A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*

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Background: Both animals and plants respond rapidly to pathogens by inducing the expression of defense-related genes. Whether such an inducible system of innate immunity is present in the model nematode *Caenorhabditis elegans* is currently an open question. Among conserved signaling pathways important for innate immunity, the Toll pathway is the best characterized. In *Drosophila*, this pathway also has an essential developmental role. *C. elegans* possesses structural homologs of components of this pathway, and this observation raises the possibility that a Toll pathway might also function in nematodes to trigger defense mechanisms or to control development.

Results: We have generated and characterized deletion mutants for four genes supposed to function in a nematode Toll signaling pathway. These genes are *tol-1*, *trf-1*, *pik-1*, and *ikb-1* and are homologous to the *Drosophila melanogaster Toll*, *dTraf*, *pelle*, and *cactus* genes, respectively. Of these four genes, only *tol-1* is required for nematode development. None of them are important for the resistance of *C. elegans* to a number of pathogens. On the other hand, *C. elegans* is capable of distinguishing different bacterial species and has a tendency to avoid certain pathogens, including *Serratia marcescens*. The *tol-1* mutants are defective in their avoidance of pathogenic *S. marcescens*, although other chemosensory behaviors are wild type.

Conclusions: In *C. elegans*, *tol-1* is important for development and pathogen recognition, as is Toll in *Drosophila*, but remarkably for the latter rôle, it functions in the context of a behavioral mechanism that keeps worms away from potential danger.

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Background

Drosophila melanogaster possesses an inducible antibacterial defense system, first described nearly 30 years ago [1], that includes the regulated production of antimicrobial peptides by the fat body [2]. The system is relatively sophisticated, and *Drosophila* is capable of distinguishing between bacterial and fungal pathogens and of mounting an appropriate response [3]. Natural infection with the fungus *Beauveria bassiana*, for example, induces the expression of drosomycin, a powerful antifungal peptide. This induction is dependent upon the nuclear import of Dif, a member of the Rel/NFκB family of transcription factors [4], that results from activation of the *spätzle/Toll/tube/pelle/cactus* regulatory gene cassette [5, 6]. This cas-

sette had been originally identified as playing a role in dorsoventral patterning during early *Drosophila* development (reviewed in [7]). The demonstration of the importance of the Toll pathway in an innate immune response, and the parallels between this pathway and the pathway of NFκB activation in higher eukaryotes [5], triggered a search for vertebrate Toll homologs. A large family of such proteins, the Toll-like receptors (TLRs) were subsequently discovered and have been shown to function in vertebrate innate immunity (reviewed in [8–11]).

Currently, it is not known whether the nematode *Caenorhabditis elegans* possesses an inducible system of defense analogous to that found in *Drosophila* [12, 13]. The functional conservation of the Toll signaling pathway from flies to man prompted us to look for structural homologs of the components of this pathway in *C. elegans*. We have identified and characterized nematode genes homologous to *Toll*, *pelle*, *cactus*, and *dTraf1* (also believed to function in NFκB signaling [14, 15]) and generated the corresponding deletion mutants. We show that the *C. elegans Toll* homolog (*tol-1*) alone is required for nematode development. The four nematode genes do not appear to be involved in an inducible system of defense. Remarkably, however, we show that *tol-1* contributes to the recognition of potential pathogens and is involved in a simple defense mechanism, avoidance.

Results

Identification of *C. elegans* homologs of Toll pathway components

One defining feature of TLRs is the presence of a conserved intracellular domain, the TIR domain ([10]; IPR000157, http://www.ebi.ac.uk/interpro/; Pfam01582, http://www.sanger.ac.uk/Software/Pfam/index.shtml). This domain is also found in a number of cytoplasmic proteins, including MyD88 [16], and plant defense proteins, including the downy mildew resistance protein RPP5 [17]. Searches of the available *C. elegans* genomic and EST sequences revealed the presence of two putative TIR domain-encoding genes. The first, F13B10.1, potentially codes for a protein that also contains a SAM domain, a protein interaction domain suggested to be involved in developmental regulation [18]. For the second, by sequencing a number of overlapping partial cDNAs and specific RT-PCR fragments, we were able to reconstruct a complete cDNA of 4077 bp. The gene covers more than 17 kb of genomic sequence and comprises 14 exons, ranging from to 1380 bp to 75 bp in size (Figures 1 and 2). Among TIR-containing proteins from other organisms, the nematode gene is most similar to members of the TLR family, and we consequently named the gene *tol-1*. This similarity extends across the entire protein; the presumptive TOL-1 extracellular domain contains 22 leucine-rich repeats (LRRs), with interspersed cysteine-rich flanking motifs [19], followed by a potential transmembrane domain. When compared to the family of *Drosophila* TLR proteins, TOL-1 is most similar overall to Toll-8, closely followed by Toll-6 (Figure 3). TOL-1, however, lacks a C-terminal extension after the TIR domain. Such an extension is found in certain *Drosophila* Toll-family proteins [20].

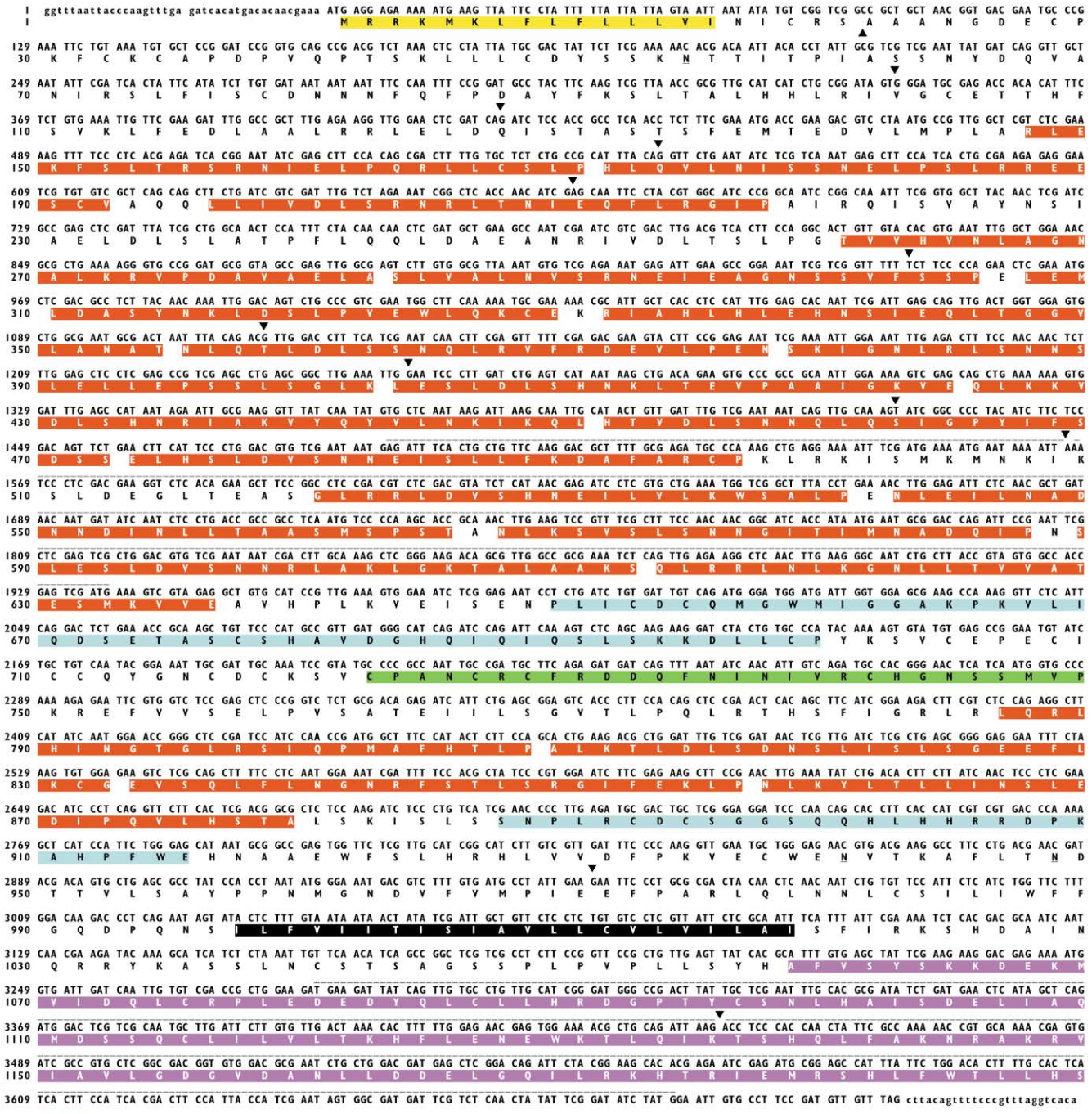
Similarly, by sequence analysis, we were able to identify genes potentially encoding structural homologs of Pelle (*pik-1*, for Pelle/IRAK kinase; [21]), Traf (*trf-1*; see GenBank AF079837; [14, 22]) and Cactus (*ikb-1*; [23]). The existence of these genes has been previously noted [12, 24, 25], and the corresponding predicted proteins are present in GenBank (K09B11.1, F45G2.6, and C04F12.3 for PIK-1, TRF-1, and IKB-1, respectively). For each of these genes, partial cDNA molecules and specific RT-PCR fragments were sequenced, which in the case of PIK-1 and TRF-1 led to a refinement of the predicted open reading frames relative to the available sequences. The most-conserved region for each of these predicted proteins is shown in Figures 3–6.

