2004; Singh and Aballay,

2006) may be related to pathogen defense. The simple well-defined anatomy and physiology of *C. elegans* facilitate the genetic dissection of the multiple ways in which a multicellular organism interacts with its environment, and I anticipate that these studies will illuminate evolutionarily conserved physiological processes by which host organisms interact with microbes.

### ACKNOWLEDGEMENTS

Deposited in PMC for immediate release.

### COMPETING INTERESTS

The author declares no competing financial interests.

### REFERENCES

- Aballay, A., Yorgey, P. and Ausubel, F. M. (2000). Salmonella typhimurium proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr. Biol.* **10**, 1539-1542.
- Aballay, A., Drenkard, E., Hilbun, L. R. and Ausubel, F. M. (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.
- Akira, S., Uematsu, S. and Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell* **124**, 783-801.
- Carty, M., Goodbody, R., Schroder, M., Stack, J., Moynagh, P. N. and Bowie, A. G. (2006). The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat. Immunol.* **7**, 1074-1081.
- Chavez, V., Mohri-Shiomi, A., Maadani, A., Vega, L. A. and Garsin, D. A. (2007). Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by *Caenorhabditis elegans*. *Genetics* **176**, 1567-1577.
- Chuang, C. F. and Bargmann, J. J. (2005). A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev.* **19**, 270-281.
- Couillault, C. and Ewbank, J. J. (2002). Diverse bacteria are pathogens of *Caenorhabditis elegans*. *Infect. Immun.* **70**, 4705-4707.
- Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J. F., Kohara, Y. and Ewbank, J. J. (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.
- Darby, C., Cosma, C. L., Thomas, J. H. and Manoil, C. (1999). Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* **96**, 15202-15207.
- Dong, C., Davis, R. J. and Flavell, R. A. (2002). MAP kinases in the immune response. *Annu. Rev. Immunol.* **20**, 55-72.
- Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V., Murray, B. E., Calderwood, S. B. and Ausubel, F. M. (2001). A simple model host for identifying Gram-positive virulence factors. *Proc. Natl. Acad. Sci. USA* **98**, 10892-10897.
- Garsin, D. A., Villanueva, J. M., Begun, J., Kim, D. H., Sifri, C. D., Calderwood, S. B., Ruvkun, G. and Ausubel, F. M. (2003). Long-lived *C. elegans* *daf-2* mutants are resistant to bacterial pathogens. *Science* **300**, 1921.
- Hodgkin, J., Kuwabara, P. E. and Corneliusen, B. (2000). A novel bacterial pathogen, *Microbacterium*

