# *CAENORHABDITIS ELEGANS*: AN EMERGING GENETIC MODEL FOR THE STUDY OF INNATE IMMUNITY

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Invaluable insights into how animals, humans included, defend themselves against infection have been provided by more than a decade of genetic studies that have used fruitflies. In the past few years, attention has also turned to another simple animal model, the nematode worm *Caenorhabditis elegans*. What exactly have we learned from the work in *Drosophila*? And will research with *C. elegans* teach us anything new about our response to pathogen attack?

MACROPHAGE
A specialized type of white blood cell that can engulf foreign particles and microorganisms.

GRAM NEGATIVE
Bacteria that cannot be coloured with Gram's stain and generally have an lipopolysaccharide-containing outer membrane.

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In contemporary language, pathogens represent a real and present danger. Although both plants and animals are protected by physical barriers that block the entry of many potential pathogens, they have to be able to recognize when microorganisms have breached these barriers, and to respond rapidly to infection by deploying a range of defensive strategies. These functions are assumed by the innate immune system, which identifies, contains and kills invading pathogens.

By the late nineteenth and early twentieth century, important advances had been made in the study of innate immunity in invertebrates; for example, it had been shown that insects have MACROPHAGE-like cells that are able to engulf microorganisms, and that they also produce a range of antimicrobial substances (reviewed in REF. 1). Subsequently, however, these seemingly primitive mechanisms were largely neglected for many years. This was partly owing to the discovery that mammals, as well as having an innate immune system, possess an adaptive immune system that is characterized by an exquisite specificity. In contrast to innate immunity, which represents a first line of defence against infection, the adaptive immune system responds slowly to a new pathogen; however, it has a 'memory' that allows the host to resist a second infection by the same pathogen more effectively, which opened the possibility of vaccination against certain pathogens — as had been shown as early as 1796 by Jenner. So, immunologists tended to focus on the adaptive defence mechanisms of mice and men, with their multiple specialized cell types and enormous repertoires of antibodies that can discriminate 'self' molecules from potentially pathogenic 'non-self'. It was only comparatively recently that attention was again turned to invertebrate immunity. This was, in part, a result of the realization that, even in vertebrates, the innate immune mechanisms are extremely important — they can often successfully block infections at an early stage, and if not, they influence the subsequent adaptive immune response[2,3].

The important role of the mammalian innate immune response in the reaction of the host against pathogens is underscored by the deleterious consequences of several different mutations that impinge on its proper functions[4,5]. Mice that are mutant at the *Bcg/Ity/Lsh* locus, which encodes the natural-resistance-associated macrophage protein (Nramp) — renamed as solute carrier family 11a member 1 (Slc11a1) — are as much as 1,000 times more susceptible to infection by a diverse range of pathogens, including *Mycobacteria*, *Salmonella* and *Leishmania*, than are wild-type mice. Similarly, *Lps*-mutant mice that are resistant to the toxic effects of bacterial lipopolysaccharide (LPS), are hypersensitive to GRAM-NEGATIVE bacteria in general. It was 15 years after a description of the *Lsh* mutant was published that the corresponding gene was cloned[6], and for *Lps*, 20 years elapsed between it being linked to a chromosome and being cloned[7]. Although mouse models have an important role in studies of innate immunity[5],

these examples illustrate one of the drawbacks of studying innate immunity in mice. With the advent of the complete mouse genome sequence and the development of better mapping approaches, the situation has improved to such an extent that forward genetic screens for immunologically relevant traits have been undertaken using mice (see for example REFS 8,9), although these approaches still require a substantial investment of time and effort.

We know that vital cellular and organismal functions are based on molecular mechanisms that have been remarkably conserved across hundreds of millions of years of evolution. Indeed, given the findings of the past five years, it is now clear that the study of innate immunity in invertebrates can aid our understanding of how mammals defend themselves against infection[10–12]. There is, therefore, every reason to study innate immunity in species that are amenable to genetic analysis. Several important insights into conserved aspects of innate immunity have been derived from research on antimicrobal defence in a diverse range of invertebrate species, including mussels, shrimps, moths and horseshoe crabs, often through biochemical approaches (see, for example, REFS 13,14). However, this review concentrates mainly on research that uses the nematode worm *Caenorhabditis elegans* and presents it in the context of more established studies of *Drosophila melanogaster*. Although it has been known for more than 30 years that *D. melanogaster* has an inducible immune system, until as recently as 1999 there had been no published reports on *C. elegans* defences against pathogens. Several different approaches are now being used to answer the question of how *C. elegans* responds to infection. These studies have been motivated by the conviction that, despite its simplicity, *C. elegans* must possess an innate immune system that, at least in part, resembles that of higher organisms. Also, although *D. melanogaster* is a good model, it is far from certain that it will be the best system for studying all aspects of host defences. Finally, the worm possesses practical attributes such as short lifespan and self fertilization that, for example, facilitate high throughput investigations and allow a two-sided approach to the study of host–pathogen interactions[15–17].

## Lessons from *Drosophila*

One starting point for the investigation of innate immunity in *C. elegans* has been to look for orthologues of genes that are known to function in host defence in *Drosophila*. This is relatively straightforward as the *C. elegans* genome is now essentially completely sequenced (A. Coulson, personal communication) and well annotated (see WormBase in online links box). If nematodes and insects are more closely related to each other than either is to humans[18] — which is still debated[19] — the results seem surprising. In both *Drosophila*[20] and vertebrates[21], nitric oxide contributes to the induction of innate immune responses. However, *C. elegans* lacks a homologue of the inducible synthase that is necessary for the production of nitric oxide. In *Drosophila*, wounding leads to the activation of prophenoloxidases and the production of a plug of melanin at the wound site. This arises through the action of a proteolytic cascade that is similar to those that are involved in blood-clotting reactions in other arthropods[22,23]. But the genes that are necessary for mounting such a response are absent from the worm genome[16]. In *Drosophila*, the relevant proteolytic cascade is under the negative control of Serpin27A — a member of a family of protease inhibitors. The level of Serpin27A is regulated by the Toll pathway[24,25] (FIG. 1), which, in turn, is negatively controlled by a serpin[26]. There are 9 serpin genes in *C. elegans*, compared to 30 in *Drosophila*, but it is unclear at present whether any or all of them have a role in host defences (G. Silverman, personal communication).

*The Toll pathway.* The Toll pathway was first described in the context of the specification of dorsoventral polarity in the *Drosophila* embryo (reviewed in REF. 27). Elements of this signalling cascade also contribute to the innate immune responses of the fly (FIG. 1). The best-characterized function of the Toll pathway in *Drosophila* innate immunity is its control of the production of antimicrobial peptides (AMPs) that are secreted into the haemolymph — the fly equivalent of blood. The pathway is triggered by infection with fungi or Gram-positive bacteria and results in upregulation of the expression of the specific AMP genes — including *drosomycin* and *cecropin A1* — through the action of Rel/nuclear factor κB (NF-κB)-like transcription factors (FIG. 1A). Various mutants in the Toll pathway have reduced levels of AMP expression after infection by fungi or Gram-positive bacteria, and are killed more rapidly by these pathogens[28,29]. As illustrated in FIG. 1A, analogous signalling pathways that involve a family of Toll-like receptors (TLRs) in mammals are responsible for the innate response to diverse pathogens (reviewed in REF. 30). In contrast to Toll, the TLRs are believed to function directly in the specific recognition of so-called pathogen-associated molecular patterns (PAMPs). These are invariant components of microorganisms, such as the LPS of Gram-negative bacteria. In mammals, most of the TLRs have been assigned a function in defence against infection[30]. Although the *Drosophila* genome encodes eight extra Toll-family members, it is unclear whether any of them have a role in innate immune signalling[31,32]. For example, although one of these TLRs —18-wheeler — had been proposed to be important for the antibacterial response of *Drosophila*, this now seems not to be the case[33].