Isolation of deletion mutants and initial phenotypic analysis

Extrapolation from the physical map positions of the *tol-1*, *trf-1*, *pik-1*, and *ikb-1* genes to the corresponding positions on the genetic map suggested that these genes do not match previously characterized mutants. Therefore, to investigate the function of the *C. elegans* Toll signaling pathway homologs, we produced deletion mutants for each gene by target-selected PCR screening of a deletion mutation library [26]. The extents of the different deletions are shown in Figure 2.

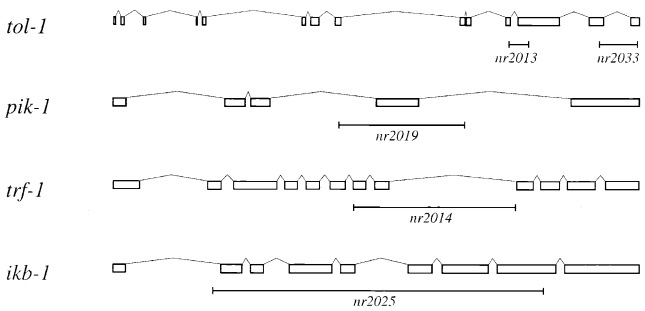
Apart from a diminution of mean life span (see below) and with the exception of *tol-1*, the mutants showed no visible phenotype. In the case of *tol-1*, two recessive alleles were obtained. *tol-1(nr2013)* potentially encodes a secreted protein comprised principally of the first 12 LRRs (Figures 1 and 2). At 25°C, *tol-1(nr2013)* homozygotes are not viable. There is a high proportion of embryonic lethality, and the worms that do hatch arrest as small, deformed larvae. At 15°C, less than 10% of *tol-1(nr2013)* mutants develop into adults that are marginally fertile, while the majority of worms arrest at different developmental stages and exhibit dramatic defects in morphogenesis (Figure 7c,d,f,g; Table 1). The observed defects could be due to abnormal elongation, as precomma-stage embryos appear normal, tissue differentiation appears to progress normally, and muscle contractions are seen even in embryos as deformed as that shown in Figure 7. There was a maternal rescue of the embryonic lethality. Thus, 0.5% dead embryos, as opposed to the expected >20%, in the progeny of *tol-1(nr2013)* heterozygous worms at 25°C (n = 1049) were obtained. This maternal effect did not extend to a complete rescue, as 15% of the progeny

Figure 1



Nucleotide sequence of the *tol-1* gene and corresponding conceptual translation. Lowercase letters correspond to 5′ and 3′ untranslated regions. The 5′ sequence in italics corresponds to the SL1 spliced leader [75]. Downward-pointing arrowheads mark intron junctions. The predicted signal peptide is highlighted in yellow, and the corresponding predicted cleavage siteis marked by an upward-pointing arrowhead. The leucine-rich repeats are shown in red, the carboxy-terminal flanking domains are in blue, the amino-terminal flanking domain is in green, the predicted transmembrane domain is in black, and the TIR domain is in purple. Predicted N-glycosylation sites are underlined, and lines indicate the extents of the 5′ *nr2013* and 3′ *nr2033* deletions.

Figure 2



Genomic organization of the *tol-1*, *pik-1*, *trf-1*, and *ikb-1* genes and their corresponding deletion alleles. Exons are represented as boxes and introns as lines, and the extents of the different deletions are shown below each gene. The scale is not the same for the four genes. The *tol-1(nr2013)* mutation is a deletion of 690 bp (positions 36,394–37,083 of cosmid C07F11), *tol-1(nr2033)* is one of 1,288 bp (1,011–2,299 of W05D2), *pik-1(nr2019)* is one of 1,137 bp (2,851–3,988 of K09B11), *trf-1(nr2014)* is one of 895 bp (25,261–26,156 of F45G2), and *ikb-1(nr2027)* is one of 1,926 bp (13,889–15,799 of C04F12), where the coordinates refer to the corresponding sequence records in GenBank.

arrested as larvae. The *tol-1(nr2013)* mutant phenotype was rescued by transformation with cosmid W05D2 (results not shown), which contains the entire *tol-1* gene together with more than 16 kb of upstream sequence. Although the maternal effect was not recapitulated (results not shown), which probably reflects the silencing of germline expression seen for transgenes in *C. elegans* [27], this result indicates that the lethality is not due to extraneous mutations. Since Cactus is a downstream suppressor of Toll mutations in *Drosophila* [28], we tested whether *ikb-1(nr2025)* could suppress the mutant phenotype of *tol-1(nr2013)*. Suppression was not observed, and this suggests either that the lethality conferred by *tol-1(nr2013)* is a nonspecific effect of the mutant protein or that *ikb-1* does not lie downstream of *tol-1(nr2013)* in a *C. elegans* signaling pathway. To distinguish between these possibilities, we inactivated *tol-1* expression by RNA interference (RNAi; [29]). Two double-stranded RNA molecules corresponding to different regions of the *tol-1* transcript (see Materials and methods) were independently injected into L4 worms. In both cases, 25% of the progeny of the injected worms arrested as embryos. Among the worms that did hatch, 40% arrested as abnormal larvae. The finding that RNAi of *tol-1* mimics the *tol-1(nr2013)* mutant phenotype suggests that *tol-1(nr2013)* is a null allele rather than being associated with a nonspecific effect and that *tol-1* function is essential for *C. elegans* development. It further suggests that *ikb-1* does not function downstream of *tol-1* in *C. elegans* development.

The second *tol-1* allele, *nr2033*, potentially encodes a protein in which 134 amino acids are deleted from a highly conserved region of the TIR domain (Figures 1 and 3). *tol-1(nr2033)* mutants are healthy and fertile but exhibit a lowly penetrant lethality, and a small but significant proportion of the mutants arrest as early larvae (Table 1). The TIR domain is thus largely dispensable for the developmental function of TOL-1.

Resistance to infection of the mutants

In *Drosophila*, *Toll* mutants show a greatly increased susceptibility to a natural fungal infection [5]. Many ecological studies have been published on the interactions between fungi and free-living Rhabditid nematodes (see for example, [30–34]), but only in the case of the endoparasitic fungus *Drechmeria coniospora* has a model of infection with *C. elegans* been established [35, 36]. Infection of *C. elegans* by *D. coniospora* starts by the adhesion of fungal spores to the head and is followed by the extension of hyphal processes into the worm's body [36]. For the first two days postinfection, worms appear healthy but become increasingly moribund until they die. There is then a very rapid proliferation of the fungus, with extension of multiple hyphae until the worm is hidden by a mass of filaments. We found no difference between the wild-type (N2) and *tol-1(nr2033)* worms in terms of the adhesion of *D. coniospora* spores to the worms, the time course of the infection, or the rate at which the fungus eventually engulfed the infected worms (Figure 8a and results not shown).

The Toll family member 18-wheeler has been suggested to be important for antibacterial defenses in *Drosophila* [37]. We therefore investigated the resistance of the different mutants to the Gram-negative enterobacterium *Pseudomonas aeruginosa*, currently the best-characterized bacterial pathogen of *C. elegans* [12]. Neither under "fast-killing" nor under "slow-killing" conditions was there a significant difference between the wild-type and the different mutants (Figure 8b and results not shown). The four viable *C. elegans* mutants were also tested with a strain of *Microbacterium nematophilum*, a Gram-positive bacterium that causes anal infection and swelling [38]. None of the mutants showed an alteration in the swelling phenotype (J. Hodgkin, personal communication). The Gram-negative enterobacterium *Serratia marcescens* is also capable of infecting *C. elegans* [39]. We tested two strains known to infect *Drosophila*: Db11 [40] (Figure 8c) and the less-virulent Db1140 [41] (Figure 8d). As in insects, Db11 was found to be more virulent against *C. elegans* than was Db1140. Although the *tol-1(nr2033)*, *trf-1*, *pik-1*, and *ikb-1* mutants all showed an increased susceptibility to both Db11 and Db1140 (Figure 8c,d), the effect was no greater than the relative change in life span of the mutants in the absence of a pathogen (Figure 8e). This suggests that their increased susceptibility to *S. marcescens* infection reflects the general weakness of these mutants rather than a specific defect in a defense mechanism.

