ISJ 9: 122-133, 2012 ISSN 1824-307X

REVIEW

Nematode antimicrobial peptides

DEK Tarr

Department of Microbiology and Immunology, Arizona College of Osteopathic Medicine, Midwestern University, 19555 N. 59th Ave., Glendale, AZ, 85308, USA

Accepted June 15, 2012

Abstract

There is currently insufficient data to determine the full spectrum of antimicrobial peptides (AMPs) that nematodes produce. Defensins, nemapores, cecropins, and caenacins/neuropeptide-like proteins have been identified, but none of these is produced universally by all nematode species, and no single species produces all AMP types. Therefore, it seems unlikely that there is a core set of AMPs that can be considered "archetypal" for nematodes. Additional information is also needed from under-represented Ecdysozoan and Lophotrochozoan taxa to clarify the evolution of AMPs. To avoid generalizations that may later prove inaccurate, caution should be used when choosing "representative" sequences or taxa, and analyses should be interpreted conservatively when limited information is available.

Key Words: antimicrobial peptide; caenacin; caenopore; defensin; nemapore; neuropeptide-like peptide

Introduction

Let's be honest-we often use the word "nematode" when we should say "*C. elegans"* (because no one actually *says* "*Caenorhabditis"*). The overwhelming majority of nematode antimicrobial peptide (AMP) research is performed using *C. elegans,* with *Ascaris suum* a distant second, and along with *Drosophila melanogaster, C. elegans* has become a popular model for the study of innate immunity. In general, this isn't because there is significant interest in how nematodes defend themselves against potential pathogens. The interest is usually in human innate immunity, but humans (and other vertebrates) have that troublesome adaptive immune system that gets in the way of studying innate immunity, so an organism lacking adaptive immunity is an attractive alternative.

Findings in *C. elegans* are often generalized to all nematodes, even though no single species should be considered completely representative of the phylum. Nematodes are an incredibly diverse group of organisms with species adapted to both free-living and parasitic lifestyles in a broad range of environments. It is possible (although I think unlikely) that there is a core set of defense molecules that comprise an archetypal nematode defense system, but the wide variety of environments has almost certainly influenced the evolution of immune-related molecules in different lineages. There may also be similarities between species of divergent lineages that have experienced similar environmental pressures.

The use of the model systems, *C. elegans* and *D. melanogaster,* naturally leads to comparisons between the two and to speculation regarding how innate immune molecules may have evolved. Nematodes and arthropods represent two phyla within Ecdysozoa (Fig. 1), a superphylum that also includes Nematomorpha (horsehair worms), Loricifera, Priapulida (penis worms), Kinorhyncha (mud dragons), Onychophora (velvet worms), and Tardigrada (water bears) (Telford *et al.*, 2008). Very little (if anything) is known about the AMPs produced by most of these phyla. Further characterization of these groups will facilitate analyses of how nematode and arthropod AMPs have evolved.

Invertebrate AMPs can be broadly classified by structure: α-helical linear peptides, peptides that contain several cysteines that form one or more disulfide bonds, peptides with a large proportion of one or two amino acids, and peptides that are processed from larger precursors that do not have antimicrobial activity (Bulet, 2004). This review will discuss four groups of AMPs identified in nematodes that represent three of these structural groups: defensins and nemapores (cysteine-stabilized

Corresponding author:
D Ellen K Tarr
Department of Microbiology and Immunology
Arizona College of Osteopathic Medicine
Midwestern University
19555 N. 59th Ave, Glendale, AZ, 85308, USA
E-mail: etarrx@midwestern.edu

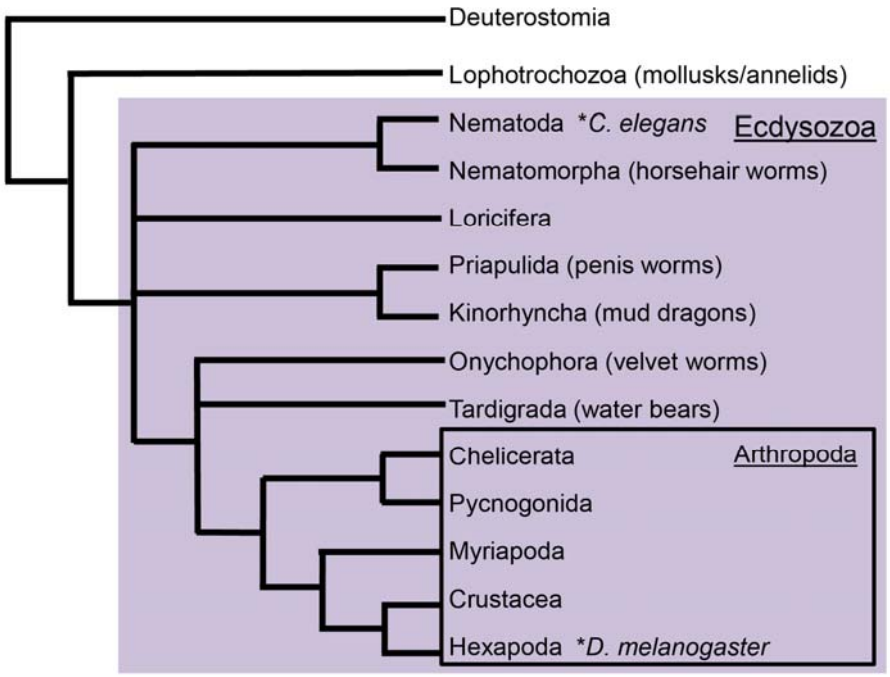

Fig. 1 Representation of Ecdysozoan phylogeny reproduced from Telford *et al.* (2008). The tree is intended to show relationships only and is not drawn to scale.

peptides), cecropins (α-helical peptides), and caenacins/neuropeptide-like proteins (glycine-rich peptides). These groups have not been studied equally-there is significantly more information on the cysteine-stabilized peptides compared to the other groups. Reviews of *C. elegans* antimicrobial peptides are published regularly; I see no need to duplicate these efforts and will refer the reader to a recent review for information regarding well-established findings. In this review, I will focus on the general structure and function of nematode AMPs, the distribution of different AMP groups across the phylum, and phylogenetic relationships.

Defensins

Defensins are by far the most studied of nematode antimicrobial peptides. The first nematode defensins identified and characterized were *Ascaris suum* antibacterial factors (ASABFs) and their homologs in *C. elegans* (Kato, 1995; Kato and Komasku, 1996; Zhang *et al.*, 2000; Kato *et al.*, 2002; Pillai *et al.*, 2003). With the exception of ASABF-6Cys-α (Minaba *et al.*, 2009), these ASABFs and Ce-ABFs have eight cysteines predicted to form four disulfide bonds. Findings regarding expression of these peptides and the signaling pathways involved have been reviewed recently (Bogaerts *et al.*, 2010). They are often still referred to as ABFs, but based on the conserved cysteine pattern, they are clearly similar to other invertebrate defensins (see below). For clarity, I will refer to the previously described ABFs and their homologs in other nematode species collectively as nematode defensins.

Structure

Nematode defensins are part of the cysteine-stabilized α-helix and β-sheet (CS-αβ) group of defensins that are evolutionarily divergent from mammalian defensins. The CS-αβ fold defines a superfamily that includes plant and invertebrate defensins, as well as arthropod toxins that target ion channels. This structural motif is characterized by six cysteines that form an α-helix and two β-sheets stabilized by three disulfide bonds (Figs 2A, C). The proposed signature sequence for the CS-αβ superfamily is as follows: C-X(2,18)-C-X(3)-C-X(2,10)-[GAPSIDERYW]-X-C-X(4,17)-CXC (Zhu *et al.*, 2005).

