Published in final edited form as:

Nat Rev Immunol. 2010 January ; 10(1): 47–58. doi:10.1038/nri2689.

Evolution of host innate defence: insights from *C. elegans* and primitive invertebrates

Javier E. Irazoqui, Jonathan M. Urbach, and Frederick M. Ausubel*

Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 USA

Preface

The genetically tractable model organism *Caenorhabditis elegans* was first used to model bacterial virulence *in vivo* a decade ago. Since then, great strides have been made in the identification of host response pathways that are involved in the defence against infection. Strikingly, *C. elegans* seems to detect and respond to infection without the involvement of its Toll-like receptor homologue, in contrast to the well-established role for these proteins in innate immunity in mammals. What, therefore, do we know about host defence mechanisms in *C. elegans*, and what can they tell us about innate immunity in higher organisms?

As metazoans evolved from simple multicellular organisms to mammals, they evolved increasingly sophisticated immune systems¹. Consequently, mammalian innate immune systems are presumably composed of elements of those systems that evolved in their now extinct invertebrate predecessors². Conversely, pathogens have evolved sophisticated mechanisms to defend themselves against metazoan antimicrobial mechanisms and to take advantage of flaws in this defence³. Therefore, to understand the evolution and function of human innate immunity, a reasonable place to start is the study of invertebrate (and, likely, more ancient) immunity. Similarly, the study of invertebrate pathogenesis models should provide new insights into conserved virulence strategies that have been successful for pathogens, irrespective of the host.³

Nematodes are the most abundant animals on Earth⁴. Microbes and nematodes have interacted and co-evolved for over 600 million years³. Some free-living soil nematodes, such as *Caenorhabditis elegans*, are bacterivores. From a bacterial perspective, the evolution of both defensive and offensive mechanisms to avoid predation by nematodes and other invertebrates may be the origin of virulence-related traits^{3, 5–7}. Conversely, the evolution of defence mechanisms in nematodes (in the form of an immune system) became essential for surviving the effects of ingesting potential pathogens.

An important discovery in evolutionary biology during the past few decades was that many of the signalling pathways involved in human development, homeostasis, and disease pathogenesis had already evolved by the time of the last common ancestor between humans and nematodes, approximately 1 billion years ago⁸. It is often observed that Nature re-uses pathways that work, assigning new additional functions to them and creating variations on a theme by molecular evolution. This is also believed to apply to innate defence mechanisms: the most successful immune strategies have probably been conserved over the ensuing billion years of separate evolution of humans and nematodes because of their high defensive value. By understanding host defence systems at the genetic and molecular levels in the simple,

*Corresponding author: Ausubel@molbio.mgh.harvard.edu.

genetically amenable organism *C. elegans*, we can learn about the fundamental pathways that are conserved in nematodes and humans. What are the underlying themes, and what can we learn about human immunity from the study of nematodes?

In this Review, we aim to discuss recent advances in the field of *C. elegans* host defence, with particular emphasis on a survey of the signalling pathways involved and the novel concepts that have emerged from integrated genetic, genomic and cell-biology approaches. Because other recent reviews cover the subjects of immune effectors and coordination among different tissues in detail^{9–11}, we attempt to integrate recent information into a broader perspective of innate host defence and emphasize some parallels with mammalian innate immunity.

***C. elegans* as a model host**

C. elegans has been a workhorse of biological exploration since its initial use in the 1970s as a model system to study the genetic control of development¹². Several features of *C. elegans* make this nematode a premier genetic model (Box 1). Many human pathogens — bacterial and fungal — cause potent intestinal infections in *C. elegans* that result in death^{13–17}. The adult *C. elegans* intestine comprises 20 intestinal epithelial cells (IECs) that are non-renewable — that is, they are not shed and do not proliferate as mammalian IECs do (Figure 1). These cells share similar morphological features with mammalian IECs, or enterocytes, thereby making *C. elegans* a powerful model in which to study the interaction of microbial pathogens with primordial epithelial cells. *C. elegans* can be infected by simply replacing the normal food source (*E. coli*; Box 1) with the pathogen of choice¹³. Because *C. elegans* is transparent, one can follow infection and disease progress in real time. Several readouts can be used to monitor pathogenesis, including quantifying nematode survival¹⁸, observing morphological and behavioural changes^{19–21}, measuring bacterial persistence and/or accumulation in the intestine²², and monitoring changes in gene expression by using microarrays, quantitative reverse transcription polymerase chain reaction (qRT-PCR)²³ and reporter constructs in transgenic worms²⁴. These methods allow the detailed study of host–pathogen interactions at the cellular, genetic and molecular levels in the context of the whole organism.

Box 1

***C. elegans* as a premier genetic model**

In the laboratory, *C. elegans* is propagated and maintained on agar plates with lawns of non-pathogenic *Escherichia coli* as a food source¹³. With a 3-day life cycle, each animal produces ~300 genetically identical progeny, facilitating the establishment and maintenance of large populations of animals that can be housed in the laboratory in refrigerator-sized incubators. *C. elegans* is diploid and hermaphroditic, although males are easy to generate and readily mate with hermaphrodites for use in genetic analysis. Gene expression in *C. elegans* can conveniently be knocked down by double-stranded (ds)RNA-mediated RNA interference (RNAi) by feeding worms live non-pathogenic *E. coli* expressing dsRNA corresponding to the appropriate *C. elegans* genes (almost 90% of the genome is available as a dsRNA expression library⁹⁷). Transgenic *C. elegans* can also be easily generated by microinjection or bombardment of DNA. *C. elegans* is transparent, greatly facilitating characterization of gene expression patterns. Adult organisms have exactly 959 somatic nuclei, corresponding to cells whose developmental lineages have been completely traced to the fertilized egg.

Since the initial description of *C. elegans* as a model for bacterial infection in 1999^{25–27}, great strides have been made in identifying the genetic components that mediate the host response at the level of intestinal and epidermal epithelia, as well as understanding the virulence mechanisms used by pathogens to defeat these defences. These studies have provided important

insights into the evolution of innate immunity. The emerging picture is one of several parallel signalling pathways being involved in host defence, in many cases depending on the route of infection and the pathogen that is causing the infection. Through incompletely understood mechanisms, these pathways are likely to interact extensively in a signalling network encompassing different tissues to integrate host physiological information with the antimicrobial defence response.

Signaling pathways in *C. elegans* innate immunity

Signalling pathways that are important during early development are frequently used again in the adult for innate immune signalling. This realization highlights links between processes such as cell growth and differentiation during tissue repair, metabolic regulation and innate immune signalling, or the suspected relationship between chronic infection (and inflammation) and dysregulated tissue repair leading to neoplastic transformation^{28, 29}.

Toll-like receptors

Work in *Drosophila melanogaster* identified the key role of Toll receptors in development and pathogen detection³⁰. The Toll receptor was originally described as a developmental gene³¹, but in the adult organism, Toll functions upstream of an evolutionarily conserved signalling cascade that is involved in innate immunity. Toll is activated by Spaetzle, a cytokine produced downstream of detection of pathogen-associated molecular patterns (PAMPs) in insects. In vertebrates, Toll-like receptors (TLRs) are activated directly by PAMPs. In both insects and vertebrates, these events lead to the downstream activation of transcription factors of the nuclear factor- κ B (NF- κ B; also known as Rel) family via signalling pathways involving Mal and MyD88-class scaffold proteins (Figure 2)³². NF- κ B proteins regulate several aspects of cell differentiation during development, as well as the transcription of host response genes that encode proteins involved in the innate immune response, such as antimicrobial peptides and vertebrate pro-inflammatory cytokines³³. In *D. melanogaster*, the Toll-dependent pathway mediates activation of NF- κ B transcription factors primarily in response to fungal and Gram-positive bacterial pathogens, whereas the parallel IMD (immune deficiency) pathway (which is related to the mammalian tumor necrosis factor receptor [TNFR] pathway) does so primarily in response to Gram-negative pathogens^{30, 34}.

Analysis of the *C. elegans* genome identified a single TLR (TOL-1) and other components related to those of the insect and mammalian TLR signalling pathways, including TRF-1 (which is related to TRAF [TNFR-associated factor]), PIK-1 (related to Pelle and IRAK [IL-1R-associated kinase]), and IKB-1 (related to I κ B [inhibitor of NF- κ B]) (Figure 2)²⁰. However, the *C. elegans* genome does not appear to encode homologues of the MyD88 scaffold protein or NF- κ B-like transcription factors (Figure 2). Moreover, *C. elegans* does not appear to carry a clear IMD homologue, suggesting that the IMD pathway is also not present.