Figure 3

The conserved region of *C. elegans* TOL-1 is aligned with Toll, Toll-6, and Toll-8. Residues that are identical in the majority of the sequences are highlighted in black, and similar residues are highlighted in gray. The percentage of identical and similar residues for different pairwise comparisons of the partial sequences shown are (TOL-1 versus Toll) 23% and 39%; (TOL-1 versus Toll-6) 28% and 47%; and (TOL-1 versus Toll-8) 27% and 47%. The TIR domain is in italics. The functionally important Boxes 1 and 2, (YDAYILY and IYGRDDY in the human IL-1 receptor, respectively) [76], are indicated with lines, and an arrowhead marks the position that corresponds to the start of the *nr2033* deletion.

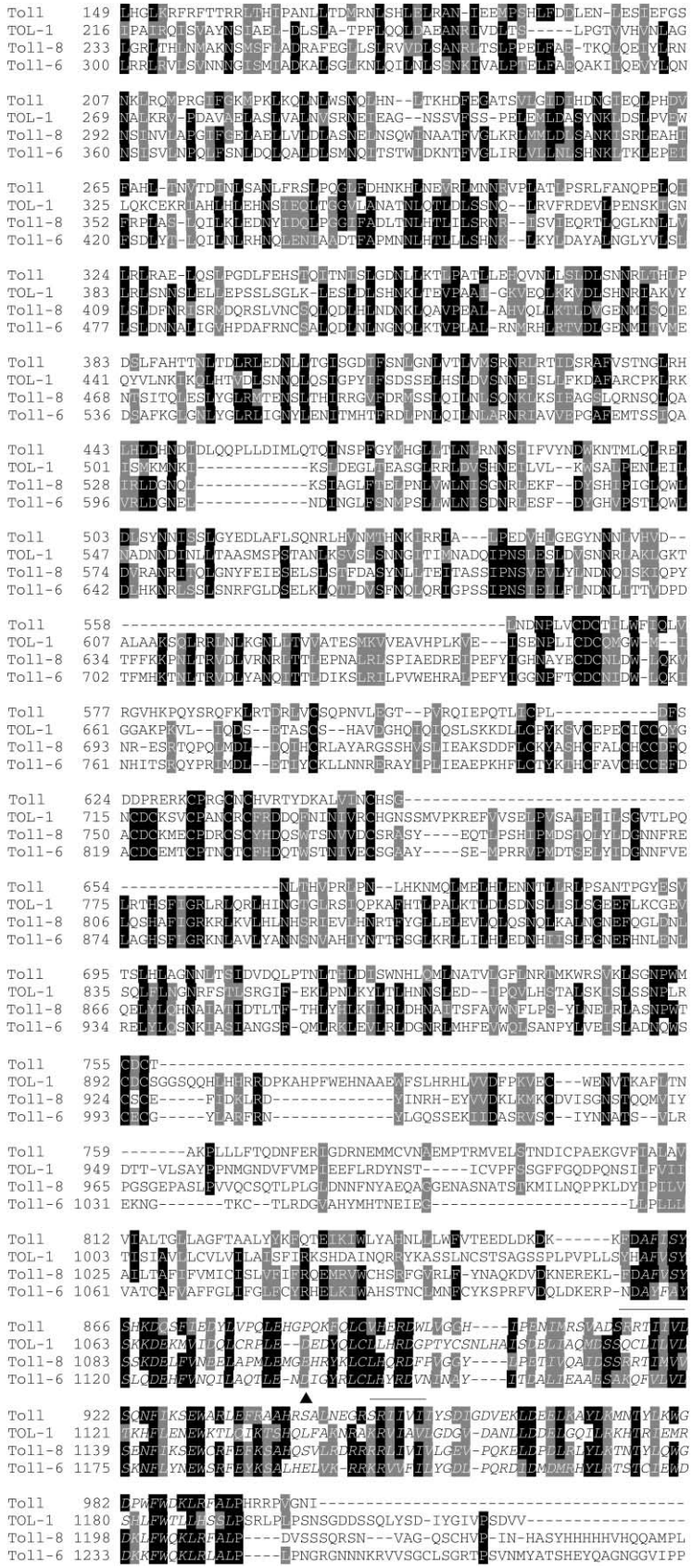
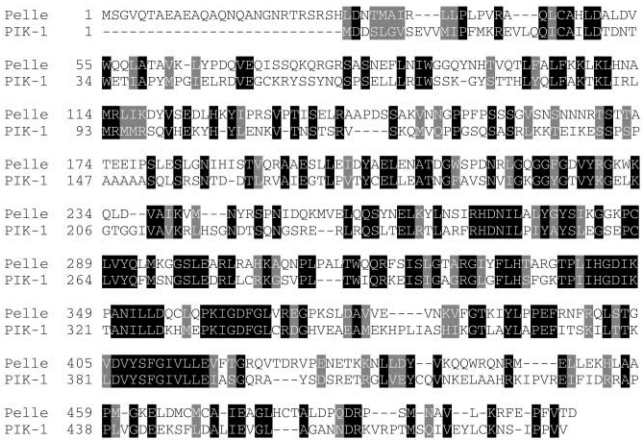


Figure 4



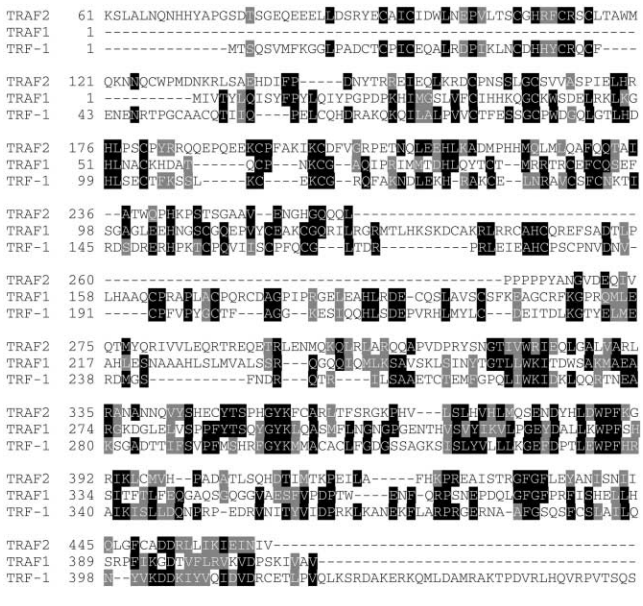
PIK-1 is aligned with Pelle. Residues that are identical in both sequences (35%) are highlighted in black, and similar residues (51%) are highlighted in gray.

During these tests, however, we observed a striking difference in the behavior of *tol-1(nr2033)* mutants when compared to N2 worms.

tol-1 mutants are deficient in pathogen avoidance

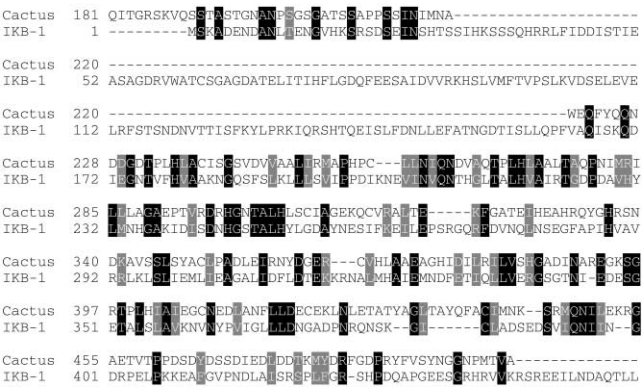
By a mechanism that is not fully understood, *C. elegans* is able to discriminate between different species of bacteria

Figure 5



The conserved region of TRF-1 is aligned with TRAF1 and TRAF2. Residues that are identical in the majority of the sequences are highlighted in black, and similar residues are highlighted in gray. The percentage of identical and similar residues for different pairwise comparisons of the partial sequences shown are (TRF-1 versus TRAF1) 20% and 35% and (TRF-1 versus TRAF2) 20% and 33%, respectively.

Figure 6



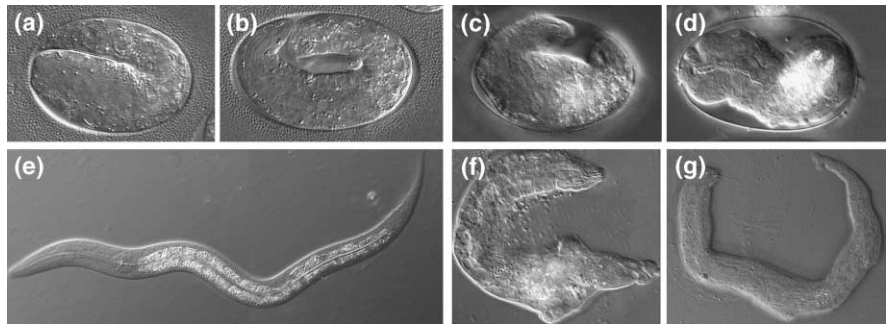
The conserved region of IKB-1 is aligned with Cactus. Residues that are identical in both sequences (12%) are highlighted in black, and similar residues (21%) are highlighted in gray.