Two smaller motifs are nested within the conserved CS-αβ fold: the cysteine-stabilized α-helix (CSH) motif and the γ-core (Fig. 2A). The CSH motif was first described from arthropod toxins and venoms that act on ion channels (Kobayashi *et al.*, 1991). The motif connects the C-X(3)-C and CXC of the above superfamily signature, which accounts for two of the three disulfide bonds of the CS-αβ fold. The γ-core is proposed to be an archetypal structural motif found in all groups of cysteine-stabilized host defense effector peptides (Yeaman and Yount, 2007). It is characterized by two anti-parallel β-sheets separated by a short turn that can be generated by three isoforms of an enantiomeric signature sequence: X(1-3)-GXC-X(3-9)-C, C-X(3-9)-CXG-X(1-3), and C-X(3-9)-GXC-X(1-3) (Yount and Yeaman, 2004). Within the proposed classification system based on this conserved motif, CS-αβ defensins are generally classified as having a γ-α structure (Yeaman and Yount, 2007).

The three disulfide bonds and conserved signatures described above are well conserved throughout the superfamily, while the presence and position of additional cysteines that form a fourth bond have been used to describe specific groups found in only some lineages. For example, the overwhelming majority of arthropod defensins described thus far have only three disulfide bonds, but drosomycin from *D. melanogaster* has a fourth disulfide bond formed from additional cysteines near the N and C termini (Fig. 2A; Landon *et al.*, 1997). Some mollusc defensins and the closely-related myticins also have eight cysteines (Hubert *et al.*, 1996; Mitta *et al.*, 1999), but the additional cysteines forming the fourth disulfide bond are located in the first β-sheet and at the C-terminus (Fig. 2A; Yang *et al.*, 2000). This pattern is sometimes referred to as "mollusc-type" to differentiate it from the six-cysteine "arthropod-type" or "insect-type" pattern.

The majority of nematode defensins have a cysteine array consistent with the "mollusc-type" pattern (Zhang and Kato, 2003; Tarr, 2012); although only the structure for ASABF-α has been experimentally determined (Fig. 2C; Aizawa *et al.*, 2001). In general, these nematode defensins have a longer n-loop than mollusc eight-cysteine defensins (Fig. 2A; Tarr, 2012). ASABF-6Cys-α is a six-cysteine defensin that is missing the first and last cysteine of the typical eight-cysteine mollusc/nematode array (Minaba *et al.*, 2009). Some rearrangement of bonding has occurred because these six cysteines do not correspond to those that form the three conserved disulfide bonds of the CS-αβ fold (Fig. 2A), but the structure for this defensin has not been experimentally determined (Minaba *et al.*, 2009). It is unknown at this time whether the ASABF-6Cys-α cysteine array is restricted to nematodes. There have also been sequences identified that are consistent with the "mollusc-type" array, but are missing only the N-terminal cysteine (Tarr, 2012). It is not clear whether only six of the seven cysteines are participating in bond formation or if the extra cysteine facilitates dimer formation as has been suggested for some plant defensins (Zhu *et al.*, 2005). In addition to "mollusc-type" defensins, nematodes have sequences predicted to encode "arthropod-type" six-cysteine defensins (Tarr, 2012; Zhu *et al.*, 2005), "arthropod-type" defensins with the addition of two N-terminal cysteines, and at least one potential drosomycin homolog (Tarr, 2012).

Invertebrate defensins are generally produced as precursor molecules with an N-terminal secretory signal peptide. Many arthropod defensins also have a pro-peptide located between the signal and mature peptides, while mollusc defensins tend to have the pro-peptide located C-terminal to the mature peptide (Mitta *et al.*, 1999; Froy and Gurevitz, 2003). The majority of nematode defensins have a signal peptide, and many of these also have a predicted C-terminal pro-peptide (Tarr, 2012). However, some nematode defensins have a predicted pro-peptide located between the signal peptide and mature peptide (Tarr, 2012), a domain organization previously thought to be found in arthropods, but not in molluscs or nematodes (Zhang and Kato, 2003; Froy, 2005; Rodriguez de la Vega and Possani, 2005). The cysteine array pattern and domain organization are not linked, *i.e.*, nematode defensins that have an arthropod-like cysteine pattern are not necessarily the same as those with an arthropod-like domain organization (Tarr, 2012).

Distribution in nematodes

There are currently 86 nematode defensins identified from 25 species (Tarr, 2012) representing clades I, III, IV, and V of the phylogenetic framework based on small subunit (SSU) ribosomal DNA sequences (Blaxter *et al.*, 1998). Although clade II (Enoplia) has been studied for phylogenetic purposes (Bik *et al.*, 2010), there are too few sequences available to determine if defensins are present in this group or not. In general, defensins from clades I and III have the "mollusc-type" cysteine pattern while the sequences from clades IV and V have more diverse cysteine patterns (Tarr, 2012). Very few nematode genomes have been sequenced, so it is premature to conclude that defensins are absent in many of the species for which a defensin sequence has not been identified yet. In spite of these limitations, there are some interesting observations when looking at the distribution of nematode defensins: (1) they are present in ascarids, but have not been identified in filarials; (2) they are present in *Xiphinema*, but have not been identified in *Trichinella* or *Trichuris*; and (3) they are present in some species of *Meloidogyne*, but not in others (Tarr, 2012).

Molecular phylogeny based on SSU generally places ascarids (large intestinal roundworms) and filarial nematodes into clade III, a grouping of zooparasitic nematodes that also includes pinworms and millipede-gut parasites (Blaxter *et al.*, 1998). Nematode defensins are consistently present in ascarids: *A. lumbricoides* (3), *A. suum* (10), *Toxascaris leonina* (2), and *Toxocara canis* (5). In contrast, no defensins have been found in *Brugia malayi, Dirofilaria immitis, Litomosoides sigmodontis, Loa loa, Onchocerca volvulus,* or *Wuchereria bancrofti* (Tarr, 2012). The lack of complete genome information can account for this in most of filarial species, but the genome for *Brugia malayi* has been completed, and the absence of ABFs was noted (Ghedin *et al.*, 2007). With defensins being widely distributed throughout nematodes, one interpretation of this observation is that defensins were lost in a filarial ancestor after the divergence of clade III from clades IV and V. However, more recent molecular phylogeny using mitochondrial genome data from 36 species has concluded that clade III is not monophyletic (Park *et al.*, 2011). Furthermore, the analyses supported placement of Ascaridida within Rhabditida, and Spirurida as a separate clade, *i.e.*, ascarids may be more closely related to *C. elegans* than to filarial nematodes (Park *et al.*, 2011). Within this context, it is not as surprising to see such a discrepancy between the groups, although it is still not clear why filarial worms do not have defensins. Two aspects of filarial biology deserve further consideration in comparison to ascarids and free-living species such as *C. elegans*: filarial nematodes have arthropod intermediate hosts and some filarial species

(although not all) have a bacterial endosymbiont. It is possible that the production of defensins would be detrimental to the nematode endosymbiont or to an endosymbiont found in the arthropod vector. Further studies should look specifically at vector-borne vs. non-vector-borne species as well as those that harbor bacterial endosymbionts vs. those that do not.