- nematophilum, induces morphological change in the nematode *C. elegans*. *Curr. Biol.* **10**, 1615-1618.
- Hoffmann, J. A., Kafatos, F. C., Janeway, C. A. and Ezekowitz, R. A.** (1999). Phylogenetic perspectives in innate immunity. *Science* **284**, 1313-1318.
- Huffman, D. L., Abrami, L., Sasik, R., Corbeil, J., van der Goot, F. G. and Aroian, R. V.** (2004). Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. *Proc. Natl. Acad. Sci. USA* **101**, 10995-11000.
- Kato, Y., Aizawa, T., Hoshino, H., Kawano, K., Nitta, K. and Zhang, H.** (2002). abf-1 and abf-2, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans*. *Biochem. J.* **361**, 221-230.
- Kim, D. H. and Ausubel, F. M.** (2005). Evolutionary perspectives on innate immunity from the study of *Caenorhabditis elegans*. *Curr. Opin. Immunol.* **17**, 4-10.
- Kim, D. H., Feinbaum, R., Alloing, G., Emerson, F. E., Garsin, D. A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M. W. et al.** (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623-626.
- Kim, D. H., Liberati, N. T., Mizuno, T., Inoue, H., Hisamoto, N., Matsumoto, K. and Ausubel, F. M.** (2004). Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. *Proc. Natl. Acad. Sci. USA* **101**, 10990-10994.
- Kim, Y., Zhou, P., Qian, L., Chuang, J. Z., Lee, J., Li, C., Iadecola, C., Nathan, C. and Ding, A.** (2007). MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. *J. Exp. Med.* **204**, 2063-2074.
- Labrousse, A., Chauvet, S., Couillault, C., Kurz, C. L. and Ewbank, J. J.** (2000). *Caenorhabditis elegans* is a model host for *Salmonella typhimurium*. *Curr. Biol.* **10**, 1543-1545.
- Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. and Hoffmann, J. A.** (1996). The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973-983.
- Liberati, N. T., Fitzgerald, K. A., Kim, D. H., Feinbaum, R., Golenbock, D. T. and Ausubel, F. M.** (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.
- Mahajan-Miklos, S., Tan, M. W., Rahme, L. G. and Ausubel, F. M.** (1999). Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell* **96**, 47-56.
- Mallo, G. V., Kurz, C. L., Couillault, C., Pujol, N., Granjeaud, S., Kohara, Y. and Ewbank, J. J.** (2002). Inducible antibacterial defense system in *C. elegans*. *Curr. Biol.* **12**, 1209-1214.
- Matsuzawa, A., Saegusa, K., Noguchi, T., Sadamitsu, C., Nishitoh, H., Nagai, S., Koyasu, S., Matsumoto, K., Takeda, K. and Ichijo, H.** (2005). ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. *Nat. Immunol.* **6**, 587-592.
- Mink, M., Fogelgren, B., Olszewski, K., Maroy, P. and Csiszar, K.** (2001). A novel human gene (SARM) at chromosome 17q11 encodes a protein with a SAM motif and structural similarity to Armadillo/beta-catenin that is conserved in mouse, *Drosophila*, and *Caenorhabditis elegans*. *Genomics* **74**, 234-244.
- Miyata, S., Begun, J., Troemel, E. R. and Ausubel, F. M.** (2008). DAF-16-dependent suppression of immunity during reproduction in *Caenorhabditis elegans*. *Genetics* **178**, 903-918.
- Mizuno, T., Hisamoto, N., Terada, T., Kondo, T., Adachi, M., Nishida, E., Kim, D. H., Ausubel, F. M. and Matsumoto, K.** (2004). The *Caenorhabditis elegans* MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J.* **23**, 2226-2234.
- Mylonakis, E., Ausubel, F. M., Perfect, J. R., Heitman, J. and Calderwood, S. B.** (2002). Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a model of yeast pathogenesis. *Proc. Natl. Acad. Sci. USA* **99**, 15675-15680.
- Nicholas, H. R. and Hodgkin, J.** (2004). The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in *C. elegans*. *Curr. Biol.* **14**, 1256-1261.
- O'Neill, L. A. and Bowie, A. G.** (2007). The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol.* **7**, 353-364.
- O'Rourke, D., Baban, D., Demidova, M., Mott, R. and Hodgkin, J.** (2006). Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum*. *Genome Res.* **16**, 1005-1016.
- Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C. et al.** (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085-2088.
- Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C. I. and Ewbank, J. J.** (2007). Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **104**, 2295-2300.
- Pujol, N., Link, E. M., Liu, L. X., Kurz, C. L., Alloing, G., Tan, M. W., Ray, K. P., Solari, R., Johnson, C. D. and Ewbank, J. J.** (2001). A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 809-821.
- Rahme, L. G., Stevens, E. J., Wolfort, S. F., Shao, J., Tompkins, R. G. and Ausubel, F. M.** (1995). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268**, 1899-1902.
- Shapira, M. and Tan, M. W.** (2008). Genetic analysis of *Caenorhabditis elegans* innate immunity. *Methods Mol. Biol.* **415**, 429-442.
- Sifri, C. D., Begun, J., Ausubel, F. M. and Calderwood, S. B.** (2003). *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. *Infect. Immun.* **71**, 2208-2217.
- Singh, V. and Aballay, A.** (2006). Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. *Proc. Natl. Acad. Sci. USA* **103**, 13092-13097.
- Sternberg, P. W.** (2001). Working in the post-genomic *C. elegans* world. *Cell* **105**, 173-176.
- Tan, M. W., Mahajan-Miklos, S. and Ausubel, F. M.** (1999a). Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl. Acad. Sci. USA* **96**, 715-720.
- Tan, M. W., Rahme, L. G., Sternberg, J. A., Tompkins, R. G. and Ausubel, F. M.** (1999b). *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proc. Natl. Acad. Sci. USA* **96**, 2408-2413.
- Tenor, J. L. and Aballay, A.** (2008). A conserved Toll-like receptor is required for *Caenorhabditis elegans* innate immunity. *EMBO Rep.* **9**, 103-109.
- Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M. and Kim, D. H.** (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet.* **2**, e183.
- Wong, D., Bazopoulou, D., Pujol, N., Tavernarakis, N. and Ewbank, J. J.** (2007). Genome-wide investigation reveals pathogen-specific and shared signatures in the response of *Caenorhabditis elegans* to infection. *Genome Biol.* **8**, R194.
- Zhang, Y., Lu, H. and Bargmann, C. I.** (2005). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438**, 179-184.