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Abstract

The natural environment of the free-living nematode *Caenorhabditis elegans* is rich in pathogenic microbes. There is now ample evidence to indicate that these pathogens exert a strong selection pressure on *C. elegans*, and have shaped its genome, physiology and behaviour. In this short review, we concentrate on how *C. elegans* stands out from other animals in terms of its immune repertoire and innate immune signalling pathways. We discuss how *C. elegans* often detects pathogens because of their effects on essential cellular processes, or organelle integrity, in addition to direct microbial recognition. We illustrate the extensive molecular plasticity that is characteristic of immune defences in *C. elegans* and highlight some remarkable instances of lineage-specific innovation in innate immune mechanisms.

Keywords

Evolution, selection, model organism, infection, pathogen, innate immunity

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Introduction

The nematode *Caenorhabditis elegans* has emerged in recent years as a tractable model for dissecting innate immune mechanisms and host-pathogen interaction. There have been many reviews of the field in the last couple of years (e.g. (Garcia-Sanchez et al. 2021; Harding and Ewbank 2021; Kim and Flavell 2020; Martineau et al. 2021; Miles et al. 2019; Penkov et al. 2019; Radeke and Herman 2021; Singh and Aballay 2020; Wani et al. 2020; Willis et al. 2021)) and we will not attempt to be exhaustive here. Rather we will mainly take an evolutionary perspective to examine in what ways innate immunity in *C. elegans* resembles that of other animals and in what ways it differs. We will purposely emphasise the ways in which *C. elegans* has its own fashion to defend itself against infection.

Any such discussion of conservation and innovation needs to be framed by phylogeny. In the case of nematodes, this remained contested for many years. Some argued, initially on the basis of morphology, that nematodes were fairly distant relatives of insects, while others, citing their shared developmental moulting, placed the two together in the clade Ecdysozoa. Surprisingly, this often-intense debate continued even after the first representative genome sequences were available (e.g. (Blair et al. 2002)). There is now, however, a relatively broad acceptance of the Ecdysozoan schema (Edgecombe et al. 2011), in which, for example, the fruitfly *Drosophila melanogaster* and *C. elegans* share a common ancestor on the Protostomial branch. Thus, it is assumed that any gene found in both humans and insects would have been present in this ancestral Ecdysozoan, and an absence from *C. elegans* reflects an event of gene loss. Conversely, any gene found in *C. elegans* but not in insects or mammals is expected to be a lineage-specific acquisition (Figure 1). From this perspective, as far as the innate immune system is concerned, and as described below, nematodes like *C. elegans* would appear to have lost many key genes, foregoing several central conserved mechanisms, while gaining alternative means to detect and respond to pathogens. This is expected to reflect the strong and unrelenting selective pressure that species, like free-living nematodes, that inhabit microbe-rich environments are likely to be subjected to.

A wild world

Despite *C. elegans* having been used in laboratories as a genetic model for several decades (Brenner 2009; Nigon and Felix 2017), very little was known of its life in natural habitats until recently. A series of ecological surveys, coupled with extensive sampling and genotyping of isolates from different locations world-wide[1] gave a picture of natural population structure and dynamics, and revealed both local genetic diversity and surprising uniformity between strains from around the globe. It was suggested that the current populations arose following successive chromosome-scale selective sweeps, so that four of the worm's six chromosomes are unexpectedly similar among many of the populations that have been sampled worldwide. The most recent such event is estimated to have occurred 600–1200 generations ago. Assuming, conservatively, just 6 generations per year (20-times slower than under optimal lab conditions), this would correspond to a time in the last two centuries. That led to the hypothesis that these selective sweeps were a consequence of human activity (Andersen et al. 2012), in line with the observation that more divergent strains can be found in remote locations (Crombie et al. 2019). It is also possible that the sweeps resulted from a global pandemic, if certain haplotypes were associated with enhanced susceptibility or resistance to infection. There are plenty of examples from other species of such effects, including in humans (Quintana-Murci 2019). Further, there is no shortage of natural pathogens for *C. elegans*. Indeed, environmental sampling suggests that life-shortening infections are the common lot of *C. elegans* (Schulenburg and Félix 2017).

As *C. elegans* can be cryopreserved, a single strain, called N2, is used in all laboratories, and its genome is taken as a reference. N2 was derived from a strain collected in Bristol, UK, that was cultured for many years before being frozen. This period of domestication was associated with the fixation of a number of alleles, not seen in wild strains, principally related to adaptation to the higher oxygen concentrations found in a Petri dish, rather than in the mildly anaerobic natural environment (Frezal and Felix 2015). Some of them affect the worm's interaction with pathogens (Chang et al. 2011; Sterken et al. 2015). Beyond this, very recent evidence suggests that in some cases, N2 lacks genes found in wild strains. Indeed, more than 350 non-overlapping genomic regions have been identified, that are hyper-

[1] See, for example, The *C. elegans* Natural Diversity Resource, and Félix lab websites [https://www.elegansvariation.org/; https://www.justbio.com/tools/worldwideworms/]

divergent from the reference isotype N2. They range in size from 9 kb to 1.35 Mb and behave as if inherited as large haplotype blocks (Lee et al. 2021a).

