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Aix Marseille Univ, CNRS, INSERM, CIML, Turing Centre for Living Systems, Marseille, France

Author for correspondence: ewbank@ciml.univ-mrs.fr

Abstract

The simple notion "infection causes an immune response" is being progressively refined as it becomes clear that immune mechanisms cannot be understood in isolation, but need to be considered in a more global context of other cellular and physiological processes. In part, this reflects the deployment by pathogens of virulence factors that target diverse cellular processes, such as translation or mitochondrial respiration, often with great molecular specificity. It also reflects molecular cross-talk between a broad range of host signalling pathways. Studies with the model animal *C. elegans* have uncovered a range of examples wherein innate immune responses are intimately connected with different homeostatic mechanisms, and can influence reproduction, ageing and neurodegeneration, as well as various other aspects of its biology. Here we provide a short overview of a number of such connections, highlighting recent discoveries that further the construction of a fully integrated view of innate immunity.

Introduction

The nematode *Caenorhabditis elegans* is often vaunted as an ideal model for multiscale investigations as it is amenable for study from the molecular to the organismal level (Figure 1). Because of this, it affords many opportunities for integrative research, bridging traditionally separate disciplines such as developmental biology and physiology. Indeed, in the two decades since the first studies addressing

host-pathogen interactions in *C. elegans* (reviewed in (1)), numerous laboratories have joined the field, often because of a previously unsuspected link between the physiological process under study and innate immunity. In this short review, we concentrate on a few examples of the sometimes-surprising, and often reciprocal, connections that exist between the innate immune system of *C. elegans* and diverse aspects of its biology. Readers interested in specific aspects of *C. elegans* immunity and host-pathogen interactions are directed to recent and more complete reviews. These cover, for example, the complex links between immunity and metabolism, or the many neuroimmune connections that we cannot hope to cover here in depth (e.g. (2-10)). Many contain diagrams that summarise the pathways involved; we have chosen not to duplicate them here.

From germline integrity to organismal resilience

A few years ago, the Schumacher group demonstrated that DNA damage in the germline of *C. elegans* leads to elevated resistance to heat and oxidative stress. This is dependent upon the activity of MPK-1 (11), a member of a large and conserved family of mitogen-activated protein kinases (MAPKs). It regulates the expression of secreted antimicrobial proteins in the gut upon bacterial infection (12, 13). Germline DNA damage was associated with an increased production of some of these antimicrobial proteins. Irradiating wild-type worms, but not *mpk-1* mutants, thus protected them from infection. This revealed a hitherto unsuspected link between genome integrity in the germline and intestinal antimicrobial defences. The innate immune response provoked by germline DNA damage was shown to activate the ubiquitin–proteasome system (UPS) in somatic tissues. This explains the observed increase in heat stress resistance, and is one illustration of the intimate relationship between different homeostatic mechanisms across tissues (11).

Connections between germline integrity, intestinal defence and longevity were also revealed through studies involving germline ablation. This causes accumulation of the FOXO transcription factor DAF-16 in the nuclei of intestinal cells, and an increased lifespan (14). DAF-16/FOXO regulates the expression of batteries of intestinal genes involved in stress resistance, ageing and innate immunity (reviewed in (15)). The nuclear translocation of DAF-16/FOXO requires the “Infection Response Gene” *irg-7* (16), so called since its expression is induced by several pathogens (17, 18). In

parallel, *irg-7* is also involved in the activation of the CREB/ATF bZIP protein ATF-7 (16). This latter transcription factor is a key regulator of the expression of many intestinal defence genes, and is itself controlled by a conserved PMK-1/p38 MAPK pathway (19). On the basis of their results, Yunger *et al*. proposed that the reproductive system might serve as a signalling centre, regulating the allocation of metabolic resources to innate immune defences upon infection (16). One way that metabolism influences immunity is through the regulation of the PMK-1/p38 - ATF-7/CREB pathway by nutrient levels. The pathway is also impacted by DAF-16/FOXO, which reduces food consumption. This thus provides a molecular link between digestion, growth, longevity and intestinal innate immunity (20).

