ISJ 7: 45-52, 2010 ISSN 1824-307X

REVIEW

# Antimicrobial peptides in *Caenorhabditis elegans*

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*Accepted January 19, 2010*

## Abstract

The nematode *Caenorhabditis elegans* is one of the most successful model species for experimental research because of its sequenced genome, the versatile genetic toolkit and the straightforward breeding among others. In natural conditions however, this tiny worm is constantly surrounded by micro-organisms, simultaneously a source of indispensable nutrition and inevitable pathogens. Lacking an adaptive immune system, the worm solely relies on its innate immune defence to cope with its challenging life style. Hence *C. elegans* is an excellent model to gain more insight in innate immunity, which is remarkably preserved between invertebrate and vertebrate animals. The innate defence consists of receptors to detect potential pathogens, a complex network of signalling pathways and last but not least, effector molecules to abolish harmful microbes. In this review, we focus on the antimicrobial peptides, a vital subgroup of effector molecules. We summarise the current knowledge of the different families of *C. elegans* antimicrobial peptides, comprising NLPs, caenacins, ABFs, caenopores, and a recently discovered group with antifungal activity among which thaumatin-like proteins.

**Key Words**: caenacins; ABFs; caenopores; insulin signalling; immunity; host-pathogen interaction

## Introduction

As a free living soil nematode, *Caenorhabditis elegans* forms an extremely interesting model to study the interaction with bacteria, its main food source and substrate. As the distribution of bacteria is mixed in natural conditions, worms should continuously search for regions in the soil where the benefit of energy-rich and benign bacteria exceeds the possible presence of harmful bacteria, be it slow or fast killers (Shtonda and Avery, 2006; Abada *et al.*, 2009). It is clear that discerning different types of bacteria and employing an effective battery of antibacterial molecules are crucial for worms. *C. elegans* has a tremendous variety of chemosensory receptors to detect both interesting and harmful bacteria, the latter provoking pathogen avoiding behaviour (Zhang et al., 2005; Pradel et al., 2007; Schulenburg and Ewbank, 2007). In contact with harmful bacteria, recognition molecules activate specific signalling pathways which ultimately induce the release of immune molecules. In this review we specifically focus on the variety of antimicrobial peptides (AMPs), produced by *C. elegans* as part of its defence system. AMPs are defined as relatively short molecules with a low molecular weight (below 5 kDa), often containing 10 up to 150 amino acids (Bulet *et al.*, 1999; Jenssen *et al.*, 2006). Their expression can be either constitutive or inducible at the time of infection (Kato *et al.*, 2002; Alegado and Tan, 2008). AMPs posses a natural antimicrobial activity often thanks to their cationic and amphipathic structure which facilitates the disruption of anionic cell walls and phospholipids membranes of microbes, although other microbicidal mechanisms have also been proposed (Bulet *et al.*, 2004; Brogden, 2005). Worms have an innate immune system which constitutively expresses certain AMPs whereas complex mixtures of AMPs are induced upon encounter with different pathogens. Note that by unfolding a specific mixture of 'antibiotics' the worm can prevent a straightforward development of resistant pathogenic strains. Therefore, studying AMPs in an experimentally favourable immunological model such as *C. elegans*, forms a lead for the development of new strategies to deal with pathogenic infections in the future. We highlight the diverse AMP families in *C. elegans* and summarise the evidence for their biological function, specificity, expression and the pathways involved as far as known.

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**Neuropeptide like proteins**

The *C. elegans* genome encodes at least 42 neuropeptide like proteins (NLPs) which can be divided into minimal 11 subgroups according to their unique bioactive motifs (Nathoo *et al.*, 2001). Although most of the *nlp* genes are translated into conventional neuropeptides, others may possess distinct or additional functions. The first indication of an antimicrobial role for *nlp* genes came from a study in 2002 by Mallo *et al.* in which they observed the induced expression of *nlp-29* upon infection with the gram-negative bacterium *Serratia marescens*. Later on in 2004, Ewbank's group demonstrated an antifungal activity for NLP-31 against *Drechmeria coniospora in vitro* (Couillault *et al.*, 2004).