In addition to TOL-1, the only other *C. elegans* protein that contains a Toll/IL-1R (TIR) protein–protein interaction domain (a salient feature of some TLR-pathway signalling components) is the scaffold protein TIR-1, which is homologous to human sterile α - and armadillo-motif-containing protein (SARM). Human SARM functions as a negative regulator of TIR-domain-containing adaptor protein inducing IFN β (TRIF)-dependent signalling downstream of TLR3 and TLR4 (Figure 2)³⁵. Because TOL-1 and TIR-1 are the only two *C. elegans* proteins with TIR domains, it seemed likely that TOL-1 might function upstream of TIR-1 in an immune signalling cascade. Surprisingly, whereas knockdown^{36, 37} or mutation³⁸ of *tir-1* results in hypersusceptibility to all pathogens tested to date, partial loss of function of *C. elegans tol-1* does not cause enhanced susceptibility to most pathogens²⁰. Two alleles of *tol-1* have been analyzed to date. The first allele represents a deletion of part of the extracellular domain, and causes severe recessive developmental abnormalities. The second

allele represents a deletion of the intracellular TIR signaling domain, lacks developmental phenotypes (thus considered a hypomorph), and is commonly considered null for conventional immune signaling.²⁰ Furthermore, reduction of *tol-1* function does not cause defects in the induction of host response genes during infection with *Staphylococcus aureus* or *Pseudomonas aeruginosa* (J. Irazoqui, E. Troemel and F. Ausubel, unpublished observations) or *Drechmeria coniospora*³⁹. *tol-1* mutants do, however, exhibit susceptibility to infection by *Salmonella enterica*, possibly owing to the decreased expression of antimicrobial peptides or developmental effects of the mutation⁴⁰. With the proviso that the *tol-1* allele used and knockdown result in partial loss of function, these studies imply that TOL-1 is not a central component of the nematode innate immune response, as it is in flies and mammals. The receptor (s) that functions upstream of TIR-1 remain unknown.

p38 mitogen-activated protein kinase pathways

An important signalling pathway downstream of TLRs in insects and mammals involves a p38 mitogen-activated protein kinase (MAPK) cascade^{41–44} (Figure 2). By screening for mutants with shortened longevity on infection with *P. aeruginosa* (but not with non-pathogenic *E. coli*), a NSY-1–SEK-1–PMK-1 p38 MAPK cascade was identified as a key component of the *C. elegans* immune response^{45–47}. *nsy-1*, *sek-1* and *pmk-1* encode the *C. elegans* orthologues of human ASK-1 (a MAPK kinase kinase [MAPKKK]), MKK3/MKK6 (a MAPKK), and p38 (a MAPK), respectively, all of which have been directly implicated in the mammalian cellular immune response⁴⁸. NSY-1, SEK-1 and PMK-1 appear to function in a linear phosphotransfer cascade^{36–45}. Intriguingly, TIR-1, but not TOL-1, functions upstream of the NSY-1–SEK-1–PMK-1 p38 MAPK cascade, as knockdown of *tir-1* blocks phosphorylation of PMK-1³⁶. By analogy with other systems, PMK-1 is likely phosphorylated by SEK-1, promoting its translocation into the nucleus, where it presumably phosphorylates target transcription factors to control gene transcription. Consistent with this view, PMK-1 phosphorylation also depends on NSY-1 and SEK-1⁴⁵.

The phosphorylation state of PMK-1 is modulated by MEK-1 (a MAPKK), a component of a parallel MAPK cassette involving KGB-1/Jun N-terminal kinase (JNK), which is activated in response to heavy metal stress⁴⁶. The phosphatase VHP-1 negatively regulates PMK-1 and KGB-1 phosphorylation, but whether VHP-1 activity is regulated remains unknown⁴⁶. The downstream phosphorylation targets of PMK-1 also remain to be elucidated, but might include the *C. elegans* programmed cell death pathway⁴⁹.

The evolution of TLR, MAPK signalling pathways

In *C. elegans*, the presence of a TIR-1–NSY-1–SEK-1–PMK-1 signalling pathway (known as the PMK-1/p38 MAPK cassette) that does not involve TOL-1 led to the hypothesis that the cassette might be an ancient ancestral immune pathway that evolved before the recruitment of NF-κB, TLRs and MyD88 as key components of immune signalling⁵⁰, and that TIR-1 (SARM) was the ancestral founding member of the TIR-domain-containing family of scaffold proteins. This was previously deemed consistent with the traditional phylogenetic placement of *C. elegans* on an evolutionary branch that diverged before the appearance of coelomates, including flies and mammals⁵⁰. Also, because a *C. elegans tol-1* null allele exhibits lethal developmental defects, it was proposed that a role for TOL-1 in immune signalling evolved more recently than its role in development. Thus, nematodes were thought to have diverged before the co-option of TLRs for immune signaling and the evolution of NF-κB and MyD88⁵⁰.

However, recent sequencing of primitive metazoan genomes has since challenged this view. Unexpectedly, the genome of the cnidarian *Nematostella vectensis* (sea anemone)⁵¹, 52 includes genes encoding TLRs, MyD88 and NF-κB (Figure 3)⁵¹. Furthermore, additional

sequencing work favours the phylogenetic placement of nematodes alongside other moulting animals, such as insects (Figure 3)⁵¹. Consequently, the favored conclusion is that the MyD88 and NF- κ B genes have been lost in the *C. elegans* lineage, which is consistent with the higher rates of evolution observed in *C. elegans* compared to vertebrates and insects⁵¹. With respect to innate immune pathways, *C. elegans* seems to be representative of nematodes in general, as analyses of other nematode genomes essentially recapitulate the findings in *C. elegans*⁵³ (J. Urbach and F. Ausubel, unpublished observations).

The presence of MyD88 and NF- κ B in *N. vectensis* does not necessarily imply a role in immunity and, until functional analysis is carried out on the cnidarian MyD88 and NF- κ B homologues, it is still possible that the immune function of TLRs evolved after nematodes branched off from the rest of bilateria. Nonetheless, the fact that the single TLR in *C. elegans* does not have a major function in immune signaling, in combination with the lack of MyD88 and NF- κ B, implies that other signalling components assume their function. It is therefore of great interest to identify these functional equivalents and to determine whether they also function as immune signalling components in other lineages, including vertebrates.

Analysis of the *Monosiga brevicollis*, *Hydra magnipapillata*, *N. vectensis* and *C. elegans* genomes has uncovered the presence of homologues of lipopolysaccharide (LPS)-binding protein (LBP). In mammals, LBP is an adaptor for LPS binding by TLR4 that is involved in sensing Gram-negative bacteria⁵². In *C. elegans*, there are at least nine putative LBP homologues (F55B12.5, T19C3.5, C06G1.1b, C05C9.1, C55C3.1, F10D11.6, F44A2.3, F01G10.10 and C06E8.5; J. Urbach and F. Ausubel, unpublished). Intriguingly, although none of these genes have been shown to be involved in immune defense in *C. elegans*, F44A2.3 is induced during infection with several pathogenic bacteria⁵⁴. *D. melanogaster* lacks LBP homologues, and instead uses peptidoglycan recognition protein L_C and -L_E (PGRP-L_C and -L_E) to activate the IMD pathway and thereby detect Gram-negative peptidoglycan³⁰. Since *C. elegans* lacks homologues of IMD pathway components, the prevailing view is that an IMD-like pathway does not function in bacterial sensing in nematodes.

Of these, only p38 MAPK signalling pathways are present in *M. brevicollis*, the sequenced single-cell eukaryote that is most closely related to metazoans (Figure 3). MAPKs are also present in unicellular fungi, in which they are involved in responding to cell-wall stress, among other functions⁵⁵. This connection with stress at the cell cortex is conserved in *C. elegans*, as the PMK-1/p38 MAPK cassette is also involved in the host response to pore-forming toxins produced by the soil pathogen *Bacillus thuringiensis*^{56–58}. Consistent with a putative role in cortical stress, the PMK-1/p38 MAPK cassette is required for defence against every intestinal bacterial pathogen that has been tested to date, most of which are known to secrete pore-forming toxins, which disrupt the cell membrane integrity of the host⁵⁹. Likewise, the cassette is required for defense against *D. coniospora*, which punctures the epidermis during infection^{60, 61}.

Regulating the PMK-1/p38 MAPK cassette

Upstream regulation of the PMK-1/p38 MAPK cassette in *C. elegans* varies according to the site of action (intestine versus epidermis) and type of cellular stress (fungal invasion versus wounding).

In the intestine

In the intestine, the protein kinase C δ (PKC δ) TPA-1 activates the protein kinase D (PKD) DKF-2 upstream of the cassette⁶² (Figure 4). The mechanism of action of these upstream components is unknown; presumably, it involves phosphorylation events. However, whether DKF-2 directly phosphorylates any of the components of the PMK-1/p38 MAPK cassette

remains unknown. The upstream signals that control TPA-1 activity also remain unknown, although a likely candidate is diacylglycerol (DAG), suggesting that upstream regulation of this pathway may also require the activity of an unidentified phospholipase C acting in the intestine^{63, 64}. The pathogen-derived signals that trigger TPA-1/PMK-1 activation in the intestine remain unknown.

