

Review

## Tackling both sides of the host–pathogen equation with *Caenorhabditis elegans*

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### Abstract

If one is interested in dissecting the complex interactions that exist between host and pathogen, the nematode worm *Caenorhabditis elegans* is perhaps not the first model host that comes to mind. In this review I will introduce 'the worm' and try to show how it is, in fact, well suited to the identification of universal virulence factors and holds great promise for the study of conserved mechanisms of innate immunity. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

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### 1. *Caenorhabditis elegans* as a model animal

*Caenorhabditis elegans* is a free-living (as opposed to parasitic) hermaphroditic nematode, which grows to 1 mm in length and is normally found in the soil (Fig. 1). Experimental study of *C. elegans* was started more than half a century ago by Dougherty, who with his colleagues Hansen, Nigon, and Nicholas, promoted its use as a model organism (reviewed in [1]). Work with *C. elegans* only really took off when it was chosen by Brenner as a genetically tractable model for the study of development and behaviour [2]. In his first genetic screens he found numerous visible mutant phenotypes, such as worms that had problems with locomotion, the *uncoordinated* or *unc* mutants, and those with *egg-laying* defects (the *egl* mutants). Over the last 20 years, the number of groups working with worms has grown constantly, and there are now probably close to 4000 researchers world-wide, all using the same strain of *C. elegans*, 'N2, Bristol'. In parallel, the number of published articles on *C. elegans* has increased exponentially over the last 20 years (Fig. 2), and continues to grow.

The experimental advantages associated with *C. elegans* include simple growth conditions and a rapid generation time. Under normal laboratory conditions, worms are cultivated on a solid agar medium in Petri dishes, and feed on a

lawn of *Escherichia coli*. Each adult hermaphrodite can lay between 250 and 300 eggs. They grow happily at temperatures up to 25 °C, when their development is complete in a couple of days. Worms have a life span of a little under 3 weeks at 25 °C, longer if the temperature is lower. Worms can be vortexed, centrifuged, and frozen. They can also be grown in liquid culture, and increasingly, in an industrial setting, are grown in 96- or 384-well plates with robotic handling. There now exists an automated sorter for *C. elegans*, similar to that described for fruit fly embryos [3], and capable of rapidly sorting worms based on their size or fluorescence.

Every adult *C. elegans* contains precisely the same number of somatic cells. These are almost invariant in identity and position between individuals. In the 1980s, Sulston (now Sir John) and colleagues determined all the cell lineages. This task was made possible by the fact that worms are fully transparent, allowing one these days to use GFP reporters in living animals to conduct dynamic studies at the cellular and intracellular level. A large number of phenotypically defined mutants are known and more than 3000 different mutant strains are available from the *Caenorhabditis* Genetics Centre (CGC, University of Minnesota). The majority of the mutations have been mapped genetically, giving high-resolution genetic maps for each of the worm's five autosomes and single sex chromosome. In addition, the *C. elegans* Genome Sequencing Consortium has completed the sequence of over 99% of the worm's genome. The Consortium makes freely available to the

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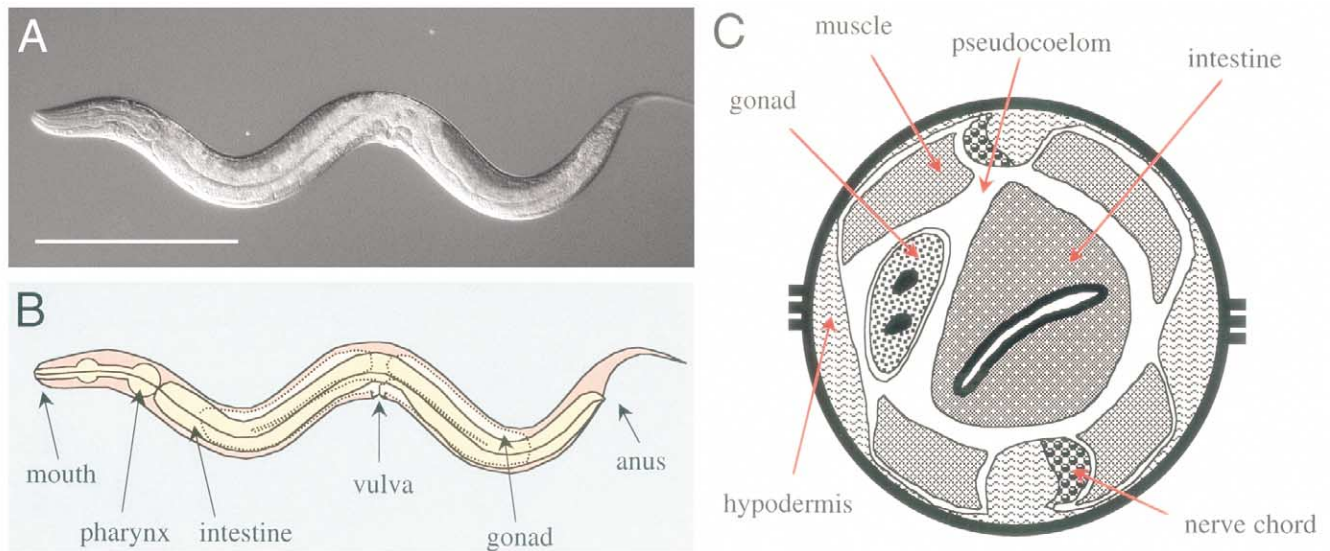