In *C. elegans,* most of the infection-inducible *nlp* genes were named as such because of their limited sequence similarity with YGGXamide neuropeptide genes, sharing YGGWG and YGGYG motifs (Nathoo *et al.*, 2001). They form a monophyletic group, found in a 12 kb region on the left arm of chromosome V, referred to as "the *nlp-29* cluster" (Pujol *et al.*, 2008b). This cluster comprises *nlp-27* to *31* and the adjacent gene *nlp-34*. Two other *Caenorhabditis* species *C. briggsae* and *C. remanei* possess orthologous genes. In *C. brigssae*, these genes are orientated in different clusters: *Cbr-nlp-27* and *Cbr-nlp-34.1, Cbr-nlp-34.2 and Cbr-nlp-34.3*. Phylogenetic analysis indicates that gene duplications driven by natural selection form the basis for the evolutionary diversification of these clusters. At the time of divergence of the different *Caenorhabditis* lineages from a common ancestor, two genes were at the nlp locus: *nlp-27* and *nlp-34*. Via gene duplication, *nlp-27* gave rise to 5 genes in *C. elegans* whilst *nlp-34* gave rise to 3 genes in *C. brigssae* (Pujol *et al*., 2008b).

Expression of the genes belonging to the *nlp-29* cluster is predominately limited to the epidermis and the intestine and is controlled by a diverse interplay of mechanisms. Besides infection, physical injury of the epidermis as well seems to have an influence on expression of *nlp-29* and *nlp-31* (Pujol *et al.*, 2008a). In both conditions, the expression levels are controlled by distinct pathways that converge in a conserved signalling cascade: the p38 mitogen activated kinase (MAPK) pathway. This pathway, involving MAPK PMK-1, MAPK kinase (MAPKK) SEK-1 and MAPKK kinase (MAPKKK) NSY-1, lies downstream of the TIR (Toll-interleukine 1 receptor) adaptor protein TIR-1, an ortholog of the human protein SARM (selective androgen receptor modulator). Involvement of TIR-1 in the control of *nlp* expression upon fungal infection was established in 2004 by Couillault *et al*. These researchers generated transgenic worms expressing GFP (green fluorescent protein) under control of the *nlp-29* promotor and observed an increased fluorescent signal in the hypodermis upon infection with *D. coniospora* and *S. marescens*. RNAi of *tir-1* in the p*nlp-29::gfp* reporter lines diminished the constitutive and infection-induced expression of p*nlp-29::gfp*. Moreover, *tir-1* (RNAi) worms are more susceptible to the deleterious effects of both fungi (*D. coniospora*) and bacteria (*S. marescens*).

Further studies have unravelled additional components of the immune signalling pathway. Expression of *nlp-29* is regulated by the upstream factor *tpa-1*, homologous to the mammalian protein kinase C (PKC) delta (Ziegler *et al.*, 2009). In the epidermal response to fungal infection, PKC delta is activated by a tribbles-like kinase NIPI-3. However, upon wounding NIPI-3 is not required for *nlp-29* induction, supporting the existence of a pathogen-specific reaction, in addition to a non-specific protective response (Pujol *et al.*, 2008a).

As mentioned above, also bacteria can trigger the *nlp* gene expression. In contrast to fungal infection, expression predominately occurs in the intestinal epithelium and is regulated by a *C. elegans* protein kinase D (DKF-2) which lies downstream of *tpa-1*, and acts in *pmk-1* dependent and independent ways (Ren *et al.*, 2009).

To make things even more complicated *nlp-28* and *nlp-29* expression is also induced as a consequence of osmotic stress and this by yet another transcriptional response which is *pmk* independent (Pujol *et al.*, 2008b).

**Caenacins**

Similarly to the neuropeptide like proteins, specific genes belonging to the caenacin family are induced upon infection with *D. coniospora* amongst other pathogens. *cnc-1* up to *cnc-5* and *cnc-11* form a genomic cluster, also situated on the left arm of chromosome V, referred to as "the *cnc-2* cluster". Mature peptides belonging to the NLP and CNC classes are rich in glycine and aromatic amino acids and most of them can be distinguished by the QWGYG motif present just C-terminal to the predicted signal sequence cleavage site. Despite the fact that they are structurally and phylogenetically related to the *nlps*, these *cnc* genes are regulated in a very distinct way (Zugasti *et al*., 2009).