[42, 43]. The standard laboratory food for *C. elegans* is the *Escherichia coli* strain OP50 [44], which exerts an attractive effect on worms. Worms migrate toward OP50 and then remain in close contact with the bacteria. The *S. marcescens* strain Db11 is more attractive to N2 worms than is Db1140 or OP50 (Table 2). Initially the worms remain within the bacterial lawn. Over time, however, Db11 had a strong tendency to repel N2 worms. With *tol-1(nr2033)* mutants, this response was markedly altered, and the mutants were much less likely to leave the bacteria. Consequently, while the eggs of N2 worms were frequently found outside the bacterial lawn, this was rarely the case for *tol-1(nr2033)* worms. To quantify this dynamic behavior,we counted the number of worms on the bacterial lawn after 48 hr four times over a 20 min period. In sharp contrast to the *tol-1(nr2033)* mutants, the majority of N2 worms were found outside the bacterial lawn for most of the time. The mutant phenotype of *tol-1(nr2033)* worms on Db11 was rescued by transformation with the cosmid W05D2 (Figure 9). The movement of the *tol-1(nr2033)* mutants was otherwise wild type (results not shown), and to test whether the observed defect reflected a general problem in chemorepulsion, we tested the worms' perception of a volatile repellent, 1-octanol, and a water-soluble one, copper sulfate, by using standard methods [45, 46]. In both cases, the *tol-1(nr2033)* mutants behaved just as N2 worms (Table 2), in contrast to the mutant *che-2(e1033)* previously shown to be insensitive to 1-octanol [45]. In addition, in the *tol-1(nr2033)* mutants the structure of the amphid and phasmid neurones were wild type as judged from dye filling (results not shown).

tol-1 expression in adults is restricted to the nervous system

To gain further insight into this intriguing phenotype, we analyzed the expression pattern of TOL-1 by using *GFP* fusion constructs. Two reporter constructs, p*Stol-1::GFP*,

Figure 7



Embryonic and larval lethality in the *tol-1(nr2013)* mutant. Shown are DIC images of **(a,b)** wild-type embryos, **(e)** a young L1 larva, and **(c,d,f,g)** mutant worms arrested at different stages of development. Bright patches, most obvious in panels (c) and (d) correspond to intestinal granules. The embryos show extremely severe morphological defects after the comma stage, during elongation (c,d). Some larvae are able to hatch but are completely deformed (f,g).

which contains 4 kb of sequence upstream of the *tol-1* initiation ATG, and p*tol-1::GFP*, which includes 5.5 kb of upstream sequence, were used. The latter gave expression in late gastrula embryos in a number of unidentified anterior ventral cells and several dorsal epithelial cells. The pattern of expression is consistent with the mutant phenotype observed in *tol-1(nr2013)* mutants and suggests a role for TOL-1 in elongation. Expression in the ALM neurones was observed during axonal elongation in late embryos. There was a progressive restriction in the expression domains of the two reporters, such that in adults pS*tol-1::GFP* gave expression in the 4 URY neurones (results not shown), and p*tol-1::GFP* showed additional expression in the six mechanoreceptor cells (ALML/R, AVM, PLML/R, and PVM) and six interneurones (ALNL/R, AVDL/R, and two neurones in the retrovesicular ganglion that remain to be identified; Figure 10). Expression was also seen in the head mesodermal cell [47]. Currently, no function is known for this cell. We analyzed the *tol-1* mutants for phenotypes associated with the mechanoreceptor neurones. They were wild type in *tol-1(nr2033)* mutants, both in terms of the position of their cell bodies and their axonal projections, as revealed by a *mec-7::GFP* reporter transgene (data not shown). Additionally, the mechanosensory behavior of the *tol-1* mutants appeared to be normal.

Discussion

TIR domain-containing proteins in both animals [11] and plants [48] are involved in signal transduction pathways important for innate immunity. In *Drosophila*, activation of the transmembrane receptor protein Toll by its presumed ligand, Spätzle, leads to the degradation of the cytoplasmic inhibitor Cactus, which thus releases the Rel protein Dif. This protein translocates to the nucleus, where it activates transcription of the gene encoding drosomycin, an antifungal peptide. Various *Drosophila* Toll pathway mutants, including *Dif*, have a heightened susceptibility to fungal, but not to bacterial, infection (reviewed in [49]). On the other hand, another Rel family member, Relish, controls the expression of antibacterial peptides. Its phosphorylation by a complex comprising an IKK-like catalytic subunit encoded by *ird-5* [50] and a regulatory subunit encoded by *kenny* [51, 52] is believed to lead to its activation by proteolytic cleavage. These mutants, as well as others for which the cloning has yet to be reported (*imd*, [53] *shadok*, and *galère*; Ferrandon et al., personal communication), exhibit a similar phenotype; they are highly susceptible to Gram-negative bacterial infection, but not to fungal infection. In other words, it would appear that an important part of insect immune responses rests on two distinct pathways that control the activation and/or the nuclear translocation of a single Rel-family target, Dif in the case of the antifungal response and Relish for the antibacterial response. In agreement with such a model, the promoter region of each *Drosophila* antimicrobial peptide contains a consensus Rel/NFκB binding site [11, 54]. The situation is in reality a little less clear cut, as, for example, defensin expression is under the control of both Dif and Relish (reviewed in [49]). But it remains true that

Table 1

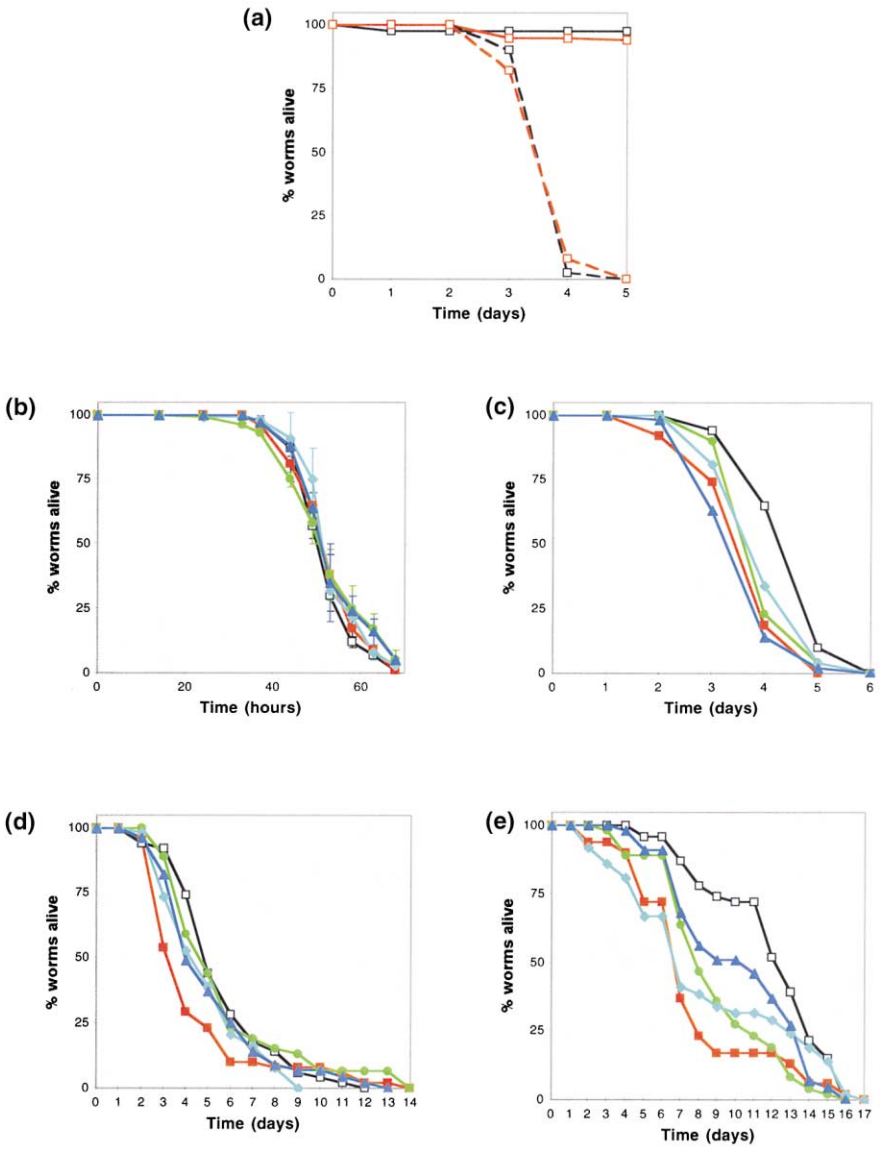
***tol-1* development phenotypes*.**

	15°C			20°C			25°C		
	wt (704)	*nr2013* (1445)	*nr2033* (854)	wt (628)	*nr2013* (2479)	*nr2033* (701)	wt (743)	*nr2013* (2173)	*nr2033* (731)
Adults	99.7	9.5	95.2	99.8	1.6	94.9	99.6	0.5	92.8
Abnormal larvae	0	24.3	2.0	0	10.9	3.3	0	7.7	1.6
Dead eggs	0.3	66.2	2.8	0.2	87.5	1.8	0.4	91.8	5.6

The number in parentheses for each column is the number of eggs tested.