In one such region, N2 contains 11 protein-coding genes, while many other isotypes contain 20. The extra genes appear to have arisen by duplication and diversification of existing *C. elegans* genes, and include several encoding G-protein coupled receptors (GPCR), a class of proteins known to be important for host-pathogen interactions (see below), as well as nematode-specific proteins. One, *F40H7.1*2, also called *ifas-1*, for "Inducible FAScin Domain containing", has been the focus of our attention recently as its expression is induced by natural fungal infection (Omi et al. 2021), and appears to be part of a poorly characterised innate immune defence mechanism (Zhang et al. 2021b). Another hyper-divergent region encompasses a cluster of paralogous genes that encode antimicrobial peptides (AMPs) of the NLP class, that show inter-species variability indicative of positive selection (Pujol et al. 2012; Pujol et al. 2008b) and, as explained below, play multiple important roles in innate immunity against natural fungal infection (Harding and Ewbank 2021). These observations corroborate the notion that these hyper-divergent regions are crucial for the capacity of *C. elegans* to adapt to its environment (Lee et al. 2021a).

There is one striking example of N2 retaining a defence pathway that has been lost from many of the *C. elegans* strains sampled in Europe. In common with most species, RNA interference (RNAi) is central to efficient antiviral defence in *C. elegans*. It requires the RIG-I homologue, DRH-1, but many strains carry a deletion polymorphism in the *drh-1* gene that compromises antiviral immunity (Ashe et al. 2013). The reasons for this widespread loss of an essential defence mechanism is far from clear. Viral infections in natural *C. elegans* populations are not that frequent (Felix and Wang 2019), so the selective impact of the *drh-1* deletion could be neutral. Or the loss could be a consequence of hitchhiking with a closely linked beneficial mutation (Ashe et al. 2013). Alternatively, loss of its RNAi pathway could be beneficial to *C. elegans* under particular environmental conditions. It should be noted that in fungi, RNAi has been lost in many independent lineages, including *Saccharomyces cerevisiae* (Billmyre et al. 2013). It appears that eliminating RNAi allows the yeast to harbour double-stranded RNA "killer viruses" and this conveys a compensatory advantage (Drinnenberg et al. 2011). What *C. elegans* might gain from loss of *drh-1* remains an open question.

In additional to these observations, and *in silico* support for the expansion and diversification of specific gene families involved in innate immunity across *Caenorhabditis* species (e.g. (Pujol et al. 2008b; Thomas 2006)), there is experimental support for negative frequency-dependent selection (i.e. phenotypic/genotypic fitness is inversely correlated to prevalence) concerning resistance to infection (Schulenburg and Ewbank 2004). This is generally taken to reflect co-evolutionary arms races between hosts and pathogens. Taken together, one can conclude that natural pathogens are undoubtedly an important factor shaping the *C. elegans* genome. As intra-species differences in defence mechanisms are only just starting to be studied, in the remainder of this review, for an inter-species comparison, we will use the well-characterised N2 genome as our reference. And although there is an ever-increasing number of *Caenorhabditis* genome sequences (Stevens et al. 2019), providing the basis for the investigation of evolutionary variation at the genus level, here will principally address changes across much greater phylogenetic distances, comparing *C. elegans* to non-nematode species.

What has been lost

Pathogen defences come at a high selective cost. It is energetically demanding to regulate, produce, and in some cases secrete, immune effector proteins, and if these effectors have relatively generic functions, they can cause collateral self-damage. For example, saposins are membrane-disrupting proteins active against pathogens, but also capable of damaging host cells. They are first synthesised as inactive pro-proteins, with an inhibitory sapA domain that is only cleaved off when needed. Curiously, in *C. elegans*, this auto-regulatory mechanism has been lost. As well as potentially being directly deleterious, this also renders worms susceptible to a specific counter-defence mechanism, with the nematophagous fungus *Drechmeria coniospora* producing sapA on its surface (Lebrigand et al. 2016). It is well known that host-adapted pathogens can also subvert defence pathways, rendering them partially or fully ineffective; several examples have been reported for *C. elegans* (e.g. (Lee et al. 2013; Vasquez-Rifo et al. 2020; Zhang et al. 2021b). Such diminished utility will lessen the selection pressure on the corresponding defence genes. It is thus conceivable that the absence from *C. elegans* of different immune pathways reflects

such processes, in addition to the more broad high selective costs of maintaining pathogen defences.

Perhaps the best-known example of a "missing" gene concerns the key immune transcription factor, NF-κB, that became apparent when the first nematode and insect genomes were completed (Rubin et al. 2000). As more and more genomes are sequenced, the evolutionary history of NF-κB and the related Rel protein family becomes clearer. Rel homology domain (RHD) proteins appeared relatively early during evolution and are found not only in both Protostomes and Deuterostomes, but also in basal phyla, including Cnidaria, Porifera and even protists. Proteins bearing all the hallmarks of Rel family proteins can be found in several Ecdysozoan lineages, including the Priapulida *Priapulus caudatus*, but so far not in Nematoda or Nematomorpha (Williams and Gilmore 2020). On the other hand, a plausible Rel family member (gb|OWA53470.1|), most similar to nuclear factor of activated T-cells 5 (NFAT-5) in vertebrates, has been described in the tardigrade *Hypsibius dujardini* (Yoshida et al. 2017). There are also homologues in the 2 other publicly available tardigrade genome sequences (*Ramazzottius varieornatus* [GAV04584], and *Paramacrobiotus* sp. [PARRI_0020628.p1 in the current annotation[2]]). Thus genes for Rel family proteins appear to have been lost in Nematoda, subsequent to their evolutionary divergence from the Tardigrada (Figure 1).