Unlike the *nlp* genes, osmotic stress has only little effect on the expression of *cnc-11* and no effect on the other members of the *cnc-2* cluster. While induction of genes, belonging to the *nlp-29* cluster, upon wounding or infection relies almost entirely on a p38 MAPK signalling cascade, only physical injury and not infection seems to have a lowering effect on induction of *cnc-2* cluster genes in *pmk-1* mutants. This indicates that the expression of *cnc* genes in the epidermis upon fungal infection is dependent on a different immune pathway (Zugasti *et al*., 2009).

As the expression of *cnc-2* was more strongly induced upon infection than upon wounding, researchers focused on this gene. They constructed transgenic strains expressing either GFP (pcnc-2::GFP) or the 'mCherry' fluorescent protein (pcnc-2::mCherry) under control of the *cnc-2* promotor and reported that the *cnc-2* gene was exclusively expressed in the epidermis. Furthermore, induced expression of reporter genes was observed upon infection with *D. coniospora* but not upon bacterial infection with *S. marescens* and *Pseudomonas aeruginosa* (Zugasti *et al*., 2009).

Searching for the specific signalling pathway involved in *cnc-2* gene expression they found that the transcription is controlled in a paracrine way by

the *C. elegans* transforming growth factor β ortholog DBL-1 as in *dbl-1* mutant worms the induced expression of the *cnc-2* reporter was much lower (Zugasti *et al*., 2009).

**Antibacterial factor (ABF) peptides**

The *C. elegans* genome encodes six homologues (ABF-1 to ABF-6) of the *Ascaris suum* antibacterial factor (ASABF) peptides, which are microbicidal factors that were first discovered in the body fluid of the parasitic nematode *A. suum* (Kato and Komatsu, 1996). Sequence identity and structural commonality reveals that these nematode ABFs are genetically related: all known ABF peptides share a cysteine-array consisting of eight conserved cysteine residues and a secretory signal sequence at the N-terminus. High homology appears in the region encompassing the eight cysteines with 25-95% similarity. In contrast, the region C-terminal to the last cysteine is divergent and varies in length (Froy, 2005). Most likely this part is cleaved off post-translationally, as was shown for ASABF-α (Kato and Komatsu, 1996; Zhang *et al.*, 2000).

Apart from their sequence similarity, several other properties support a direct role for ABFs in the innate immune response of *C. elegans*. Recombinant ABF-2 exhibits antimicrobial activity *in vitro* against a broad range of gram-positive, gram-negative and fungal pathogens. However, gram-positive bacteria tend to be more sensitive and some gram-negative and fungal strains are resistant to ABF-2 (Kato *et al*., 2002). ASABF-α possesses a similar antimicrobial specificity (Zhang *et al.*, 2000). The exact bactericidal mechanism of ABF peptides remains to be elucidated, but based upon similarities in primary structure and antimicrobial effects, the killing of microbes could be caused by disruption of their cytoplasmic membrane (Zhang *et al*., 2000; Kato, 2007).

ABF peptides are constitutively expressed under normal growth conditions when *C. elegans* is cultivated on a lawn of the non-pathogenic strain *E. coli* OP50, as was shown for ABF-1, ABF-2 and ABF-3 (Kato *et al*., 2002; Alper *et al*., 2007; Alegado and Tan, 2008). The pharyngeal tissue represents the main production site for ABF-1 and ABF-2 (Kato *et al.*, 2002). Together with ABF-3, ABF-1 is also produced in the intestine (Alper *et al*., 2007). In this way, the constitutive expression of ABFs may be part of a general defence mechanism in the worm that protects the digestive tract from microbial infection, an important threat since *C. elegans* mainly feeds on bacteria. Expression of ABF-2 and ABF-3 was also observed in the excretory cells of the worm, presumably to protect the openings to the exterior (e.g., anus and excretory pores) that are continuously in contact with potential pathogens from the environment (Kato *et al.*, 2002; Alper *et al.*, 2007).