Molecular phylogeny has generally supported placement of *Xiphinema* (a plant parasite, Dorylaimida), and *Trichuris* and *Trichinella* species (vertebrate parasites, Trichinellida) in clade I, which represents one of the earliest branches of nematodes (Blaxter *et al.*, 1998; Bik *et al.*, 2010). Taxon sampling within this clade is minimal and conclusions should be drawn only tentatively, but within this framework, it is possible that defensins are present in Dorylaimida and absent in Trichinellida. Defensins from Dorylaimida have been found only in *X. index,* and there is no evidence yet from the other clade I lineages, Mononchida and Mermithida. Therefore, it is also possible that defensins have been gained by *X. index* (or an ancestor) relatively recently. Absence of defensins from Trichinellida is supported by the completion of a draft genome for *T. spiralis* (Mitreva *et al.*, 2011), but again, there is not enough information to determine whether this is species specific or the general rule across Trichinellida. As with molecular phylogenies of clade III, it is not clear that clade I is monophyletic (Bik *et al.*, 2010; Park *et al.*, 2011), complicating interpretation of the findings. Complete genomes from additional taxa are needed to determine where defensins have been lost or gained in clade I lineages.

Sequences encoding predicted defensins have been identified in *Meloidogyne hapla* and *M. javanica,* but not yet from *M. arenaria, M. artiellia, M. chitwoodi, M. incognita,* or *M. paranensis* (Tarr, 2012). Genomes have been completed and published for both *M. hapla* and *M. incognita,* making it less likely the absence of defensins in *M. incognita* is due to lack of information. It is unclear why the presence of defensins is variable within this genus, but it does not seem to be consistent with molecular phylogeny based on SSU rDNA sequences (Holterman *et al.*, 2009), or mitotic vs. meiotic parthenogenesis (*M. incognita* and *M. javanica* are both mitotic parthenogenetic species).

A possible limitation on the identification of nematode defensins is the potential that the current consensus of conserved cysteines may not accurately reflect what is required for defensin function. While attempting to identify nematode defensins, there were cysteine-rich sequences that didn't have the conserved invertebrate defensin pattern. Structural characterization and antimicrobial testing of novel, cysteine-rich peptides will determine whether these are additional groups that are unrelated to defensins or if our current definition of "defensin" should be revised to include more variable cysteine patterns.

Activity

Previous work tested the activity of purified and recombinant ASABF-α, and recombinant CeABF-2, and showed that these peptides have the greatest activity against Gram-positive bacteria, with less activity against Gram-negative bacteria and yeast (Kato, 1995; Kato and Komasku, 1996; Zhang *et al.*, 2000; Kato *et al.*, 2002). The antimicrobial activity of defensins is probably due to their ability to form pores in microbial membranes. There are multiple models of pore formation, and the mechanism of action may differ between defensins or with membrane composition (Ganz, 2003; Pálffy *et al.*, 2009). This is consistent with the proposed generalization that peptides with the γ-core motif interact with lipid membranes, resulting in pore formation or disruption of ion channels. Differences in mechanisms of pore formation may be due to subtle differences within the γ-core as well as structural differences outside this motif (Yeaman and Yount, 2007).

The three well-conserved disulfide bonds in the CS-$\alpha\beta$ motif play a structural role in folding and stability. A fourth disulfide bond is present in some members of this superfamily, and is much more variable in its location. For MGD-1 from *M. galloprovincialis*, the fourth bond is suggested to contribute additional stability in a high-osmolarity environment such as seawater (Yang *et al.*, 2000). In contrast, drosomycin from *D. melanogaster* has a fourth disulfide bond that differs in its placement from that in MGD-1, and may contribute to its antifungal activity compared to the antibacterial activity observed in most insect defensins (Dimarcq *et al.*, 1998; Zhu *et al.*, 2005). The fourth disulfide bond in ASABF-α is consistent with that in MGD-1, suggesting a role in increased stability, although this has not been experimentally verified.

In addition to antibacterial and antifungal activities, some defensins have shown anti-*Plasmodium* activity that may correlate with a five-residue motif in the m-loop (Fig. 2A; Tian *et al.*, 2008; Gao *et al.*, 2009). This motif was first identified as GRSGG (the last G corresponds to the GXC of the γ-core motif) from the *Aeschna cyanea* anti-*Plasmodium* defensin AcDEF (Shahabuddin *et al.*, 1998). Future studies should include testing activity against *Plasmodium* in addition to antibacterial and antifungal activity to verify this motif.

As noted above, many nematode defensins that have the "mollusc-type" cysteine pattern have a longer n-loop than seen in mollusc defensins/myticins (Fig. 2A). This region is upstream of the CS-$\alpha\beta$ fold and the functional significance is not known at this time. Similarly, the defensins from *X. index* and some from *Caenorhabditis* have an extended region between the conserved CXC and the last cysteine (Tarr, 2012). The impact on the function of defensins with this C-terminal extension is also unknown.

Many nematodes produce several predicted defensins (Tarr, 2012), but only the activities of ASABF-α and CeABF-2 have been characterized (see above). The number of defensins per species may reflect differences in antimicrobial spectrum, differences in tissue localization, or both. There is some evidence of differential tissue expression for both ASABFs (Kato *et al.*, 2002; Pillai *et al.*, 2003; Minaba *et al.*, 2009) and CeABFs (Kato *et al.*, 2002), but neither activity nor expression have been investigated for most nematode defensins.

Phylogenetic relationships

The initial characterization of ABFs showed that they are most similar to mollusc defensins and myticins. This led to a series of papers that suggested a common ancestor for mollusc and nematode defensins (Zhang and Kato, 2003), then convergent evolution of these groups (Froy, 2005), and finally a lack of sufficient information to definitively establish the evolutionary relationship (Rodriguez de la Vega and Possani, 2005). Unfortunately, these studies were based on a limited number of nematode and mollusc sequences that underestimated defensin diversity in both groups. Although the ABFs represented two species traditionally considered to be relatively divergent that live in completely different environments (*A. suum* and *C. elegans*), these two species are still not completely representative of the phylum and as mentioned earlier, may not be as divergent as traditionally thought (Park *et al.*, 2011).

Several reports show that both molluscs and nematodes clearly have defensins with the "arthropod-type" cysteine pattern in addition to the "mollusc-type" pattern (Charlet *et al.*, 1996; Zhu *et al.*, 2005; De Zoysa *et al.*, 2010; Xu and Faisal, 2010; Tarr, 2012). Defensins with these different patterns are not distributed equally in nematodes. As stated previously, all ascarid and *Xiphinema* defensins have a "mollusc-type" cysteine array (the spacing for ASABF-6Cys-α is still more similar to the mollusc spacing than the arthropod spacing). In contrast, clades IV and V have more diverse cysteine patterns, and none of the sequences identified from *Meloidogyne* have the eight-cysteine "mollusc-type" array (Tarr, 2012). A recent analysis included defensins from across nematode taxa, but was unable to definitively resolve phylogenetic relationships and did not support a clear "nematode" clade, suggesting a common origin for nematode and other invertebrate defensins (Tarr, 2012). In contrast to previous studies that used either MGD-1 (Froy and Gurevitz, 2003; Froy, 2005) or myticin (Minaba *et al.*, 2009) as the mollusc representative, this analysis used both, and did not place them in the same clade (Tarr, 2012). The myticins are considered a different family of antimicrobial peptides based on lack of sequence similarity to mussel defensins (Mitta *et al.*, 1999), but the cysteine pattern is nearly identical and the myticins are clearly part of the CS-$\alpha\beta$ superfamily (Fig. 2A; Zhu *et al.*, 2005). In addition to mollusc defensins and myticins, there are mytilins, mytimycin, and mytimacins that seem to have conserved cysteine spacings that could indicate a CS-$\alpha\beta$ fold (Charlet *et al.*, 1996; Gerdol *et al.*, 2012). These families should be investigated further to identify nematode homologs and determine their relationship to identified defensins. Additional mollusc sequences as well as defensins from Ecdysozoan taxa not currently represented in analyses should facilitate resolution of nematode phylogenetic relationships.