Surprisingly, the Toll pathway seems not to be conserved in *C. elegans*. Although sequence comparisons show that the worm possesses homologues of certain component of the Toll/TLR pathways, there are some important absences in a putative nematode TLR cascade. Most strikingly, there is no obvious NF-κB homologue[34] (FIG. 1A). As it is relatively simple to knock out genes in *C. elegans* using random mutagenesis followed by a polymerase chain reaction (PCR) screen[35,36] (see Michael R. Koelle's laboratory in online links box), mutants for several genes of a putative Toll/TLR pathway have been generated[34]. In contrast to *Toll* in *Drosophila* — or, for example, *Tlr4* in mice[7] — the single *C. elegans* TLR gene *tol-1*, has not yet been shown to have a direct role in

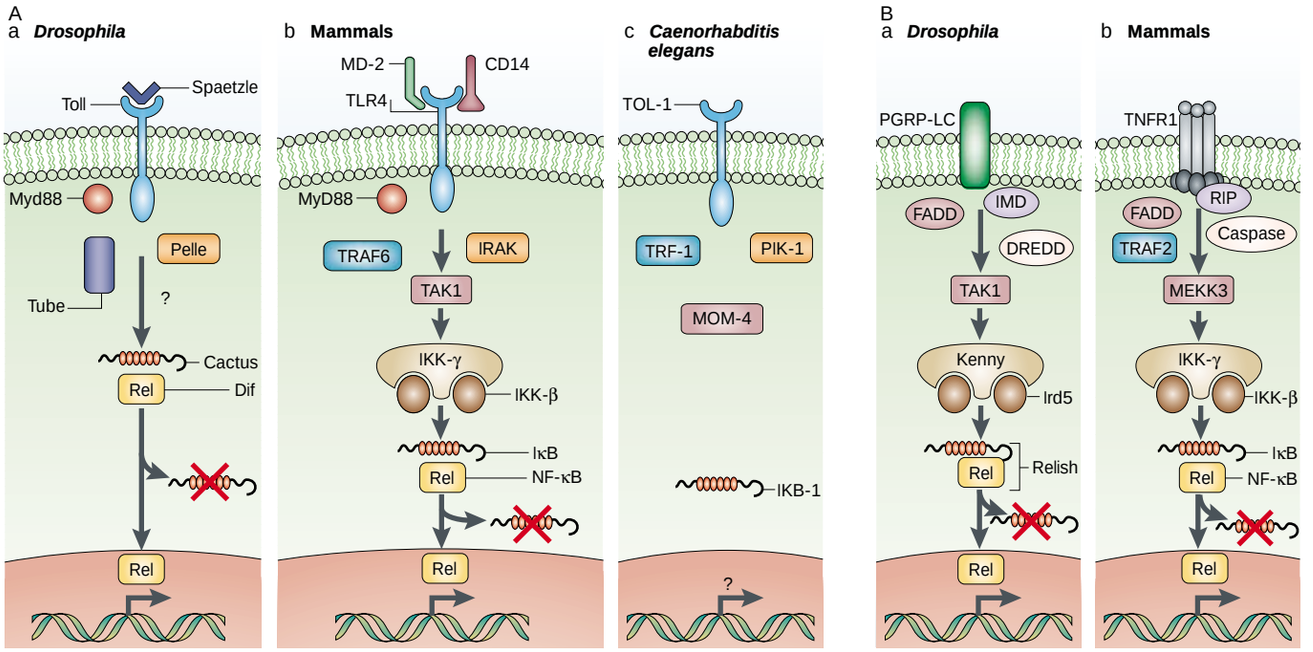


Figure 1 | **The Toll and Imd pathways. A** | A simplified Toll signalling pathway in *Drosophila* (**a**) compared to the mammalian Toll-like receptor 4 (TLR4) pathway (**b**). Homologues of some, but not all, of these proteins can be found in *Caenorhabditis elegans* (**c**). **B** | A simplified Imd signalling pathway in *Drosophila* (**a**) compared to the mammalian tumour necrosis factor (TNF) pathway (**b**). Activation of the *Drosophila* Toll and Imd pathways leads to the nuclear import of Relish (Rel)-type transcription factors. Although the two pathways are often presented as being distinct, in reality, the situation is more complex (BOX 1), with, for example, cross-stimulation of the Toll and Imd pathways by certain pathogens, linked to promiscuous activation of peptidoglycan recognition protein-short-A (PGRP-SA) and peptidoglycan-recognition protein long-C (PGRP-LC)[125] (J. Royet, personal communication). Red crosses indicate the degradation of Cactus/inhibitor of nuclear factor κB (IκB). CD, cluster of differentiation; Dif, dorsal-related immunity factor; DREDD, death-related cell death abnormality-3 (*ced-3*)/Nedd2-like; FADD, Fas-associated death domain protein; Ird, immune response deficient; IMD, immune deficiency; IRAK, interleukin 1 receptor associated kinase; IRD, immune response deficient; MEKK, mitogen-activated protein kinase kinase; MOM, more of MS; MyD, myeloid differentiation primary response gene; NF, nuclear factor; PIK, Pelle/IRAK homologue; RIP, receptor interacting protein; TAK, TGFβ activitated kinase; TOL, Toll homologue; TRAF, TNF receptor associated factor; TRF, TRAF homologue.

resistance to either fungal or bacterial pathogens[34,37]. However, adult worms have a tendency to avoid the pathogenic bacterium *Serratia marcescens* and *tol-1* is necessary for this behaviour[34]. In the adult worm, the domain of *tol-1* expression is almost entirely restricted to the nervous system, and includes a group of putative sensory neurons. This observation led to the prediction that the protein TOL-1 could be part of a mechanism that allows worms to discriminate between bacteria, and might, therefore, be directly involved in the response of worms to *S. marcescens*[34].

*The Imd pathway.* In *Drosophila*, Gram-negative bacterial PAMPs trigger a distinct signalling cascade called the immune deficiency (Imd) pathway[38,39] that is analogous to the mammalian tumour-necrosis-factor (TNF) signalling pathway (reviewed in REF. 30). One of the outcomes of the activation of this pathway is the upregulation of the expression of specific AMP genes, through the action of the NF-κB family member Relish (FIG. 1B). Given the absence of relevant homologues, the existence of a *C. elegans* Imd pathway seems highly unlikely. For example, as well as the already mentioned absence of an NF-κB homologue, there are no obvious homologues of peptidoglycan recognition protein (PGRP)-LC[40–42] and the TNF receptor of mammals, or Imd and its mammalian counterpart (FIG. 1B). There is a nematode homologue of tak1/TAK-1 (transforming growth factor-β (TGF-β)-activated kinase), which is an important component in the cascade, but its known function is not related to innate immunity[43] (see later for further discussion).

The apparent absence of equivalents of the Toll and Imd pathways in *C. elegans* is surprising given their importance in *Drosophila*. If both pathways are inactivated genetically — for example, as is the case in *imd;Toll* double mutants — no AMPs are produced and flies are hypersusceptible to both fungal and bacterial infection[44]. Recently, the importance of the AMPs was formally demonstrated, as it was shown that the expression of a single AMP could, in some cases, restore a wild-type level of resistance against infection to the otherwise hyper-susceptible mutants[45]. *C. elegans* has been shown to express several antimicrobial peptides that are related to the *Ascaris suum* antibacterial factor (ASABF) peptides of *A. suum*[46,47], but whether they have a role in inducible defence mechanisms against infection is, at present, an open question.

The immune response of flies involves not just the upregulation of AMP expression, it also affects the expression of many proteins — *Drosophila* immune-induced molecules (DIMs)[48] — with confirmed or putative roles in defence. Two microarray studies have shown that there are several hundred *Drosophila* immune-regulated genes (DIRGs)[49,50]. More recently, a further microarray analysis by De Gregorio *et al.*[51] confirmed the important regulatory roles of the Toll and Imd pathways. By analysing the response of *Relish; Toll* and *Relish*;*Spaetzle* double mutants they showed that the infection-associated induction or repression of two-thirds of 283 DIRGs that were examined required one or both of the pathways[51]. It is too early to tell whether innate defences in *C. elegans* will have such restricted foundations, as the first molecular descriptions of its immune system have only recently been published.

## Worm immunity: genetic approaches

The natural environment of *C. elegans* is the soil, which is full of potential pathogens and parasites. Even if *C. elegans* has apparently lost NF-κB-based inducible defences, it would be expected to be able to defend itself. One approach to address the question of the kind of innate immunity that *C. elegans* possesses involves genetic screens. The types of genetic screen that are possible in *C. elegans* have been recently described by Jorgensen and Mango[52]. Relatively few mutations give rise to dominant phenotypes in *C. elegans*, so screens for visible phenotypes are generally carried out on the second generation ($F_2$) after a mutagenesis. As worms are self-fertilizing hermaphrodites, mutagenized individuals are simply allowed to reproduce. One-quarter of the $F_2$ progeny will be homozygous for a given mutation and can be screened, in this case, for mutants with an altered susceptibility to infection.

*A priori*, a promising screen would find mutant worms with an enhanced susceptibility to pathogens (an Esp phenotype), as they would be predicted to have compromised defences. A generally applicable, but relatively laborious, approach would be to conduct a clonal $F_1$ screen, in which the post-infection survival of a small sample (for example, about a dozen) of the 250–300 $F_2$ progeny of a large number of individual $F_1$ animals is followed. If one-quarter of the dozen $F_2$ worms were hypersusceptible to infection, it would indicate that the F1 parent had been heterozygous for a potentially interesting mutation. As most of the $F_2$ siblings would not have been infected, homozygous worms could be easily isolated by one or two rounds of selection in the following generations, by identifying individuals that give 100% Esp progeny.