Recent evidence indicates that on top of the general defences against various microbes ABFs also participate in a specific immune response induced upon infection (Alper *et al*., 2007; Alegado and Tan, 2008; Means *et al*., 2009). In *A. suum* a number of the ASABF peptides were demonstrated to be induced after injection of heat-killed bacteria in the pseudocoelom (Pillai *et al*., 2003; Minabi *et al*., 2009). This induction was also proven for some of the ASABF-type homologues in *C. elegans*. Worms infected with the gram-negative pathogen *Salmonella typhimurium* displayed an increase in *abf-2* transcript levels by a hundred-fold. Deficiencies in *abf-2* after RNAi treatment correlated with a significantly higher bacterial load in the intestine after exposure to *S. typhimurium* in comparison with control animals. These findings suggest that *C. elegans* induces the expression of *abf-2* as part of an immune response to *S. typhimurium* infection that is essential for limiting bacterial growth in the worm's digestive tract. However, transcript levels were initially indistinguishable within the first 24h of exposure which implies that the induced AMP response may not be due to direct sensing of pathogenic microbes but due to later events such as the production of specific bacterial factors or damage to host tissues (Alegado and Tan, 2008). Upregulation of *abf-1* and *abf-2* was also observed in wild type *C. elegans* after infection with the yeast *Cryptococcus neoformans (Means et al., 2009)* and exposure to the gram-positive bacteria *Staphylococcus aureus* elicited a weak induction of *abf-3* (Alper *et al*., 2007). Therefore, we can conclude that nematode ABFs play a crucial role in the general and more specific induced immune response to pathogenic attack.

The signalling pathways responsible for the upregulation of ABF peptides upon infection of *C. elegans* have not yet been fully elucidated. TOL-1, the single homologue of the Toll-like receptor encoded in the worm's genome, seems to be required for the correct expression of *abf-2* since quantitative reverse transcription-PCR analysis indicates a decreased level of *abf-2* transcripts in *tol-1* mutants (Tenor and Aballay, 2008). Moreover *tol-1* mutants display a reduced lifespan on *Salmonella enterica* due to a rapid invasion of the pharynx, which is the main expression site for the antibacterial ABF-2 peptide (Tenor and Aballay, 2008). These results demonstrate that TLR-mediated signalling probably contributes to the elicitation of a specific immune response in *C. elegans* on top of its established role in the pathogenic avoiding behaviour of the worm (Pujol *et al*., 2001). In addition neurons expressing G protein-coupled receptors (GPCRs) also participate in the regulation of *abf* expression levels. A deficiency in the neural circuit involving *npr-1*, which encodes a GPCR related to the mammalian neuropeptide Y receptors, reduces the expression level of *abf-1* in response to infection by *P. aeruginosa (*Styer *et al., 2008)*. Recently an evolutionary conserved pathway consisting of CED-1 and C03F11.3, orthologs of the mammalian scavenger receptors SCARF1 and CD36, was shown to activate antimicrobial peptides including *abf-1* and *abf-2* upon yeast-infection (Means *et al.*, 2009).

Up till now, the phylogenetic relationship between nematode ABFs and other metazoan AMPs remains unclear. ASABF-type peptides contain a cysteine-stabilised α/β (CSα/β) consensus motif consisting of an α-helix and two antiparallel β-