In the epidermis

Wounding or infection by the natural fungal pathogen *Drechmeria coniospora* triggers a host epidermal response that involves the expression of antimicrobial genes encoding neuropeptide-like peptides (NLPs) and caenacins (CNCs)^{61, 64, 65}. The PMK-1/p38 MAPK cassette is required for NLP but not CNC expression.^{39, 64} The proximal upstream signals that are sensed to activate the PMK-1 cassette during wounding are unknown; however, upstream regulation involves repression by the death-associated protein kinase DAPK-1 acting in the epidermis (Figure 4)⁶⁶.

Surprisingly, during infection by *D. coniospora*, *nlp* gene activation in the epidermis by the PMK-1/p38 MAPK cassette involves a different upstream pathway to that involved in wounding. Upstream regulation of PMK-1 for the induction of *nlp*s in the epidermis involves protein kinase C paralogs TPA-1 (identified as No Induction of Peptide after *Drechmeria* Infection 1, or *nipi-1*) and PKC-3, the phospholipase C paralogs EGL-8 and PLC-3, the heterotrimeric G-protein subunits GPA-12 and RACK-1, and a protein related to human Tribbles-like kinase, NIPI-3^{61, 64}. Not all steps in this complex pathway are clearly delineated yet, although NIPI-3 seems to function in parallel with GPA-12 and RACK-1, the PLC proteins and the PKC proteins to activate the PMK-1/p38 cassette during infection but not wounding (Figure 4). Thus, NIPI-3 is involved in the transduction of an infection-specific signal to the PMK-1/p38 MAPK cassette. Although NIPI-3 activity is required in the epidermis for PMK-1 regulation, whether the other upstream components also function cell-autonomously in the epidermis is unknown. The same study showed that DKF-2, which functions downstream of TPA-1 to regulate PMK-1 in the intestine, is not required in the epidermis for the induction of intestinal genes, and neither is its paralogue DKF-1⁶⁴. Thus, it is possible that TPA-1 regulates PMK-1/p38 MAPK in the epidermis directly, or through an unidentified kinase other than DKF-1 and DKF-2.

The induction of genes encoding CNCs during *D. coniospora* infection requires a non-canonical TGF β signalling pathway that is composed of the TGF β receptor subunits SMA-6 and DAF-4, as well as the downstream signalling component SMA-3 (Figure 4). These gene products function cell-autonomously in the epidermis, responding to a DBL-1/TGF β signal emanating from the nervous system³⁹. Importantly, these observations highlight the role of the nervous system in the induction of host defenses. Similar observations involve insulin-mediated signaling (see below). Human epithelial cells secrete TGF β in response to bacterial stimulation, priming lymphocytes to adopt a tolerogenic phenotype⁶⁷. Human TGF β has dual effects on innate immunity and inflammation, having both pro- and anti-inflammatory activity, presumably depending on poorly understood contextual influences⁶⁸. Thus, *C. elegans* provides a valuable opportunity to study the role of TGF β in the induction of epidermal host responses to infection via neuroendocrine mechanisms.

Insulin signalling

The highly conserved DAF-2–DAF-16 insulin-like signalling pathway regulates metabolism, reproduction, development, lifespan and resistance to a variety of environmental stresses in *C. elegans*⁶⁹, and also has an important role in innate immunity⁷⁰. The insulin receptor DAF-2 inhibits the downstream transcription factor DAF-16 by phosphorylation through a kinase cascade that includes the phosphoinositide 3-kinase AGE-1, the phosphoinositide-dependent

kinase PDK-1, AKT-1/2 and the serum/glucocorticoid-regulated kinase SGK-1⁷¹ (Figure 4). Mutations in *daf-2* or *age-1* constitutively activate DAF-16, prolonging lifespan, delaying ageing, and enhancing resistance to killing by both Gram-positive and Gram-negative bacterial pathogens⁷⁰. It is clear that the enhanced resistance of these mutants is not simply a consequence of their enhanced longevity. First, DAF-16 upregulates the transcription of a variety of genes with homology to antimicrobial defence genes^{72, 73}. Second, the extent of the increase in longevity of *daf-2* and *age-1* mutants does not correlate with the degree of increase in pathogen resistance^{70, 74} (for example, *daf-2* mutants live about twice as long as their wild-type counterparts on an *E. coli* diet but are five-fold more resistant to *S. aureus*⁷⁰). And, finally, lifespan regulation and stress resistance have been shown to be genetically separable from antimicrobial resistance⁷⁵. Thus, the prevailing view is that inactivation of DAF-2 induces several parallel responses, including stress resistance, metabolic regulation, and antimicrobial defense, all cumulatively resulting in enhanced longevity.

Transcriptional profiling identified a large number of DAF-16 target genes with predicted functions in stress resistance and host defence^{73, 76}, leading to the hypothesis that DAF-16 must be an important component of host defence during infection. However, *daf-16* null mutants do not exhibit the phenotype that would be expected if this hypothesis were correct — that is, they are not significantly more susceptible than wild-type to pathogen-mediated killing by a broad array of pathogens^{23, 24, 70}. Moreover, even at late stages of infection in wild-type *C. elegans*, when host damage is apparent by light and electron microscopy, DAF-16 remains localized to the cytosol (not the nucleus, where it is active)^{24, 74}. Furthermore, *daf-16* mutants are not defective in the induction of host response genes during infection with *P. aeruginosa* or *S. aureus*^{23, 24, 74}. These observations led to the prevailing view that, although constitutive activation of DAF-16 enhances pathogen resistance, DAF-16 is not normally activated during pathogen infection in wild-type *C. elegans*. This implies that DAF-16-independent pathways may be involved in the host response to intestinal infection and that the considerable cellular damage observed during infection does not result in DAF-16 activation. The molecular mechanism(s) responsible for this phenomenon are currently unclear, but might involve active repression of DAF-16 by crosstalk with other pathogen-induced host defence signalling pathways or physiological pathways, such as reproduction⁷⁴. For example, infection with *P. aeruginosa* leads to the induction of insulin-like INS-7 in the nervous system, which activates DAF-2 and downregulates DAF-16 in the intestine⁷⁷. In contrast to these observations, it was recently shown that infection with enteropathogenic *E. coli* causes DAF-16 activation, leading to the expression of a “conditioned” phenotype that protects *C. elegans* from subsequent pathogenic challenge.⁷⁸ In summary, the involvement of DAF-16-mediated immune defense appears to be triggered by unknown pathogen-specific signals.

Mutations in *daf-2* and *pmk-1* cause opposing phenotypes (pathogen resistance and pathogen susceptibility, respectively). Genetic epistasis analysis shows that *daf-2;pmk-1* double mutants exhibit the *pmk-1* phenotype²³. The formal interpretation of this analysis is that *pmk-1* functions downstream of, or parallel to, *daf-2*. Additionally, transcriptional profiling shows there to be essentially no overlap between those genes regulated downstream of DAF-2 by PMK-1 and those regulated by DAF-16²³. Thus, the simplest interpretation is that PMK-1 and DAF-16 function in parallel pathways; most likely, the PMK-1/p38 MAPK pathway functions in parallel to insulin signalling²³. The reason that *daf-2;pmk-1* mutants exhibit a *pmk-1* phenotype must therefore reflect the fact that PMK-1 signalling is indispensable for the establishment of immunity, irrespective of the activation of other immune pathways. Similar observations have been made in regard to a recently identified pathway involving BAR-1/β-catenin²⁴ (see below).

***P. aeruginosa* and *S. aureus* activate distinct responses**

Global microarray analysis has identified host response genes that are activated when *C. elegans* is exposed to various pathogens, including the human pathogens *P. aeruginosa* and *S. aureus*^{23, 24, 79}. During early infection (at 4–8 hours), *P. aeruginosa* activates some of the same immune effectors identified downstream of PMK-1 and DAF-16, but most *P. aeruginosa*-induced genes do not include those induced by PMK-1 or DAF-16²³. This observation implies that *P. aeruginosa* activates previously unidentified signalling pathways that function in parallel to the PMK-1/p38 MAPK and DAF-2–DAF-16 pathways. Similarly, *S. aureus* response genes are also activated independently of PMK-1 and DAF-16 at the same early stage of infection.²⁴ Moreover, there seems to be minimal overlap, in terms of induction of gene expression, between the early host responses to *S. aureus* and *P. aeruginosa*, indicating that *C. elegans* discriminates between different pathogens and mounts pathogen-specific responses (J. Irazoqui, E. Troemel and F. Ausubel, unpublished observations). Alternatively, the bacterial pathogens may be able to manipulate the host response to their advantage, resulting in differences at the level of transcript abundance of host response genes. Using a different set of pathogens, similar observations were made at both early and late stages of infection (24 hours)^{54, 80}. In addition, a set of genes encoding proteins involved in necrosis is commonly induced at late stages by several different pathogens⁵⁴. These transcriptional profiling data, combined with the genetic data described above, indicate that *C. elegans* has several parallel immune response pathways that activate similar categories of defence response genes, but that there is relatively little overlap among the particular genes activated by each of the pathways. One major focus in the field has been to identify additional *C. elegans* signalling pathways involved in the host response to infection, some of which are outlined below.