* The percentage of worms showing different phenotypes under each condition is shown.

Figure 8



Survival of *C. elegans* strains in the presence of different pathogens. Starting with L4 larvae, between 40 and 50 worms were tested under each condition. **(a)** The percentage of worms alive at a given time for N2 (black) and *tol-1*(*nr2033*) (red) mutants, for control animals (unbroken line) or animals infected with the fungus *D. coniospora* (dashed line) is shown. **(b)** The percentage of worms alive at a given time for wild-type (open squares), *tol-1*(*nr2033*) (red squares), *trf-1* (green circles), *pik-1* (light-blue diamonds), and *ikb-1* mutants (dark-blue triangles) after they were placed in contact with *P. aeruginosa* PA14 under slow-killing conditionsis shown. The error bars represent the standard error from three independent trials. **(c,d,e)** The percentage of worms alive at a given time for different strains in the presence of (c) Db11, (d) Db1140, and (e) OP50; the symbols are as in (b). The results of one representative trial with 50 worms under each condition are shown.

Rel/NFκB proteins are of central importance in *Drosophila* innate immunity. They also play this role in vertebrates [9]; they are involved, for example, in the response to bacterial lipopolysaccharide (LPS). The Toll pathway also acts during the establishment of the dorsal-ventral axis in the *Drosophila* embryo [7], a fact that provokes questions concerning the evolutionary emergence of two different aspects of multicellularity: the recognition of nonself and cell identity [55].

Our initial interest in the four *C. elegans* genes described here was driven by sequence analyses that suggested that the nematode might too possess such a conserved signaling cascade involved in an innate immune response or in development. Such a putative pathway must, however, differ substantially from that of *Drosophila*. With the level of resolution possible with current search tools (see Materials and methods), *C. elegans* appears not to possess a Rel/NFκB family member, an IKK-like protein, or a homolog of Spätzle. Additionally, not one of the promoter regions of the six known genes encoding putative antimicrobial peptides in *C. elegans* [12] contains a consensus Rel/NFκB binding site. Furthermore, while in *Drosophila* there are 9 members of the TLR family, in vertebrates more than 20 [49, 56] and in *Arabidopsis*, 85 TIR-NB-LRR resistance genes [57]; in *C. elegans*, there is but one, TOL-1. In this context, it is interesting to note that TIR domain-encoding genes appear to be absent from cereal genomes [58].

Abrogation of the function of any of the genes of the

Table 2

Chemosensation in *tol-1* mutants*.

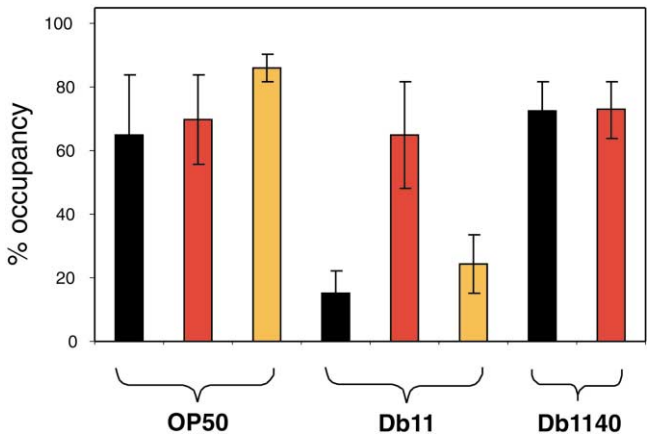
	Isoamyl alcohol	1-Octanol	Copper sulphate	Db11 versus OP50	Db1140 versus OP50	Db11 versus Db1140
wild type	0.62 ±0.16 (1926)	−0.65 ±0.08 (711)	0.03 ±0.016 (286)	0.64 ±0.01 (223)	0.64 ±0.11 (385)	0.63 ±0.01 (268)
tol-1(nr2033)	0.75 ±0.08 (1715)	−0.70 ±0.13 (864)	0.015 ±0.02 (500)	0.60 ±0.03 (313)	0.70 ±0.25 (335)	0.72 ±0.04 (239)
che-2(e1033)	−0.015 ±0.03 (481)	0.07 ±0.1 (368)	ND	ND	ND	ND

* The number in parentheses for each column is the number of worms tested. With the exception of copper sulphate, for which the aversion index [46] is shown, a chemotaxis index [45] with the average and standard deviation of at least two independent trials is presented. "ND" stands for "not determined." The chemotaxis index can vary between +1 (perfect attraction) to −1 (complete repulsion). One calculates the aversion index by dividing the number of worms that crawled through a line of copper sulphate by the total number of worms. Thus, the more closely the index approaches zero, the greater the aversion.

spätzle/Toll/tube/pelle/cactus cassette has strong deleterious effects in *Drosophila* [7]. In stark contrast, putative null alleles of the *pelle* (*pik-1*) and *cactus* (*ikb-1*) homologs in *C. elegans* exhibited no obvious phenotypes, and this result clearly indicate that these genes are not critical for nematode development. Although there is currently no *Drosophila* mutant corresponding to the *dTraf1* gene, it has been also proposed to act in a Rel/NFκB pathway [14, 15]. Again, the corresponding *C. elegans* mutant showed no obvious defect in development. When *tol-1* function was completely abrogated, on the other hand, a severe developmental phenotype was observed. Given the near wild-type phenotype of *tol-1(nr2033)*, an intact TIR domain is largely dispensable for TOL-1's role in development. This suggests that the protein might function at the level of the cell surface, where, for example, it might contribute to correct cell-cell adhesion. This would be consistent with the expression pattern of TOL-1 early in development.

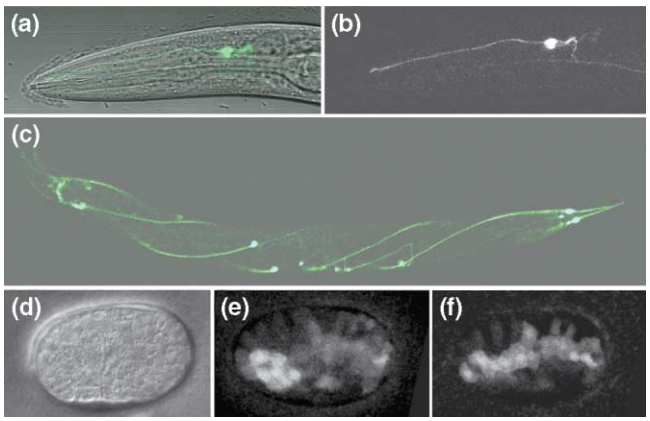
As the null allele of *tol-1* is lethal, we were not able to

Figure 9



Wild-type and *tol-1*(*nr2033*) worms behave differently when confronted by a pathogen. The percentage of N2 (black bars), *tol-1*(*nr2033*) (red bars), and rescued *tol-1*(*nr2033*)*frEx*[W05D2; pNP21] (orange bars) worms present after 48 hr on a bacterial lawn of OP50, Db11, and Db1140. The results represent the average and standard deviation from at least two independent trials with five plates of ten worms. One hour after the start of the tests, 100% of worms were present on the bacterial lawns under all conditions; at 24 hr, this was the case for >75% of the worms.

Figure 10



Expression pattern of the *tol-1* gene. **(a–c,e,f)** Confocal and **(d)** DIC images of a p*tol-1::GFP* transgenic strain; anterior is left. (a,b) Head of an adult worm showing expression in a URY neurone. These neurones have characteristically flattened endings at the anterior end of the pharynx, somewhat similar to but clearly distinct from the ciliated endings of the chemosensory neurones. (c) Expression in the six mechanoreceptors (ALML/R, PLML/R, AVM, and PVM); "nr" indicates a nerve ring. (d,e) Ventral view of a late gastrula embryo; some anterior cells show expression of GFP. (f) Dorsal view; two rows of epithelial cells express GFP.

address directly the role of TOL-1 in nematode defenses. Rather, we used the *tol-1(nr2033)* allele, which, by analogy with the homologous proteins in other species, would be predicted to be a strong loss-of-function allele. Using a number of different models of infection, we were unable to demonstrate a role for the *tol-1*, *trf-1*, *pik-1*, and *ikb-1* genes in modulating the susceptibility of *C. elegans* to pathogens. We believe that the difference seen with *S. marcescens*, for which the infection process is comparatively lengthy, reflects intrinsic differences in the health of the mutants. If a pathogen is more quickly acting, as is the case with *P. aeruginosa*, these differences do not have time to manifest themselves (Figure 8). There remains the possibility that *tol-1*, *trf-1*, *pik-1*, and *ikb-1* do participate in a defense-related signaling pathway but that we have not yet identified a pathogen that is able to trigger its activation. Since, in contrast to *tol-1*, *pik-1* is expressed ubiquitously (results not shown), it seems likely that in the nematode the function of these two genes is uncoupled.