Fig. 1. Basic anatomy of *C. elegans*. A. Photomicrograph of a young adult hermaphrodite worm. The scale bar corresponds to approximately 250  $\mu\text{m}$ . B. Schematic representation of the worm in A, showing the disposition of the major organs. C. Diagram of a posterior cross-section through an adult hermaphrodite (adapted from [72]). The three lateral projections on each side are cuticle specialisations, the alae.

research community ordered cosmids and YACs that encompass the entire genome.

The combination of good genetic maps, complete physical coverage of the genome and an efficient system for the generation of transgenic animals [4], makes it relatively straightforward to positionally clone a gene responsible for a mutant phenotype. There are also sophisticated tools available for the *in vivo* study of gene function, including protocols for target-selected gene inactivation [5,6], that are being applied at the genome scale (reviewed in [7]). Currently, other than the standard worm books [8,9], the best starting points for the novice are the two main worm

web sites, Wormbase ([www.wormbase.org](http://www.wormbase.org)) and the server maintained by L. Avery ([elegans.swmed.edu](http://elegans.swmed.edu)).

## 2. *C. elegans* as a host

*C. elegans* is considered to be benign, but many other nematode species are obligate plant parasites and represent important agricultural pests. There has been a long-standing interest in the pathogens of nematodes, since they represent potential biological control agents (see for example, [10] and references therein). This is especially the case these days as replacements for chemical nematicides are being sought. Thus for plant parasitic nematodes, a number of pathogens have been characterised (see for example, <http://sacs.cpes.peachnet.edu/nemabc/>). Certain known nematode-trapping fungi are also capable of capturing *C. elegans* [11]. Until recently, however, only one infection had been described in any detail for *C. elegans*: that by the endoparasitic fungus *Drechmeria coniospora* (formerly *Meria coniospora*) [12].

*D. coniospora* spores can adhere to the head of a nematode, then extend hyphae into the body, via the open ends of sensory neurons [13,14]. For the first day or two, the worms appear healthy, but then die and rapidly become engulfed by the hyphae that sprout from the inside (Fig. 3). Due to practical difficulties associated with the cultivation of the fungus, and the absence of molecular genetic tools for its study, this model has remained something of a curiosity. The remainder of this review will focus entirely on the very active field of investigation concerning interactions between *C. elegans* and bacterial pathogens.

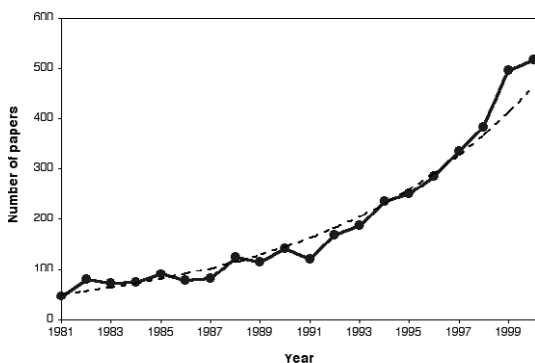


Fig. 2. Exponential rise in the number of *C. elegans* publications. The graph shows the number of papers published on *C. elegans* over the last 20 years (from data kindly provided by T. Stiernagle of the *Caenorhabditis* Genetics Center). The dashed line is the closest fit exponential curve.