The activity of DAF-16/FOXO is controlled by insulin signalling. More precisely, it is negatively regulated by the insulin-like receptor (ILR) DAF-2. As animals age, the PMK-1/p38 - ATF-7/CREB pathway plays a diminishing role in immunity (21), and insulin signalling becomes more important (22). But eventually this too declines. Thus, age-dependent increases in the levels of the insulin-like peptide INS-7 activate DAF-2/ILR, and this down-regulates DAF-16/FOXO activity. As *ins-7* is regulated negatively by DAF-16/FOXO, this further increases *ins-7* expression, an effect reinforced by the action of the bZIP transcription factor ZIP-10 (23).

In addition to being regulated by insulin-like peptides, there is growing evidence for a regulatory role for the neurotransmitter GABA in the control of the DAF-16/FOXO pathway (24), and of PMK-1/p38, perhaps as part of a biological trade-off between immunity and longevity (25). One can imagine that these regulatory connections that link food intake, immunity and ageing also reflect the complex and changing environment in which *C. elegans* exists in nature (26), and there is increasing experimental evidence to support this idea (27, 28). The species evolutionary fitness will depend on maximizing egg production and progeny survival in the presence of potential pathogens, sometimes by unexpected means (29). Past the reproductive age, there may be no selective benefit to maintaining immune defences. Indeed in *C. elegans*, death could even be adaptive (30).

Surveillance of processes and organelles

We mentioned above the UPS, a broadly conserved set of mechanisms that are important for intracellular homeostasis and that play an important role in innate

immunity in animals, including *C. elegans* (reviewed in (31)). The UPS can be triggered as part of an immune response if virulence factors interfere with proteostasis. Conversely, activation of the UPS can itself elicit host defence mechanisms. For example, treatment of *C. elegans* with proteasome inhibitors, as well as experimental down-regulation of genes for ubiquitin or proteasomal subunits, switches on the UPS. And this then induces a transcriptional response similar to that seen upon infection with the natural microsporidial pathogen *Nematocida parisii* (32). This is one aspect of what has been termed the "intracellular pathogen response" (IPR). It is not currently known what activates the IPR following microsporidial infection. The IPR is also elicited by the positive-sense single-stranded RNA virus known as the Orsay virus (33). Recent work has shown that in this case, DRH-1, the worm RIG-I homologue, is required (34).

The IPR is an example of a surveillance mechanism, set-off by so-called 'homeostasis-altering molecular processes' (HAMPs) (35), rather than microbe-associated molecular patterns (MAMPs) such as lipopolysaccharide and peptidoglycan, or damage-associated molecular patterns (DAMPs), cellular components released inappropriately, including extra-cellular ATP, and mitochondrial DNA in the cytoplasm. A slew of other surveillance mechanisms have now been described in *C. elegans*, involving for example, the monitoring of cytoplasmic DNA (36), translation, and mitochondrial function (reviewed in (37, 38)). Interestingly, the RNA interference (RNAi) machinery that, in conjunction with 3' uridylation of the viral genomic RNA (39), is central to anti-viral defence in *C. elegans* (40), appears to target mRNA during active translation (41), suggesting potential cross-talk between RNAi and translation surveillance. In the context of antiviral RNAi, DRH-1/RIG-I processes dsRNA into small interfering RNAs (42), whereas in its role in the IPR, DRH-1/RIG-I appears to act as a sensor of viral RNA-dependent RNA polymerase activity (34). Further, through a process involving a cullin-RING ligase complex, the IPR promotes thermotolerance (43), again highlighting the intimate link between the different homeostatic mechanisms brought into play following biotic and abiotic stresses in *C. elegans*.