strands stabilised by four internal disulfide bridges. Recently, a novel member of ASABF-type peptides was discovered in *A. suum* containing only six cysteines. So far this AMP forms the single exception and probably arose after the divergence of Ascaridida and Rhabditida (Minaba *et al.*, 2009). Structural similarities between nematode ABFs and invertebrate defensins suggest a common ancestry in the evolution of these antimicrobial factors. First of all, the CSα/β motif of ASABF-type peptides is also found within invertebrate defensins that are further classified according to the number of cysteine residues contributing to the intramolecular disulfide bonds: the cysteine array of insect/arthropod defensins typically comprises six cysteine residues, mollusk defensins are characterized by eight cysteines (Froy, 2005). Secondly, the primary structure of ASABF-type peptides includes an insect/arthropod consensus sequence (Cys1-[…]-Cys2-Xaa-Xaa-Xaa-Cys3-[…]-Gly-Xaa-Cys4-[…]-Cys5-Xaa-Cys6) with six conserved cysteines and one glycine residue (Kato and Komatsu, 1996; Kato *et al*., 2002). As a third argument Zhang and Kato (2003) showed that nematode ABFs share even more characteristics with two mollusk defensins: myticin and an AMP isolated from the Mediterranean mussel *Mytilus galloprovincialis* (MGD-1). ASABF-type peptides and mollusk defensins both contain eight cysteine residues with an identical pairing and a similar precursor organization consisting of an N-terminal secretory signal sequence, followed by the mature polypeptide and a cleavable "pro-region " at the C-terminus. In classical CSα/β type AMPs, such as insect defensins, this "pro-region" is located directly after at the N-terminus. In summary, nematode ABFs and mollusk defensins share several structural properties and could therefore be generated from a common ancestor. However, the absence of highly reliable evidence such as a significant sequence similarity or a conserved genomic organization (exon-intron structure) cannot exclude that these two groups of AMPs developed through convergent evolution (Froy, 2005). Hopefully, the identification of new CS$\alpha/\beta$ type AMPs in different phyla will clarify the evolutionary trajectory of nematode and invertebrate defensins (Froy, 2005; Rodriguez de le Vega and Possani, 2005).

**Caenopores**

Caenopores are the saposin-like proteins (SPP) of *C. elegans*. Saposins form a multifarious protein family characterized by an alpha helix bundle stabilized by 3 unique disulphide bonds and the ability to interact with phospholipid membranes (see for review Bruhn, 2005). Based on these hallmarks, Patthy (1996) designated the putative protein product of gene T07C4.4 as the first *C. elegans* saposin-like protein. Two years later, 5 additional SPP genes were found in this nematode. All six predicted SPPs appeared similar to the amoebapores of *Entamoeba histolytica* and granulysin from human cytotoxic T lymphocytes as they consist of a secretory signal peptide followed by a single saposin-like domain (Banyai and Patthy, 1998). Note that amoebapore-like SPPs might have been the first antimicrobial peptides since this protein family emerged even before the advent of metazoans (Leippe 1999). Banyai and Patthy (1998) recombinantly expressed T07C4.4 (SPP-1) which allowed to demonstrate the characteristic helix bundle structure and an antibacterial effect on *E. coli* (JM-109). In addition, the three-dimensional structure of SPP-5 was studied in great detail by Mysliwy *et al*. (in press). They confirmed that SPP-5 has five amphiphatic helices, connected by three disulfide bonds, arranged in a folded leaf typical for the saposin-like protein family.

Further examination of the worm's genome lead to the discovery of 28 different *spp* genes coding for 33 saposin-like proteins which were named caenopores because of their structural and functional resemblance with amoebapores (Roeder *et al*., 2010). Indeed, like amoebopores, at least SPP-5 was shown to display pore-forming activity capable of killing bacteria by permeabilizing their cytoplasmic membrane. A phylogenetic analysis of the 33 SPPs shows different clusters. In case of the cluster comprising SPP-*2* to SPP-*6*, all corresponding genes are located in the same chromosomal region, consistent with a series of gene duplications. Roeder *et al*. (2010) investigated the functional significance of SPP-1, SPP-3, SPP-4, SPP-5 and SPP-6 by means of RNAi-mediated gene silencing. One gene, *spp-5*, significantly affected the overall fitness of worms measured as the number of laid eggs and the deposition of fat tissue. Silencing this same gene, contrary to the other genes tested, had a huge impact on the number of *E. coli* that could survive in the intestinal lumen (Roeder *et al*., 2010).

When grown on standard culture medium NGM, wild type worms consequently expressed *spp-5* for all the bacteria/conditions tested. The *spp-3* gene, on the other hand, was induced only when confronted with *B. megaterium*, *M. luteus* and starvation. Further analyses of SPP-5 learnt that it is as potent as SPP-1 against the gram-positive *B. megaterium* and even more active against the gram-negative *E. coli* (Roeder *et al*., 2010). The genome wide microarray analysis of Wong *et al*. (2007) searched for pathogen specific signatures in the immune response of *C. elegans*. No expression change of any *spp* gene was observed against *Erwinia carotovora*, whereas *spp-18* was upregulated upon infection with *Photorhabdus luminescens* as well as *spp-5*, *spp-8*, *spp-14* and *spp-21* in case of an *Enterococcus faecalis* infection (Wong *et al*. 2007). Moreover, SPP-1 was shown to be induced upon infection with *S. typhimurium*. It was found that *Salmonella* strains lacking, among others, the virulence factor SPI-2 had difficulties to persist in *C. elegans* intestine. Interestingly, such persistence deficiencies could be rescued when *spp-1* of the worm was reduced by RNAi (Alegado and Tan, 2008). However, Evans *et al*. (2008) showed that *spp-1* is repressed upon infection with *P. aeruginosa*. Next, they showed that downregulation of *spp-1* expression, among others, is a key strategy to overcome the immune system of its host. This downregulation depends on the response regulator GacA and the quorum sensing