β-catenin signalling and host defence

A reverse genetic approach identified the β-catenin gene, *bar-1*, a component of the *C. elegans* canonical Wnt signalling pathway (Box 2), as a critical determinant of susceptibility to *S. aureus*²⁴. BAR-1 (β-catenin) is involved in post-embryonic vulva formation and neuroblast migration, and in oxidative stress resistance, in addition to host defense^{81–83}. EGL-5, a homeobox (HOX) transcription factor, is a downstream mediator of BAR-1, and is also involved in the regulation of the host response to *S. aureus* infection²⁴. Some *S. aureus*-induced genes require BAR-1 and EGL-5 for proper regulation. Others require only BAR-1, and yet others are BAR-1 and EGL-5 independent. These results hint at the existence of additional host defence pathways that are a) BAR-1-dependent but EGL-5-independent, and b) BAR-1-independent²⁴.

Box 2

A role for β-catenin signaling in epithelial immunity?

In human epithelial cells, β-catenin and EGL-5/HOX homologues modulate nuclear factor κB (NF-κB)-dependent transcription downstream of Toll-like receptor (TLR)2 and inhibitor of NF-κB kinase (IKK) activation^{24, 98, 99}, implying that a putative β-catenin-dependent host defence pathway might crosstalk with TLR-dependent NF-κB function^{100–104}. It is incompletely understood how β-catenin is regulated during bacterial infection in human epithelial cells, although reactive oxygen species and Wnt signalling appear to be involved upstream^{105–110}.

Are β-catenin target genes involved in host defence in humans? Human antimicrobial peptides called defensins, produced in the intestinal epithelium, are direct targets of β-catenin signalling^{111, 112}. Reduced defensin expression owing to defective β-catenin-dependent transcription correlates with increased susceptibility to Crohn's disease^{109, 113}. Furthermore, it is possible that β-catenin regulates the expression of human EGL-5-like

homeobox genes that have roles in immunity, as in *C. elegans*. Supporting this view, the human homeobox transcription factors Cdx1 and Cdx2 are transcriptional targets of β -catenin in intestinal epithelia, where they control the expression of antimicrobial factors^{114–117}. The *D. melanogaster* homologue *Caudal* positively drives the constitutive, NF- κ B-independent production of antimicrobial peptides in barrier epithelia such as the salivary glands¹¹⁸. In contrast, *Caudal* also negatively regulates NF- κ B in the fly intestinal epithelium, where it modulates the expression of antimicrobial agents and, thereby, the composition of the microbiota¹¹⁹. Thus, β -catenin and HOX seem to have significant roles in epithelial host defence in worms, flies and humans.

Epistasis analysis has shown that BAR-1 and EGL-5 function in parallel to the PMK-1/p38 MAPK cassette during host defence²⁴. Similar to PMK-1, loss of *bar-1* in *daf-2* mutants causes the *bar-1* susceptibility phenotype; *daf-16* mutation does not affect the host response to *S. aureus*. These observations suggest that BAR-1 functions downstream of, or parallel to, DAF-2 in a DAF-16-independent manner. The upstream signals that regulate BAR-1 function during the host response remain unknown; it is also unclear whether other components of the canonical Wnt signalling cascade are involved in host defence. In this regard, EGL-5 was independently shown to be required for the host rectal epithelial swelling response to infection by *M. nematophilum*; this response might constitute the worm equivalent to tissue inflammation^{84, 85}. EGL-5 functions during normal development of the hindgut, specifying cellular competence to respond to unknown bacterial signals triggering the swelling response mediated by an ERK-like MAPK signaling cascade that involves MPK-1/ERK⁸⁶. It is unknown whether EGL-5 also functions post-developmentally as a component of immune-response pathways⁸⁴. These studies spurred the identification of β -catenin and HOX proteins as important modulators of innate immune signalling downstream of TLR2 signalling in humans (see Box 2)²⁴.

Interestingly, *S. aureus* primarily activates genes regulated by the BAR-1 signalling pathway, whereas *P. aeruginosa* activates genes regulated by the PMK-1/p38 MAPK pathway²⁴. This observation suggests that the BAR-1 pathway might be more important in conferring resistance to *S. aureus* than the PMK-1 pathway and *vice versa* for *P. aeruginosa*. Indeed, *pmk-1* mutants are relatively more susceptible to *P. aeruginosa* than to *S. aureus*, and *bar-1* mutants are relatively more susceptible to *S. aureus* than to *P. aeruginosa*²⁴. These results indicate that pathogen specificity of the host response may be achieved through pathogen-specific signalling pathways in *C. elegans*. These distinct pathways might potentially be regulated by different upstream receptors, and considerable effort is being invested in their identification. Two putative upstream transmembrane receptors that regulate the *C. elegans* response to infection have been identified^{87, 88}.

FSHR-1 as a putative immune receptor

Cell-surface molecules that contain extracellular leucine-rich repeat (LRR) domains are good candidates for immune receptors on the basis that this domain is present in major classes of pathogen receptors in plants and animals (for example, TLRs, NLRs, the plant flagellin receptor FLS2). As discussed above, TOL-1, the single TLR homologue in *C. elegans*, does not have a central role in innate immunity. However, loss of function of FSHR-1, one of 14 candidate transmembrane LRR receptors in *C. elegans*, results in a high degree of susceptibility to killing by *P. aeruginosa*, *S. aureus* and *Enterococcus faecalis*, but not in a reduced lifespan with non-pathogenic *E. coli*⁸⁷. This putative immune receptor is the sole *C. elegans* LGR (LRR-containing G-protein-coupled receptor). Genetic analysis indicates that FSHR-1 functions in the intestine in a separate pathway from PMK-1 and DAF-2⁸⁷. Further, qRT-PCR analysis showed that FSHR-1 and the PMK-1/p38 MAPK cassette regulate the induction of overlapping, but nonidentical, sets of *P. aeruginosa*-induced genes. Thus, FSHR-1 and PMK-1/

p38 MAPK pathways function in parallel to each other but converge on a common set of target effector genes in response to infection by *P. aeruginosa*.

How is FSHR-1 involved in mediating the *C. elegans* host response? We currently lack evidence that FSHR-1 can sense infection directly. The canonical ligand for this class of GPCR in mammals is the heterodimeric glycopeptide hormone follicle-stimulating hormone (FSH) α/β . Worms do not have an identifiable FSH α subunit, and the gene that most closely resembles an FSH β subunit does not seem to have an important role in *C. elegans* immunity⁸⁷. However, it still remains a possibility that FSHR-1 is activated by as-yet-unidentified non-canonical host ligands. Because FSHR-1 has important roles in the development of the nervous system and the germline, it is likely that endogenous ligands exist.^{89, 90}

CED-1 and the unfolded protein response

In their efforts to identify upstream immune receptors in *C. elegans*, Aballay and coworkers found that *ced-1* mutants are hypersusceptible to *S. enterica* infection⁸⁸. These mutants are also hypersusceptible to the fungal pathogen *Candida albicans*⁹¹. CED-1 functions as a receptor during phagocytic uptake of apoptotic cells in *C. elegans*⁹². Microarray analysis of *ced-1* mutants showed that CED-1 functions upstream of presumptive signalling pathways that regulate a non-canonical unfolded protein response (UPR), thereby implicating this response in host defence against *S. enterica*⁸⁸. Consistent with this idea, mutants defective in components of this UPR are hypersusceptible to *S. enterica*⁸⁸. It is possible that the *Salmonella*-derived trigger for CED-1-dependent regulation of the UPR is a pore-forming toxin; these virulence factors are well known to be secreted by bacterial pathogens. In support of this hypothesis, Cry5B, a pore-forming toxin made by *Bacillus thuringiensis* that targets *C. elegans*, triggers a canonical UPR-mediated response that is required for survival⁵⁶. Importantly, the UPR in this case is activated downstream of the PMK-1/p38 MAPK cassette during attack by Cry5B⁵⁶. It remains to be determined whether CED-1 functions upstream of the PMK-1/p38 MAPK cassette during *S. enterica* and Cry5B-mediated *B. thuringiensis* attack to trigger these distinct UPRs. These and other results^{93, 94} suggest that monitoring cell damage or damage-associated molecular patterns⁹⁵ (such as membrane integrity and protein homeostasis) is a key component of *C. elegans* immunity.