One of the conserved canonical pathways that lead to activation of NF-κB starts with cell-surface receptors of the Toll family. In flies, Toll binds endogenous cytokine-like proteins, while in vertebrates, different members of the Toll-like receptor (TLR) family recognise a panoply of microbial moieties, including lipopolysaccharide and double-stranded RNA (dsRNA). In both cases, the result is an increased expression of genes encoding immune effectors that contribute to host defence (Kawai and Akira 2010; Lemaitre and Hoffmann 2007). Although lacking NF-κB, there is one TLR gene in *C. elegans*, *tol-1*. In addition to an essential role in cell adhesion during early development ((Pujol et al. 2001) and NP, unpublished observations), *tol-1* is required for the terminal differentiation of a class of CO_2-sensing neurons that are involved in governing aversive behaviour in the presence of pathogenic bacteria (Brandt and Ringstad 2015; Pradel et al. 2007). *C. elegans* is sensitive to even small changes in

[2] https://figshare.com/projects/Metazoan_TPS-TPP_gene_identification/36410; https://figshare.com/articles/dataset/Paramacrobiotus_sp_TYO_gene_pep_fa_gz/6854120

the CO_2/O_2 balance (Carrillo et al. 2013), and the resulting neuronal signals feed into a neuropeptide circuit that also influences whether worms clump together or remain isolated (de Bono and Bargmann 1998). The responsiveness to O_2 is modulated by interleukin signalling. Specifically, an IL17-like protein acts via its receptors (ILCR-1 and ILCR-2) and the associated downstream pathway to alter O_2-escape behaviours, as well as suppressing immunity. In this context, it is striking that homologues of proteins involved in TLR/NF-κB signal transduction in other species, such as ACT1, IκB, and the TIR-domain adapter protein SARM1, called in *C. elegans* ACTL-1, IKB-1 and TIR-1, respectively, are all required (Flynn et al. 2020). Further, there are conceptual similarities between this role for IL17 in *C. elegans* and its neuronal function in mammals (Rua and Pujol 2020).

In mice and flies, Akirin links RHD proteins to chromatin remodellers of the SWItch/Sucrose Non-Fermentable family. In flies, for example, Akirin acts downstream of peptidoglycan recognition proteins to regulate AMP gene expression (Tartey and Takeuchi 2015). Nematodes have lost peptidoglycan recognition proteins (Kurz and Ewbank 2003). In the epidermis of *C. elegans*, Akirin appears rather to act downstream of a GPCR, DCAR-1, and a p38 MAPK signalling cascade, described further below, and to bridge a POU-class transcription factor to the NuRD chromatin remodelling complex (Figure 2A), thereby controlling AMP gene expression upon fungal infection (Polanowska et al. 2018).

Another prominent example of evolutionary *bricolage* concerns STAT signalling. Central to immune defences across animal species, STAT transcriptional activation typically relies on JAK, a kinase that has also been lost from nematodes. Instead, in *C. elegans*, one of its two STAT homologues, STA-2, associates with an atypical putative transceptor, SNF-12, via a large C-terminal domain conserved among nematodes, but unlike any SLC6 family proteins in other species. By a mechanism that has yet to be fully elucidated, this association is essential for AMP gene expression in the epidermis following fungal infection (Dierking et al. 2011; Taffoni et al. 2020). It is important to note that a quarter of the ca. 300 genes identified in a pan-genomic RNAi screen for positive regulators of this same antifungal response are nematode-specific (Zugasti et al. 2016), and a similar situation applies to effector genes too (Figure 2B). Collectively, these and other studies have provided considerable insight into the evolution of lineage-specific signalling pathways,

revealing a remarkable molecular plasticity in *C. elegans* innate immune signalling. Notably, STA-2 also appears to act as a transcriptional repressor for some genes, including *ifas-1*, mentioned above. A specific fungal enterotoxin from *Drechmeria coniospora* can antagonise STA-2 function, leading to decreased AMP gene expression but increased *ifas-1* expression. This dual function for STA-2 may therefore represent an evolutionary-recent counter-defensive strategy against fungal enterotoxins (Zhang et al. 2021b), potentially restricted to *Caenorhabditis* nematodes. It is likely subject to on-going selective pressure in *C. elegans*, since wild isolates have different numbers of *ifas-1* paralogues (Lee et al. 2021a).