regulators LasR and RhlR; and interferes with the insulin-like signalling via the DAF-2/DAF-16 axis, which is important for the regulation of stress response, longevity and immune function (Evans *et al*., 2008). This discovery correlates perfectly with the observation that *spp-1* (and *spp-12*) expression is downregulated by loss of insulin signalling. Decreasing the expression of either of these *spp* genes by RNAi reduced the lifespan of *C. elegans* on *E. coli* (Murphy *et al*., 2003). The exact mechanism of how the diverse *spp* genes are controlled is still not known. The only additional information currently available is that expression of *spp-9* and *spp-18*, along with more than 80 defence-related genes, appears to be regulated by the protein kinase D (Ren *et al.,* 2009).

Worth mentioning is that *spp-5* is exclusively expressed in the gut (Roeder *et al*., 2010). The same accounts for *spp-1,* but *spp-7* is also expressed in the pharyngeal muscles and head neurons (Alper *et al*., 2007). Given that most caenopore genes (except *spp-2*, *spp-12*, *spp-16* and *spp-19*) have the intestine specific transcription factor ELT-2 binding domain in their putative promotor regions, it can be assumed that most caenopores will be expressed in the intestine. This finding prompted Roeder *et al*. (2010) to state that "Caenopores or SPPs are most likely the only candidates to tackle the diverse mixture of microbes *C. elegans* is confronted with in the natural environment". In their opinion, other antimicrobial peptides described for *C. elegans* are either not numerous enough (ABFs), or they are expressed at the wrong location (hypodermis: CNCs and NLPs). Although Roeder is perhaps correct when postulating that the SPPs are the most significant group of AMPs, this statement should be relativized as the importance of other types of AMPs, functioning alone or in cooperation with other defence molecules of the immune system of *C. elegans*, should not be underestimated.

**Other potential AMPs**

Besides the above mentioned members of the NLP, CNC, ABF and SPP families, which are induced upon infection with different types of pathogens (Troemel *et al.*, 2006; Muir *et al.*, 2008), Pujol *et al*. (2008b) found other previously uncharacterised genes that seem to be specifically induced upon fungal challenge. Hence they were named: Fungus-Induced Proteins (FIP), FIP Related Proteins (FIPR) and Glycine-Rich Secreted Protein 2 (GRSP-2). A comparison to peptides with known antimicrobial activity (Fjell *et al.*, 2007) revealed that each of the *fip*, *fipr* and *grps* genes could potentially encode AMPs (Pujol *et al*., 2008b). However, further biochemical and functional analysis will be required to confirm this statement.

Recently, homologs of thaumatins and other pathogenesis-related plant proteins have been discovered in the *C. elegans* genome (Brandazza *et al*., 2004; Shatters *et al*., 2006). Thaumatins were originally isolated from the fruits of *Thaumatococcus danielli* and extensively studied because of its sweetening properties. Later studies have demonstrated antifungal activity for thaumatin-like proteins such as stimulating microbial membrane permeability (Vigers *et al*., 1991), beta-1,3-glucanase activity (Grenier *et al*., 1999) and alpha-amylase inhibiting properties (Svensson *et al*., 2004). Moreover, recent work from the Tan group has implicated an antimicrobial role for a member of the thaumatin family: *thn-2*. Knockdown of *thn-2* by RNAi enhances the susceptibility of *C. elegans* to a *P. aeruginosa* infection (Evans *et al*., 2008). As for *spp-1* (see above), the expression of *thn-2* is down-regulated upon infection with this pathogen (Shapira *et al*., 2006; Evans *et al*., 2008). These findings suggest that homologs of these plant proteins could well represent potential antimicrobial proteins in *C. elegans*. Note that thaumatins are not indisputably antimicrobial *peptides* as they count around 200 amino acid residues.