One other recent addition to the list of surveillance mechanisms concerns peroxisomes, for which knockdown of a peroxisomal matrix import receptor alters peroxisomal lipid metabolism and is linked to the expression of innate immunity genes. This process that has been termed peroxisomal retrograde signalling (PRS)

(44). Indeed infection of *C. elegans* by *P. aeruginosa* leads to some of the signature changes of the PRS. In common with the response to infection (45-48) and to LK56, a small molecule that promotes resistance by switching on innate immunity (49), activation of the PRS involves the nuclear hormone receptor NHR-49, homologue of PPARα, and its co-activator MDT-15, homologue of the mediator complex component MED15 (44). The mediator complex is a highly conserved part of the RNA polymerase II transcription system, linking transcriptional responses to regulatory factors. As well as controlling transcriptional initiation, particularly of stress response genes, it is connected to the nuclear pore and likely plays a role in the coordination of chromatin organisation and gene expression (50). Collectively, these findings suggest a tight functional choreography of diverse organelles and processes during the innate immune response.

Notably, MDT-15/MED15 is also involved in regulating gene expression changes provoked by mitochondrial dysfunction, in concert with another nuclear hormone receptor, NHR-45 (51). This suggests that there is a convergence of signalling pathways and a common response to different perturbations of cellular homeostasis, one that overlaps with detoxification mechanisms triggered by xenobiotics (37) (see also (52)). This may reflect the fact that individual pathogens deploy multiple effectors to maximise their capacity to infect *C. elegans*. In the case of *P. aeruginosa*, these include exotoxin A that inhibits elongation factor-2 and thereby blocks translation (53), extendable protein polymers, called R-bodies, that may cleave ribosomes (38, 54), and pyoverdine, a siderophore that captures host iron, causing mitochondrial dysfunction and reductive stress. The latter leads to activation of the protective Ethanol and Stress Response Element (ESRE) network (55, 56). In what is likely to be part of a continual evolutionary arms race, *P. aeruginosa* can manipulate the host's energy status and suppress mitochondrial stress signalling (57, 58), but this can actually boost ESRE network activation (59).

Perhaps also because of this plethora of pathogenic strategies, on the host side, a variety of inputs modulate innate immune mechanisms, as part of the broad "Integrated Stress Response" (reviewed in (60)). Thus, in addition to GABA controlling DAF-16/FOXO and PMK-1/p38 signalling (see above), tyramine, acting through its neuronal receptor, and downstream serotonergic transmission, exerts a systemic control of mitochondrial states that influences resistance against pathogenic bacteria. This also involves 2 specific insulin-like peptides (61), as well the

neuronally-expressed nematode follicle-stimulating hormone G protein-coupled receptor homologue, FSHR-1 (62). In the case of *Staphylococcus aureus* infection, a major part of host defences are regulated by neuronally-released acetylcholine. This stimulates muscarinic signalling in the gut, leading to expression of Wnt and, via a canonical Wnt pathway, activation of the transcription factor HLH-30/TFEB. This then increases the expression of antimicrobial proteins (63). In parallel, an independent mechanism involving NHR-49/PPARα (see above) provides an important contribution to the host's resistance to infection (48). As a further example, in contrast to non-pathogenic bacteria, pathogens provoke a distension of the gut lumen (64, 65). Recent studies have shown that intestinal bloating activates neuroendocrine signalling and HLH-30/TFEB-dependent expression of innate immune genes, even in the absence of pathogenic bacteria (66, 67). This suggests that *C. elegans* is able to assess the physical state of its digestive system and mount a defensive response when it is perturbed.

Innate immunity, development and inheritance

Just as there appears to be a balance between immunity and reproduction (see above), development and defence are inter-related in *C. elegans*. One early example came from the study of mutants deficient for the Unfolded Protein Response (UPR), a conserved pathway that functions to restore homeostasis when misfolded proteins destined for secretion accumulate in the endoplasmic reticulum (ER). It was found that in the absence of the UPR, worms could not develop in the presence of *P. aeruginosa*, unless the PMK-1/p38 pathway was also knocked out. Both development and the innate immune response involve high levels of protein secretion. It was hypothesised that animals could accommodate the loss of the UPR during development, only if there was not the additional burden associated with the production of secreted antimicrobial proteins (68). Further studies continue to give insights into the molecular basis of ER homeostasis during an immune response, as well as revealing links that exist between the UPR and the UPS (69).