Studies of the CS-$\alpha\beta$ and γ-core motifs have suggested that sequences with these motifs are descended from a common ancestor (Zhu *et al.*, 2005; Yeaman and Yount, 2007). Three additional findings support this. First, an ASABF-like sequence has been identified in the sponge *Suberites domuncula,* making it the first defensin described from a poriferan. This peptide shows antimicrobial activity against Gram-positive bacteria and hemolytic activity, as well as activity against a gastropod that sometimes grazes on the sponge (Wiens *et al.*, 2011). Second, several groups of defensin-like peptides in fungi have been identified (Zhu, 2008). This study used a framework that proposes three basic groups of invertebrate defensins: a group of defensins found only in neopteran insects referred to as classical insect-type defensins (CITDs), a group with broader taxonomic distribution called ancient invertebrate-type defensins (AITDs), and a group that includes drosomycin called antifungal plant/insect-type defensins (PITDs) (Dimarcq *et al.*, 1998; Froy and Gurevitz, 2003). This study concluded that fungi and animals have all three groups of defensins, with the PITDs being the only group that is also found in plants (Zhu, 2008). This framework was based largely on arthropod defensins, and should be re-evaluated to determine if it is still applicable with the addition of mollusc and nematode sequences. The recent analysis of nematodes included several arthropod sequences, but did not support these distinct clades (Tarr, 2012). The last finding that provides evidence for a common ancestor of the CS-$\alpha\beta$ defensins is the identification of two myxobacterial defensin-like peptides that might represent this ancestor (Zhu, 2007). These defensin-like peptides from *Anaeromyxobacter dehalogenans* (AdDLP) and *Stigmatella aurantiaca* (SdDLP) have the CSH motif, and a domain organization with an N-terminal signal peptide and C-terminal pro-peptide (Zhu, 2007). Recombinant AdDLP has no antibacterial or antifungal activity, but has a predicted antiparasitic motif and activity against *Plasmodium falciparum* (Gao *et al.*, 2009).

In addition to the above findings that contribute to developing a coherent evolutionary history of the CS-$\alpha\beta$ superfamily, an ASABF-like peptide (HKABF) has been reported from the seahorse *Hippocampus kuda* (Wang *et al.*, 2008). This represents the only CS-$\alpha\beta$ defensin in vertebrates, and it is unclear whether it is the result of horizontal gene transfer (and if so, from what organism), or if there could have been a nematode parasite contaminating the source RNA.

Nemapores

Caenopores are antimicrobial peptides from *C. elegans* with similarity to amoebapores from *Entamoeba histolytica,* naegleriapores from *Naegleria fowleri,* and mammalian NK-lysin and granulysin (Leippe *et al.*, 1991; Leippe, 1995, 1999; Banyai and Patthy, 1998; Herbst *et al.*, 2002). The caenopores are encoded by 28 *spp* genes, several of which are involved in responses to various pathogens (see review by Bogaerts *et al.*, 2010; Roeder *et al.*, 2010; Hoeckendorf *et al.*, 2012). "Nemapore" is a more general term used to describe similar peptides produced by any nematode species (Tarr, 2012). As peptides from additional species are characterized, they may be given more specific names (ascaripores, brugiapores, etc.), but at this time, only caenopores have been studied experimentally.

Structure

Nemapores are cysteine-rich antimicrobial peptides that belong to the saposin-like protein (SAPLIP) superfamily. In addition to the antimicrobial peptides listed above, the saposin domain is also found in other membrane-interacting proteins, such as saposins and surfactant proteins, and can act either independently or as part of a multidomain protein (for a review of SAPLIPs, see Bruhn, 2005). The saposin fold has a core of hydrophobic amino acids and six cysteines that form three disulfide bonds (Figs 2B, D; Bruhn, 2005). In contrast to the CS-$\alpha\beta$ fold, the saposin fold does not have a clear γ-core motif, so this may not be conserved among all cysteine-stabilized AMPs. A general consensus for the conserved cysteines differs substantially from that of the defensins: C-X(2)-C-X(21-31)-C-X(7-16)-C-X(19-29)-C-X(3-7)-C (Tarr, 2012). The bonding between C1-C6, C2-C5, and C3-C4 stabilizes a structure that is generally composed of five α-helices that form two bundles (Bruhn, 2005). In nemapores, this structure has been confirmed experimentally only for caenopore-5 (Fig. 2D; Mysliwy *et al.*, 2010). Two sequences from prokaryotes, the bacteriocin AS-48 from *Enterococcus faecalis* and plasmid *Achromobacter* secretion (PAS) from *Vibrio vulnificus,* do not have the conserved cysteines, but appear to maintain the saposin fold structure (González *et al.*, 2000; Lee *et al.*, 2006), suggesting the cysteines may have been a later addition to further stabilize the fold. Another deviation from the canonical saposin domain is the "swaposin" domain found in some plant aspartic proteinases. The "swaposin" domain is a circular permutation of the saposin domain, composed of the C-terminal half of one saposin domain linked to the N-terminal half of a second saposin domain (Ponting and Russell, 1995).

Distribution

Nemapores have been identified from 46 species representing all clades (except clade II due to lack of sequence information), and in every nematode species with a completed genome except *M. incognita* (Tarr, 2012). The discrepancy between *M. hapla* and *M. incognita* was noted above, and the reason for the difference is no clearer for the nemapores than for the defensins. Sequences encoding potential "swaposin" domains have not been investigated in nematodes. It is possible that these are present in *M. incognita,* although this would still not explain the discrepancy between the two closely-related species. The only other *Meloidogyne* species with identified nemapores thus far is *M. chitwoodi* (Tarr, 2012).

Caenopores have been proposed to be the best candidates for defense of *C. elegans* against microbes based on the large number of variants with varying antimicrobial spectra that are induced by exposure to different pathogens and active at an acidic pH (Roeder *et al.*, 2010). Several species have a large number of predicted nemapores, including all *Caenorhabditis* species with completed genomes and *Pristionchus pacificus.* In contrast to defensins, filarials as well as ascarids have nemapores, although clade III nematodes do not seem to have the high numbers found in some clade IV and V species (Tarr, 2012).

Identified nemapores are predicted to have one to four saposin domains, with sequences predicted to have three or four domains found only in clade I and V species thus far (Tarr, 2012). *T. spiralis* secretes a prosaposin homolog with four saposin domains and a single glycosylation site in the first domain (Selkirk *et al.*, 2004).

Activity

The antimicrobial activity of caenopores has only been investigated for SPP-1, SPP-5, and SPP-12. All three are active against *Bacillus megaterium*, but only SPP-5 shows significant activity against *Escherichia coli* (Roeder *et al.*, 2010; Hoeckendorf *et al.*, 2012). SPP-12 is also active against *B. thuringiensis* and is involved in *C. elegans* resistance to this pathogen (Hoeckendorf *et al.*, 2012). The pore-forming activity of all three has been confirmed using liposomes, and in *B. megaterium, Saccharomyces cerevisiae,* and *Dictyostelium discoideum* for SPP-1 and SPP-12 (Roeder *et al.*, 2010; Hoeckendorf *et al.*, 2012). Similar to amoebapores, SPP-1, 5, and 12 are more active at acidic pH (Leippe *et al.*, 1991; Roeder *et al.*, 2010; Hoeckendorf *et al.*, 2012).