There is an important consideration that needs to be taken into account for such screens in worms. It is known that the age of a worm has an important influence on its susceptibility to pathogens[16]. For example, worms of the last larval stage (L4) are much more susceptible than adults to the toxic effects of the *Pseudomonas aeruginosa* strain PA14 (REF. 53). By contrast, whereas pre-L4 worms are resistant to infection with the *S. marcescens* strain Db11, after the L4-stage, worms become progressively more susceptible[54]. Equally, adult worms are killed more rapidly by PA14 infection, as opposed to intoxication, than are L4 worms[55]. Therefore, any screen that uses post-infection survival as the basis of selection needs to start with worms of the same age. Tightly synchronized populations can be generated relatively easily — treating gravid hermaphrodites with an alkaline bleach solution kills the adults but releases their eggs, which can be left to hatch. Also, in the absence of food the larvae arrest their development. As soon as food is given to the larvae they resume their normal development synchronously. In this way, large populations of worms of the same age can be obtained. *C. elegans* eggs are also resistant to many pathogens and can be recovered from infected cadavers to give rise to viable progeny. So, it is possible to conduct direct $F_2$ screens for worms that die more rapidly when in contact with a pathogen. Obviously, this approach will only work if the pathogen in question does not render worms sterile or kill them before they are fertile. Among the well-characterized pathogens of *C. elegans*, the *P. aeruginosa* strain PA14 is ideally suited to this approach, and has been used in such a screen[56].

*A screen for enhanced susceptibility.* Kim and colleagues directly screened an estimated 14,000 haploid mutagenized genomes and isolated 10 Esp mutants that were more susceptible to PA14 infection. Whereas under the screen conditions, wild-type worms that were infected with PA14 started to die after 34 hours, the Esp mutants died after as little as 16 hours. One important barrier for any potential pathogen, such as PA14, which normally colonizes the nematode intestine, is the grinder — a chitinous structure in the terminal bulb of the pharynx[16]. Indeed, in 1 of the 10 Esp mutants, which was not studied further, the function of the grinder was compromised. Kim *et al.* focused on

### Box 1 | Imd/Toll crosstalk and complexity

Both the Toll and Imd pathways (FIG. 1) can be simultaneously activated by a sterile injury, such as pricking with a clean needle, and are involved in MELANIZATION. Furthermore, certain *Drosophila* immune-induced molecules (DIMs)[48], including Relish (Rel) itself[101], are under the control of the Toll and Imd pathways, and the various Rel-type transcription factors can heterodimerize to give combinations that differentially regulate target-gene expression[102]. The response of a fly to infection will, therefore, partly involve a balance between the activation of these two complementary pathways[103]. Toll also seems to act in more than one Dorsal- and Dif-independent manner, adding extra branches to the signalling cascades that are activated on infection. For example, it can control the activity of a JAK/STAT-mediated response[101] (BOX 2). To further complicate matters, the observed mechanisms change during development and are tissue specific[104–108].

MELANIZATION
A reaction of invertebrates to infection that leads to the production of antimicrobial phenol derivatives and that can involve the encapsulation of a potential pathogen in a melanin envelope.

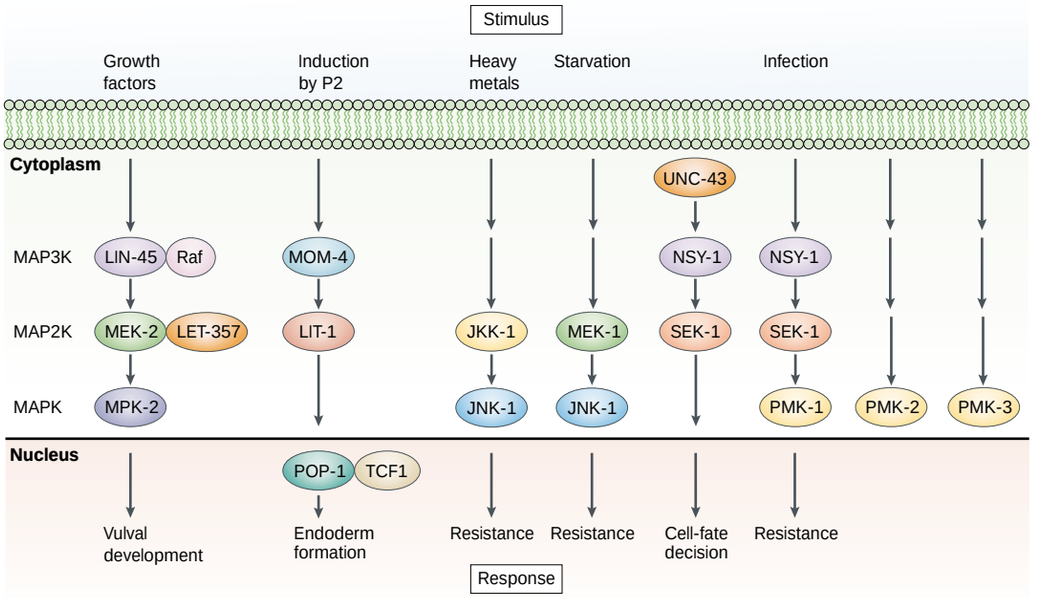


Figure 2 | **Mitogen-activated protein kinase (MAPK) signalling pathways in *Caenorabditis elegans* are involved in multiple processes.** JKK, JNK kinase homologue; JNK, c-Jun N-terminal kinase; LET, lethal; LIN, abnormal cell lineage; LIT, loss of intestine; MEK, MAP kinase kinase or Erk Kinase; MOM, more of MS; MPK, MAP kinase homologue; NSY, neuronal symmetry; PMK, P38 MAP kinase family; POP, posterior pharynx defect; SEK, SAPK/ERK kinase; TCF, T-cell factor; UNC, uncoordinated.

the two most susceptible mutants — *esp-2* and *esp-8*. Importantly, they showed that although these two mutants were also hypersusceptible to a second pathogen, *Enterococcus faecalis*, they had a lifespan that was comparable to that of wild-type worms if cultivated on the standard worm diet of *Eschericia coli*.

High-resolution single-nucleotide polymorphism (SNP) mapping, the sequencing of candidate genes and transformation rescue were used to clone *esp-2* and *esp-8* (REF. 56). They were shown to correspond to two known genes, *sek-1* and *nsy-1*, respectively, and to therefore be part of a mitogen-activated protein kinase (MAPK) signalling cascade. In species ranging from yeast to mammals, these cascades act downstream of receptors or sensors that transduce extracellular stimuli, and transform these signals into the appropriate intracellular response. Three main signalling cascades have been defined: the p38 mitogen-activated protein kinase (p38 MAPK), the extracellular-signal-regulated protein kinase (ERK) and the JUN kinase (JNK) signalling pathways. Each cascade consists of three classes of protein kinase: MAPK, MAPK kinase (MAPKK or MAP2K) and MAPK kinase kinase (MAPKKK or MAP3K). So, *esp-2*/*sek-1* and *esp-8*/*nsy-1* encode a MAP2K and a MAP3K, respectively[56]. These kinases had previously been shown to act downstream of the $Ca^{2+}$/calmodulin-dependent protein kinase II UNC-43 (FIG. 2) to control the asymmetric expression of an olfactory receptor gene in one of a pair of sensory neurons[57,58]. Although, in this seemingly esoteric and potentially nematode-specific context, the downstream MAPK target has yet to be identified, Kim *et al.* were able to show by RNA INTERFERENCE (RNAi) of the two *C. elegans* p38 homologues *pmk-1* and *pmk-2* that *sek-1* and *nsy-1* act in a *pmk-1*-dependent manner to mediate resistance to *P. aeruginosa* infection[56]. They also showed that this resistance does not involve *unc-43*.

The knockdown of *pmk-1* function by RNAi also rendered *C. elegans* more susceptible to infection with a different Gram-negative pathogen — *Salmonella typhimurium*. *Salmonella* infection is known to trigger programmed cell death or apoptosis in *C. elegans*[59]. Analysis of freely available worm mutants (see the *Caenorhabditis* Genetics Center in online links box), which had been isolated in many previous screens that have helped to delineate apoptotic pathways[60], showed that apoptosis contributes to the resistance of *C. elegans* to *Salmonella* infection. It has now been established that the involvement of an apoptotic pathway in this type of resistance depends on *sek-1*, *nsy-1* and *pmk-1* (REF. 37).

Curiously, although *P. aeruginosa* activates the p38 MAPK pathway, it has been reported not to provoke apoptosis[59], which indicates that there might be different possible outcomes following activation of the pathway by different pathogens. This might simply reflect the fact that *P. aeruginosa* kills worms much faster than *S. typhimurium*[61,62]. A definitive answer might be obtained by looking at apoptosis in worms that have been infected with attenuated strains of *P. aeruginosa*. Such strains already exist. This example illustrates one of the advantages of using the worm as a model for the study of innate immunity — it can be used in conjunction with large-scale genetic screens of the pathogen to identify the factors that contribute to virulence.

RNA INTERFERENCE (RNAi). The specific inactivation of gene expression by a double-stranded RNA molecule.