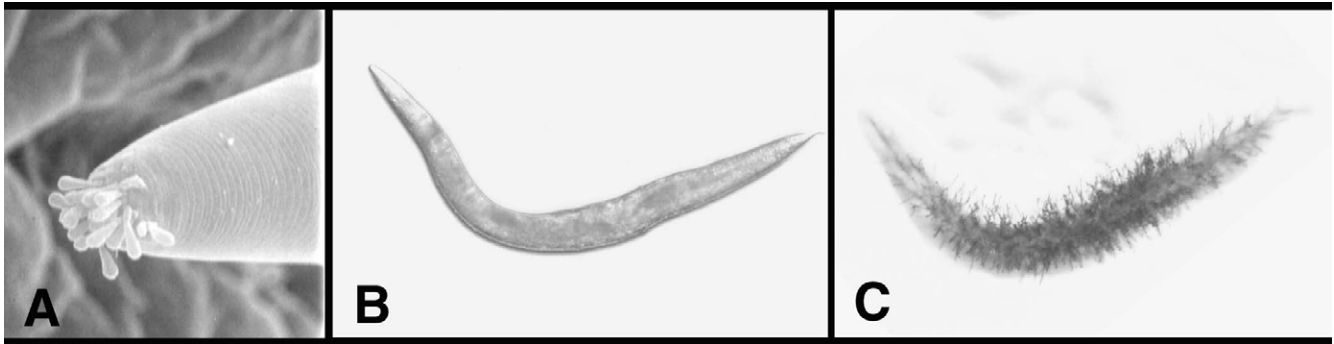


Fig. 3. Infection of nematodes by *D. coniospora*. A. Electron micrograph showing the adhesion of *D. coniospora* spores to the head of the nematode *Panagrellus redivivus* (image kindly provided by H.-B. Jansson; [13]). B. Photomicrograph of an adult *C. elegans* killed by *D. coniospora* 3 d after being put in contact with the spores. C. The worm of (B) viewed 9 h later, showing the extensive growth of fungal hyphae.

### 3. *C. elegans* as a model for defining bacterial virulence factors

It has become clear that for certain pathogens capable of infecting a broad range of organisms, there exist universal virulence factors, necessary for full pathogenicity regardless of the host. This has been most clearly demonstrated in the case of the human opportunistic pathogen *Pseudomonas aeruginosa* (reviewed in [15]). As a consequence, one can use non-mammalian model systems, such as *C. elegans*, to assay for such virulence factors. As a first step, one can test whether a given bacterium is pathogenic for *C. elegans* relatively simply. As worms eat bacteria, their normal food source, *E. coli*, can be replaced by the candidate pathogen. Some pathogens under particular culture conditions produce fast-acting nematocidal toxins (see below). Other pathogens that are relatively virulent with regards to *C. elegans*, such as certain strains of *P. aeruginosa* [16] and *Serratia marcescens* [17], establish intestinal infections, and provoke obvious signs of sickness: locomotory problems, distension of the intestine and cell lysis. These are followed by the precocious death of the worms. If a pathogen is less virulent, and does not provoke such obvious signs, such as is the case with *Shewanella massilia* (JJE and C. Couillault, unpublished results), the life span of individual worms can be measured and compared with that of worms cultivated on non-pathogenic bacteria. The Gram-positive bacterium *Microbacterium nematophilum* falls into a further category as it does not provoke a lethal infection. Rather the bacteria adhere to the cuticle of the worm, just behind the anus and provoke a pronounced swelling [18]. Altogether, a respectable list of known pathogens of *C. elegans*, provoking a range of diseases can now be drawn up (Table 1).

If a particular bacterium proves to be pathogenic for the worm, one approach to uncovering the underlying virulence factors is to conduct screens for bacterial mutants attenuated in their virulence. Practically speaking, this is easiest if the parental strain kills *C. elegans* sufficiently rapidly such that it does not permit the worm's reproduction. This was the basis for a screen of *P. aeruginosa* mutants carried out in the Ausubel laboratory. In what constituted a 'proof of prin-

ciple' of the general approach, they showed that the majority of *P. aeruginosa* mutants isolated on the basis of an attenuation of their virulence in the *C. elegans* infection model also displayed reduced virulence in a mouse model [19]. Further, using the worm, they were able to identify genes that had hitherto not been characterised as playing a role in pathogenesis (reviewed in [16]). These experiments opened the possibility of conducting similar screens using other pathogens. Results from our laboratory indicate that *C. elegans* can be used to identify candidate universal virulence factors even if one is interested in pathogens, such as *S. marcescens*, that are less virulent and support the reproduction of worms (see below). *C. elegans* is well suited to high-throughput screening methods, and the general applicability of the approach to other worm pathogens will probably be limited by two factors on the bacterial side: the