Naegleriapores are produced as precursors with multiple saposin domains that are processed to produce multiple mature peptides, not all of which have pore-forming activity (Herbst *et al.*, 2004). Some non-antimicrobial SAPLIPs are also produced as precursors, and in general, glycosylation seems to play a role in peptide stability (reviewed in Bruhn, 2005). The only multi-domain nemapore that has been studied is the *T. spiralis* prosaposin, but there is currently no evidence regarding processing of the precursor (Selkirk *et al.*, 2004).

In contrast to defensins, nemapores are hypothesized to have an additional role in nematode biology. Bacteria aren't just a potential source of infection for nematodes, but are a food source for many species. Therefore, molecules that participate in bacterial killing, especially those expressed in the intestine, may be contributing to nutrition as well as to host defense. *C. elegans spp-5* mutants do not store sufficient fat for normal egg production, providing evidence for SPP-5 contributing to digestion (Roeder *et al.*, 2010). With the large number of nemapores expressed in some species, there is the potential that the sequences have diverged, with some playing a defensive role and others involved in nutrition.

Phylogenetic relationships

Previous analyses of caenopores (Roeder *et al.*, 2010) and nemapores (Tarr, 2012) have been unable to resolve phylogenetic relationships of the saposin domains, due at least in part to the short length of the domain sequences. With the exception of mammalian sequences, phylogenetic analyses do not clearly separate nematode sequences from other eukaryotic sequences. They also do not provide evidence for defensive vs. digestive clades of nemapores (Tarr, 2012). However, the analyses do suggest that the domains of multi-domain sequences are not the result of recent duplication of one of the domains (Roeder *et al.*, 2010; Tarr, 2012). Phylogenetic analyses have not been

performed that include the related bacterial sequences lacking the conserved cysteines.

Cecropins

Cecropins are an example of why it is necessary to look beyond *C. elegans* when characterizing nematode AMPs. *A. suum* cecropin P1 has been extensively characterized compared to other nematode AMPs, probably because it was originally isolated from pig intestine and thought to be a porcine cecropin (Lee *et al.*, 1989). This also highlights the potential difficulty of separating host DNA from that of potential parasites, infections, and symbionts. Cecropin P1 was later realized to be from the pig intestinal roundworm, *A. suum* (Andersson *et al.*, 2003), and a total of four cecropins are induced in response to pathogen injection into the pseudocoelom (Pillai *et al.*, 2005).

Structure

In contrast to the defensins and nemapores, cecropins have an amphipathic α-helical structure that is not stabilized by disulfide bonds (Sipos *et al.*, 1992). Therefore, a consensus sequence based on the number and spacing of cysteine residues is not possible for identification of new cecropins. A signature sequence proposed based only on insect cecropins (Tamang and Saier, 2006) was recently updated to include mosquito and nematode cecropins (Tarr, 2012):
[KRDEN]-[KRED]-[LIVMR]-[ED]-[RKGHN]-X(0,1)-[IVMALT]-[GVIK]-[QRKHA]-[NHQRK]-[IVTA]-[RKFAS]-[DNQKE]-[GASV]-[LIVSATG]-[LIVEAQKG]-[RKQSGIL]-[ATGVSFIY]-[GALIVQN]
This sequence appears to be cecropin-specific, but should not be used as the only criteria for identification of new cecropins. This proposed signature sequence is significantly different from the signature sequence currently defined in PROSITE (Sigrist *et al.*, 2010):
W-X(0,2)-[KDN]-{Q}-{L}-K-[KRE]-[LI]-E-[RKN]
This signature (PS00268) is not currently cecropin-specific and begins upstream of the previous one, but may facilitate cecropin identification if the other signature is found to be too stringent. The PROSITE signature includes a tryptophan that is usually found as the first or second residue of the cecropin mature peptide, but is known to be absent from some cecropins (Bulet *et al.*, 2004).

Similar to many nematode defensins, nematode cecropins are predicted to be produced as a precursor with an N-terminal signal peptide and a C-terminal pro-peptide (Pillai *et al.*, 2005; Tarr, 2012). This domain structure is more similar to styelins from tunicates (Zhao *et al.*, 1997) than to insect cecropins, which are more likely to have a short pro-peptide immediately N-terminal to the mature peptide (Boman and Boman, 1989). For cecropin P4, the C-terminal pro-peptide has an inhibitory effect on the antibacterial activity of the mature peptide (Ueno *et al.*, 2008).

Distribution

Nematode cecropins have only been identified in *Ascaris* and *Toxocara* (Pillai *et al.*, 2005; Tarr, 2012). These previous analyses used BLAST searches that did not rely on proposed signature sequences for identification, so it is unlikely that additional nematode cecropins have been missed because they deviate only slightly from these signatures. The presence of cecropins in such a limited group of nematodes suggests that a cecropin gene was acquired by an ancestor of *Ascaris* and *Toxocara,* but there is currently no evidence for the source of this transferred gene.

Activity

Cecropins P1-P4 from *A. suum* show activity against Gram-positive and Gram-negative bacteria, with weaker activity against yeast (Pillai *et al.*, 2005). Studies of cecropin P1 suggest that it forms pores in bacterial membranes using a “carpet” mechanism in which the peptides are oriented parallel to the membrane, resulting in membrane destabilization once a threshold concentration is reached (Pouny *et al.*, 1992; Gazit *et al.*, 1996).

Phylogenetic relationships

Previous analyses have shown that nematode cecropins are monophyletic and may be more closely related to styelins from the tunicate *Styela clava* than insect cecropins, although this relationship is not always conserved with changes in alignment parameters (Tarr, 2012). In general, nematode cecropins seem to be more similar to dipteran than lepidopteran cecropins (Tarr, 2012). Within nematodes, clear orthologs are found between species, suggesting that more than one cecropin was already present in an ancestor of *Ascaris* and *Toxocara* (Pillai *et al.*, 2005).

The cecropin family is part of the cecropin superfamily, which also includes the pleurocidin and dermaseptin families of toxic peptides (Tamang and Saier, 2006). Sequences predicted to encode members of these families have not been found in nematodes (Tarr, 2012). Cecropins may have evolved from peptides found in bacterial ribosomal proteins. Specifically, peptides from *Helicobacter pylori* ribosomal protein L1 are similar to cecropins A and B from *Hyalophora cecropia* (Pütsep *et al.*, 1999). Whether the different families of the cecropin superfamily may have evolved from the same ribosomal peptides has not been investigated.

Caenacins and neuropeptide-like proteins

The caenacins (CNCs) and neuropeptide-like proteins (NLPs) are related groups of glycine-rich peptides that have been most studied in *C. elegans*. Two subgroups of these genes, the “*cnc-2* cluster” (*cnc-1* to *cnc-5* and *cnc-11*) and the “nlp-29 cluster” (nlp-27 to nlp-31 and nlp-34), are induced by wounding and infection, but regulated by different signal transduction pathways, which have been characterized extensively (reviewed in Bogaerts *et al.,* 2010). I will refer to all NLPs predicted to be antimicrobial as “antimicrobial NLPs” to differentiate them from other NLP families that are not currently predicted have a role in innate immunity.