What has been retained

These examples of lineage-specific gene loss do not mean that nematodes, including *C. elegans*, do not share some conserved immune mechanisms with other species. Indeed, the core of its AMP gene regulation in the epidermis, and defence gene expression in the gut, is a common, conserved, p38 MAPK signalling cascade that also involves TIR-1/SARM (reviewed in (Kim and Ewbank 2018)). There are parallels between this pathway and stress-response pathways even in yeast, including an important role for protein-protein interactions through SAM-domains (Pujol et al. 2008a). The conservation with vertebrates extends beyond this, as the upstream pseudokinase NIPI-3 functions in concert with its binding partner, the bZIP transcription factor CEBP-1 (Kim et al. 2016; McEwan et al. 2016), as do their respective orthologues, Tribbles and C/EBP (CCAAT/enhancer-binding protein) in mammals (Eyers et al. 2017).

TIR-1/SARM acts upstream of the MAP3K NSY-1. As well as directly controlling AMP expression in epithelial cells, NSY-1 plays roles in several processes that are related to the interaction of *C. elegans* with pathogens. It controls the exact nature of the nematode's surface coat (Foley et al. 2019), which in turn determines whether or not a microbe can adhere and initiate an infection (e.g. (Gravato-Nobre et al. 2011; Rouger et al. 2014)). It is required for the normal left-right asymmetry of a pair of chemosensory neurons (called "AWC"). Unlike vertebrates that express a single chemoreceptor gene per neuron, *C. elegans* expresses several of its greatly expanded family (>1500) of chemoreceptor genes in a small number (<70) of chemosensory neurons. In the absence of NSY-1, worms lose the expression of some

chemoreceptors and hence the ability to sense the corresponding odours. As a consequence, their capacity to sense their environment and to distinguish between nutrients and potential pathogens will be compromised (Alqadah et al. 2016); smelling and tasting are vitally important for worms (Ferkey et al. 2021). Indeed, there are a growing number of examples where detection of a specific microbial metabolite either attracts or repels *C. elegans*, influencing its risk of infection (reviewed in (Kim and Flavell 2020)). NSY-1 is also required in another pair of chemosensory neurons (called ADF) for the upregulation of serotonin biosynthesis that can be triggered by pathogen exposure. Serotonin mediates food-odour associative learning (Nuttley et al. 2002) and is required for the aversive olfactory learning induced by pathogenic bacteria (Zhang et al. 2005). Surprisingly, in this context, it acts via a serotonin-gated cation channel, LGC-50, with ligand binding and ion selectivity properties different from previously described neurotransmitter receptors (Morud et al. 2021). Serotonin signalling will affect whether worms ingest harmful microbes and thus have a direct impact on survival. It also controls egg-laying behaviour (Shivers et al. 2009), as well as protective gene expression in offspring (Das et al. 2020), so can affect future generations too. Notably, all these effects can be influenced by microbial metabolites that act as serotonergic agonists (Chen et al. 2020), opening a way for manipulation of host physiology by pathogens. Interestingly, for these different roles, the regulatory factors upstream and downstream of NSY-1 can differ (e.g. (Foster et al. 2020; Pagano et al. 2015)), which must place particular constraints on how the central MAPK cassette can evolve to deal with a changing microbial environment.

What has been gained

Selective pressures have also led to various types of gene acquisition. In addition to several genes encoding the type of lysozyme found in other invertebrate, *C. elegans* has a gene family coding for lysozymes that are most similar to those found in amoebae (Mallo et al. 2002; Schulenburg and Boehnisch 2008). These genes, several of which are induced by infection, were presumably acquired by horizontal gene transfer. The same is true for the sapA-domainless saposin mentioned above; it is most like amoeboid proteins (Lebrigand et al. 2016). For another class, the thaumatins that resemble plant proteins that inhibit fungal growth and sporulation, the

evolutionary history is less clear, as examples are found scattered across a range of Ecdysozoan species (Shatters et al. 2006). In other cases, there has been extensive gene duplication, both at the species level, or as outlined above, even in individual *C. elegans* lineages (Lee et al. 2021a), leading to large families encoding either effector proteins, or proteins potentially involved in pathogen recognition (Garcia-Sanchez et al. 2021; Pees et al. 2016; Reboul and Ewbank 2016). The former category includes various classes of AMPs, including the neuropeptide-like (NLP) family, so called because of their similarity to phylogenetically more ancient *bona fide* neuropeptides. As well as possessing direct antimicrobial activity (Couillault et al. 2004) these peptides play other physiological roles (Harding and Ewbank 2021), as described below, placing singular constraints on their potential evolutionary trajectories.