The activity of the PMK-1/p38 MAPK pathway is regulated across multiple tissues during development by the orthologue of Tribbles (70). It plays a role in intestinal immune surveillance (71), as well as in more generic stress resistance (72). PMK-1/p38 activity in the intestine is also repressed by a neuronal signal, and this

repression favours development (73). Additionally, PMK-1/p38 is regulated directly by the caspase CED-3, well known for its role in apoptosis. CED-3 can cleave PMK-1/p38, again reducing expression of infection- and stress-responsive genes, and promoting developmental gene expression (74). Something similar has been reported for the regulation of the IPR, with 2 antagonistic paralogues of unknown function controlling the balance between pathogen resistance and growth (75). There are likely to be other mechanisms that prioritise defence over development when worms are faced by a hostile environment.

It should not be forgotten that the entire trajectory of development in *C. elegans* is controlled by environmental signals. When conditions are unfavourable, for example in the absence of bacteria, worms enter an alternative "dauer" pathway rather than progressing towards the reproductive adult stage. Dauer larvae are non-feeding and highly resistant to environmental stress (76) and pathogens (77). In what could be an anticipatory protective mechanism, chronic exposure to certain pathogenic bacteria, including *P. aeruginosa*, causes a small proportion of progeny to enter the dauer stage, in a process dependent upon a single microRNA gene (78).

Small RNAs, both in *C. elegans* and from *P. aeruginosa*, are also involved in a remarkable instance of transgenerational inheritance of avoidance behaviour that helps protect worms from infection (79). Perhaps even more surprisingly, this transgenerational "memory" can be transferred to naïve animals when they are exposed to capsids purified from conditioned animals. It is hypothesised that these capsids, produced by an endogeneous retrotransposon, contain one or more species of small RNAs that mediate the effect (80). The effects seen with *P. aeruginosa* are currently somewhat exceptional. For example, the aversive behaviour elicited by *Serratia marcescens* (81) is not passed on to subsequent generations (79).

While earlier studies indicated that the antiviral RNAi triggered by Orsay virus is not passed on to subsequent generations (40), more recent work indicates that both abiotic and biotic treatments, including viral and microsporidial infection, that provoke an IPR can prime the defences of the subsequent generation, making them more resistant to infection (82). The molecular details are still being worked out, but this is clearly an exciting area for future research.

The response to oomycete infection

The protective mechanisms discussed above involve pathogens that infect *C. elegans per os*. This includes the coryneform bacterium, *M. nematophilum* that after passage through the intestine (83) adheres to the cuticle at the rectal and peri-anal regions. There it switches on an ERK MAP kinase cascade that mediates a protective tail swelling response (12). In the case of the oomycete, *Myzocytiopsis humicola*, infection of *C. elegans* is initiated following adhesion of mature zoospores to the nematode cuticle. The pathogen then penetrates its host's epidermis and grows and multiplies inside (84). Oomycete infection, or simply sensing by worms of an extract from infected animals, leads to the induction of multiple host genes encoding chitinase-like (CHIL) proteins (85). This is believed to lead, indirectly, to a modification of the worm's cuticle. As a consequence, *M. humicola* can no longer attach normally to its host, thus providing a protective mechanism against infection (84). For the time being, *chil* gene expression is the unique hallmark of oomycete infection. Indeed, neither infection with various pathogens nor exposure to abiotic stresses (heat shock, ER stress, starvation, mechanical damage of the cuticle, or osmotic shock) was found to up-regulate a canonical *chil* reporter gene (84). There are an ever-expanding number of natural pathogens described for *C. elegans*. Understanding the specificity of the host response remains a major challenge.