Structure

The *C. elegans* “*nlp-29* cluster” (*nlp-27* to *nlp-31* and *nlp*-34) overlaps with the previously identified

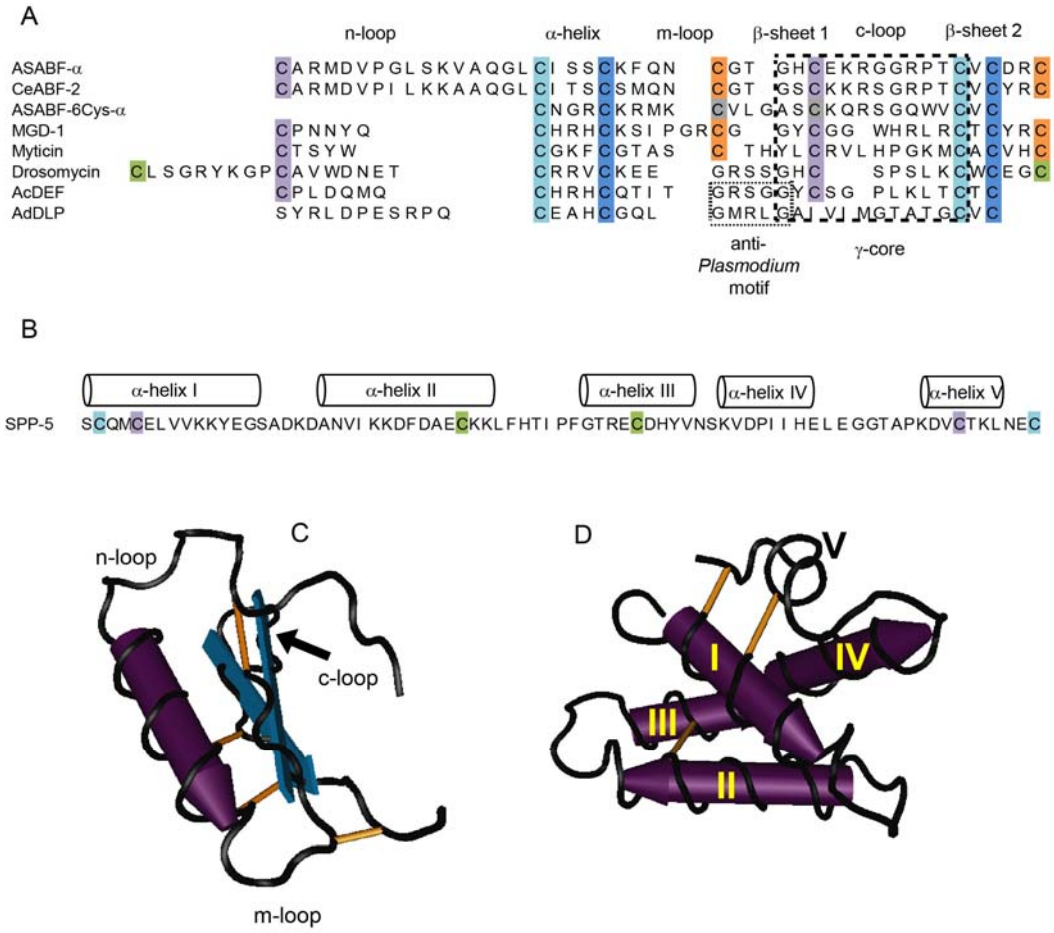

Fig. 2 Comparison of cysteine patterns and tertiary structures of nematode cysteine-stabilized antimicrobial peptides. **A**) The cysteine arrays for invertebrate defensins and a bacterial defensin-like peptide are aligned for comparison. Cysteines that form disulfide bonds are color coded: the four cysteines that form the conserved disulfide bonds of the CSH motif are shaded blue, the cysteines forming the remaining disulfide bond of the CS-αβ motif are shaded purple, the cysteines forming the fourth disulfide bond in nematode and mollusc sequences are shaded orange, the cysteines forming the fourth disulfide bond in drosomycin are shaded green, and the two non-canonical cysteines of ASABF-6Cys-α are shaded grey. The predicted structure is indicated above the alignment. The predicted γ-core and anti-*Plasmodium* motifs are outlined. Accession numbers: ASABF-α (BAA89497), CeABF-2 (NP_491252), ASABF-6Cys-α (BAC41496), MGD-1 (P80571), Myticin (P82103), Drosomycin (P41964), AcDEF (P91793), AdDLP sequence from Gao *et al.* (2009). **B**) The cysteine array of caenopore-5 (SPP-5) is shown with color-coded cysteines indicating the bonding pattern. The locations of α-helices as determined by Mysliwy *et al.* (2010) are shown above the sequence. Accession number for SPP-5: NP_509238. **C**) 3-D structure of ASABF-α (PDB ID: 2D56) with the location of the loops labeled. The α-helix is colored purple, β-sheets are colored blue, and the disulfide bonds are colored gold. **D**) 3-D structure of caenopore-5 (PDB ID: 2JSA). The α-helices are colored purple and labeled to correspond with helices shown in 2B, and disulfide bonds are colored gold.

YGGWamide family, initially defined as *nlp-24, nlp-25,* and *nlp*-27 through *nlp-32* (Nathoo *et al.*, 2001), but later updated to include nlp-*24* through *nlp-33* (McVeigh *et al.*, 2008). This family of antimicrobial peptides is characterized by an N-terminal signal peptide, arginine cleavage site, and a conserved YGGYG motif (Nathoo *et al.*, 2001; McVeigh *et al.*, 2008). The "*cnc-2* cluster" is a subset of genes encoding caenacins that have been studied at the transcriptional level, but have not been the subject of investigations to clarify motifs defining specific families within this larger group. A cursory look at the amino acid sequences suggests the caenacins may also have the YGGYG motif, but this has not been addressed directly in caenacin studies. In contrast to the other groups of peptides, studies of CNCs and NLPs have not focused on determining peptide tertiary structure.

Distribution

A study of NLP diversity in nematodes was unable to identify sequelogs, a neutral term used by

the authors to indicate sequence similarity without implying common ancestry (Varshavsky, 2004), of individual *C. elegans* YGGWamide NLPs (McVeigh *et al.*, 2008). However, NLPs with the conserved YGGYG motif are present in *Anisakis simplex, Bursaphalenchus xylophilus, B. macronatus, C. remanei, Globodera rostochiensis, M. incognita, Pristonchus pacificus,* and *Strongyloides ratti* (McVeigh *et al.*, 2008). The study found only one or two sequelogs in each of these species, although some of these were predicted to encode several peptides (McVeigh *et al.*, 2008).

The initial study that characterized the YGGWamide NLPs identified a potential sequence in *B. malayi* (AI079056) that was not found in the more recent study (Nathoo *et al.*, 2001; McVeigh *et al.*, 2008), making it unclear whether CNC/NLP antimicrobial peptides are found in filarials, or in the majority of clade III nematode species (*A. simplex* was the only clade III representative in the study). NLP antimicrobial peptides are thus far the only group to be found in *M. incognita* but not in *M. hapla,* although this study may have been completed prior to publication of the *Meloidogyne* genomes (McVeigh *et al.*, 2008). The *T. spiralis* genome had also not been published at the time this study was performed. Only one NLP was identified in *T. spiralis,* but it was not an antimicrobial NLP (McVeigh *et al.*, 2008). As pointed out in the study, antimicrobial NLPs (and be extension, the related CNCs) may be restricted to only a few species, but the data for most species are too incomplete to draw this conclusion at this time (McVeigh *et al.*, 2008).