Feeling sick

We outlined above how the nervous system can mediate the responses of *C. elegans* to pathogens. It can also affect the nature of transcriptional response to infection-manifested by the intestine and epidermis (e.g. (Foster et al. 2020)) since it is the origin of a wide-range of secreted modulatory peptides and other small molecules that affect distant tissues. For example, biotic and abiotic factors that unbalance mitochondrial homeostasis, including bacterial toxins that disrupt oxidative phosphorylation, initiate the mitochondrial unfolded protein response (UPR^{mt}). In addition to the expression of mitochondrial chaperones, activation of the UPR^{mt} in the gut leads to the expression of antimicrobial proteins (Naresh and Haynes 2019), influenced by a complex network of both host and microbe factors (e.g. (Deng et al. 2019; Mahmud et al. 2020)). When it is triggered in neurons, it spreads to peripheral tissues through the action of Wnt (Zhang et al. 2018) that is known to regulate defence gene expression in the gut (Labed et al. 2018). Remarkably, the UPR^{mt} can also be associated with elevated levels of mitochondrial DNA that can be maternally inherited, so that there is the potential for a transgenerational transmission of a primed and protective state (Zhang et al. 2021a). Surveillance mechanisms that are tied to immune defence have been described for other organelles and cellular processes, including peroxisomes (Rackles et al. 2021), ribosomes (Vasquez-Rifo et al. 2020), the nucleolus (Tiku et al. 2018), translation (Troemel 2012) and purine metabolism

(Tecle et al. 2021). They undoubtedly interact during infection within a tissue and across tissues (see for example (Runkel et al. 2013)). But how they collectively contribute to resisting a given infection remains to be fully explored.

Another major actor in regulating cellular and organismal homeostasis is insulin signalling (Murphy and Hu 2013). Insulin and insulin-like peptides (INS) have conserved functions in regulating ageing and immunity (Fabian et al. 2021), acting via the FOXO transcription factor, DAF-16. While humans have 10 insulin-like genes, there are four times as many in *C. elegans*, acting synergistically or antagonistically (Fernandes de Abreu et al. 2014; Ritter et al. 2013; Zheng et al. 2018), affecting the physiology of *C. elegans* in many ways (Tissenbaum 2018). Here, we will only mention a few related to immunity. As a first example, the aversive olfactory learning referred to above is controlled by several *ins* genes. Some, like *ins-6* and *ins-7*, are expressed in neurons. *ins-6* promotes learning by stopping the expression of *ins-7*, an inhibitory factor (Chen et al. 2013). As worms grow old, a feed-forward mechanism modulates the expression of *ins-7*, thereby regulating immune ageing and behaviour (Lee et al. 2021b). Others, such as *ins-11,* are only expressed in adult worms following infection, either in the epidermis or the intestine, depending on the pathogen and its route of infection (Lee et al. 2018). Intestinal *ins-11* expression negatively regulates aversive learning, and has thus been suggested to play a role in a negative feedback loop connecting the gut and nervous system that allows *C. elegans* to navigate successfully in complex environments (Lee and Mylonakis 2017). Lastly, a recent report indicates that INS-31 levels are boosted by GABAergic signalling in neuromuscular junctions. As *ins-31* inhibits resistance to infection, this means that there is a connection between muscle synapse activity and intestinal innate immunity (Zheng et al. 2021). Despite this progress, the large number of different *ins* genes still represents a significant challenge for any attempt to model comprehensively the effect of insulin signalling on the interaction of *C. elegans* with pathogens.

This is made more difficult as other factors impact the same defence pathways. Thus, for example, neuronally-secreted TGF-β activates its receptor in the epidermis and this elicits secondary signalling, thereby inducing aversive learning (Zhang and Zhang 2012). Further, peptides previously assumed to act as direct antimicrobial factors have recently been shown to have other activities. Thus, NLP-29, which was among the

first family of AMPs in *C. elegans* to be shown to be induced by fungal infection of the epidermis (Couillault et al. 2004), regulates both neurodegeneration (E et al. 2018) and sleep (Sinner et al. 2021). Importantly, expression of *nlp-29* is controlled during development by a caspase that directly cleaves the p38 MAPK PMK-1, thereby also affecting the expression of hundreds of other genes, in what appears to be a mechanism to balance the opposing requirements for development and innate immunity (Weaver et al. 2020), mirroring the dual roles of NIPI-3 and CEBP-1 (Tribbles/C/EBP) (Kim et al. 2016). There is thus a tangled web of intra- and inter-cellular communication affecting multiple aspects of *C. elegans* biology (Harding and Ewbank 2021), impacting the nematode's capacity to cope with pathogens. Once again, this raises fundamental questions regarding the evolvability of any particular immune gene, pathway or mechanism, when they are so intimately embedded in other physiological processes.

Recognising infection

Although surveillance mechanisms play a central role in *C. elegans* defence, there has long been speculation about its capacity for direct and specific recognition of pathogens, in part because of the very distinct transcriptional responses triggered by different microbes (e.g. (Engelmann et al. 2011; Wong et al. 2007)). As alluded to above, however, *C. elegans* has lost many of the best-characterised pathogen-sensing receptors found in other organisms (Kurz and Ewbank 2003), including the cGAS-STING antiviral system (Cai and Imler 2021). It has been suggested that some of the nematode's expanded repertoire of 283 C-type lectin-like domain (CTLD) proteins might be involved in microbial detection (Pees et al. 2016; Schulenburg et al. 2004), especially as at least two of them can direct bind bacteria (Miltsch et al. 2014). Generally, however, although several CTLD proteins do play a role in resistance to infection, this appears not to involve direct pathogen recognition (Pees et al. 2017; Pees et al. 2021; Yunger et al. 2017). On the other hand, some very recent work suggests that there may be at least one exception (see below).