Table 1  
Known bacterial pathogens of *C. elegans*

Species	Reference
Gram-positive	
<i>Bacillus megaterium</i>	[28]
<i>Bacillus thuringiensis</i>	[30]
<i>Enterococcus faecalis</i>	[73]
<i>Microbacterium nematophilum</i>	[18]
<i>Staphylococcus aureus</i>	[73]
<i>Streptococcus pneumoniae</i>	[73]
Gram-negative	
<i>Burkholderia pseudomallei</i>	[27]
<i>B. thailandensis</i>	[27]
<i>B. cepacia</i>	[16,27]
<i>Pseudomonas aeruginosa</i>	[16]
<i>P. fluorescens</i>	[25,26]
<i>Salmonella typhimurium</i>	[39,40]
<i>Serratia marcescens</i>	[17,37]
<i>Aeromonas hydrophila</i>	a
<i>Agrobacterium tumefaciens</i>	a
<i>Erwinia chrysanthemi</i>	a
<i>E. carotovora carotovora</i>	a
<i>Shewanella massilia</i>	a
<i>S. putrefaciens</i>	a
<i>Yersinia</i> spp.	b

<sup>a</sup> JJE and C. Couillault, unpublished results.

<sup>b</sup> C. Darby, personal communication.

facility of generating transposon-induced bacterial mutants and availability of the complete genomic sequence.

Obviously, not all bacterial genes necessary for full pathogenicity in the worm will be universal virulence factors. Some may be specific in their action against the worm, and not play a role in the infection of other animals or plants. As mentioned above, many nematode species are plant parasites and provoke enormous economic losses for agriculture. Others cause serious disease in animals, including humans. The identification of bacterial genes that contribute to the nematocidal activity of a given pathogen may in the long term lead to better nematode management strategies.

#### 4. Disease models

Of the pathogens listed in Table 1, at least half a dozen are the object of active research. There follows a brief description of these disease models, starting with the most benign.

##### 4.1. *M. nematophilum*

A number of different bacterial pathogens are capable of provoking cuticular lesions in a range of nematode species [20]. The fascinating story of the chance isolation of two coryneform bacterial strains capable of attaching themselves to *C. elegans*, and consequently called *M. nematophilum*, has been recounted by Hodgkin et al. [18]. These bacteria adhere to a specific post-anal region of the worm's cuticle, replicate there and provoke a pronounced swelling of the underlying cells. Other than this, the most obvious symptom of the infection is constipation, which anticipates, but does not explain, the subsequent swelling. This induced constipation is probably responsible for the fact that infected worms develop slowly.

It is not clear whether the swelling constitutes a host defence mechanism or the subversion of a host cellular process by the bacteria. The Hodgkin laboratory is currently undertaking an analysis of the molecular basis of the swelling. They carried out a large screen of previously characterised worm mutants and showed that certain mutants with an altered surface antigenicity (the *srf-2*, *-3* and *-5* mutants) are resistant to the bacteria. The most likely explanation for this is that the bacteria are simply unable to adhere to the abnormal cuticle [18]. Genetic screens for bacterially unswollen (*bus*) mutants are currently underway (J. Hodgkin, personal communication). The molecular cloning of the *bus* genes may shed light on the different steps of the infection.

##### 4.2. *P. aeruginosa*

As explained above, and reviewed in [16,21,22], *P. aeruginosa* was the first bacterial pathogen of *C. elegans*

to be well characterised. The Ausubel group has concentrated on the clinical isolate PA14 [23]. Under conditions of high osmolarity the bacterium produces low-molecular-weight toxins that kill worms in 4–24 h, so-called 'fast killing'. These are thought to act via the generation of free-radicals and consistent with this, *C. elegans* mutants resistant to oxidative stress are more resistant to fast killing than wild-type worms. Exposure of young worms to a sub-lethal environmental stress, such as a mild heat shock, or low concentrations of sodium hypochlorite, subsequently protected them against fast killing. Conversely, worms that lack functional P glycoprotein efflux pumps are hypersensitive to fast killing [24]. Otherwise, under conditions of low osmolarity, the bacterium colonises the worm intestine and kills the animals over the course of several days [25]. Out of 2400 clones tested, eight bacterial mutants were found that were greatly attenuated for their virulence in this slow-killing model. Remarkably, seven out of the eight were also attenuated for their virulence in a murine model of *P. aeruginosa* pathogenesis [16].