Despite the comparative structural simplicity of its nervous system, *C. elegans* has highly complex sensory behaviors [59–62]. We have shown that wild-type worms are able to discriminate between *E. coli* and *S. marcescens*. Although initially strongly attracted by the bacteria, with time worms avoid the *S. marcescens* strain Db11. This avoidance behavior is lost in the *tol-1(nr2033)* mutant, and this finding suggests that TOL-1 functions in a sensory pathway. In *C. elegans*, three classes of dopaminergic neurones are known to contribute to the perception of a mechanosensory stimulus from bacteria [62]. We observed expression of *tol-1* in certain (non-dopaminergic) mechanosensory neurones. Thus, the mutant phenotype could conceivably result from a defect in mechanosensation. On the other hand, it may be that the URY neurones are responsible for the altered behavior of the *tol-1(nr2033)* mutant. Based on their morphology and connectivity, they have previously been suggested to function as sensory receptors [63]. The role of the head mesodermal cell is also unknown, and it could also contribute to the observed phenotype. We propose that TOL-1 contributes to the recognition of a specific bacterial component and results in a change in *C. elegans* behavior such that the nematode has a tendency to avoid potentially harmful bacteria. This avoidance phenotype was not observed with the related *S. marcescens* strain Db1140.

Db1140 is a mutated derivative of Db11 and was selected for its reduced secretion of proteases [41]. It is attenuated in its virulence during infection in insects, probably due in part to the fact that it is more sensitive than Db11 to the anti-bacterial peptides and proteins found in insect immune haemolymph [41]. This increased sensitivity could be linked to the reduced secretion of proteases by the bacteria. On the other hand, certain of the peptides and proteins found in the haemolymph of infected insects act against bacteria through direct interaction with LPS (see, for example, [64, 65]). The increased sensitivity of Db1140 to immune haemolymph could therefore also reflect a change in the structure of its LPS. If this is so, it raises the intriguing possibility that LPS is a ligand for TOL-1. In such a model, TOL-1 would be responsible for the discrimination between Db11 and Db1140 through their respective LPS structures. Recently, it has been shown that bacterial flagellin, which has an immunostimulatory effect in *Drosophila* [3] and in mammals [66], elicits defense responses in *Arabidopsis* via activation of an LRR receptor-like kinase, FLS2 [67]. Flagellin therefore represents another candidate for a putative TOL-1 ligand. Consistent with this is the fact that while Db11 is motile, Db1140 is not (S. Chauvet and J.J.E., unpublished data). Since, however, other motile bacteria, including *P. aeruginosa*, do not repel *C. elegans* (M.-W.T., unpublished data), further experiments will be needed to clarify the basis of this phenotype.

Conclusions

The Toll signaling pathway is involved in development and innate immunity and is conserved from *Drosophila* to mammals. We have characterized four *C. elegans* genes homologous to genes encoding components of this pathway. The data presented here indicate that three of these genes have no apparent function in nematode development, nor do they contribute to the resistance of the nematode to various infections. On the other hand, the fourth, *tol-1*, which encodes a TLR, is essential for nematode development. It also functions in pathogen recognition, as do the homologous proteins in other organisms, but in an entirely unexpected neuronal context, enabling *C. elegans* to avoid a potential pathogen.

Materials and methods

General methods and strains

The wild-type N2 Bristol strain, *tol-1(nr2033)* LGI, *tol-1(nr2013)/szT1[lon-2(e678)]* LGI; +/*szT1* LGX, *pik-1(nr2019)* LGIV, *trf-1(nr2014)* LGIII, *ikb-1(nr2027)* LGI, and *che-2(e1033)* LGX (a gift from B. Lakowski) and *muIs32* [*mec-7::GFP*] LGII (a gift from Q.L. Ch'ng and C. Kenyon) were grown at 20°C or 25°C and maintained as described [44]. *tol-1(nr2013)* was maintained at 15°C. The *E. coli* strain OP50 was obtained from the *Caenorhabditis* Genetics Center, and *S. marcescens* strains Db11 and Db1140 were a gift from D. Ferrandon.

tol-1::GFP *expression*

To monitor *tol-1* expression, we constructed a translational p*tol-1::GFP* fusion, pNR64. This contained 5.5 kb of genomic sequence upstream of the start codon of *tol-1*, (amplified with primers FP4 5′-AACCAATCT GCAGCAGACAGGCCTGAGGCAGGC-3′ and RSP1 5′-CTAGCCA CTAGTCGTTGTGTCATGTGATCTGG-3′ harbouring restriction sites for Pst I and Spe I at the 5′ and 3′ ends, respectively) fused to the *GFP* coding region of pPD117.01 (a gift from A. Fire). A second construct, p*Stol-1::GFP*, containing 4 kb of upstream sequence was made in a similar fashion, but with the primers F15 5′-GCGCGCTGCCGCAACT GATTTTGCAGGGTTAAGACTTGCTG-3′ and R19 5′-GCGCGTCGA CATTTCGGGCATTCGTCACCGTTAGCAGC-3′, which contain Sph I and Sal I restriction sites, respectively. Transgenic arrays were generated with standard techniques [68]. Transgenic animals were identified by

the dominant Roller phenotype conferred by coinjected plasmid pRF4 (that contains *rol-6(su1006)* DNA).

Isolation of deletion mutants and rescue of tol-1

Chemically mutagenized nematode populations were screened by PCR for deletion mutations in the desired target gene as previously described [26]. Mutants in *tol-1* (alleles *nr2013* and *nr2033)*, *pik-1(nr2019)*, *trf-1(nr2014)*, and *ikb-1(nr2027)* were obtained. The *tol-1(nr2013)*, *tol-1(nr2033)*, and *pik-1* strains were backcrossed twice and confirmed by PCR. The breakpoints of each deletion were determined by sequencing. Transformation rescue of *tol-1(nr2013)* was obtained by coinjection into the balanced strain of an injection mix containing pRF4 at 150 ng/μl and cosmid W05D2 at 15 ng/μl. Nonrolling transgenic worms containing W05D2 were obtained by coinjection into *tol-1(nr2033)* worms of pNP21 [69] at 200 ng/μl and W05D2 at 5 ng/μl.

RNAi of tol-1

DNA templates for RNA synthesis were either the cDNA clone yk412a2, corresponding to the last two exons of the *tol-1* gene, or a PCR amplicon, corresponding to 1319 bp of genomic DNA from the 12th exon of the *tol-1* gene, flanked by T7 polymerase promoter sequences (amplified with primers TL1-T7/TL2 5′-TAATACGACTCACTATAGGCGACGAA GGTCTCACAGAAG/TCGTATCGTTCGTCAGGAAG-3′ and TL2-T7/ TL1 5′-TAATACGACTCACTATAGGTCGTATCGTTCGTCAGGAAG/ CGACGAAGGTCTCACAGAAG-3′). Single-stranded (sense and antisense) RNA molecules were synthesized in vitro with T3 or T7 polymerase and purified on QIAquick columns (Qiagen). Double-stranded RNA was generated by the mixing of sense and antisense RNA in water at 4°C prior to injection at a concentration of 9 mg/ml into L4 worms.

Developmental and behavioral tests

Adults of the desired genotype were raised at 15°C, 20°C, or 25°C, were allowed to lay eggs for one day, and were then removed. The numbers of unhatched eggs, morphologically abnormal larvae, and adults were counted on subsequent days. To test the importance of maternal expression of *tol-1*, we crossed *tol-1(nr2013)* hermaphrodites with wild-type males and analyzed the progeny of the resulting heterozygotes at 25°C as above. The relative attraction and repulsion of worms to volatile compounds (iso-amyl alcohol and 1-octanol, both diluted 1/10 in ethanol) was scored as previously described, with ethanol as the counterattractant [45]. The relative attraction of worms to different bacteria was scored in a similar manner. Briefly, worms were placed between small spots of two bacterial strains at opposite sides of an otherwise empty 10 cm NGM agar plate, and the number of worms in each spot after 6 hr was counted. Additionally, to measure the balance between repulsion and attraction of worms by different bacteria, we placed worms at the center of a small spot of bacteria on an NGM agar plate. Worms were transferred to fresh plates after 24 hr. The number of worms within and outside the circumference of the spot after 48 hr was counted four times, with a 5 min interval between each count, and an average percentage occupancy was calculated. Repulsion from 150 mM copper sulfate was measured essentially as described [46]. The response to body touch was assayed as previously described [70]. The axonal morphology of the mechanoreceptor cells was observed in a *tol-1(nr2033)*; *muIs32* strain obtained by mating.