A second class of proteins that can trigger immune responses in various invertebrates is GPCRs (Reboul and Ewbank 2016). In *C. elegans*, some appear to important for the recognition of specific signals produced by bacteria, including surfactants from *Serratia marcescens* (Pradel et al. 2007) and 1-undecene from *Pseudomonas*

aeruginosa (Prakash et al. 2021). In the latter case, 1-undecene can induce both a behavioural and a transcriptional response, tying together these two types of protective mechanisms.

One of the many GPCRs, DCAR-1, is activated by the tyrosine-derivative hydroxyphenyllactic acid (HPLA), and structurally-related compounds, switching on the p38 MAPK pathway in the epidermis and expression of AMP genes. HPLA levels go up when worms are infected with *Drechmeria coniospora*, but also in mutants with defects in their cuticle. Since fungal infection involves penetration of the cuticle, it is assumed that although crucial for the capacity to respond to fungal infection, DCAR-1 does not actually recognise the pathogen, rather its binding of the endogenous ligand HPLA is a type of surveillance mechanism (Zugasti et al. 2014). Indeed, AMP genes are also switched on when worms are injured (Belougne et al. 2020; Pujol et al. 2008a; Taffoni et al. 2020). Interestingly, disruption of the cuticle causes at least 2 other stress pathways to be activated, by a putative damage sensor that remains to be identified (Dodd et al. 2018; Pujol et al. 2008b).

Infection of *C. elegans* by the oomycete *Myzocytiopsis humicola* has very distinct consequences, characterised by an induction in the expression of multiple chitinase-like genes, as part of the oomycete recognition response (ORR). This alters the structure of the cuticle and reduces the capacity of *M. humicola* to attach, decreasing the pathogenic burden and favouring the worm's survival (Osman et al. 2018). The ORR can be induced, via chemosensory neurons, by exposing worms to an innocuous extract derived from animals infected with *M. humicola*. In this case, contrary to initial indications for the involvement of a GPCR` (Fasseas et al. 2021), it now appears likely that two of the many worm C-type lectin domain proteins mediate this neuronal recognition of an oomycete-derived moiety (Michalis Barkoulas, personal communication).

During infection by the single-stranded RNA Orsay virus, DRH-1, the worm RIG-I homologue mentioned above, appears to act as a sensor of viral RNA-dependent RNA polymerase activity (Sowa et al. 2020). RNA is also involved in a recently described and unprecedented form of precise pathogen recognition by *C. elegans*. Bacteria make a range of non-coding RNAs (ncRNAs). A particular 137-nt ncRNA called P11 that is specific to the genus *Pseudomonas* appears to be taken up by *C. elegans*, then processed by the intestinal Dicer and germline PIWI-interacting RNA machinery. P11 has a short stretch of sequence that matches the gene *maco-1*. As a consequence of its

processing, P11 down-regulates *maco-1* and this, indirectly, provokes pathogen avoidance. Remarkably, this adaptive response that keeps *C. elegans* out of harm's way is transmitted to progeny over four generations (Kaletsky et al. 2020). Perhaps even more extraordinary, this propensity for pathogen avoidance can be transmitted from P11-exposed animals to naïve animals, altering behaviour in a heritable manner. The horizontal and vertical transfer of learned pathogen-avoidance behaviour appears to be mediated by secreted Cer1 retrotransposon particles that need to be present in both donor and recipient animals. Interestingly, not all wild *C. elegans* strains carry copies of *Cer1* in their genome, so this precise capacity to share environmental information is not ubiquitous; other mechanisms may also exist. Although the exact nature of the signal transmitted by these virus-like particles has not been determined, it is assumed to be an RNA molecule (Moore et al. 2021). This then represents a novel paradigm for pathogen recognition, currently unique to *C. elegans* (Figure 3).

Concluding remarks

While some authors emphasise structural and functional conservation for genes and pathways involved in nematode innate immunity (e.g. (Fabian et al. 2021)), as we have tried to illustrate here, it is perhaps the differences between *C. elegans* and other branches of the animal phylogenetic tree that are most striking. Although this undoubtedly diminishes the relevance of *C. elegan*s for clinically-oriented research, it offers rich ground for those interested in exploring fundamental questions related to the origins and evolution of host defence mechanisms at a time when rapid environmental changes are altering long-standing balances between pathogens and hosts; the worm has still much to teach us.

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Figure legends

Figure 1. Simplified phylogenetic tree showing the evolutionary position of *C. elegans* (in Nematoda, in bold) relative to selected Metazoan lineages. The black square marks the base of the Ecdysozoa, the red the Protostomia/Deuterostomia junction. Deuterostomia contains 2 clades, the chordates (e.g. vertebrates, including mammals) and ambulacrarians (e.g. echinoderms, including starfish). The dashed line leads to the Lophotrochozoa (not shown). The names of the branches that include species containing at least one predicted Rel homology domain (RHD) protein are in green. The pattern of conservation supports several independent losses of RHD genes, including one on the Nematoda lineage.