A different strain of *P. aeruginosa*, PAO1, provokes a neuromuscular paralysis of *C. elegans* within seconds of contact. The paralytic action apparently requires the production of a diffusible compound, and is not seen with mutants deficient for the Rhl and Las quorum-sensing systems [26], the latter being important also for slow killing. One *C. elegans* mutant, *egl-9*, has been shown to be resistant to lethal paralysis. The molecular cloning of the gene unfortunately failed to give any hints as to its cellular role [26].

##### 4.3. *Burkholderia* spp.

The potential utility of *C. elegans* as a model has not been lost on the US army. In a recent report, a team from Fort Detrick investigated the killing of worms by the 'potential bioware agent' *Burkholderia pseudomallei* [27]. Again, killing appears to be mediated by a toxin, which was shown to be UV-labile, but gamma-ray resistant. The authors also showed that a number of characterised mutations, including those affecting the Amr multidrug efflux system, LPS O-antigen synthesis, the general protein secretion machinery (*gspD*), and the flagella (*fliC*) had little effect on this toxin-based killing. *B. thailandensis* also produces a paralysing toxin, and for both *Burkholderia* species the toxin appears to act via a disruption of calcium signalling. Thus mutations affecting the functional integrity of the worms' L-type voltage gated Ca<sup>2+</sup> channels (*egl-19* and *unc-36*) provoke an increased susceptibility to the paralysis. Interestingly, the *C. elegans* mutant *egl-9*, resistant to lethal paralysis by PAO1, was found to be somewhat more resistant than wild-type worms to *B. pseudomallei* killing [27], presumably reflecting a common mechanism for the two toxins. The Gram-positive *Bacillus megaterium* also appears to produce a fast-acting nematocidal toxin [28], but in this case, nothing is known about its mode of action.

#### 4.4. *Bacillus thuringiensis*

One group of bacterial toxins that has been much in the news recently is that produced by *B. thuringiensis* [29]. These  $\delta$ -endotoxins, known collectively as 'Bt toxin', are widely used as insecticides, but also have a nematocidal activity and are toxic for *C. elegans*. A number of different strains that kill worms have been identified [30], and the intoxication process has been studied in some detail [31–33]. The direct effect of the toxins appears to be limited to the worms' intestinal cells, and results in a pronounced distension of the intestinal lumen. Despite regression of the microvilli, and a dramatic shrinkage of the cells, with concomitant destruction of intracellular organelles, including the nucleus, both the apical and lateral intestinal membranes remain intact and no cell lysis is observed [32]. More recently, studies have been undertaken using one specific toxin, Cry5B, that alone provokes destruction of the intestine [34]. To understand better the action of this toxin, Aroian and colleagues have undertaken genetic screens for worms resistant to Cry5B. Five such *bre* mutants (for *Bacillus*-toxin resistant) were isolated. Significantly, they are not more resistant than wild-type worms to the structurally unrelated nematocidal Cry6A toxin [34]. The cloning of one of these resistance genes, *bre-5*, has now been reported. It encodes a protein homologous to mammalian  $\beta$ 1,3-galactosyltransferases. As the gene is normally expressed in gut cells, and as many bacterial pathogens and their toxins bind to carbohydrates present on host cell surfaces [35], *bre-5* may be needed for the constitution of a Cry5B target site [36]. Characterisation of the remaining *bre* mutants may give further insights into the cellular targets of the Cry5B toxin.

#### 4.5. *S. marcescens*

*S. marcescens* is a human opportunistic pathogen, increasingly associated with life threatening nosocomial infections. It is an extracellular pathogen with a very broad host range, being able to infect plants, insects and worms. A screen of 2000 transposon-induced bacterial mutants has been carried out [37]. Even though the infection is less rapid than that with *P. aeruginosa*, 1000 clones can be screened manually per month by one person. Some 10% of mutant bacterial clones were found to be partially attenuated in their virulence against *C. elegans*. Among them, the majority are also attenuated during infection of *Drosophila*, and a number were also less virulent when tested in a human cell culture model (Kurz et al., in preparation). These represent candidate universal virulence factors. One such mutant corresponds to an insertion in the *shl* operon (Kurz et al., in preparation), previously shown to contribute to the haemolytic activity of *S. marcescens* [38].

On the host side, screens for EMS-induced *C. elegans* mutants resistant to *S. marcescens* infection have so far led to the identification of nine mutants that fall into five

complementation groups (JJE and S. Chauvet, unpublished results). One will have to await the molecular identification of the underlying mutation to determine whether these different worm strains are resistant to infection for physical reasons, or because of changes in inducible defences (see below).

