Studying host-pathogen interactions and innate immunity in Caenorhabditis elegans

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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 (Lemaître 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 innate 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. Infection with 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 defense function (Hodgkin et al., 2000). Second, Drechmeria coniospora is a member of the agriculturally important nematophagous fungi that capture C. elegans and kill the worms through conidial penetration of the outer cuticle and epidermal layers of C. elegans (Coulillaud 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.

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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 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-κB, 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
Innate immunity in *C. elegans*

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 (Miyan 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.

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**References**