Figure 2. (A) Comparing Akirin function in *Drosophila* and *C. elegans*. In *Drosophila* (left), binding of peptidoglycan to the appropriate peptidoglycan recognition protein (PGRP) activates the IMD pathway, leading to a Rel family transcription factor. Akirin bridges chromatin remodellers of the SWItch/Sucrose Non-Fermentable family (SWI/SNF) to the Rel protein and acts as a positive regulator of defence gene expression. In *C. elegans*, activation of a G-protein coupled receptor (GPCR) by an endogenous ligand (HPLA) triggers a downstream p38 MAPK cascade. Akirin here interacts with the NuRD chromatin remodelling complex, including the protein LIN-40, and a POU-class transcription factor. Akirin needs to dissociate from its target loci if defence genes are to be expressed. Figure modified from one kindly provided by O. Zugasti. (B) Clustering of 280 genes that act as positive regulators of the antifungal innate immune response (left) and of 144 genes that are up-regulated upon fungal infection (right; adapted from (Thakur et al. 2021)) on the basis of their patterns of conservation (red indicates the presence of an orthologue) across 113 species (coloured bar at bottom), ranging from Archaea and bacteria grey; left) to chordates, including human (brown; right). Among the invertebrates (yellow), the 5 *Caenorhabditis* species are indicated by the pink box.

Figure 3. Three examples of innate immune recognition in *C. elegans*. Left: An as yet uncharacterised compound from pathogenic oomycetes acts via a pair of proteins containing C-type lectin domains that function non-redundantly in specific chemosensory neurons (M. Barkoulas, personal communication) to up-regulate the

expression of genes including a family of chitinase-like (*chil*) that alter the properties of the cuticle. Middle: a breach of the cuticle by physical injury, mutation of specific cuticle components, or by infection with *Drechmeria coniospora*, increases the level of HPLA. This activates the GPCR DCAR-1 and the downstream p38 MAPK pathway, leading to increased expression of AMPs such as NLP-29, and the insulin-like protein INS-11. These contribute either directly to defence or signal to other tissues as part of a coordinated response to infection. Right: A specific small RNA (sRNA) called P11 and produced by *Pseudomonas aeruginosa* is taken up by intestinal cells and processed. This results in the generation and transmission of successive signal(s) to the germline and then from the germline to the neurons involved in pathogen aversive behaviour. The formation of virus-like particles by the Cer1 retrotransposon, hypothesised to carry a specific RNA molecule, allows a worm exposed to P11 to affect the behaviour of naïve worms and progeny through both vertical and horizontal transmission.

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FIGURE 1

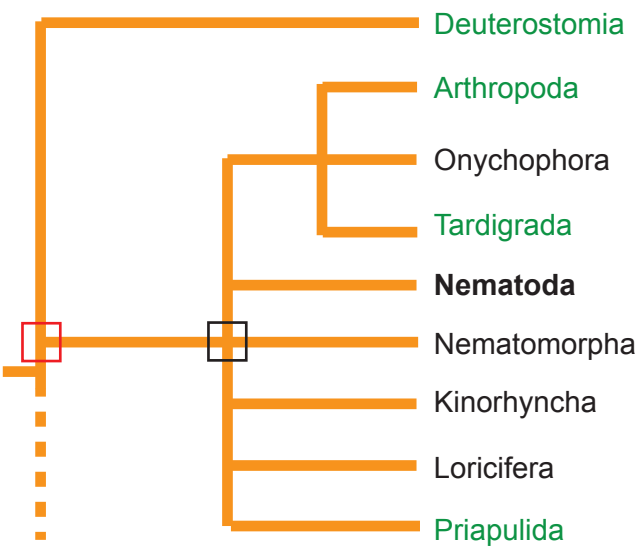

Deuterostomia
Arthropoda
Onychophora
Tardigrada
Nematoda
Nematomorpha
Kinorhyncha
Loricifera
Priapulida