#### 4.6. *Salmonella typhimurium*

*Salmonella enterica* serovar *typhimurium*, a pathogen seemingly well adapted to its intracellular lifestyle in mice, is also capable of infecting and killing *C. elegans* [39,40]. Unlike in the murine host, throughout the course of the infection, the bacteria remain extracellular. Certain *S. typhimurium* mutants, however, that are less virulent in the mouse, including *phoP/phoQ* [39] and mutants that affect acid tolerance [40] are also attenuated for their virulence in the worm. As it is unlikely that modern-day *S. typhimurium* strains are nematode pathogens under natural conditions, this may well reflect the conservation of survival and virulence mechanisms acquired long before the bacterium developed its present host tropism. In this context, it is interesting to note that the *bacA* gene is functionally important for *Brucella abortus*, an intracellular mammalian pathogen, and for *Rhizobium meliloti*, a plant symbiont, during their chronic infection of their respective hosts [41]. This underscores the notion that from a bacterium's point of view all eukaryotic cells, regardless of their origin, are more alike than dissimilar and that any pathogen will be confronted by the same obstacles and opportunities in a wide range of hosts.

The Ausubel group has continued to investigate the interaction between *S. typhimurium* and worms. Starting from the observation that infected worms produced dramatically fewer progeny, potentially a consequence of increased germ cell apoptosis, they went on to show that the programmed cell death genes *ced-3* and *ced-4* partially protect the worms from the lethal consequences of infection [42]. Apoptosis in the nematode germline is also upregulated during starvation. Cell death may thus constitute a general mechanism of protection against stressful situations. Although the trigger has not been identified, the response to *S. typhimurium* appears to be relatively specific, as it is not provoked by the avirulent *phoP/phoQ* mutant, or by *P. aeruginosa* [42].

In *Drosophila*, the *ced-3*-related caspase Dredd has been lately described as contributing to the fly's defences against Gram-negative bacterial infection [43]. In this case, Dredd appears to act through activation of an NF- $\kappa$ B pathway, probably absent from worms (see below). Nevertheless, programmed cell death appears to be among the most ancient of host defence mechanisms, being seen both in plant and in higher animals [44,45]. Whether using *C. elegans* as a model will reveal hitherto unexpected facets to an ancient defence system remains to be seen.

## 5. Nematode defence strategies

In the preceding sections, mention has been made of a number of genes that appear to be important for the resistance or susceptibility of *C. elegans* to particular pathogens. But in some regards, *C. elegans* seems very poorly equipped to defend itself against infection, especially when compared with other species. In diverse invertebrates, coelomocytes (or haemocytes) are important mediators of cellular defence reactions. They are generally motile, have strong phagocytic activity and are present in variable numbers, arising from the proliferation of a stem cell population [46,47]. The coelomocytes in *C. elegans*, found in the pseudocoelom (Fig. 1), share none of these properties. They do not move more than a few microns and then only passively, and they do not appear to be capable of phagocytosis (H. Fares, personal communication). Additionally, in hermaphrodite worms, there are always just three pairs of cells, anterior and ventral, medial and ventral, and posterior and dorsal. Consequently, *C. elegans* is extremely susceptible to the intra-pseudocoelomic injection of even small numbers of non-pathogenic *E. coli* (H. Fares, personal communication). It must be assumed that under natural circumstances, pathogens do not gain access to the pseudocoelom. From the outside, worms are protected by their multi-layered cuticle. Under sterile conditions, worms can be pierced through with a fine needle without suffering any apparent ill effect, even though such wounds do not provoke the type of melanization reactions seen in many other invertebrates. Indeed the necessary genes for mounting such a response, those of the pro-phenoloxidase cascade, also implicated in blood-clotting reactions in arthropods [48,49], seem to be absent from the worm's genome. Instead, it would appear that the worm's internal hydrostatic pressure is sufficient to seal small punctures.

The weak points in this cuticular armour are the different openings to the outside world: the mouth and anus, which give access to the intestine, the vulva, which leads to the gonad, and the tips of the amphid neurones. As explained above, it is via the latter that *D. conosporia* appears to penetrate the worm. *M. nematophilum* is able to enter through the anus, while the other bacterial pathogens that establish intestinal infections listed above gain access through the mouth. Currently nothing is known about intra-uterine defences, but one would predict the gonad to be a particularly hostile environment for microbes.