*Screens for avirulent bacterial mutants.* Screens for avirulent bacterial mutants generally involve the production of a bank of mutant bacterial clones that can be tested individually to identify those with reduced virulence — in other words, those that support the growth and survival of worms for longer than the parental strain. Such screens are technically highly feasible[15–17], because not only can pathogenic bacteria be used as the sole food source for the worms, but worms can also be grown in 24- or 96-well plates, which makes large-scale screens possible. Screens for bacterial virulence factors were first performed using PA14 (reviewed in REF. 63), but have also been reported for *E. faecalis*[64], *Burkholderia pseudomallei*[65], *Yersinia pseudotuberculosis*[66] and, most recently, *S. marcescens*[54]. As worms can be grown in liquid culture, with robotized handling (see Marseille-Génopole post-genomics centre in online links box), high-throughput screens can now be envisaged for any of the bacteria on the ever-growing list of known pathogens of *C. elegans*[16,67].

By screening several *Salmonella* mutants that had been isolated on the basis of their reduced virulence, Aballay *et al.* showed that intact LPS in the bacterial outer membrane is important not only to establish a permanent intestinal infection but also to trigger apoptosis[37]. Together with the finding that the p38 MAPK pathway is required for *Salmonella*-induced apoptosis, these results raise the possibility that there is a linear cascade that links LPS recognition to apoptosis in *C. elegans*. However, Aballay *et al.* showed that *C. elegans* TOL-1, which is by analogy with other TLRs a candidate LPS receptor, does not act upstream of the MAPK-dependant cell-death pathway, which reduces the probability that it acts as a PAMP receptor. They also failed to show directly that LPS could trigger the p38 MAPK pathway, which indicated that the simultaneous activation of a second — as yet unidentified — signalling pathway might be necessary for its activation.

### Resistance to infection and stress in *C. elegans*

This putative second MAPK activation pathway could be part of a more general stress response, as the *sek-1–nsy-1–pmk-1* pathway also regulates the stress response of nematodes to arsenic (K. Matsumoto, personal communication). If true, this would indicate that *C. elegans* possesses a MAPK-based integrated stress-signalling network, as seems to be the case in plants and insects (BOX 2), for which pathogen response is only one of its functions.

At present, the best-characterized stress-resistance pathway in *C. elegans* involves DAF-2 (DAUER formation defective), an insulin/insulin-like growth factor receptor homologue that is conserved in higher organisms (see, for example, REFS 68, 69). *daf-2* mutants have an increased resistance to heat, ultraviolet light, hypoxia[70] and heavy metals[71]. The increased resistance requires the activity of DAF-16, a forkhead transcription factor, because *daf-16*;*daf-2* double mutants are not stress resistant (see for example REF. 70). *scl-1* has been identified as one target of *daf-16*. It encodes a putative secretory protein with an SCP domain (see Protein Families Database of Alignments and HMMs (Pfam) entry PF00188) — hence the name *scl-1* (SCP-like). As would be expected, the expression of *scl-1* is upregulated by various types of stress, including heat and starvation, and its inactivation by RNAi abolishes the increased stress resistance of *daf-2* mutants[72]. Recently, it has been found that *daf-2* mutants are also highly resistant to bacterial pathogens, particularly those that are Gram-positive, and that this resistance depends on *daf-16* (D. Garsin and F. M. Ausubel, personal communication). Together, these results reinforce the common sense notion that resistance to infection is, in part, a consequence of general mechanisms that protect an organism from stress, probably by acting at the level of cell viability. The recent description of a link between amino-acid sufficiency and resistance to oxidative stress provides a clear precedent for such a mechanism[73]. The control of *daf-16* activity is complex[74], and it is possible that DAF-16 is a direct substrate for a p38 MAPK, especially as there are several consensus phosphorylation sites for p38 MAPK in the DAF-16 sequence, which are conserved in its mammalian homologues Forkhead box 1 (FoxO1), FoxO3 and FoxO4.

### Other MAP3K pathways in worm defence?

As mentioned previously, *Tak1* is important in *Drosophila* for the Imd signalling pathway. It encodes a MAP3K and its mammalian homologue acts upstream of both JNK and p38 MAPK (reviewed in REF. 75), as well as the IκB kinase (IKK) SIGNALOSOME (FIG. 1A). The nematode homologue of TAK1 is MOM-4, which transduces anterior/posterior polarity signals through a MAPK-like signalling mechanism[76] and acts, in cooperation with the WNT pathway, in endoderm formation during embryonic development[77,78] —probably downstream of a $Ca^{2+}$/calmodulin-dependent protein kinase[79]. At present, there is no evidence to indicate that it has a role in stress resistance or innate immunity, in contrast with certain other of the nematode MAPK pathway genes (FIG. 2). For example, worms with mutations in the *mek-1* gene, which encodes a homologue of the mammalian MAP2K MKK7 (an activator of JNK), are hypersensitive to heavy metals[80], as are *jnk-1* mutants themselves[81] (FIG. 2).

### TGF-β pathway is important in worm defence

The screen for *C. elegans* mutants that are hypersusceptible to PA14 showed that a TGF-β signalling pathway also contributes to worm defences against infection[82]. This is particularly interesting in the context of recent microarray-based experiments in which several nematode genes that were robustly upregulated following infection with *S. marcescens* were identified. These included genes that encode lysozymes and lectins that are known to be important for defence against pathogens in other species[83]. Among these, a small fraction had been shown to be under the positive control of the TGF-β-related gene *dbl-1* (REF. 84) — previously known as *cet-1* (REF. 85) — but not the gene *F46F2.3*, which was erroneously included among these genes in the original publication. This fraction includes *lys-8*, a gene that

DAUER
An alternative larval stage that is able to survive adverse conditions.

SIGNALOSOME
A protein complex that is involved in signal transduction.

encodes a lysozyme and would be predicted to have a direct antibacterial activity. Consistent with this, *dbl-1* mutants are more susceptible than wild-type worms to infection with *S. marcescens*[83] and *P. aeruginosa*[82].

During the infection of *C. elegans* by *S. marcescens* and *P. aeruginosa*, and by most other known pathogens[16], the bacteria are confined to the intestine. We have proposed[83] that the defence proteins that are expressed predominantly in the intestinal epithelium are secreted into the intestinal lumen, perhaps through an exocytotic mechanism that is analogous to the export of secretory lysosomes from cytotoxic T-lymphocytes. It is interesting to note that *dbl-1* mutants are shorter lived than wild-type worms if grown on live, but not dead, *E. coli*. This indicates that eliminating part of the antibacterial arsenal of the worms reveals a latent pathogenicity of *E. coli*[83]. Indeed, old worms often succumb to *E. coli* infection[86], probably as a result of an age-related decline in antimicrobial defences (reviewed in REF. 16).

It is interesting to speculate on how the nematode TGF-β pathways might be connected to defence mechanisms. The DBL-1 pathway is one of several nematode TGF-β signalling cascades (FIG. 3). They are activated by soluble factors that, together with the Toll ligand Spaetzle, have a cystine knot structure[87,88]. In many species, TGF-β-like ligands are synthesized as precursors

## Box 2 | MAPK pathways and stress responses in plants and insects

In vertebrates, cellular stress and inflammatory cytokines commonly activate JUN kinases (JNKs) and p38 mitogen-activated protein kinases (MAPKs), whereas extracellular-signal-regulated protein kinases (ERKs) often mediate cell proliferation in response to growth factors. This broad functional classification does not necessarily hold in other organisms. For example, all known plant MAPK genes — there are 23 in *Arabidopsis thaliana*[109] — belong to the ERK subfamily[110]. As well as certain plant-specific roles (such as AUXIN signalling), MAPK cascades in plants have important roles in the signal-transduction pathways that underlie cell-cycle regulation, in stress responses and in defence against infection (reviewed in REFS 109,110). Plants possess relatively complex defence systems that link the specific recognition of an invading microbe with intra- and inter-plant signalling. This can lead to the production of antimicrobial compounds and/or the apoptosis of the cells that surround the infection site, which prevents the pathogen from spreading (reviewed in REF. 111). For example, in *A. thaliana*, recognition by FLS2 (flagellin sensing) of bacterial flagellin — a pathogen-associated molecular pattern (PAMP) that also activates mammalian Toll-like receptor 5 (TLR5)[112] — triggers a complete MAPK signalling cascade (consisting of the MAP3K AtMEKK1, the redundant MAP2Ks AtMKK4 and AtMKK5, and the MAPKs AtMPK3 and AtMPK6) and leads to the activation of the transcription factors WKRY22 and WKRY29 (REF. 113). WRKY proteins are specific to flowering plants and are known to activate the transcription of many defence-related genes. Activation of the AtMEKK1 cascade confers resistance to both fungal and bacterial pathogens, which indicates a convergence of signals that is initiated by the different microbes[113]. As several of the MAPKs that are involved in plant defence —including AtMPK3 and AtMPK6 — are also activated in response to wounding, extreme temperature and/or high doses of ultraviolet radiation[109,110], MAPK-based anti-pathogen-defence signalling might be part of a larger integrated stress-signalling network[114].