Conclusions and perspectives

It is clear that *C. elegans* mounts pathogen-specific host defence responses upon pathogenic attack, as other animals do. In other systems, TLR-mediated control of NF- κ B activity has a central role in this defence; it is therefore surprising to find that *C. elegans* has the capacity to mount specific host responses in the absence of NF- κ B and independently of TLR. How does this happen? Several signalling pathways (some identified, some not) are involved. Presumably, during the evolutionary divergence of nematodes from the basal bilaterian ancestor, the NF- κ B gene was lost owing to functional redundancy with some unknown factor (s). Likewise, although we do not know yet whether TLRs perform immune functions in basal metazoans like the cnidarians, it is possible that additional pathways superseded TLR signalling in the worm as central immune pathways. It is also possible that these additional immune pathways evolved in primitive metazoans such as cnidarians and have been conserved throughout metazoan evolution, including mammalian lineages, acting in concert with TLR pathways. *C. elegans* seems to have several parallel pathways that can carry out such roles; these different pathways might be important for mediating the appropriate host response to distinct pathogens. It is tantalizing to think that by studying *C. elegans*, which does not rely on NF- κ B for innate immunity, it might be possible to identify as-yet-unknown mammalian host defence pathways that function in addition to the canonical TLR pathways.

Further work is needed to elucidate the bacterially-derived signals that trigger morphological and transcriptional host responses to infection in *C. elegans*. Important areas that require further study include extensive characterization of the signalling networks that influence the outcome of infection and host response, and the cell types in which they function. At the whole organism level, different tissues and organ functions are likely to be coordinated during infection through systemic endocrine signals that remain to be fully delineated. Further insight will be gained by precise examination of the actual mechanisms involved in pathogenesis for each pathogen type and infection process. How many of these important answers will apply to mammals as well, and to what extent? What pathways have been conserved, which have been modified, and which have been lost altogether in the splits and radiations from the common eumetazoan ancestor?

Insights gained from the study of host defence strategies can be applied to the design of better therapeutic approaches, which are desperately needed in view of human overpopulation and antibiotic resistance. Conversely, it is imperative that the molecular mechanisms used by pathogens to exploit their hosts be fully understood. The use of model hosts will be instrumental in understanding the molecular functions of virulence factors and their regulation during infection *in vivo*. *C. elegans* provides a means to quickly test hypotheses related to fundamental features of host epithelial cells in the whole organism context, and identify points of weakness that bacteria have evolved to so expertly exploit. The ability to carry out automated high-throughput chemical screens on whole infected nematodes⁹⁶ provides an opportunity to identify new ways to deprive pathogenic bacteria of their highly evolved weapons.

At A Glance

- The nematode *C. elegans* was developed as a model for studying bacterial virulence and innate immunity in 1999. *C. elegans* does not have circulating cells and appears to rely almost exclusively on epithelial immunity to combat pathogen attack. Several parallel immune response pathways have been identified that activate distinct but partially overlapping sets of immune effectors. Despite its simplicity, the *C. elegans* immune response is highly pathogen specific and different pathogens activate distinct immune response pathways.
- Although *C. elegans* has a single Toll-Like-Receptor (TLR), MyD88 and NF- κ B are not encoded in the *C. elegans* genome or in the genomes of other nematode species. Moreover, the single *C. elegans* TLR does not appear to play an important role in the immune response. Because some cnidarians (e.g., the sea anemone *Nematostella vectensis*) have TLRs, MyD88 and NF- κ B, it appears that TLR signaling has been lost in the nematode lineage.
- A highly conserved p38 MAPK signalling cascade plays a central role in the *C. elegans* immune response as it does in mammals. The p38 pathway is required for the activation of a set of immune effectors that are required to maintain a basal level of immune function.
- The p38 MAPK signalling pathway is active during both infection and wounding and functions in at least the intestine, neurons, and epidermis in response to pathogen attack.
- Several highly conserved metazoan signalling pathways play dual roles, functioning as important components of the *C. elegans* immune response. It is of interest to determine whether the same pathways function in immune signalling throughout metazoan evolution, including acting in concert with TLR pathways in mammals.

- In addition to its role in stress resistance, lifespan extension, and metabolic regulation in *C. elegans*, the DAF-2/DAF-16 insulin signalling pathway confers resistance to a wide variety of pathogens when DAF-16 is constitutively activated.
- The *C. elegans* β -catenin homologue BAR-1 and the downstream EGL-5/HOX protein play central roles in activating the *C. elegans* immune response to infection by *Staphylococcus aureus* but not *Pseudomonas aeruginosa*. Roles for β -catenin and HOX in immune signaling in flies and mammals has also been recently demonstrated.
- The G protein coupled receptor FSHR-1 is the first candidate immune receptor to be identified in *C. elegans*.

GLOSSARY

Hermaphrodite	An organism that has both male and female reproductive organs
RNA interference	The silencing of gene expression by the introduction of double-stranded RNA that triggers the specific degradation of a homologous target mRNA, often accompanied by a concomitant decrease in production of the encoded protein
Microarrays	A technique for measuring the transcription of genes. It involves hybridization of fluorescently labelled cDNA prepared from a cell or tissue of interest to glass slides or other surfaces dotted with oligonucleotides or cDNA, representing all genes in the species
Quantitative reverse transcription polymerase chain reaction (qRT-PCR)	A quantitative PCR method that is used to measure relative or absolute mRNA concentrations
Scaffold protein	A protein that functions as a support to assemble a multiprotein complex
Gram-negative pathogens	Bacteria that present as pink-red after exposure to Gram stain. The Gram stain is useful to distinguish faintly staining bacteria (Gram-negative), which possess an extracellular cell wall composed of an outer membrane and a periplasmic space separating it from the plasma membrane. A relatively thin layer of peptidoglycan resides in the periplasmic space and provides structural stability to the cell wall. In contrast, darkly staining bacteria (Gram-positive) lack an outer membrane, but are surrounded by a thick layer of peptidoglycan
Coelomates	Animals that possess an internal body cavity derived from the mesoderm
Epistasis	An interaction between non-allelic genes, such that one gene masks, interferes with or enhances the expression of the other gene
Reverse genetic approach	A genetic approach that proceeds from genotype to phenotype by gene-manipulation techniques, such as homologous recombination in embryonic stem cells and RNA interference

Homeobox genes	Genes that contain a 180-base-pair sequence coding for the homeodomain and are involved in the regulation of animal and plant development. This sequence encodes a DNA-binding helix–turn–helix motif (the homeodomain), indicating that homeobox-containing gene products function as transcription factors
Leucine-rich repeat (LRR) domains	Domains that contain LRRs have a conserved solenoid structure, typically of 20–29 residues and containing an 11 amino-acid consensus sequence, LXXLXLXX(N/C)XL, where X denotes any amino acid. These domains lack considerable identity or similarity in the amino acids surrounding this structure, both between and among families. Sequence substitutions in LRR-containing proteins are associated with changes in specificity and relative affinity towards LRR domain binding partners
Neuropeptide-like peptides	Family of short peptides, with sequence homology to YGGWamide neuropeptides, which can be induced during infection
Caenacins	Family of peptides related to neuropeptide-like peptides, which can be induced during infection
Apical junctions	Junctional multiprotein complexes localized to the apical junctions of intestinal epithelial cells in <i>C. elegans</i> . The <i>C. elegans</i> apical junctions can be viewed as functionally equivalent to the cell junctions from <i>Drosophila</i> epithelia and the tight junctions, zonula adherens and desmosomes from vertebrate epithelia