Any potential pathogen wanting to reach the worm's gut via the mouth must first confront a formidable physical barrier: the grinder (Fig. 4). This ridged tri-lobed structure made of cuticle, secreted by the underlying muscle cells, is situated in the terminal bulb of the pharynx. It serves to break down bacteria, such that in young healthy worms, bacteria do not normally pass intact into the intestine. As worms get older, its efficiency decreases, which may in part explain why old worms are in general more sensitive to bacterial pathogens. Similarly, genetic abrogation of its



Fig. 4. The grinder of *C. elegans*. An electron micrograph of a cross-section through the head of a worm, at the level of the pharyngeal terminal bulb (image kindly provided by D. Hall; <http://www.aecom.yu.edu/wormem/>). The three serrated lobes of the grinder are clearly visible in the centre of the image.

function, for example in the mutant *phm-2* (for *pharynx morphology defective*), render worms hypersensitive to *Salmonella* [40] and other infections (JJE and C. Couillault, unpublished results).

The grinder, though important, is not indispensable for nematode survival. The *phm-2* mutants live happily on a diet of *E. coli*. The worm intestine contains potent degradative activities and the mutants are still capable of breaking down bacteria. The nematode gut is a cylindrical tube of 20 polyploid epithelial cells. It is bounded on the apical side by a peritrophic-like membrane that resembles those found in insects [50], and that is likely to act as a further physical barrier to pathogen attack. The intestinal cells contain abundant autofluorescent secondary lysosomes [51], and are known to be the exclusive site of expression for a range of different genes, including certain lectins [52], and aspartic [53] and cysteine proteases [54]. Worms defecate about once a minute and in doing so void entirely their intestinal contents, so the antibacterial activities present in the intestine must be rapidly acting. The worm possesses a number of saposin-like proteins [55], members of the amoebapore cytolytic superfamily [56], as well as several peptides with an antibacterial activity (Y. Kato, personal communication), related to the ASABF-type antimicrobial peptides of *Ascaris suum* [57,58]. Whether these different proteins act constitutively to aid digestion or have a role in inducible defence mechanisms against infection is currently an open question.

## 6. Inducible defences in *C. elegans*

### 6.1. The Toll pathway

The best-characterised innate immunity pathway in *Drosophila* is the Toll pathway. It was originally characterised because of its essential role in dorso-ventral patterning during early fly development. In adult flies, it is activated following exposure to fungi and leads to the nuclear import of a Rel/NF- $\kappa$ B protein. This in turn triggers the expression of potent anti-fungal peptides, protecting the fly from infection. Thus, flies deficient for this pathway are hypersensitive to infection. The pathway has been highly conserved through evolution and is a central part of vertebrate innate immunity [59,60]. For example, the murine Toll-like receptor-4 (TLR4) plays a fundamental role in an LPS-triggered signalling pathway that acts through NF- $\kappa$ B, and TLR4 knockout mice are hypersensitive to Gram-negative bacterial infections [61]. *C. elegans* appears not to possess a Rel/NF- $\kappa$ B family member. There are, however, genes homologous to three genes in the pathway, *Toll*, *pelle* and *cactus* as well as a homologue of *dTraf1*, the latter also believed to function in Rel/NF- $\kappa$ B signalling. This suggested that the Toll pathway might be conserved in part in the nematode [62]. Mutants for these four worm genes (*tol-1*, *pik-1*, *ikb-1* and *trf-1*, respectively) have now been generated and analysed [17]. For *tol-1*, null mutants show a highly penetrant temperature-sensitive lethality, possibly due to a defect in elongation. This would be consistent with the observed expression of *tol-1::GFP* reporter constructs in a subset of dorsal epithelial cells in late gastrula embryos. Mutants homozygous for a partial loss-of-function allele of *tol-1*, *nr2033*, potentially encoding a protein in which 80% of the intracellular TIR domain is missing, are healthy and fertile. Thus, in contrast to *Drosophila* Toll, the TIR domain is largely dispensable for TOL-1's developmental function. The other three genes have no apparent function in *C. elegans* development, underlining a functional divergence between the insect and nematode pathways. Further, and again in contrast to *Drosophila*, none of the four genes appear to contribute to the resistance of the nematode to various infections [17].

On the other hand, by a mechanism that is not fully understood, *C. elegans* is able to discriminate between different species of bacteria [17,28,63]. The standard laboratory food for *C. elegans* is the *E. coli* strain OP50 [2]. Worms migrate towards OP50 and then remain in close contact with the bacteria. The *S. marcescens* strain Db11 is more attractive to naïve worms than OP50. Over time, however, Db11 has a strong tendency to repel wild-type worms. With *tol-1(nr2033)* mutants, this response is greatly diminished, and they are never repelled by the pathogen.