Activity

To my knowledge, there have been no direct tests of the antimicrobial activity of purified or recombinant CNCs or antimicrobial NLPs. These groups have been studied in the *C. elegans* epidermal response to the nematophagous fungus *Drechmeria coniospora,* but direct antifungal activity has not been demonstrated and no mechanism has been proposed (Bogaerts *et al.*, 2010; Engelmann and Pujol, 2010).

Phylogenetic relationships

The phylogenetic relationships between all nematode CNCs and antimicrobial NLPs have not been investigated, but an analysis of *C. elegans* CNCs and NLPs has been published. This analysis supports a common ancestor of NLP-24 to NLP-34 (with the exception of NLP-26) and the 11 CNCs compared to the other NLPs (Pujol *et al.*, 2008). It is unclear at this time whether the CNCs would be more appropriately named as additional antimicrobial NLPs or vice versa, but the analysis does not support a clear division between these two groups (Pujol *et al.*, 2008).

Conclusion

In spite of the lack of complete genome information for most nematode species, it is clear that none of the antimicrobial peptide groups studied thus far are expressed universally by nematodes. Conversely, current results suggest that no single species produces all types of antimicrobial peptides. These observations suggest that there isn't a single "archetypal" nematode innate immune system. Understanding how different nematode species defend themselves against potential pathogens in their environment(s) will necessitate characterization of the defense molecules from the species of interest instead of reliance on a general description derived from *C. elegans*.

The cell membrane is the common target of three groups of antimicrobial peptides (defensins, nemapores, and cecropins). This may become the general rule for nematode antimicrobial peptides, but structure and activity have not been investigated for the caenacins and neuropeptide-like proteins. Although the target is the same, the precise mechanism of pore formation has not been established experimentally for most peptides.

In addition to greater characterization of nematode AMPs, information from Ecdysozoan taxa not currently represented in phylogenetic analyses and from Lophotrochozoan taxa for comparison will help establish a more complete picture of AMP evolution. Identification of AMPs is complicated by the large number of groups from different taxa that have been given names that may or may not reflect their similarity to other groups. As the key features of different AMP groups are clearly established, some changes in nomenclature may be warranted.

References

Aizawa T, Hoshino H, Fujitani N, Koganesawa N, Matsuura A, Miyazawa M*, et al.* Structural analysis of an antibacterial peptide derived from a nematode. In: Shiroiri T (ed), Peptide science 2000, The Japanese Peptide Society, pp 269-272, 2001.

Andersson M, Boman A, Boman HG. *Ascaris* nematodes from pig and human make three antibacterial peptides: isolation of cecropin P1 and two ASABF peptides. Cell. Mol. Life Sci. 60: 599-606, 2003.

Banyai L, Patthy L. Amoebapore homologs of *Caenorhabditis elegans.* Biochim. Biophys. Acta 1429: 259-264, 1998.

Bik HM, Lambshead PJD, Thomas WK, Lunt DH. Moving towards a complete molecular framework of the Nematoda: a focus on the Enoplida and early-branching clades. BMC Evol. Biol. 10: 353, 2010.

Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A*, et al.* A molecular evolutionary framework for the phylum Nematoda. Nature 392: 71-75, 1998.

Bogaerts A, Beets I, Schoofs L, Verleyen P. Antimicrobial peptides in *Caenorhabditis elegans.* Inv. Surv. J. 7: 45-52, 2010.

Boman HG, Boman IA. Chemical synthesis and enzymic processing of precursor forms of cecropins A and B. J. Biol. Chem. 264: 5852-5860, 1989.

Bruhn H. A short guided tour through functional and structural features of saposin-like proteins. Biochem. J. 389: 249-257, 2005.

Bulet P, Stocklin R, Menin L. Anti-microbial peptides: from invertebrates to vertebrates. Immunol. Rev. 198: 169-184, 2004.

Charlet M, Chernysh S, Philippe H, Hetru C, Hoffmann JA, Bulet P. Innate immunity. Isolation of several cyteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis.* J. Biol. Chem. 271: 21808-21813, 1996.

De Zoysa M, Whang I, Lee Y, Lee S, Lee J-S, Lee J. Defensin from disk abalone *Haliotis discus discus:* Molecular cloning, sequence characterization and immune response against bacterial infection. Fish Shellfish Immunol. 28: 261-266, 2010.

Dimarcq J-L, Bulet P, Hetru C, Hoffmann J. Cysteine-rich antimicrobial peptides in invertebrates. Biopolymers 47: 465-477, 1998.

Elsworth B, Wasmuth J, Blaxter M. NEMBASE 4: the nematode transcriptome resource. Int. J. Parasitol. 41: 881-894, 2011.

Engelmann I, Pujol N. Innate immunity in *C. elegans.* In: Söderhäll K (ed), Invertebrate immunity, Landes Bioscience and Springer Science+Business Media, LLC, 105-121, 2010.

Froy O. Convergent evolution of invertebrate defensins and nematode antibacterial factors. Trends Microbiol. 13: 314-319, 2005.

Froy O, Gurevitz M. Arthropod and mollusk defensins-evolution by exon-shuffling. Trends Genet. 19: 684-687, 2003.

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3: 710-720, 2003.

Gao B, del Carmen Rodriguez M, Lanz-Mendoza H, Zhu S. AdDLP, a bacterial defensin-like peptide, exhibits anti-*Plasmodium* activity. Biochem. Biophys. Res. Commun. 387: 393-398, 2009.

Gazit E, Miller IR, Biggin PC, Sansom MSP, Shai Y. Structure and orientation of the mammalian antibacterial peptide cecropin P1 within phospholipid membranes. J. Mol. Biol. 258: 860-870, 1996.

Gerdol M, De Moro G, Manfrin C, Venier P, Pallavicini A. Big defensins and mytimacins, new AMP families of the Mediterranean mussel *Mytilus galloprovincialis.* Dev. Comp. Immunol. 36: 390-399, 2012.

Ghedin E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J*, et al.* Draft genome of the filarial nematode *Brugia malayi*. Science 317: 1756-1760, 2007.

González C, Langdon GM, Bruix M, Gálvez A, Valdivia E, Maqueda M*, et al.* Bacteriocin AS-48, a microbial cyclic polypeptide structurally and functionally related to mammalian NK-lysin. Proc. Natl. Acad. Sci. USA 97: 11221-11226, 2000.

Herbst R, Marciano-Cabral F, Leippe M. Antimicrobial and pore-forming peptides of free-living and potentially highly pathogenic *Naegleria fowleri* are released from the same precursor molecule. J. Biol. Chem. 279: 25955-25958, 2004.

Herbst R, Ott C, Jacobs T, Marti T, Marciano-Cabral F, Leippe M. Pore-forming polypeptides of the pathogenic protozoon *Naegleria fowleri.* J. Biol. Chem. 277: 22353-22360, 2002.

Hoeckendorf A, Stanisak M, Leippe M. The saposin-like protein SPP-12 is an antimicrobial polypeptide in pharyngeal neurons of *Caenorhabditis elegans* and participants in defence against a natural bacaterial pathogen. Biochem. J. 2012.

Holterman M, Karssen G, van den Elsen S, van Megen H, Bakker J, Helder J. Small subunit rDNA-based phylogeny of the Tylenchida sheds light on relationships among some high-impact plant-parasitic nematodes and the evolution of plant feeding. Phytopathology 99: 227-235, 2009.