Innate immunity, sleep and neurodegeneration

The transcriptional response to *M. humicola* infection overlaps partially with the response to *Drechmeria coniospora* infection. This fungus also infects via the cuticle, sending vegetative hyphae into the epidermis. The commonly induced genes include several encoding antimicrobial peptides (AMPs). These AMPs are members of a remarkably diverse family, expanded in a lineage-specific manner in *Caenorhabditis* species, and exhibit a relatively broad spectrum of activity (86-88). Their regulation has been the subject of extensive investigation, with most attention focused on a single gene, *nlp-29* (e.g. (89, 90)) and serves as a good example of the degree to which immune defences in *C. elegans* have diverged from those of other animals (e.g. (91, 92)).

In contrast to *chil* genes, the expression of *nlp-29* is induced by physical damage in a PMK-1/p38-dependent manner (93-95). This requires microtubule-dependent recruitment of signalling molecules to the site of injury, in a process that is

coordinated with wound closure (96). Actin plays an important role in the process. But, unexpectedly, this relies on its interaction with the ARP2/3 complex, rather than with myosin as seen in other species (96, 97). This further highlights how divergent *C. elegans* is in some aspects of its biology, even those involving fundamental cellular processes.

Again in contrast to *chil* genes, expression of *nlp-29* is also induced by osmotic stress, but via a distinct PMK-1/p38-independent mechanism (89, 98). Moreover, it has been suggested that, in part, *nlp-29* expression is regulated by a putative cuticle-associated damage sensor that coordinates three distinct environmental stress responses (99), reinforcing the notion of an intimate link between immunity and other homeostatic and physiological mechanisms in *C. elegans*.

Indeed, AMPs have recently been shown to be important regulators of sleep in worms. An epidermal tolloid/bone morphogenic protein (BMP)-1-like protein, NAS-38, promotes entry of *C. elegans* into a quiescent state during larval moulting. NAS-38/BMP activates PMK-1/p38 and transforming growth factor beta signalling pathways leading to AMP gene expression. Several AMPs act as somnogens, and are presumed to be secreted from the epidermis, to signal across tissues to the sleep-active neuron called RIS. Thus NLP-29 acts via its cognate receptor NPR-12, in locomotion-controlling neurons that are presynaptic to RIS, depolarize RIS and induce sleep. Significantly, sleep increases the chance of *C. elegans* surviving injury. This suggests that peripheral wounds can signal to the nervous system to increase protective quiescence in *C. elegans*, also linking innate immunity to sleep (100).

Despite an overall age-related decrease in the activity of the PMK-1/p38 immune pathway (see above), NLP-29 levels actually increase in post-reproductive aged worms, possibly because of a deterioration of the cuticle's barrier function. High levels of NLP-29 trigger dendrite degeneration in ageing *C. elegans*, acting through NPR-12. Infection with the fungus *D. coniospora* also boosts NLP-29 expression and as a consequence, can also cause dendrite degeneration. Thus in addition to its antimicrobial and somnogenic roles, NLP-29 antagonises neuronal function in *C. elegans* (101). The physiological relevance of these findings has not yet been clearly elucidated, but this link between infection and neurodegeneration might confer an adaptive advantage, if it reduces pathogen spread by making worms less motile.

Defence and counter-attack

Given the importance of AMPs like NLP-29 in these different aspects of host biology, it is perhaps not surprising that its regulation is targeted by *D. coniospora*. The fungal genome encodes hundreds of predicted secreted virulence factors ((102); see also (103)). Only a very few have been studied in any detail. Among them one enterotoxin (DcEntA) appears to interact with multiple host proteins and thereby interfere with *nlp-29* expression in several different ways. It affects the cytoskeleton, which, as explained above, is required for the recruitment of signalling proteins needed to switch on AMP gene expression. DcEntA also interferes with endocytosis, and thereby innate immune signalling, imposes a general block on translation and stops a key STAT-like transcription factor from entering the nucleus, directly preventing AMP genes from being transcribed (104).