Evidence is accumulating for an analogous system in *Drosophila melanogaster*. Both p38 and JNK MAPKs are activated by environmental stress in a MAP3K-dependent fashion[115–117]. The two p38 MAPKs (D-p38) might also have a role in fly immunity, as overexpression of *D-p38* downregulates the transcription of two AMP genes — *Attacin* and *Cecropin* — in flies that have been exposed to bacteria[118]. For the *Drosophila* JNK (DJNK) pathway, a direct role in triggering part of the coordinated response to infection seems more certain. This would be one of several characterized functions, as the DJNK pathway has an essential role during embryogenesis and is required for the generation of tissue polarity. It also participates in the concerted epithelial-cell movements that underlie DORSAL CLOSURE (reviewed in REF. 119). As for the role of the JNK pathway in immune defences, adding lipopolysaccharide (LPS) to SL2 cells — which resemble embryonic HAEMOCYTES — provokes *Relish* (*Rel*)-dependent upregulation of AMP genes and *Rel*-independent but *Tak1*-dependent expression of proapoptotic proteins and cytoskeleton components. This response also depends on the JNK kinases (MAP2Ks) *Mkk4* and *hep*, which indicates that there is a bifurcation of the Imd pathway downstream of TAK1, with one branch leading to NF-κB-mediated AMP gene expression and the other to a JNK-mediated response[101]. This is similar to the branching of the TLR/IL-1 pathways that is seen in vertebrates (reviewed in REF. 75), with TAK1 being able to phosphorylate inhibitor of nuclear factor κB (IκB) kinase β (IKKβ) and MKK6, leading to the activation of NF-κB and of JNK and p38, respectively[101]. Interestingly, in flies, in which this dual response has been confirmed genetically *in vivo*, the DJNK-dependent response is faster than the response that involves NF-κB[101]. This might be because the DJNK pathway is important for wound healing[120], and damaged tissue needs to be repaired rapidly if the entry of potential pathogens is to be prevented, especially as *Drosophila* spends most of its life in rotting fruits, steeped in an unsavoury microbial mulch. As well as the early DJNK-dependent response, there is a distinct later JAK/STAT-controlled pathway. This governs, for example, the induction of *Turandot M* (*TotM*)[101], a gene that is related to the LPS- and stress-inducible gene *TotA*[121]. Surprisingly, this infection-induced upregulation of *TotM* seems to depend on both *Toll* and *Rel*, but not on *tube* and *cactus*[101]. As blocking *tak1* expression by RNA interference (RNAi) reduced the level of induction and repression of all known LPS-regulated genes[101], there might be an alternative signalling cascade that leads from Toll to Tak1 to Relish, as well as the canonical Toll pathway. An important role for the MAP3K TAK1 in *Drosophila* innate immunity has been directly shown, as *tak1* mutants were isolated from a screen for flies that were hypersensitive to Gram-negative bacterial infection[103]. In this study, however, it was suggested that if Tak1 was involved in a DJNK-dependent response, there would have to be another redundant, as yet unidentified, MAP3K-mediated signal[103]. These apparently conflicting results could reflect different degrees of redundancy in MAP3K pathways in embryonic cells and adult flies.

AUXINS
Plant hormones that control growth.

DORSAL CLOSURE
The concerted movement of epidermal cells that encloses the embryo during early development.

HAEMOCYTES
Specialized blood cells that are important for defence.

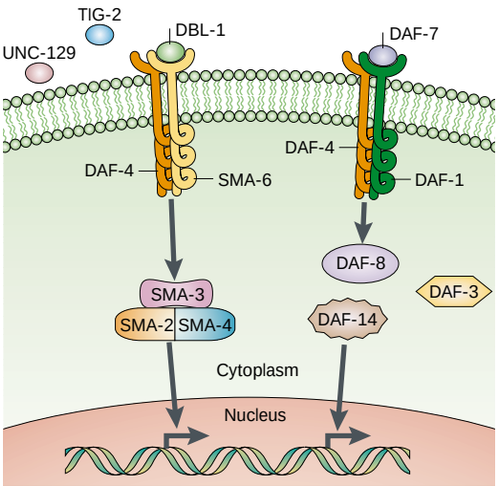


Figure 3 | **TGF-β ligands and signalling pathways in *Caenorhabditis elegans*.** Transforming growth-factor-β (TGF-β) ligands and signalling pathways in *Caenorhabditis elegans* are involved in many different processes, including the control of polyploidy and the morphology of the mail tail. Specific roles of *dbl-1* and *daf-7* are discussed in the main text. The orphan ligand transforming growth factor-β (TIG-2) is the least well characterized. Although *tig-2* mutants have been obtained, they have not yet helped to clarify the function of the gene (R. Padgett, personal communication). UNC-129 is involved in axon guidance, but little is known about its signalling partners or its regulation, apart from the fact that its expression is controlled by the forkhead transcription factor UNC-130 (REF. 126), which is also responsible for the generation of chemosensory neuron diversity in worms[127]. Figure modified with permission from REF. 92. DAF, abnormal dauer formation; DBL, decapentaplegic/bone morphogenetic protein-like; SMA, small; UNC, uncoordinated.

that undergo a proteolytic maturation; however, this has not yet been shown for DBL-1 or any of the other three *C. elegans* TGF-β family members (TIG-2, UNC-129 and DAF-7). Of these ligands, the function of DAF-7 has been well studied[89]. In conditions of low food availability and high population density, *C. elegans* can enter a quiescent dauer state. This special larval stage is characterized by changes in morphology, behaviour and physiology that allow extended survival under adverse conditions. Two extra pathways influence this developmental decision: a cyclic nucleotide pathway and the DAF-2 insulin-related pathway mentioned previously (for reviews, see REFS 90,91). If food is available and the population density is low, DAF-7 is secreted by the AMPHID SENSORY NEURONS. It binds to the DAF-1/DAF-4 receptor, which causes the subsequent activation of the SMAD family members DAF-8 and DAF-14, both of which activate target-gene transcription on translocation to the nucleus. (reviewed in REF. 92). Together with SMA-6 (small), DAF-4 also forms the DBL-1 receptor. DBL-1 was originally described as a regulator of body length[85]. It is produced by several neurons[85,93] and having bound its receptor SMA-6/DAF-4, which is expressed on HYPODERMAL and intestinal epithelium cells, it activates the Smads encoded by *sma-2*, *sma-3* and *sma-4* (REF. 92) (FIG. 3). Hypodermal expression of *sma-6* is sufficient to rescue the small size of *sma-6* mutants[94].

Studies have shown that as well as *dbl-1* mutants, *sma-2*, *sma-3*, *sma-4* and *sma-6* mutants are also hypersusceptible to *P. aeruginosa* infection[82], perhaps as a result of the direct downregulation of antibacterial genes. Curiously, one of the known downstream targets of DBL-1 — *lon-1*, a negative regulator of body length and hypodermal ploidy — encodes a molecule with sequence similarity to the plant-defence protein PR-1 (REF. 95), which also contains a SCP domain. It is expressed in the hypodermis and in the intestine[84], and hypodermal expression of *lon-1* is necessary and sufficient to restore the normal size to *lon-1* mutant animals[96]. These results indicate that DBL-1 signalling in the intestine is not required for body-length regulation and raises the possibility of a second, direct or indirect, antimicrobial role for LON-1 in the intestine. If true, this would indicate that *dbl-1–lon-1* pathways might be used in different tissues for two functions that *a priori* seem unrelated — there does not seem to be a general correlation between body length and longevity[97], or to resistance to *P. aeruginosa* (M.-W. Tan, personal communication) or *S. marcescens* infection (C.L.K., unpublished observations). Which of these roles is more ancient is, for the moment, obscure. We speculated previously on a functional link between MAPK and DAF-2 pathways; it is possible that the MAPK and *dbl-1* pathways might also be interconnected (BOX 3).

### Box 3 | A MAPK- TGF-β link?

Given that both mitogen-activated protein kinase (MAPK) and transforming growth factor-β (TGF-β) pathways contribute to the resistance of *Caenorhabditis elegans* to *Pseudomonas aeruginosa* infection, it is reasonable to ask whether the two pathways might be linked mechanistically. In *Drosophila*, activation of the MAPK kinase MAP2K TAK1 activates the *Drosophila* JUN kinase (DJNK) pathway[101]. The *Drosophila* homologue of TGF-β decapentaplegic (dpp) acts downstream of DJNK during embryonic morphogenesis[122], and is induced following immune challenge[49,50]. So, although dpp seems not to be involved during wound healing[120], it might be under the control of DJNK and be involved in the regulation of a subset of *Drosophila* immune-regulated genes (DIRGs). Such a possibility is now being tested (J. Royet, personal communication). Similarly, as far as its function in nematode immune defences is concerned, the TGF-β-related gene *dbl-1* might be directly downstream of a MAPK pathway. Several alternative scenarios, however, can be envisaged. As an extreme example, *dbl-1* could be upstream of the p38 homologue *pmk-1*, as TGF-β can act upstream of p38 in a SMAD-dependent[123] or -independent[124] manner. Fortunately, the *C. elegans* mutants that are required to delineate the different pathways are available.