**Conclusion**

To feed on and fight off a smorgasbord of bacteria and pathogens, *C. elegans* developed a diverse armory of immune defence molecules. This feed versus fight paradox is most certainly an intriguing issue. Whilst on the one hand worms are completely dependent on bacteria/fungi for their survival, some of these micro-organisms might on the other hand contribute to their death. Numerous chemoreceptors allow *C. elegans* to distinguish between benign and harmful bacteria. However, a continuous cost-benefit analysis is necessary for making the correct choice between for example a slightly pathogenic bacterium with a high nutritional value or a non-pathogenic bacterium with low nutritional value.

The AMPs constitute the most numerous and versatile group of immune effector molecules, allowing specific and effective responses against different pathogens. Even the expression of related AMP genes can be regulated by very distinct pathways, providing the host specific alternative defence possibilities against the potentially detrimental effects of different types of pathogen invasions. The important role of AMPs in the early immune response has already been proven by the isolation of numerous animal AMPs, mostly from higher organisms such as vertebrates and arthropods. Nonetheless, the *C. elegans* AMPs still form a poorly studied group. As described earlier in this review many putative AMPs were yet identified thanks to their induced expression upon infection or their structure/sequence similarities with closely related AMPs (Kato *et al.*, 2002; Mallo *et al.*, 2002; Couillault *et al.*, 2004). The strategy of homology-based searches starting from known AMP sequences (e.g. vertebrate or arthropod AMPs), however, is limited due to the short length and rapid molecular evolution of these peptides (Kato *et al.*, 2002). This constant evolution of the immune defences compensates for the renewing virulence mechanisms of a pathogen and is a necessity to ensure the survival of a species. In fact, to our knowledge, none of the natural occurring AMPs were yet directly purified from *C. elegans*. Therefore, we assume that not all *C. elegans* AMPs are identified yet which makes the identification of additional AMPs a first great challenge to better

understand the worm's innate immunity. In addition, peptidomics based approaches for the identification of *C. elegans* AMPs might help to address the question of which AMPs might cooperate and contribute to target specific pathogens.

Since multiple AMPs are expressed in response to infection by a single pathogen a certain level of cooperativity might exist between different AMPs and/or other defense molecules. Cases of cooperative activity among AMPs and/or other immune effectors have already been reported in mammals, e.g. the synergy between different murine defensins or the enhanced antibacterial effects of human β-defensin and lyzosyme (Chen *et al.*, 2005; Wu *et al.*, 2009). In *C. elegans* the field of synergistic effects in innate immunity thus remains an important research objective.

To date, many aspects of the regulation of AMP expression and their mode/site of action remain elusive. It was shown that *C. elegans* can activate multiple immune pathways upon encountering a single pathogen (Alper *et al.*, 2007; Schulenberg *et al.*, 2007) and assumed that this network of interacting signalling cascades results in the expression of an appropriate set of antimicrobial genes. Indeed, another type of pathogen can elicit the expression of a different set of AMPs indicating that *C. elegans* distinguishes between pathogens and suppresses infections in a specific manner (Alper *et al.*, 2007; Wong *et al.*, 2007). Yet, the exact role of many putative upstream regulators of AMP expression, as suggested by e.g. genome-wide transcriptomic analyses, has not yet been characterised. Given that *C. elegans* is a versatile model with an extended experimental toolbox, e.g. the possibility to perform (large-scale) RNAi and fluorescence studies (Couillault *et al.*, 2004; Alper *et al.*, 2007), this organism is ideally suited to address these and other challenges in innate immunity research.

During evolution, *C. elegans* managed to survive a daily confrontation with bacteria, many of which can be harmful for humans. Therefore, it is expected that future efforts will result in a deeper insight in how this worm resists bacteria in a sustainable way.

**Acknowledgments**

PV benefits a post-doctoral fellowship and AB and IB a Ph. D. fellowship of the Research Foundation - Flanders (FWO-Vlaanderen).

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