C. elegans *killing assays with* D. coniospora

D. coniospora was cultured and used to infect *C. elegans* essentially as previously described [36]. L4 stage hermaphrodite worms were placed in the presence of fungal spores for 4 hr and then picked to clean plates. Plates were incubated at 25°C and scored for live and dead worms each day. A worm was considered dead when it no longer responded to touch. Any worms that died as a result of getting stuck to the wall of the plate were excluded from the analysis. To follow the growth of the fungal hyphae, we picked dead worms to plates without bacteria after three days and observed them by video time-lapse microscopy for 15 hr.

C. elegans *killing assays with bacteria*

Assays of *P. aeruginosa* "fast killing" and "slow killing" were performed as previously described [71]. Briefly, 10 μl of an overnight *P. aeruginosa* PA14 culture grown in King's B broth was spread on a 3.5 cm diameter NGM agar plate and incubated at 37°C for 24 hr. After 12–24 hr at room temperature (23–25°C), each plate was seeded with 40–50 L4 stage hermaphrodite worms from the test strains. Plates were incubated at 25°C and scored for live and dead worms every 4–8 hr as above. *E. coli* OP50 was used as a negative control. Assays for *S. marcescens* killing were conducted in a similar manner, except that the bacteria were grown in LB and spread on NGM agar plates. Ten L4 stage hermaphrodite worms from the test strains were placed on each plate. Plates were incubated at 25°C and scored for live and dead worms every 24 hr as above. During the first few days, worms were transferred to fresh plates daily.

Gene identification and characterization

BLAST searches [72] were performed by the use of the relevant *Drosophila* and vertebrate proteins as queries against *C. elegans* genomic DNA and EST sequence databases (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml and http://www.ddbj.nig.ac.jp/c-elegans/html/CE_BLAST.html, respectively). Additional BLAST searches against the GenBank nonredundant databases of protein and nucleotides (http://www.ncbi.nlm.nih.gov/BLAST/) with the identified *C. elegans* genes as the query sequences were used for confirming the supposed relationships. Partial cDNA molecules, kindly provided by Y. Kohara, and specific RT-PCR fragments were sequenced. This led to the refinement of the predicted structure of TOL-1, TRF-1, and PIK-1. The sequences of *tol-1*, *pik-1*, *trf-1*, and *ikb-1* have been deposited at GenBank with accession numbers AF348166, AF348167, AF348168, and AF348169, respectively.

Sequence analysis

The sequences were further analyzed as described at http://tagc.univ-mrs.fr/bioinfo/cDNAguided/; MAP multiple sequence alignments [73] were performed at http://tagc.univ-mrs.fr/bioinfo/seqUtil/options/map.html; sequence conversions took place at http://bioweb.pasteur.fr/seqanal/interfaces/readseq-simple.html; and shading with Baron and Hofmann's BOXSHADE was performed at http://www.ch.embnet.org/software/BOX_form.html. As no NFκB/Rel/dorsal family member was found with BLAST searches, a more thorough search was undertaken. All known family members contain the short motif F-R-Y-x-C-E-G (see InterPro entry IPR000451). Direct searches with G. Mennessier's BioMotif (ftp://ftp.lpm.univ-montp2.fr:7084/pub/BioMotif) of Wormpep19, and of the entire available *C. elegans* genomic sequence for a DNA sequence potentially encoding this motif, were unproductive. In the case of IKK homologs, no *C. elegans* predicted protein that was more similar to a known IKK sequence than to another unrelated kinase was found. It is very unlikely that the missing homologs exist given that currently about 500 kb of genomic sequence remains to be determined (A. Coulson, personal communication). No nematode MyD88 homolog was identified either. Searches for consensus Rel/NFκB binding sites (GGGRNNY YCC or GRGAAANCC) in the predicted promoter regions of the six putative antimicrobial peptides in the *C. elegans* genome [12] were performed within the ACeDB database [74]. Genome-wide searches revealed the presence of 9,248 and 19,531 such sites, respectively.

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References

1. Boman HG, Nilsson I, Rasmuson B: **Inducible antibacterial defence system in *Drosophila***. *Nature* 1972, **237:**232-235.
2. Meister M, Lemaitre B, Hoffmann JA: **Antimicrobial peptide defense in *Drosophila***. *Bioessays* 1997, **19:**1019-1026.
3. Lemaitre B, Reichhart JM, Hoffmann JA: ***Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms.** *Proc Natl Acad Sci USA* 1997, **94:**14614-14619.
4. Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, Gonzalez-Crespo S, *et al.*: **Dif, a dorsal-related gene that mediates an immune response in *Drosophila***. *Cell* 1993, **75:**753-763.
5. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA: **The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults.** *Cell* 1996, **86:**973-983.
6. Meng X, Khanuja BS, Ip YT: **Toll receptor-mediated *Drosophila* immune response requires Dif, an NF- kappaB factor.** *Genes Dev* 1999, **13:**792-797.
7. Morisato D, Anderson KV: **Signaling pathways that establish the dorsal-ventral pattern of the *Drosophila* embryo.** *Annu Rev Genet* 1995, **29:**371-399.
8. Janeway CA Jr: **Presidential address to the American Association of Immunologists. The road less traveled by: the role of innate immunity in the adaptive immune response.** *J Immunol* 1998, **161:**539-544.
9. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA: **Phylogenetic perspectives in innate immunity**. *Science* 1999, **284:**1313-1318.
10. Kopp EB, Medzhitov R: **The Toll-receptor family and control of innate immunity**. *Curr Opin Immunol* 1999, **11:**13-18.
11. Anderson KV: **Toll signaling pathways in the innate immune response.** *Curr Opin Immunol* 2000, **12:**13-19.
12. Tan MW, Ausubel FM: ***Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis.** *Curr Opin Microbiol* 2000, **3:**29-34.
13. Chauvet S, Ewbank JJ: ***Caenorhabditis elegans*: un modèle d'étude des interactions hôte-organisme pathogène.** *M/S médecine sciences* 2000; **16:**912-916.
14. Medzhitov R, Janeway C Jr: **Innate immune recognition: mechanisms and pathways**. *Immunol Rev* 2000, **173:**89-97.
15. Zapata JM, Matsuzawa S, Godzik A, Leo E, Wasserman SA, Reed JC: **The *Drosophila* tumor necrosis factor receptor-associated factor-1 (DTRAF1) interacts with Pelle and regulates NFkappaB activity.** *J Biol Chem* 2000, **275:**12102-12107.
16. Hultmark D: **Macrophage differentiation marker MyD88 is a member of the Toll/IL-1 receptor family.** *Biochem Biophys Res Commun* 1994, **199:**144-146.
17. Parker JE, Coleman MJ, Szabo V, Frost LN, Schmidt R, van der Biezen EA, *et al.*: **The Arabidopsis downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6**. *Plant Cell* 1997, **9:**879-894.
18. Schultz J, Ponting CP, Hofmann K, Bork P: **SAM as a protein interaction domain involved in developmental regulation**. *Protein Sci* 1997, **6:**249-253.
19. Buchanan SG, Gay NJ: **Structural and functional diversity in the leucine-rich repeat family of proteins.** *Prog Biophys Mol Biol* 1996, **65:**1-44.
20. Imler JL, Hoffmann JA: **Toll and Toll-like proteins: an ancient family of receptors signalling infection.** *Rev Immunogenet* 2000, **2:**294-304.
21. Shelton CA, Wasserman SA: **pelle encodes a protein kinase required to establish dorsoventral polarity in the Drosophila embryo.** *Cell* 1993, **72:**515-525.
22. Liu H, Su YC, Becker E, Treisman J, Skolnik EY: **A *Drosophila* TNF-receptor-associated factor (TRAF) binds the ste20 kinase Misshapen and activates Jun kinase.** *Curr Biol* 1999, **9:**101-104.
23. Geisler R, Bergmann A, Hiromi Y, Nusslein-Volhard C: **cactus, a gene involved in dorsoventral pattern formation of Drosophila, is related to the I kappa B gene family of vertebrates.** *Cell* 1992, **71:**613-621.
24. Wajant H, Muhlenbeck F, Scheurich P: **Identification of a TRAF (TNF receptor-associated factor) gene in *Caenorhabditis elegans*.** *J Mol Evol* 1998, **47:**656-662.
25. Rich T, Allen R, Trowsdale J: **How low can toll go?** *Trends Genet* 2000, **16:**292-294.
26. Liu LX, Spoerke JM, Mulligan EL, Chen J, Reardon B, Westlund B, *et al.*: **High-throughput isolation of *Caenorhabditis elegans* deletion mutants**. *Genome Res* 1999, **9:**859-867.
27. Mello C, Fire A: **DNA transformation**. In: *Methods in Cell Biology. Caenorhabditis elegans: Modern Biological Analysis of an Organism*, vol. 48. Edited by Epstein HF, Shakes DC, pp. 451-482. San Diego: Academic Press; 1995:451-482.
28. Belvin MP, Anderson KV: **A conserved signaling pathway: the *Drosophila* toll-dorsal pathway**. *Annu Rev Cell Dev Biol* 1996, **12:**393-416.
29. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC: **Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*.** *Nature* 1998, **391:**806-811.
30. Linford MB: **Stimulated activity of natural enemies of nematodes.** *Science* 1937, **85:**123-124.
31. Drechsler C: **Some hyphomycetes parasitic on free-living terricolous nematodes.** *Phytopathology* 1941, **31:**773-802.
32. Sohlenius B: **Influence of microorganisms and temperature upon some Rhabditid nematodes.** *Pedobiologia* 1968, **8:**137-145.
33. Barron GL: **Nematophagous fungi: new species of the Langenidiales endoparasitic on *Rhabditis*.** *Antonie van Leeuwenhoek* 1976, **42:**131-139.
34. Mendoza De Gives PM, Davies KG, Clark SJ, Behnke JM: **Predatory behaviour of trapping fungi against *srf* mutants of *Caenorhabditis elegans* and different plant and animal parasitic nematodes.** *Parasitology* 1999, **119:**95-104.
35. Jansson HB, Jeyaprakash A, Zuckerman BM: **Differential adhesion and infection of nematodes by the endoparasitic fungus *Meria coniospora* (*Deuteromycetes*).** *Appl Envir Microbiol* 1985, **49:**552-555.
36. Jansson HB: **Adhesion of conidia of *Drechmeria coniospora* to *Caenorhabditis elegans* wild type and mutants.** *J Nematol* 1994, **26:**430-435.
37. Williams MJ, Rodriguez A, Kimbrell DA, Eldon ED: **The 18-wheeler mutation reveals complex antibacterial gene regulation in *Drosophila* host defense.** *EMBO J* 1997, **16:**6120-6130.
38. Hodgkin J, Kuwabara PE, Corneliussen B: **A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*.** *Curr Biol* 2000, **10:**1615-1618.
39. Kurz CL, Ewbank JJ: ***Caenorhabditis elegans* for the study of host-pathogen interactions.** *Trends Microbiol* 2000, **8:**142-144.
40. Flyg C, Kenne K, Boman HG: **Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to Cecropia immunity and a decreased virulence to *Drosophila*.** *J Gen Microbiol* 1980, **120:**173-181.
41. Flyg C, Xanthopoulos KG: **Insect pathogenic properties of *Serratia marcescens*. Passive and active resistance to insect immunity studied with protease-deficient and phage-resistant mutants.** *J Gen Microbiol* 1983, **129:**453-464.
42. Andrew PA, Nicholas WL: **Effect of bacteria on dispersal of *Caenorhabditis elegans* (Rhabditidae).** *Nematologica* 1976, **22:**451-461.
43. Grewal PS, Wright DJ: **Migration of *Caenorhabditis elegans* (Nematoda: Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus.** *Fundamental and Applied Nematology* 1992, **15:**159-166.
44. Brenner S: **The genetics of *Caenorhabditis elegans*.** *Genetics* 1974, **77:**71-94.
45. Bargmann CI, Hartwieg E, Horvitz HR: **Odorant-selective genes and neurons mediate olfaction in *C. elegans*.** *Cell* 1993, **74:**515-527.
46. Wicks SR, de Vries CJ, van Luenen HG, Plasterk RH: **CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*.** *Dev Biol* 2000, **221:**295-307.
47. Hedgecock EM, Culotti JG, Hall DH, Stern BD: **Genetics of cell**