AMPHID SENSORY NEURON
A specialized anterior chemosensory neuron.

HYPODERMIS
The external epidermal cell layer.

## Conclusions

It seems that *C. elegans* possesses a relatively complex innate immune system, that is without analogy to the best known parts of the antimicrobial defences of *Drosophila*. Although we have focused on the interaction of *C. elegans* with a few Gram-negative bacteria, and thereby illustrated some of the specificity that is inherent to interactions of the worms with pathogens, it should not be forgotten that, in their natural environment, worms are also confronted by Gram-positive and fungal[98,99] pathogens (reviewed in REF. 16). It might be predicted that they too could elicit specific defence mechanisms. This has been proposed for *Microbacterium nematophilum*, which produces a characteristic swelling of the hypodermal tissue in the perianal area[100]. Genetic screens for bacterial unswollen (*bus*) mutants are now underway (J. Hodgkin, personal communication) and might contribute to a better understanding of this disease model.

The investigation of innate immunity in *Drosophila* has shown that close parallels exist between insect and mammalian defences against infection, and aided our comprehension of the latter. *C. elegans* is a complementary model for studying the dialogue between an organism and its biotic environment. Unravelling the interconnections of the different host-signalling cascades should contribute to a deeper understanding of innate immunity and its evolutionary origins. That future discoveries with *C. elegans* could be applied productively to our understanding of human innate immunity is a tantalizing prospect.


1. Hoffmann, J. A. & Reichhart, J. M. *Drosophila* immunity. *Trends Cell. Biol.* **7**, 309–316 (1997).
2. Medzhitov, R. & Janeway, C. A. Innate immune recognition and control of adaptive immune responses. *Semin. Immunol.* **10**, 351–353 (1998).
   **An early review on the role of the innate immune system in the clonal selection of lymphocytes and the activation of the subsequent adaptive response.**
3. Barton, G. M. & Medzhitov, R. Control of adaptive immune responses by Toll-like receptors. *Curr. Opin. Immunol.* **14**, 380–383 (2002).
4. Malo, D. & Skamene, E. Genetic control of host resistance to infection. *Trends Genet.* **10**, 365–371 (1994).
5. Buer, J. & Balling, R. Mice, microbes and models of infection. *Nature Rev. Genet.* **4**, 195–205 (2003).
   **A recent review on the use of mice as models for the study of host–pathogen interactions.**
6. Vidal, S. M., Malo, D., Vogan, K., Skamene, E. & Gros, P. Natural resistance to infection with intracellular parasites: isolation of a candidate for *Bcg*. *Cell* **73**, 469–485 (1993).
7. Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088 (1998).
   **A genetic study that shows a role for TLR4 in LPS signalling.**
8. Flaswinkel, H. *et al.* Identification of immunological relevant phenotypes in ENU mutagenized mice. *Mamm. Genome* **11**, 526–527 (2000).
9. Miosge, L. A., Blasioli, J., Blery, M. & Goodnow, C. C. Analysis of an ethylnitrosourea-generated mouse mutation defines a cell intrinsic role of nuclear factor κB2 in regulating circulating B-cell numbers. *J. Exp. Med.* **196**, 1113–1119 (2002).
   **A technical tour de force, in which forward genetics in used in mice.**
10. Hoffmann, J. A., Kafatos, F. C., Janeway, C. A. & Ezekowitz, R. A. Phylogenetic perspectives in innate immunity. *Science* **284**, 1313–1318 (1999).
    **An excellent overview that illustrates the relevance of invertebrate model systems for the understanding of mammalian immunity.**
11. Medzhitov, R. & Janeway, C. Innate immune recognition: mechanisms and pathways. *Immunol. Rev.* **173**, 89–97 (2000).
12. Hoffmann, J. A. & Reichhart, J. M. *Drosophila* innate immunity: an evolutionary perspective. *Nature Immunol.* **3**, 121–126 (2002).
    **An update on reference 10.**
13. Iwanaga, S. The molecular basis of innate immunity in the horseshoe crab. *Curr. Opin. Immunol.* **14**, 87–95 (2002).
14. Lavine, M. D. & Strand, M. R. Insect hemocytes and their role in immunity. *Insect Biochem. Mol. Biol.* **32**, 1295–1309 (2002).
15. Kurz, C. L. & Ewbank, J. J. *Caenorhabditis elegans* for the study of host–pathogen interactions. *Trends Microbiol.* **8**, 142–144 (2000).
16. Ewbank, J. J. Tackling both sides of the host–pathogen equation with *Caenorhabditis elegans*. *Microbes Infect.* **4**, 247–256 (2002).
    **An introduction to the worm and a description of the best characterized pathogen models.**
17. Aballay, A. & Ausubel, F. M. *Caenorhabditis elegans* as a host for the study of host–pathogen interactions. *Curr. Opin. Microbiol.* **5**, 97–101 (2002).
18. Aguinaldo, A. M. *et al.* Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* **387**, 489–493 (1997).
19. Blair, J. E., Ikeo, K., Gojobori, T. & Hedges, S. B. The evolutionary position of nematodes. *BMC Evol. Biol.* **2**, 7 (2002).
    **Contradicts the conclusions of Aguinaldo *et al.* (reference 18) and affirms that insects are genetically and evolutionarily closer to humans than to nematode worms.**
20. Foley, E. & O'Farrell, P. H. Nitric oxide contributes to induction of innate immune responses to Gram-negative bacteria in *Drosophila*. *Genes Dev.* **17**, 115–125 (2003).
21. Wei, X. Q. *et al.* Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* **375**, 408–411 (1995).
22. Sritunyalucksana, K. & Soderhall, K. The proPO and clotting system in crustaceans. *Aquaculture* **191**, 53–69 (2000).
23. Nagai, T. & Kawabata, S. A link between blood coagulation and prophenol oxidase activation in arthropod host defense. *J. Biol. Chem.* **275**, 29264–29267 (2000).
    **On the basis of data from the horseshoe crab, the authors suggest that blood coagulation and prophenol oxidase activation might have evolved from a common ancestral protease cascade.**
24. Ligoxygakis, P. *et al.* A serpin mutant links Toll activation to melanization in the host defence of *Drosophila*. *EMBO J.* **21**, 6330–6337 (2002).
25. De Gregorio, E. *et al.* An immune-responsive Serpin regulates the melanization cascade in *Drosophila*. *Dev. Cell.* **3**, 581–592 (2002).
26. Levashina, E. A. *et al.* Constitutive activation of Toll-mediated antifungal defense in serpin-deficient *Drosophila*. *Science* **285**, 1917–1919 (1999).
27. Belvin, M. P. & Anderson, K. V. A conserved signaling pathway: the *Drosophila* Toll-Dorsal pathway. *Annu. Rev. Cell. Dev. Biol.* **12**, 393–416 (1996).
28. Lemaitre, B., Reichhart, J. M. & Hoffmann, J. A. *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl Acad. Sci. USA* **94**, 14614–14619 (1997).
    **This study clearly shows specificity at the level of effector molecules in *Drosophila* innate immunity.**
29. Rutschmann, S., Kilinc, A. & Ferrandon, D. The Toll pathway is required for resistance to Gram-positive bacterial infections in *Drosophila*. *J. Immunol.* **168**, 1542–1546 (2002).
30. Hoffmann, J. A. & Reichhart, J. M. *Drosophila* innate immunity: an evolutionary perspective. *Nature Immunol.* **3**, 121–126 (2002).
31. Tauszig, S., Jouanguy, E., Hoffmann, J. A. & Imler, J. L. Toll-related receptors and the control of antimicrobial peptide expression in *Drosophila*. *Proc. Natl Acad. Sci. USA* **97**, 10520–10525 (2000).
32. Imler, J. L. & Hoffmann, J. A. Toll receptors in innate immunity. *Trends Cell. Biol.* **11**, 304–311 (2001).
33. Ligoxygakis, P., Bulet, P. & Reichhart, J. M. Critical evaluation of the role of the Toll-like receptor 18-wheeler in the host defense of *Drosophila*. *EMBO Rep.* **3**, 666–673 (2002).
34. Pujol, N. *et al.* A reverse genetic analysis of components of the Toll signalling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 809–821 (2001).
35. Jansen, G., Hazendonk, E., Thijssen, K. L. & Plasterk, R. H. Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans*. *Nature Genet.* **17**, 119–121 (1997).
36. Liu, L. X. *et al.* High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res.* **9**, 859–867 (1999).
37. Aballay, A., Drenkard, E., Hilbun, L. R. & Ausubel, F. M. *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 (2003).
    **An elegant example of the two-sided approach that can be taken using worms.**
38. Lemaitre, B. *et al.* A recessive mutation, immune deficiency (*imd*), defines two distinct control pathways in the *Drosophila* host defense. *Proc. Natl Acad. Sci. USA* **92**, 9465–9469 (1995).
    **Early evidence to show that *Drosophila* distinguishes bacterial from fungal infection, with the first description of the *imd* gene.**
39. Georgel, P. *et al. Drosophila* immune deficiency (imd) is a death domain protein that activates antibacterial defense and can promote apoptosis. *Dev. Cell.* **1**, 503–514 (2001).
40. Choe, K. M., Werner, T., Stoven, S., Hultmark, D. & Anderson, K. V. Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in *Drosophila*. *Science* **296**, 359–362 (2002).
41. Gottar, M. *et al.* The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. *Nature* **416**, 640–644 (2002).
42. Ramet, M., Manfruelli, P., Pearson, A., Mathey-Prevot, B. & Ezekowitz, R. A. Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. *Nature* **416**, 644–648 (2002).
43. Shin, T. H. *et al.* MOM-4, a MAP kinase kinase kinase-related protein, activates WRM-1/LIT-1 kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Mol. Cell.* **4**, 275–280 (1999).
44. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M. & Hoffmann, J. A. The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983 (1996).
    **With more than 500 citations, this classic paper is still highly useful.**
45. Tzou, P., Reichhart, J. M. & Lemaitre, B. Constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient *Drosophila* mutants. *Proc. Natl Acad. Sci. USA* **99**, 2152–2157 (2002).
46. Kato, Y. & Komatsu, S. ASABF, a novel cysteine-rich antibacterial peptide isolated from the nematode *Ascaris suum*: purification, primary structure, and molecular cloning of cDNA. *J. Biol. Chem.* **271**, 30493–30498 (1996).
47. Kato, Y. *et al. abf-1* and *abf-2*, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans*. *Biochem. J.* **361**, 221–230 (2002).
48. Uttenweiler-Joseph, S. *et al.* Differential display of peptides induced during the immune response of *Drosophila*: a matrix-assisted laser desorption ionization time-of-flight mass spectrometry study. *Proc. Natl Acad. Sci. USA* **95**, 11342–11347 (1998).