The observed defect is specific and does not reflect a general problem in chemorepulsion nor in locomotion. In adults, *tol-1* expression is almost entirely restricted to the nervous system. We have proposed that TOL-1 contributes

to the recognition of a specific bacterial component and results in a change in *C. elegans* behaviour such that the nematode avoids the pathogenic strain Db11 [17]. The basis of such a behaviour remains to be elucidated. It is interesting to note that worms also avoid *B. megaterium* [28], *Dictyostelium* [64], as well as the nematocidal Basidiomycete *Hericium coralloides* [65], so for the worm, evasion may be an effective form of defence.

### 6.2. Using microarrays to identify inducible genes

As in *C. elegans* the Toll receptor has been recruited to a function that appears not to involve the activation of antimicrobial gene expression. To address the question of whether worms possess inducible defence mechanisms, we have been using high-density cDNA arrays [52]. By comparing gene expression in infected and non-infected populations, we have found a number of host genes are indeed up-regulated upon *S. marcescens* infection (Mallo et al., submitted). Among them, other than nematode-specific genes for which no homologue exists in the publicly available sequence databases, several have significant sequence similarity to lectins. In *C. elegans*, the lectins represent a very large class of proteins; there are, for example, predicted to be at least 125 C-type lectins [66]. Significantly, in both vertebrates [67] and invertebrates [68], certain lectins are known to play key roles in innate immunity. Although the sugar-binding properties of certain nematode lectins have been studied in great detail (see [69], for example), nothing is known about their *in vivo* function. Our results suggest that a subset of nematode lectins may well play a role in host defence.

We have also found that the transcription of certain nematode lysozyme genes can be induced upon infection. Lysozymes have long been recognised as playing an important role in innate defence reactions. Unlike vertebrates and other invertebrates, such as *Drosophila* [70], *C. elegans* does not appear to possess chicken-type lysozymes, nor homologues of the other well-characterised lysozyme protein families (see <http://www.sanger.ac.uk/Software/Pfam/>), but rather has a family of lysozymes homologous to those of the amoeboid protozoon *Entamoeba histolytica* [71]. These results strongly suggest that the worm does indeed react to infection by the induction of defence-related genes (Mallo et al., submitted). The future challenge will be to dissect the underlying molecular basis for this response. Eventually, one would hope to be able to characterise the nematode antimicrobial response in all its aspects, from the recognition of a pathogen to its elimination by the host. This may lead to the identification of evolutionary conserved mechanisms of innate immunity.

## 7. Concluding remarks

The worm has already proved its worth as an *in vivo* model for the identification of universal bacterial virulence

factors, via the isolation of attenuated mutants. Nematode defence mechanisms are currently being unravelled, and specific *C. elegans* mutants that are compromised in their antimicrobial defences are being characterised. The analysis of the combination of different host and pathogen mutants will allow one to investigate, in a systematic way, the underlying interactions at the molecular level. Thus genetically accessible model systems, such as *C. elegans*, have a great potential to aid the comprehension of host–pathogen interactions, and may eventually contribute to the elaboration of new strategies to combat infectious diseases.

#### • Note added in proof

Two important reports of studies using microarrays to analyse global patterns of gene expression in *C. elegans* and to follow defence gene induction in *Drosophila* have recently been published.

S.K. Kim, J. Lund, M. Kiraly, K. Duke, M. Jiang, J.M. Stuart, A. Eizinger, B.N. Wylie, G.S. Davidson, A gene expression map for *Caenorhabditis elegans*, *Science* 293 (2001) 2087–2092.

E. De Gregorio, P.T. Spellman, G.M. Rubin, B. Lemaitre, Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays, *Proc. Natl. Acad. Sci. USA* 98 (2001) 12590–12595.

A complementary microarray analysis of *Drosophila* immune responses has also appeared.

P. Irving, L. Troxler, T.S. Heuer, M. Belvin, C. Kopczyński, J.M. Reichhart, J.A. Hoffmann, C. Hetru, A genome-wide analysis of immune responses in *Drosophila*, *Proc. Natl. Acad. Sci. USA* 98 (2001) 15119–15124.

The *P. aeruginosa* PAO1 toxin has been identified.

L.A. Gallagher, C. Manoil, *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning, *J. Bacteriol.* 183 (2001) 6207–6214.

And the description of the worm's antimicrobial peptides has now been published.

Y. Kato, T. Aizawa, H. Hoshino, K. Kawano, K. Nitta, H. Zhang, *abf-1* and *abf-2*, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans*, *Biochem. J.* 361 (2002) 221–230.

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