Intriguingly, expression in the *C. elegans* epidermis of a second secreted fungal enterotoxin, DcEntB, actually promotes nuclear localisation of the STAT transcription factor that drives AMP gene expression. In this case, since DcEntB has a profound effect on nucleolus morphology, it was suggested that the heightened immune response could reflect an as yet uncharacterised surveillance mechanism. This would then represent a host counter-measure against the pathogen (104).

Concluding remarks

It is clear that we are still a very long way from being able to describe in detail the remarkably complex dynamics that play out during an actual infection, not just because of the extensive molecular arsenals deployed by pathogens, but also because the innate immune system is so intimately connected to the whole of host biology (Figure 2). Despite the impressive advances that have been made in recent years, unravelling these links and obtaining a comprehensive understanding of host-pathogen interactions remains, even with *C. elegans*, a daunting challenge.

Perspectives

- The use of *C. elegans* as a model host provides an unrivalled opportunity to understand, at the molecular and cellular levels, how innate immune mechanisms are imbricated in organismal physiology.

- *C. elegans* can be used to understand pathogen virulence strategies, and thanks to the powerful genetic tools available, to address host defence too.
- Increasingly, questions about immunity in *C. elegans* are being addressed from an integrative perspective. These studies hold the promise of providing a synthetic understanding of host-pathogen interactions, for which the broad lines should be relevant across species.

Figure Legend.

Figure 1. *C. elegans* is readily amenable to study at multiple scales. **A**. Top panels: from the whole animal to the cellular scale; photomicrographs, merging differential interference contrast and fluorescence images, of adult transgenic *C. elegans* infected by *D. coniospora*. The worms express dsRed in the epidermis under the control of the constitutive *col-12* promoter and GFP under the control of the infection-inducible *nlp-29* promoter (94). On the left, fungal spores are mainly concentrated on the tail, where bright green fluorescence can be seen. The worm on the right has been infected principally on the head and vulva (not visible), and the green fluorescence is strong throughout most of the epidermis but not in the tail, which therefore appears red. Bottom panel: from the whole animal to the macromolecular scale; tiling assembly of confocal images of an adult transgenic *C. elegans* expressing in the epidermis chimeric proteins labelling actin (red) and microtubules (green). The insert is a higher magnification of the region bounded by the dotted white rectangle, showing the complex architecture of the cytoskeleton; individual actin filaments and microtubules are visible. In all images, the worm's head is to the left; adult worms are approximately 1 mm in length. Images kindly provided by Sebastien Mailfert (bottom panel; CIML imaging facility) and Nathalie Pujol (all panels). **B**. Schematic representation of the different topics covered in the review, with an indication of the scale(s) of their study.

Abbreviations

AMP	Anti-microbial peptide
ARP2/3	Actin-related proteins-2/3

ATF	Activating transcription factor
bZIP	Basic leucine zipper
CHIL	Chitinase-like
ER	Endoplasmic-reticulum
ERK	Extracellular signal regulated kinase
FOXO	Forkhead box O protein
GABA	Gamma-aminobutyric acid
ILR	Insulin-like receptor
MAPK	Mitogen-activated protein kinases
PPARα	Peroxisome proliferator-activated receptor alpha
STAT	Signal Transducer and Activator of Transcription
UPS	Ubiquitin–proteasome system
TFEB	Transcription factor EB
Wnt	Wingless and Int-1

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Authors' contributions

BH and JJE defined an outline, JJE wrote a first draft, BH and JJE edited and revised the manuscript.