49. Irving, P. *et al.* A genome-wide analysis of immune responses in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 15119–15124 (2001).
50. De Gregorio, E., Spellman, P. T., Rubin, G. M. & Lemaitre, B. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. *Proc. Natl Acad. Sci. USA* **98**, 12590–12595 (2001).
51. De Gregorio, E., Spellman, P. T., Tzou, P., Rubin, G. M. & Lemaitre, B. The Toll and Imd pathways are the major regulators of the immune response in *Drosophila*. *EMBO J.* **21**, 2568–2579 (2002).
52. Jorgensen, E. M. & Mango, S. E. The art and design of genetic screens: *Caenorhabditis elegans*. *Nature Rev. Genet*. **3**, 356–369 (2002).
**A lucid review on the use of *C. elegans* as a genetic model.**
53. Mahajan-Miklos, S., Tan, M. W., Rahme, L. G. & Ausubel, F. M. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*–*Caenorhabditis elegans* pathogenesis model. *Cell* **96**, 47–56 (1999).
**A landmark paper that established worms as a model host.**
54. Kurz, C. L. *et al.* Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by *in vivo* screening. *EMBO J.* **22**, 1451–1460 (2003).
55. Tan, M. W., Mahajan-Miklos, S. & Ausubel, F. M. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc. Natl Acad. Sci. USA* **96**, 715–720 (1999).
56. Kim, D. H. *et al.* A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623–626 (2002).
**Genetic evidence for a role of MAPK signalling in worm defences.**
57. Sagasti, A. *et al.* The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* **105**, 221–232 (2001).
58. Tanaka-Hino, M. *et al.* SEK-1 MAPKK mediates $Ca^{2+}$ signaling to determine neuronal asymmetric development in *Caenorhabditis elegans*. *EMBO Rep.* **3**, 56–62 (2002).
59. Aballay, A. & Ausubel, F. M. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proc. Natl Acad. Sci. USA* **98**, 2735–2739 (2001).
60. Bargmann, C. & Hodgkin, J. Accolade for *elegans*. *Cell* **111**, 759–762 (2002).
**An entertaining historical view of the worm and the *C. elegans* community.**
61. Aballay, A., Yorgey, P. & Ausubel, F. M. *Salmonella typhimurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans*. *Curr. Biol.* **10**, 1539–1542 (2000).
62. Labrousse, A., Chauvet, S., Couillault, C., Kurz, C. L. & Ewbank, J. J. *Caenorhabditis elegans* is a model host for *Salmonella typhimurium*. *Curr. Biol.* **10**, 1543–1545 (2000).
63. Tan, M. W. & Ausubel, F. M. *Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis. *Curr. Opin. Microbiol.* **3**, 29–34 (2000).
64. Garsin, D. A. *et al.* A simple model host for identifying Gram-positive virulence factors. *Proc. Natl Acad. Sci. USA* **98**, 10892–10897 (2001).
65. Gan, Y. H. *et al.* Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. *Mol. Microbiol.* **44**, 1185–1197 (2002).
66. Darby, C., Hsu, J. W., Ghori, N. & Falkow, S. *Caenorhabditis elegans*: plague bacteria biofilm blocks food intake. *Nature* **417**, 243–244 (2002).
67. Couillault, C. & Ewbank, J. J. Diverse bacteria are pathogens of *Caenorhabditis elegans*. *Infect. Immun.* **70**, 4705–4707 (2002).
68. Tran, H. *et al.* DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. *Science* **296**, 530–534 (2002).
69. Holzenberger, M. *et al.* IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**, 182–187 (2002).
70. Scott, B. A., Avidan, M. S. & Crowder, C. M. Regulation of hypoxic death in *C. elegans* by the insulin/IGF receptor homolog DAF-2. *Science* **296**, 2388–2391 (2002).
71. Barsyte, D., Lovejoy, D. A. & Lithgow, G. J. Longevity and heavy metal resistance in *daf-2* and *age-1* long-lived mutants of *Caenorhabditis elegans*. *FASEB J.* **15**, 627–634 (2001).
72. Ookuma, S., Fukuda, M. & Nishida, E. Identification of a DAF-16 transcriptional target gene, *scl-1*, that regulates longevity and stress resistance in *Caenorhabditis elegans*. *Curr. Biol.* **13**, 427–431 (2003).
73. Harding, H. P. *et al.* An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell.* **11**, 619–633 (2003).
74. Lin, K., Hsin, H., Libina, N. & Kenyon, C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nature Genet.* **28**, 139–145 (2001).
75. Janssens, S. & Beyaert, R. A universal role for MyD88 in TLR/IL-1R-mediated signaling. *Trends Biochem. Sci.* **27**, 474–482 (2002).
76. Shin, T. H. *et al.* MOM-4, a MAP kinase kinase kinase-related protein, activates WRM-1/LIT-1 kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Mol. Cell.* **4**, 275–280 (1999).
77. Meneghini, M. D. *et al.* MAP kinase and Wnt pathways converge to downregulate an HMG-domain repressor in *Caenorhabditis elegans*. *Nature* **399**, 793–797 (1999).
78. Ishitani, T. *et al.* The TAK1–NLK–MAPK-related pathway antagonizes signalling between β-catenin and transcription factor TCF. *Nature* **399**, 798–802 (1999).
79. Ishitani, T. *et al.* The TAK1–NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/$Ca^{2+}$ pathway to antagonize Wnt/β-catenin signaling. *Mol. Cell. Biol.* **23**, 131–139 (2003).
80. Koga, M., Zwaal, R., Guan, K. L., Avery, L. & Ohshima, Y. A *Caenorhabditis elegans* MAP kinase kinase, MEK-1, is involved in stress responses. *EMBO J.* **19**, 5148–5156 (2000).
**Evidence of a role for a JNK pathway in an organismal stress response.**
81. Villanueva, A. *et al. jkk-1* and *mek-1* regulate body movement coordination and response to heavy metals through *jnk-1* in *Caenorhabditis elegans*. *EMBO J.* **20**, 5114–5128 (2001).
82. Tan, M. W. Genetic and genomic dissection of host–pathogen interactions using a *P. aeruginosa*–*C. elegans* pathogenesis model. *Pediatr. Pulmonol.* **32**, 96–97 (2001).
83. Mallo, G. V. *et al.* Inducible antibacterial defence system in *C. elegans*. *Curr. Biol.* **12**, 1209–1214 (2002).
**The first indication that worms have inducible innate immune responses, with the identification of genes that are upregulated by infection.**
84. Mochii, M., Yoshida, S., Morita, K., Kohara, Y. & Ueno, N. Identification of transforming growth factor-β-regulated genes in *Caenorhabditis elegans* by differential hybridization of arrayed cDNAs. *Proc. Natl Acad. Sci. USA* **96**, 15020–15025 (1999).
85. Morita, K., Chow, K. L. & Ueno, N. Regulation of body length and male tail ray pattern formation of *Caenorhabditis elegans* by a member of TGF-β family. *Development* **126**, 1337–1347 (1999).
86. Herndon, L. A. *et al.* Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* **419**, 808–814 (2002).
87. Bergner, A. *et al.* Horseshoe crab coagulogen is an invertebrate protein with a nerve growth factor-like domain. *Biol. Chem.* **378**, 283–287 (1997).
88. Bork, P. The modular architecture of a new family of growth regulators related to connective tissue growth factor. *FEBS Lett.* **327**, 125–130 (1993).
89. Ren, P. *et al.* Control of *C. elegans* larval development by neuronal expression of a TGF-β homolog. *Science* **274**, 1389–1391 (1996).