FIGURE 2

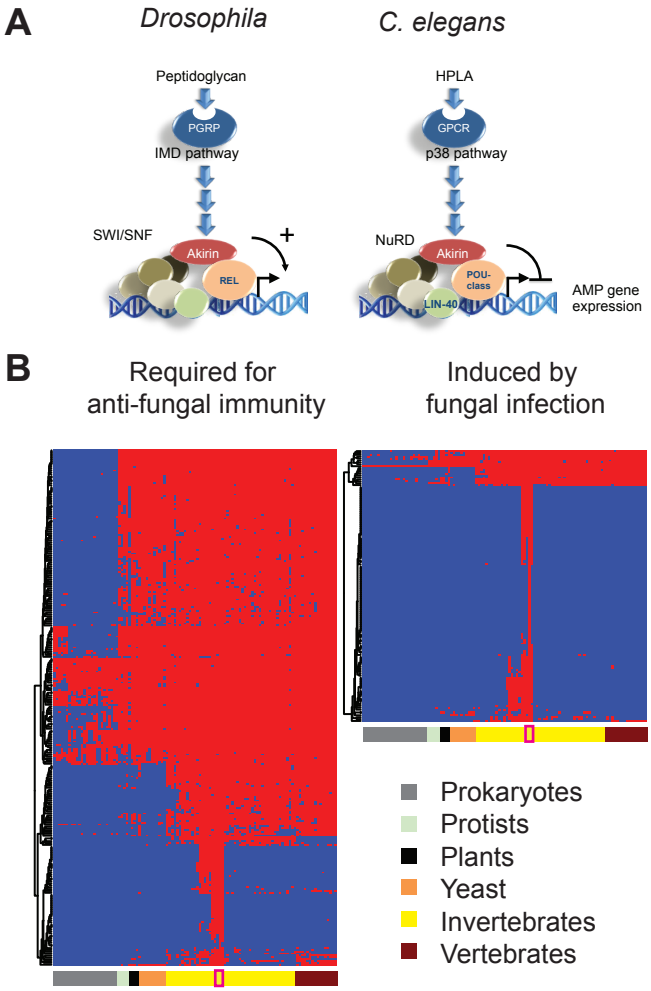
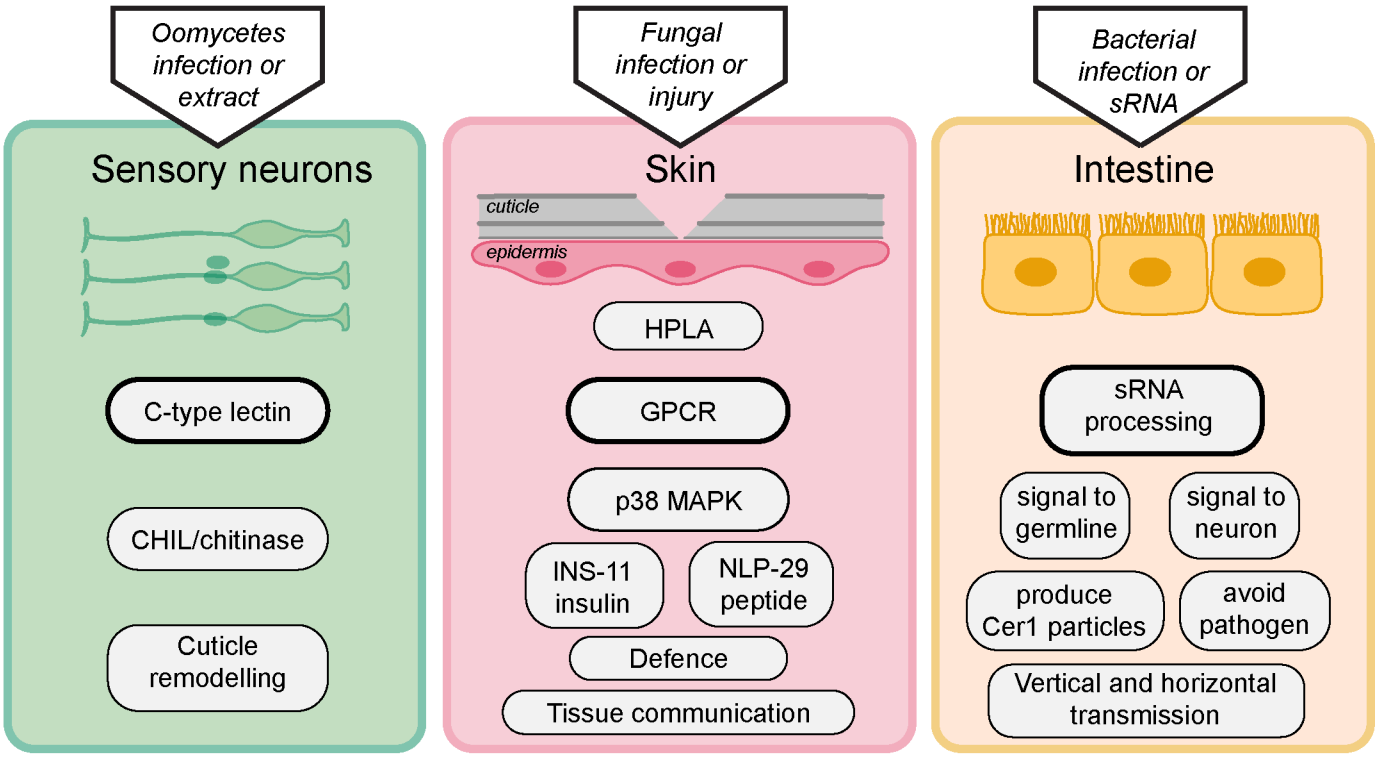

A
Drosophila
C. elegans
Peptidoglycan
PGRP
IMD pathway
SWI/SNF
Akirin
REL
+
HPLA
GPCR
p38 pathway
NuRD
Akirin
POU-class
LIN-40
AMP gene expression
B
Required for anti-fungal immunity
Induced by fungal infection
Prokaryotes
Protists
Plants
Yeast
Invertebrates
Vertebrates

FIGURE 3


Oomycetes infection or extract
Sensory neurons
C-type lectin
CHIL/chitinase
Cuticle remodelling
Fungal infection or injury
Skin
cuticle
epidermis
HPLA
GPCR
p38 MAPK
INS-11 insulin
NLP-29 peptide
Defence
Tissue communication
Bacterial infection or sRNA
Intestine
sRNA processing
signal to germline
signal to neuron
produce Cer1 particles
avoid pathogen
Vertical and horizontal transmission