Competing interests

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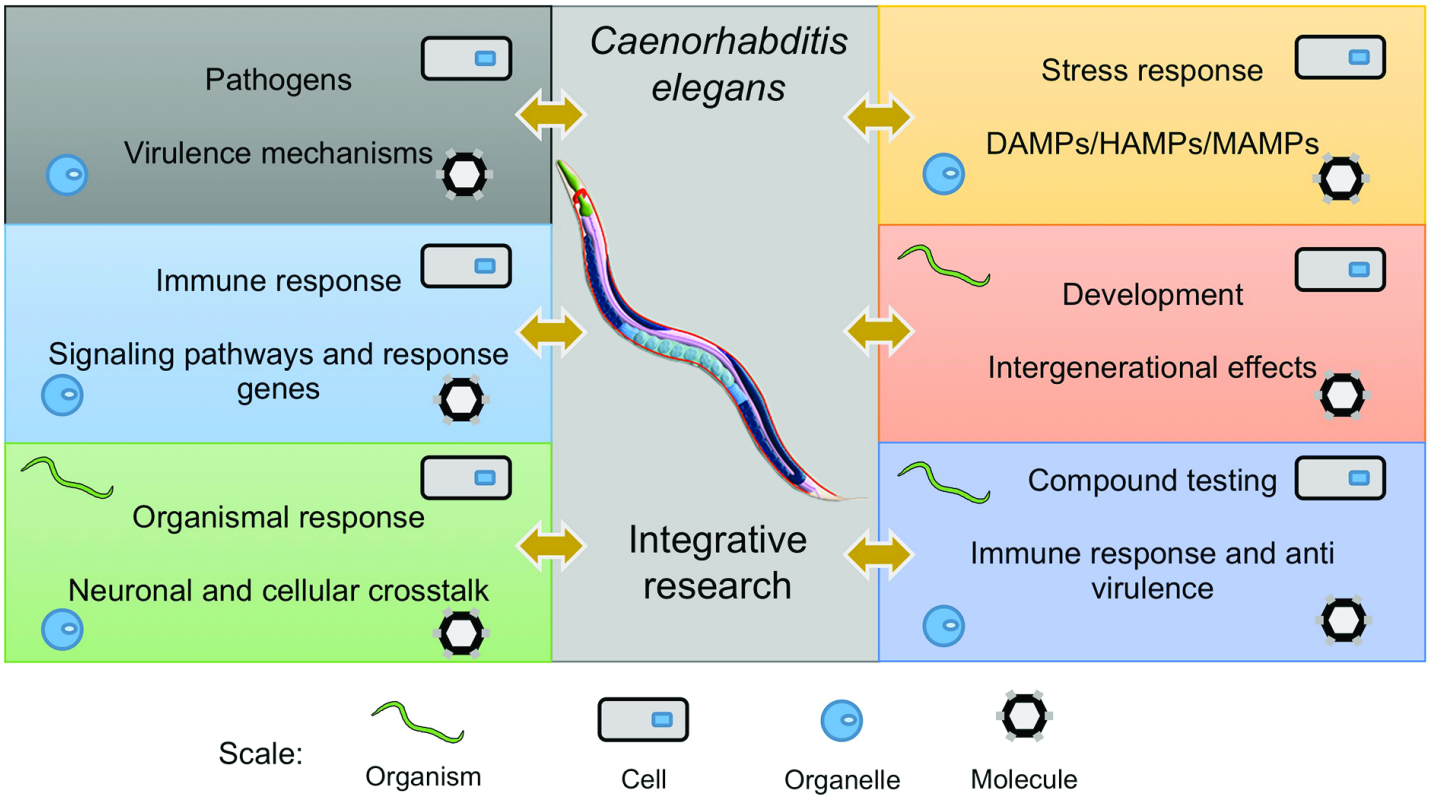
Pathogens
Virulence mechanisms
Caenorhabditis elegans
Stress response
DAMPs/HAMPs/MAMPs
Immune response
Signaling pathways and response genes
Development
Intergenerational effects
Organismal response
Neuronal and cellular crosstalk
Integrative research
Compound testing
Immune response and anti virulence
Scale:
Organism
Cell
Organelle
Molecule