90. Guarente, L. & Kenyon, C. Genetic pathways that regulate ageing in model organisms. *Nature* **408**, 255–262 (2000).
91. Hekimi, S., Burgess, J., Bussiere, F., Meng, Y. & Benard, C. Genetics of lifespan in *C. elegans*: molecular diversity, physiological complexity, mechanistic simplicity. *Trends Genet.* **17**, 712–718 (2001).
92. Patterson, G. I. & Padgett, R. W. TGF β-related pathways: roles in *Caenorhabditis elegans* development. *Trends Genet.* **16**, 27–33 (2000).
93. Suzuki, Y. *et al.* A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. *Development* **126**, 241–250 (1999).
94. Yoshida, S., Morita, K., Mochii, M. & Ueno, N. Hypodermal expression of *Caenorhabditis elegans* TGF-β type I receptor SMA-6 is essential for the growth and maintenance of body length. *Dev. Biol.* **240**, 32–45 (2001).
95. Morita, K. *et al.* A *Caenorhabditis elegans* TGF-β, DBL-1, controls the expression of LON-1, a PR-related protein, that regulates polyploidization and body length. *EMBO J.* **21**, 1063–1073 (2002).
96. Maduzia, L. L. *et al. lon-1* regulates *Caenorhabditis elegans* body size downstream of the *dbl-1* TGFβ signaling pathway. *Dev. Biol.* **246**, 418–428 (2002).
97. McCulloch, D. & Gems, D. Body size, insulin/IGF signaling and aging in the nematode *Caenorhabditis elegans*. *Exp. Gerontol.* **38**, 129–136 (2003).
98. Park, J.-O., El-Tarabily, K. A., Ghisalberti, E. L. & Sivasithamparam, K. Pathogenesis of *Streptoverticillium albireticuli* on *Caenorhabditis elegans* and its antagonism to soil-borne fungal pathogens. *Lett. Appl. Microbiol.* **35**, 361–365 (2002).
99. Mylonakis, E., Ausubel, F. M., Perfect, J. R., Heitman, J. & Calderwood, S. B. Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a model of yeast pathogenesis. *Proc. Natl Acad. Sci. USA* **99**, 15675–15680 (2002).
100. Hodgkin, J., Kuwabara, P. E. & Corneliussen, B. A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. *Curr. Biol.* **10**, 1615–1618 (2000).
101. Boutros, M., Agaisse, H. & Perrimon, N. Sequential activation of signaling pathways during innate immune responses in *Drosophila*. *Dev. Cell.* **3**, 711–722 (2002).
**An extensive study of fly immunity that combines microarray studies, RNAi and the analysis of mutants, indicating a link between the control of tissue repair and the induction of antimicrobial proteins.**
102. Han, Z. S. & Ip, Y. T. Interaction and specificity of Rel-related proteins in regulating *Drosophila* immunity gene expression. *J. Biol. Chem.* **274**, 21355–21361 (1999).
103. Vidal, S. *et al.* Mutations in the *Drosophila dTAK1* gene reveal a conserved function for MAPKKKs in the control of rel/NF-κB-dependent innate immune responses. *Genes Dev.* **15**, 1900–1912 (2001).
**A rare direct screen for fly mutants with an altered susceptibility to infection.**
104. Ferrandon, D. *et al.* A drosomycin-GFP reporter transgene reveals a local immune response in *Drosophila* that is not dependent on the *Toll* pathway. *EMBO J.* **17**, 1217–1227 (1998).
105. Manfruelli, P., Reichhart, J. M., Steward, R., Hoffmann, J. A. & Lemaitre, B. A mosaic analysis in *Drosophila* fat body cells of the control of antimicrobial peptide genes by the Rel proteins Dorsal and DIF. *EMBO J.* **18**, 3380–3391 (1999).
106. Meng, X., Khanuja, B. S. & Ip, Y. T. Toll receptor-mediated *Drosophila* immune response requires Dif, an NF-κB factor. *Genes Dev.* **13**, 792–797 (1999).
107. Petersen, U. M. *et al.* Serpent regulates *Drosophila* immunity genes in the larval fat body through an essential GATA motif. *EMBO J.* **18**, 4013–4022 (1999).
108. Tzou, P. *et al.* Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. *Immunity* **13**, 737–748 (2000).
109. Jonak, C., Okresz, L., Bogre, L. & Hirt, H. Complexity, cross talk and integration of plant MAP kinase signalling. *Curr. Opin. Plant Biol.* **5**, 415–424 (2002).
110. Tena, G., Asai, T., Chiu, W. L. & Sheen, J. Plant mitogen-activated protein kinase signaling cascades. *Curr. Opin. Plant Biol.* **4**, 392–400 (2001).
111. Cohn, J., Sessa, G. & Martin, G. B. Innate immunity in plants. *Curr. Opin. Immunol.* **13**, 55–62 (2001).
112. Hayashi, F. *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**, 1099–1103 (2001).
113. Asai, T. *et al.* MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**, 977–983 (2002).
114. Innes, R. W. Mapping out the roles of MAP kinases in plant defense. *Trends Plant Sci.* **6**, 392–394 (2001).
115. Han, S. J., Choi, K. Y., Brey, P. T. & Lee, W. J. Molecular cloning and characterization of a *Drosophila* p38 mitogen-activated protein kinase. *J. Biol. Chem.* **273**, 369–374 (1998).
116. Botella, J. A. *et al.* The *Drosophila* cell shape regulator c-Jun N-terminal kinase also functions as a stress-activated protein kinase. *Insect Biochem. Mol. Biol.* **31**, 839–847 (2001).
117. Inoue, H. *et al.* A *Drosophila* MAPKKK, D-MEKK1, mediates stress responses through activation of p38 MAPK. *EMBO J.* **20**, 5421–5430 (2001).
118. Han, Z. S. *et al.* A conserved p38 mitogen-activated protein kinase pathway regulates *Drosophila* immunity gene expression. *Mol. Cell. Biol.* **18**, 3527–3539 (1998).
119. Noselli, S. & Agnes, F. Roles of the JNK signaling pathway in *Drosophila* morphogenesis. *Curr. Opin. Genet. Dev.* **9**, 466–472 (1999).
120. Ramet, M., Lanot, R., Zachary, D. & Manfruelli, P. JNK signaling pathway is required for efficient wound healing in *Drosophila*. *Dev. Biol.* **241**, 145–156 (2002).
**Describes the use of flies to understand how an organism responds to physical injury.**
121. Ekengren, S. *et al.* A humoral stress response in *Drosophila*. *Curr. Biol.* **11**, 714–718 (2001).
**The first description of the Turandot family.**
122. Sluss, H. K. & Davis, R. J. Embryonic morphogenesis signaling pathway mediated by JNK targets the transcription factor JUN and the TGF-β homologue decapentaplegic. *J. Cell. Biochem.* **67**, 1–12 (1997).

123. Takekawa, M. *et al.* Smad-dependent GADD45β expression mediates delayed activation of p38 MAP kinase by TGF-β. *EMBO J.* **21**, 6473–6482 (2002).
124. Yu, L., Hebert, M. C. & Zhang, Y. E. TGF-β receptor-activated p38 MAP kinase mediates Smad-independent TGF-β responses. *EMBO J.* **21**, 3749–3759 (2002).
125. Werner, T. *et al.* A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **97**, 13772–1377 (2000).
**The identification of the PGRP family that has subsequently been shown to have important roles in fly defences.**
126. Nash, B., Colavita, A., Zheng, H., Roy, P. J. & Culotti, J. G. The forkhead transcription factor UNC-130 is required for the graded spatial expression of the UNC-129 TGF-β guidance factor in *C. elegans*. *Genes Dev.* **14**, 2486–2500 (2000).
127. Sarafi-Reinach, T. R. & Sengupta, P. The forkhead domain gene *unc-130* generates chemosensory neuron diversity in *C. elegans*. *Genes Dev.* **14**, 2472–2485 (2000).

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