and axon migrations in *Caenorhabditis elegans*. *Development* 1987, **100:**365-382.
48. Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND: **Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily.** *Plant J* 1999, **20:**317-332.
49. Imler JL, Hoffmann JA: **Signaling mechanisms in the antimicrobial host defense of *Drosophila*.** *Curr Opin Microbiol* 2000, **3:**16-22.
50. Lu Y, Wu LP, Anderson KV: **The antibacterial arm of the *Drosophila* innate immune response requires an IkappaB kinase.** *Genes Dev* 2001, **15:**104-110.
51. Rutschmann S, Jung AC, Hetru C, Reichhart JM, Hoffmann JA, Ferrandon D: **The Rel protein DIF mediates the antifungal but not the antibacterial host defense in *Drosophila*.** *Immunity* 2000, **12:**569-580.
52. Rutschmann S, Jung AC, Zhou R, Silverman N, Hoffmann JA, Ferrandon D: **Role of *Drosophila* IKKγ in a Toll-independent antibacterial immune response.** *Nat Immunol* 2000, **1:**342-347.
53. Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, Georgel P, *et al.*: **A recessive mutation, immune deficiency (*imd*), defines two distinct control pathways in the *Drosophila* host defense.** *Proc Natl Acad Sci USA* 1995, **92:**9465-9469.
54. Engstrom Y, Kadalayil L, Sun SC, Samakovlis C, Hultmark D, Faye I: **kappa B-like motifs regulate the induction of immune genes in *Drosophila*.** *J Mol Biol* 1993, **232:**327-333.
55. Wasserman SA: **Toll signaling: the enigma variations.** *Curr Opin Genet Dev* 2000, **10:**497-502.
56. Tauszig S, Jouanguy E, Hoffmann JA, Imler JL: **Toll-related receptors and the control of antimicrobial peptide expression in *Drosophila*.** *Proc Natl Acad Sci USA* 2000, **97:**10520-10525.
57. The Arabidopsis Genome Initiative: **Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*.** *Nature* 2000, **408:**796-815.
58. Pan Q, Wendel J, Fluhr R: **Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes.** *J Mol Evol* 2000, **50:**203-213.
59. Chou JH, Troemel ER, Sengupta P, Colbert HA, Tong L, Tobin DM, *et al.*: **Olfactory recognition and discrimination in *Caenorhabditis elegans*.** *Cold Spring Harb Symp Quant Biol* 1996, **61:**157-164.
60. Colbert HA, Bargmann CI: **Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*.** *Learn Mem* 1997, **4:**179-191.
61. Mori I: **Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*.** *Annu Rev Genet* 1999, **33:**399-422.
62. Sawin ER, Ranganathan R, Horvitz HR: ***C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway.** *Neuron* 2000, **26:**619-631.
63. White JG, Southgate E, Thomson JN, Brenner S: **The structure of the nervous system of the nematode *C. elegans*.** *Philos Trans R Soc Lond B Biol Sci* 1986, **314B:**1-340.
64. Carlsson A, Nystrom T, de Cock H, Bennich H: **Attacin–an insect immune protein–binds LPS and triggers the specific inhibition of bacterial outer-membrane protein synthesis.** *Microbiology* 1998, **144:**2179-2188.
65. Axen A, Carlsson A, Engstrom A, Bennich H: **Gloverin, an antibacterial protein from the immune hemolymph of Hyalophora pupae.** *Eur J Biochem* 1997, **247:**614-619.
66. Ciacci-Woolwine F, McDermott PF, Mizel SB: **Induction of cytokine synthesis by flagella from gram-negative bacteria may be dependent on the activation or differentiation state of human monocytes.** *Infect Immun* 1999, **67:**5176-5185.
67. Gomez-Gomez L, Boller T: **FLS2: an LRR receptor–like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*.** *Mol Cell* 2000, **5:**1003-1011.
68. Mello CC, Kramer JM, Stinchcomb D, Ambros V: **Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences.** *EMBO J* 1991, **10:**3959-3970.
69. Pujol N, Torregrossa P, Ewbank JJ, Brunet JF: **The homeodomain protein CePHOX2/CEH-17 controls antero-posterior axonal growth in *C. elegans*.** *Development* 2000, **127:**3361-3371.
70. Chalfie M, Sulston J: **Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*.** *Dev Biol* 1981, **82:**358-370.
71. 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:**715-720.
72. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215:**403-410.
73. Huang X: **On global sequence alignment.** *Comput Appl Biosci* 1994, **10:**227-235.
74. Eeckman FH, Durbin R: **ACeDB and macace.** *Methods Cell Biol* 1995, **48:**583-605.
75. Blumenthal T: **Trans-splicing and polycistronic transcription in *Caenorhabditis elegans*.** *Trends Genet* 1995, **11:**132-136.
76. Slack JL, Schooley K, Bonnert TP, Mitcham JL, Qwarnstrom EE, Sims JE, *et al.*: **Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways.** *J Biol Chem* 2000, **275:**4670-4678.