Bibliography

1. Hemmrich G, Miller DJ, Bosch TCG. The evolution of immunity: a low-life perspective. *Trends Immunol* 2007;28:449–54. [PubMed: 17855167]
2. Salzet M. Vertebrate innate immunity resembles a mosaic of invertebrate immune responses. *Trends Immunol* 2001;22:285–8. [PubMed: 11377277]
3. Waterfield NR, Wren BW, Ffrench-Constant RH. Invertebrates as a source of emerging human pathogens. *Nat Rev Micro* 2004;2:833–41.
4. Adams B, et al. Biodiversity and systematics of nematode-bacterium entomopathogens. *Biol Control* 2006;37:32–49.
5. Lee DG, et al. Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. *Genome Biol* 2006;7:R90. [PubMed: 17038190]
6. Pedersen AL, Nybroe O, Winding A, Ekelund F, Bjørnlund L. Bacterial Feeders, the Nematode *Caenorhabditis elegans* and the Flagellate *Cercomonas longicauda*, have different Effects on Outcome of Competition among the *Pseudomonas* Biocontrol Strains CHA0 and DSS73. *Microb Ecol* 2009;57:501–9. [PubMed: 18975025]
7. Hilbi H, Weber SS, Ragaz C, Nyfeler Y, Urwyler S. Environmental predators as models for bacterial pathogenesis. *Environ Microbiol* 2007;9:563–75. [PubMed: 17298357]
8. Ruvkun G, Hobert O. The taxonomy of developmental control in *Caenorhabditis elegans*. *Science* 1998;282:2033–41. [PubMed: 9851920]
9. Schulenburg H, Hoepfner MP, Weiner J, Bornberg-Bauer E. Specificity of the innate immune system and diversity of C-type lectin domain (CTLN) proteins in the nematode *Caenorhabditis elegans*. *Immunobiology* 2008;213:237–50. [PubMed: 18406370]
10. Zhang X, Zhang Y. Neural-Immune Communication in *Caenorhabditis elegans*. *Cell Host & Microbe* 2009;5:425–9. [PubMed: 19454346]
11. Shivers RP, Youngman MJ, Kim DH. Transcriptional responses to pathogens in *Caenorhabditis elegans*. *Curr Opin Microbiol* 2008;11:251–6. [PubMed: 18567532]

12. Brenner S. In the beginning was the worm. *Genetics* 2009;182:413–5. [PubMed: 19506024]
13. Powell JR, Ausubel FM. Models of *Caenorhabditis elegans* infection by bacterial and fungal pathogens. *Methods Mol Biol* 2008;415:403–27. [PubMed: 18370168]
14. Aballay A, Ausubel FM. *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. *Current Opinion in Microbiology* 2002;5:97–101. [PubMed: 11834377]
15. Couillault C, Ewbank JJ. Diverse bacteria are pathogens of *Caenorhabditis elegans*. *Infect Immun* 2002;70:4705–7. [PubMed: 12117988]
16. Kurz CL, Ewbank JJ. *Caenorhabditis elegans* for the study of host-pathogen interactions. *Trends in Microbiology* 2000;8:142–4. [PubMed: 10707068]
17. Sifri CD, Begun J, Ausubel FM. The worm has turned--microbial virulence modeled in *Caenorhabditis elegans*. *Trends in Microbiology* 2005;13:119–27. [PubMed: 15737730]
18. Tan MW, Mahajan-Miklos S, Ausubel FM. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci USA* 1999;96:715–20. [PubMed: 9892699]
19. Hodgkin J, Kuwabara PE, Corneliussen B. A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. *Curr Biol* 2000;10:1615–8. [PubMed: 11137017]
20. Pujol N, et al. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr Biol* 2001;11:809–21. [PubMed: 11516642]
21. Zhang Y, Lu H, Bargmann CI. Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* 2005;438:179–84. [PubMed: 16281027]
22. Aballay A, Yorgey P, Ausubel FM. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr Biol* 2000;10:1539–42. [PubMed: 11114525]
23. Troemel ER, et al. p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* 2006;2:e183. [PubMed: 17096597]
24. Irazoqui JE, Ng A, Xavier RJ, Ausubel FM. Role for beta-catenin and HOX transcription factors in *Caenorhabditis elegans* and mammalian host epithelial-pathogen interactions. *Proc Natl Acad Sci U S A* 2008;105:17469–74. [PubMed: 18981407]
25. Tan MW, Rahme LG, Sternberg JA, Tompkins RG, Ausubel FM. *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proc Natl Acad Sci USA* 1999;96:2408–13. [PubMed: 10051655]
26. Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell* 1999;96:47–56. [PubMed: 9989496]
27. Darby C, Cosma CL, Thomas JH, Manoil C. Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 1999;96:15202–7. [PubMed: 10611362]
28. DeNardo DG, Johansson M, Coussens LM. Inflaming gastrointestinal oncogenic programming. *Cancer Cell* 2008;14:7–9. [PubMed: 18598939]
29. Karin M. The I κ B kinase - a bridge between inflammation and cancer. *Cell Res* 2008;18:334–42. [PubMed: 18301380]
30. Ferrandon D, Imler JL, Hetru C, Hoffmann JA. The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. *Nat Rev Immunol* 2007;7:862–74. [PubMed: 17948019]
31. Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the *Drosophila* embryo: genetic studies on the role of the Toll gene product. *Cell* 1985;42:779–89. [PubMed: 3931918]
32. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783–801. [PubMed: 16497588]
33. Vallabhapurapu S, Karin M. Regulation and function of NF- κ B transcription factors in the immune system. *Annu Rev Immunol* 2009;27:693–733. [PubMed: 19302050]
34. Gottar M, et al. The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. *Nature* 2002;416:640–4. [PubMed: 11912488]

35. Carty M, et al. The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat Immunol* 2006;7:1074–81. [PubMed: 16964262]
36. Liberati NT, et al. Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *Proc Natl Acad Sci USA* 2004;101:6593–8. [PubMed: 15123841]
37. Couillault C, et al. TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat Immunol* 2004;5:488–94. [PubMed: 15048112]
38. Shivers RP, Kooistra T, Chu SW, Pagano DJ, Kim DH. Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in *C. elegans*. *Cell Host & Microbe* 2009;6:321–30. [PubMed: 19837372]
39. Zugasti O, Ewbank JJ. Neuroimmune regulation of antimicrobial peptide expression by a noncanonical TGF-beta signaling pathway in *Caenorhabditis elegans* epidermis. *Nat Immunol* 2009;10:249–56. [PubMed: 19198592]
40. Tenor JL, Aballay A. A conserved Toll-like receptor is required for *Caenorhabditis elegans* innate immunity. *EMBO Rep* 2008;9:103–9. [PubMed: 17975555]
41. Zhong J, et al. GCK is essential to systemic inflammation and pattern recognition receptor signaling to JNK and p38. *Proc Natl Acad Sci USA* 2009;106:4372–7. [PubMed: 19246396]
42. Dong C, Davis RJ, Flavell RA. MAP KINASES IN THE IMMUNE RESPONSE. *Annu Rev Immunol* 2002;20:55–72. [PubMed: 11861597]
43. Lu HT, et al. Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (Mkk3)-deficient mice. *EMBO J* 1999;18:1845–57. [PubMed: 10202148]
44. Tobiume K, et al. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO Rep* 2001;2:222–8. [PubMed: 11266364]
45. Kim DH, et al. A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* 2002;297:623–6. [PubMed: 12142542]
46. Kim DH, et al. 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* 2004;101:10990–4. [PubMed: 15256594]
47. Mizuno T, et al. The *Caenorhabditis elegans* MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J* 2004;23:2226–34. [PubMed: 15116070]
48. Takeda K, Noguchi T, Naguro I, Ichijo H. Apoptosis signal-regulating kinase 1 in stress and immune response. *Annu Rev Pharmacol Toxicol* 2008;48:199–225. [PubMed: 17883330]
49. Aballay A, Ausubel FM. Programmed cell death mediated by ced-3 and ced-4 protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proc Natl Acad Sci USA* 2001;98:2735–9. [PubMed: 11226309]
50. Kim DH, Ausubel FM. Evolutionary perspectives on innate immunity from the study of *Caenorhabditis elegans*. *Current Opinion in Immunology* 2005;17:4–10. [PubMed: 15653303]
51. Putnam NH, et al. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* 2007;317:86–94. [PubMed: 17615350]
52. Miller DJ, et al. The innate immune repertoire in cnidaria--ancestral complexity and stochastic gene loss. *Genome Biol* 2007;8:R59. [PubMed: 17437634]
53. Abad P, et al. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nat Biotechnol* 2008;26:909–15. [PubMed: 18660804]
54. Wong D, Bazopoulou D, Pujol N, Tavernarakis N, Ewbank JJ. Genome-wide investigation reveals pathogen-specific and shared signatures in the response of *Caenorhabditis elegans* to infection. *Genome Biol* 2007;8:R194. [PubMed: 17875205]
55. Chen RE, Thorner J. Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 2007;1773:1311–40. [PubMed: 17604854]
56. Bischof LJ, et al. Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. *PLoS Pathog* 2008;4:e1000176. [PubMed: 18846208]
57. Huffman DL, et al. Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. *Proc Natl Acad Sci USA* 2004;101:10995–1000. [PubMed: 15256590]

58. Huffman DL, Bischof LJ, Griffiths JS, Aroian RV. Pore worms: using *Caenorhabditis elegans* to study how bacterial toxins interact with their target host. *Int J Med Microbiol* 2004;293:599–607. [PubMed: 15149037]
59. Gal-Mor O, Finlay BB. Pathogenicity islands: a molecular toolbox for bacterial virulence. *Cell Microbiol* 2006;8:1707–19. [PubMed: 16939533]
60. Jansson HB. Adhesion of *Conidia* of *Drechmeria coniospora* to *Caenorhabditis elegans* Wild Type and Mutants. *J Nematol* 1994;26:430–5. [PubMed: 19279912]
61. Pujol N, et al. Distinct Innate Immune Responses to Infection and Wounding in the *C. elegans* Epidermis. *Curr Biol* 2008;18:481–489. [PubMed: 18394898]
62. Ren M, Feng H, Fu Y, Land M, Rubin CS. Protein kinase D is an essential regulator of *C. elegans* innate immunity. *Immunity* 2009;30:521–32. [PubMed: 19371715]
63. Shirai Y, Saito N. Activation mechanisms of protein kinase C: maturation, catalytic activation, and targeting. *Journal of Biochemistry* 2002;132:663–8. [PubMed: 12417013]
64. Ziegler K, et al. Antifungal innate immunity in *C. elegans*: PKCdelta links G protein signaling and a conserved p38 MAPK cascade. *Cell Host & Microbe* 2009;5:341–52. [PubMed: 19380113]
65. Pujol N, et al. Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. *PLoS Pathog* 2008;4:e1000105. [PubMed: 18636113]
66. Tong A, et al. Negative regulation of *Caenorhabditis elegans* epidermal damage responses by death-associated protein kinase. *Proc Natl Acad Sci U S A* 2009;106:1457–61. [PubMed: 19164535]
67. Zeuthen LH, Fink LN, Frokiaer H. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. *Immunology* 2008;123:197–208. [PubMed: 17655740]
68. Wahl SM. Transforming growth factor-beta: innately bipolar. *Current Opinion in Immunology* 2007;19:55–62. [PubMed: 17137775]
69. Wolkow CA, Kimura KD, Lee MS, Ruvkun G. Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 2000;290:147–50. [PubMed: 11021802]
70. Garsin DA, et al. Long-lived *C. elegans* *daf-2* mutants are resistant to bacterial pathogens. *Science* 2003;300:1921. [PubMed: 12817143]
71. Antebi A. Genetics of aging in *Caenorhabditis elegans*. *PLoS Genet* 2007;3:1565–71. [PubMed: 17907808]
72. Lee SS, Kennedy S, Tolonen AC, Ruvkun G. DAF-16 target genes that control *C. elegans* life-span and metabolism. *Science* 2003;300:644–7. [PubMed: 12690206]
73. Murphy CT, et al. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 2003;424:277–83. [PubMed: 12845331]
74. Miyata S, Begun J, Troemel ER, Ausubel FM. DAF-16-dependent suppression of immunity during reproduction in *Caenorhabditis elegans*. *Genetics* 2008;178:903–18. [PubMed: 18245330]
75. Evans EA, Chen WC, Tan MW. The DAF-2 insulin-like signaling pathway independently regulates aging and immunity in *C. elegans*. *Aging Cell* 2008;7:879–93. [PubMed: 18782349]
76. McElwee JJ, Schuster E, Blanc E, Thomas JH, Gems D. Shared transcriptional signature in *Caenorhabditis elegans* Dauer larvae and long-lived *daf-2* mutants implicates detoxification system in longevity assurance. *J Biol Chem* 2004;279:44533–43. [PubMed: 15308663]
77. Evans EA, Kawli T, Tan MW. *Pseudomonas aeruginosa* Suppresses Host Immunity by Activating the DAF-2 Insulin-Like Signaling Pathway in *Caenorhabditis elegans*. *PLoS Pathog* 2008;4:e1000175. [PubMed: 18927620]
78. Anyanful A, Easley KA, Benian GM, Kalman D. Conditioning Protects *C. elegans* from Lethal Effects of Enteropathogenic *E. coli* by Activating Genes that Regulate Lifespan and Innate Immunity. *Cell Host & Microbe* 2009;5:450–62. [PubMed: 19454349]
79. Shapira M, et al. A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc Natl Acad Sci USA* 2006;103:14086–91. [PubMed: 16968778]
80. O'rouke D, Baban D, Demidova M, Mott R, Hodgkin J. Genomic clusters, putative pathogen recognition molecules, and antimicrobial genes are induced by infection of *C. elegans* with *M. nematophilum*. *Genome Research* 2006;16:1005–16. [PubMed: 16809667]

81. Eisenmann, DM. WormBook. 2005. Wnt signaling; p. 1-17.
82. Eisenmann DM, Kim SK. Protruding vulva mutants identify novel loci and Wnt signaling factors that function during *Caenorhabditis elegans* vulva development. *Genetics* 2000;156:1097–116. [PubMed: 11063687]
83. Eisenmann DM, Maloof JN, Simske JS, Kenyon C, Kim SK. The beta-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* 1998;125:3667–80. [PubMed: 9716532]
84. Nicholas HR, Hodgkin J. The *C. elegans* Hox gene *egl-5* is required for correct development of the hermaphrodite hindgut and for the response to rectal infection by *Microbacterium nematophilum*. *Dev Biol* 2009;329:16–24. [PubMed: 19232338]
85. Gravato-Nobre MJ, et al. Multiple genes affect sensitivity of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. *Genetics* 2005;171:1033–45. [PubMed: 16079230]
86. Nicholas HR, Hodgkin J. The ERK MAP kinase cascade mediates tail swelling and a protective response to rectal infection in *C. elegans*. *Curr Biol* 2004;14:1256–61. [PubMed: 15268855]
87. Powell JR, Kim DH, Ausubel FM. The G protein-coupled receptor FSHR-1 is required for the *Caenorhabditis elegans* innate immune response. *Proc Natl Acad Sci U S A* 2009;106:2782–7. [PubMed: 19196974]
88. Haskins KA, Russell JF, Gaddis N, Dressman HK, Aballay A. Unfolded protein response genes regulated by CED-1 are required for *Caenorhabditis elegans* innate immunity. *Developmental Cell* 2008;15:87–97. [PubMed: 18606143]
89. Sieburth D, et al. Systematic analysis of genes required for synapse structure and function. *Nature* 2005;436:510–7. [PubMed: 16049479]
90. Cho S, Rogers KW, Fay DS. The *C. elegans* glycopeptide hormone receptor ortholog, FSHR-1, regulates germline differentiation and survival. *Curr Biol* 2007;17:203–12. [PubMed: 17276913]
91. Means TK, et al. Evolutionarily conserved recognition and innate immunity to fungal pathogens by the scavenger receptors SCARF1 and CD36. *The Journal of Experimental Medicine* 2009;206:637–53. [PubMed: 19237602]
92. Chung S, Gumienny TL, Hengartner MO, Driscoll M. A common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in *C. elegans*. *Nat Cell Biol* 2000;2:931–7. [PubMed: 11146658]
93. Mohri-Shiomi A, Garsin DA. Insulin signaling and the heat shock response modulate protein homeostasis in the *Caenorhabditis elegans* intestine during infection. *J Biol Chem* 2008;283:194–201. [PubMed: 17951251]
94. Singh V, Aballay A. Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. *Proc Natl Acad Sci USA* 2006;103:13092–7. [PubMed: 16916933]
95. Medzhitov R. Approaching the asymptote: 20 years later. *Immunity* 2009;30:766–75. [PubMed: 19538928]
96. Moy TI, et al. High-throughput screen for novel antimicrobials using a whole animal infection model. *ACS Chem Biol* 2009;4:527–33. [PubMed: 19572548]
97. Boutros M, Ahringer J. The art and design of genetic screens: RNA interference. *Nat Rev Genet* 2008;9:554–66. [PubMed: 18521077]
98. Trivedi CM, Patel RC, Patel CV. Differential regulation of HOXA9 expression by nuclear factor kappa B (NF-kappaB) and HOXA9. *Gene* 2008;408:187–95. [PubMed: 18068911]
99. Trivedi CM, Patel RC, Patel CV. Homeobox gene HOXA9 inhibits nuclear factor-kappa B dependent activation of endothelium. *Atherosclerosis* 2007;195:e50–60. [PubMed: 17586512]
100. Sun J, et al. Crosstalk between NF-kappaB and beta-catenin pathways in bacterial-colonized intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2005;289:G129–37. [PubMed: 15790758]
101. Sun J, Hobert ME, Rao AS, Neish AS, Madara JL. Bacterial activation of beta-catenin signaling in human epithelia. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G220–7. [PubMed: 14764450]
102. Franco AT, et al. Activation of beta-catenin by carcinogenic *Helicobacter pylori*. *Proc Natl Acad Sci USA* 2005;102:10646–51. [PubMed: 16027366]

103. Ye Z, Petrof EO, Boone D, Claud EC, Sun J. Salmonella effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination. *Am J Pathol* 2007;171:882–92. [PubMed: 17690189]
104. Deng J, et al. Crossregulation of NF-kappaB by the APC/GSK-3beta/beta-catenin pathway. *Mol Carcinog* 2004;39:139–46. [PubMed: 14991743]
105. Pereira C, Schaer DJ, Bachli EB, Kurrer MO, Schoedon G. Wnt5A/CaMKII signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. *Arterioscler Thromb Vasc Biol* 2008;28:504–10. [PubMed: 18174455]
106. Kumar A, et al. Commensal bacteria modulate cullin-dependent signaling via generation of reactive oxygen species. *EMBO J* 2007;26:4457–66. [PubMed: 17914462]
107. Blumenthal A, et al. The Wingless homolog WNT5A and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood* 2006;108:965–73. [PubMed: 16601243]
108. Gregorieff A, et al. Expression pattern of Wnt signaling components in the adult intestine. *Gastroenterology* 2005;129:626–38. [PubMed: 16083717]
109. Koslowski MJ, et al. Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) putative promoter region are associated with small intestinal Crohn's disease. *PLoS ONE* 2009;4:e4496. [PubMed: 19221600]
110. Wehkamp J, et al. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007;179:3109–18. [PubMed: 17709525]
111. van Es JH, et al. Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat Cell Biol* 2005;7:381–6. [PubMed: 15778706]
112. Andreu P, et al. Crypt-restricted proliferation and commitment to the Paneth cell lineage following Apc loss in the mouse intestine. *Development* 2005;132:1443–51. [PubMed: 15716339]
113. Simms LA, et al. Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. *Gut* 2008;57:903–10. [PubMed: 18305068]
114. Lengerke C, et al. BMP and Wnt specify hematopoietic fate by activation of the Cdx-Hox pathway. *Cell stem cell* 2008;2:72–82. [PubMed: 18371423]
115. Lickert H, et al. Wnt/(beta)-catenin signaling regulates the expression of the homeobox gene Cdx1 in embryonic intestine. *Development* 2000;127:3805–13. [PubMed: 10934025]
116. Benahmed F, et al. Multiple regulatory regions control the complex expression pattern of the mouse Cdx2 homeobox gene. *Gastroenterology* 2008;135:1238–1247. 1247.e1–3. [PubMed: 18655789]
117. Wang ML, et al. Regulation of RELM/FIZZ isoform expression by Cdx2 in response to innate and adaptive immune stimulation in the intestine. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G1074–83. [PubMed: 15576623]
118. Ryu JH, et al. The homeobox gene Caudal regulates constitutive local expression of antimicrobial peptide genes in Drosophila epithelia. *Mol Cell Biol* 2004;24:172–85. [PubMed: 14673153]
119. Ryu JH, et al. Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. *Science* 2008;319:777–82. [PubMed: 18218863]

Biographies

Javier E. Irazoqui. Javier E. Irazoqui received his Ph.D. in 2003 from Duke University, North Carolina, USA (for research carried out with D. J. Lew). After his postdoctoral training with F. M. Ausubel at the Department of Molecular Biology, Massachusetts General Hospital, USA, he became an Assistant Professor at the Program in Developmental Immunology, Department of Pediatrics, Massachusetts General Hospital. His research focuses on studying the function of the BAR-1/EGL-5 pathway in host defense and the pathogenesis of *S. aureus* infection.

Jonathan Urbach. Jonathan Urbach received his Ph.D. at Harvard Medical School in the laboratory of Jack W. Szostak where his work focused on in vitro selection of functional RNA molecules. Since 2000, he has worked as a bioinformatician in the Department of Molecular

Biology at Massachusetts General Hospital. Current research interests include the pathogen *Pseudomonas aeruginosa*, and the evolution of NBS-LRR and NLR innate immunity receptor proteins.

Frederick M. Ausubel. Fred Ausubel has been Professor of Genetics at Harvard Medical School and a member of the Department of Molecular Biology at Massachusetts General Hospital since 1982. His research focuses on host-microbe interactions and innate immunity, using the model genetic hosts *Caenorhabditis elegans* and *Arabidopsis thaliana*.

Figure 1. Schematic representation of the *C. elegans* intestine

The *C. elegans* intestine is composed of 20 intestinal epithelial cells. These cells are organized in 9 rings: ring 1 contains four cells and rings 2–9 contain two cells each. The apical surface of each of the intestinal epithelial cells forms the microvillar brush border and faces the intestinal lumen. The intestinal epithelium is the major interface of interaction between *C. elegans* and ingested microbes.

Figure 2. Parts of mammalian TLR signalling are conserved in *C. elegans*

a | model of Toll-like receptor (TLR)-dependent signalling. Engagement of TLRs by cognate ligands such as lipopolysaccharide (LPS) trigger the recruitment of scaffolding proteins, such as MyD88. MyD88 and interleukin-1 receptor-associated kinase (IRAK) recruit and activate the scaffold tumor necrosis factor receptor-associated factor (TRAF)6, which, in turn, activates transforming growth factor- β (TGF β)-activated kinase 1 (TAK1). TAK1 activates inhibitor of nuclear factor κ B (NF- κ B) kinase (IKK β / γ), which phosphorylates inhibitor of NF- κ B (I κ B) resulting its degradation, thus releasing NF- κ B from inhibition. Free NF- κ B translocates into the nucleus and drives the transcription of inflammatory host responses, including cytokines and defensins. TAK1 also activates the p38 mitogen-activated protein kinase (MAPK) cascade, which comprises ASK-1/MAPK kinase kinase (MAPKKK), MKK3/6/4/7/MAPKK, and p38 and Jun N-terminal kinase (JNK). p38 and JNK target the activator protein-1 (AP-1) transcription factor to drive the transcription of host response genes. **b** | *C. elegans* has homologues for some components of TLR signalling, but notably lacks MyD88, IKK β / γ and NF- κ B homologues. MOM-4 (TAK1) functions during embryonic development in non-canonical Wnt signalling. The NSY-1–SEK-1–PMK-1 cassette is a central regulator of host defence, and depends on the upstream scaffold TIR-1 for activity. In mammals, the TIR-1 homologue sterile α - and armadillo-motif-containing protein (SARM) negatively regulates TLR signalling, showing how the same protein can have opposite roles in different organisms.

Figure 3. Components of Toll-like receptor signalling in bilaterians and cnidarians

Cnidarians and bilaterians diverged from the common eumetazoan ancestor (**E**) ~600–630 million years ago (2). One cnidarian branch, represented by *N. vectensis*, conserves nuclear factor κ B (NF- κ B), whereas a second branch, represented by *H. magnipapillata*, does not. Deuterostomes (including vertebrates) and protostomes (including nematodes and arthropods) diverged from the common bilaterian ancestor (**B**) ~570–582 million years ago.^{91, 92} Because *D. melanogaster* and *N. vectensis* both possess NF- κ B and MyD88, the most likely scenario is the loss of NF- κ B and MyD88 from the nematode lineage. The presence or absence of other components of Toll-like receptor (TLR) signalling, including sterile α - and armadillo-motif-containing protein (SARM), inhibitor of NF- κ B (I κ B) and p38 mitogen-activated protein kinase (MPAK), is indicated.

Figure 4. Parallel signalling pathways in the induction of *C. elegans* host defence

The transforming growth factor (TGF)- β homologue DBL-1 signals to SMA-3 in the epidermis through the heterodimeric receptor DAF-4–SMA-6 for the induction of caenacin (CNC) genes during *D. coniospora* infection. Unknown upstream signals induce the transcription of neuropeptide-like peptide (NLP) genes in the epidermis through a signalling cascade involving the heterotrimeric G-protein subunits GPA-12 and RACK-1, the phospholipase C proteins PLC-3 and EGL-8, the protein kinase C proteins TPA-1 and PKC-3 and PMK-1 during *D. coniospora* infection. During wounding, death-associated protein kinase (DAPK-1) functions as an upstream negative regulator of the PMK-1 cassette in the epidermis; however, the exact point of input is unknown. In the intestine, TPA-1 and DKF-2 (protein kinase D) activate the PMK-1 cassette for the induction of host defence genes during infection by *P. aeruginosa*. The MAPKK MEK-1 increases PMK-1 phosphorylation. The phosphatase VHP-1 downregulates PMK-1 by dephosphorylation. Insulin (such as INS-7, which is upregulated during *P. aeruginosa* infection) activates the insulin receptor DAF-2, which sequentially activates AGE-1 (phosphoinositide 3-kinase), PDK-1 (phosphoinositide-dependent kinase-1), AKT-1, AKT-2, and SGK-1 (protein kinase B) to phosphorylate, and thereby inhibit, the FOXO transcription factor DAF-16.