Hubert F, Noël T, Roch P. A member of the arthropod defensin family from edible Mediterranean mussels (*Mytilus galloprovincialis)*. Eur. J. Biochem. 240: 302-306, 1996.

Kato Y. Humoral defense of the nematode *Ascaris suum*: antibacterial, bacterioloytic, and agglutinating activities in the body fluid. Zoolog. Sci. 12: 225-230, 1995.

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

Kato Y, Komasku S. ASABF, a novel cysteine-rich antibacterial peptide isolated from the nematode *Ascaris suum*. J. Biol. Chem. 271: 30493-30498, 1996.

Kobayashi Y, Takashima H, Tamaoki H, Kyogoku Y, Lambert P, Kuroda H*, et al.* The cysteine-stabilized α-helix: A common structural motif of ion-channel blocking neurotoxic peptides. Biopolymers 31: 1213-1220, 1991.

Landon C, Sodano P, Hetru C, Hoffmann J, Ptak M. Solution structure of drosomycin, the first inducible antifungal protein from insects. Protein Sci. 6: 1878-1884, 1997.

Lee J-Y, Boman A, Chuanxin S, Andersson M, Jornvall H, Mutt V*, et al.* Antibacterial peptides from pig intestine: Isolation of a mammalian cecropin. Proc. Natl. Acad. Sci. USA 86: 9159-9162, 1989.

Lee JH, Yang S-T, Rho S-H, Im YJ, Kim SY, Kim YR*, et al.* Crystal structure and functional studies reveal that PAS factor from *Vibrio vulnificus* is a novel member of the saposin-fold family. J. Mol. Biol. 355: 491-500, 2006.

Leippe M. Ancient weapons: NK-lysin is a mammalian homolog to pore-forming peptides of a protozoan parasite. Cell 83: 17-18, 1995.

Leippe M. Antimicrobial and cytolytic polypetides of amoeboid protozoa-effector molecules of primitive phagocytes. Dev. Comp. Immunol. 23: 267-279, 1999.

Leippe M, Ebel S, Schoenberger OL, Horstmann RD, Müller-Eberhard HJ. Pore-forming peptide of pathogenic *Entamoeba histolytica.* Proc. Natl. Acad. Sci. USA 88: 7659-7663, 1991.

McVeigh P, Alexander-Bowman S, Veal E, Mousley A, Marks NJ, Maule AG. Neuropeptide-like protein diversity in phylum Nematoda. Int. J. Parasitol. 38: 1493-1503, 2008.

Minaba M, Ueno S, Pillai A, Kato Y. Evolution of ASABF (*Ascaris suum* antibacterial factor)-type antimicrobial peptdies in nematodes: Putative rearrangement of disulfide bonding patterns. Dev. Comp. Immunol. 33: 1147-1150, 2009.

Mitreva M, Jasmer DP, Zarlenga DS, Wang Z, Abubucker S, Martin J*, et al.* The draft genome of the parasitic nematode *Trichinella spiralis*. Nat. Genet. 43: 228-235, 2011.

Mitta G, Hubert F, Noël T, Roch P. Myticin, a novel cystein-rich antimicrobial peptide isolated from haemocytes and plasma of the mussel *Mytilus galloprovincialis.* Eur. J. Biochem. 265: 71-78, 1999.

Mitta G, Vandenbulcke F, Hubert F, Roch P. Mussel defensins are synthesised and processed in granulocytes then released into the plasma after bacterial challenge. J. Cell Sci. 112: 4233-4242, 1999.

Mysliwy J, Dingley AJ, Stanisak M, Jung S, Lorenzen I, Roeder T*, et al.* Caenopore-5: The three-dimensional structure of an antimicrobial protein from *Caenorhabditis elegans*. Dev. Comp. Immunol. 34: 323-330, 2010.

Nathoo AN, Moeller RA, Westlund BA, Hart AC. Identification of *neuropeptide-like protein* gene families in *Caenorhabditis elegans* and other species. Proc. Natl. Acad. Sci. USA 98: 14000-14005, 2001.

Pálffy R, Gardlík R, Behuliak M, Kadasi L, Turna J, Celec P. On the physiology and pathophysiology of antimicrobial peptides. Mol. Med. 15: 51-59, 2009.

Park J-K, Sultana T, Lee S-H, Kang S, Hyong KK, Min G-S*, et al.* Monophyly of clade III nematodes is not supported by phylogenetic analysis of complete mitochondrial genome sequences. BMC Genomics 12: 392, 2011.

Pillai A, Ueno S, Zhang H, Kato Y. Induction of ASABF (*Ascaris suum* antibacterial factor)-type antimicrobial peptide by bacterial injection: novel members of ASABF in the nematode *Ascaris suum*. Biochem. J. 371: 663-668, 2003.

Pillai A, Ueno S, Zhang H, Lee JM, Kato Y. Cecropin P1 and novel nematode cecropins: a bacteria-inducible antimicrobial peptide family in the nematode *Ascaris suum*. Biochem. J. 390: 207-214, 2005.

Ponting CP, Russell RB. Swaposins: circular permutaions within genes encoding saposin homologues. Trends Biochem. Sci. 20: 179-180, 1995.

Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes. Biochemistry 31: 12416-12423, 1992.

Pujol N, Zugasti O, Wong D, Couillault C, Kurz CL, Schulenburg H*, et al.* Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. PLoS Pathog. 4: 2008.

Pütsep K, Normark S, Boman HG. The origin of cecropins; implications from synthetic peptides derived from ribosomal protein L1. FEBS Lett. 451: 249-252, 1999.

Rodriguez de la Vega RC, Possani LD. On the evolution of invertebrate defensins. Trends Genet. 21: 330-332, 2005.

Roeder T, Stanisak M, Gelhaus C, Bruchhaus I, Grotzinger J, Leippe M. Caenopores are antimicrobial peptides in the nematode *Caenorhabditis elegans* instrumental in nutrition and immunity. Dev. Comp. Immunol. 34: 203-209, 2010.

Selkirk ME, Hussein AS, Chambers AE, Goulding D, Gares M-P, Vásquez-Lopez C*, et al. Trichinella spiralis* secretes a homologue of prosaposin. Mol. Biochem. Parasitol. 135: 49-56, 2004.

Shahabuddin M, Fields I, Bulet P, Hoffmann J, Miller LH. *Plasmodium gallinaceum:* Differential killing of some mosquito stages of the parasite by insect defensin. Exp. Parasitol. 89: 103-112, 1998.

Sigrist CJA, Cerutti L, de Castro E, Langendijk-Genevaux PS, Bulliard V, Bairoch A*, et al.* PROSITE, a protein domain database for functional characterization and annotation. Nucleic Acids Res. 38: D161-D166, 2010.

Sipos D, Andersson M, Ehrenberg A. The structure of the mammalian antibacterial peptide cecropin P1 in solution, determined by proton-NMR. Eur. J. Biochem. 209: 163-169, 1992.

Tamang DG, Saier MH, Jr. The cecropin superfamily of toxic peptides. J. Mol. Microbiol. Biotechnol. 11: 94-103, 2006.

Tarr DEK. Distribution and characteristics of ABFs, cecropins, nemapores, and lysozymes in nematodes. Dev. Comp. Immunol. 36: 502-520, 2012.

Telford MJ, Bourlat SJ, Economou A, Papillon D, Rota-Stabelli O. The evolution of the Ecdysozoa. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 363: 1529-1537, 2008.

Tian C, Gao B, del Carmen Rodriguez M, Lanz-Mendoza H, Ma B, Zhu S. Gene expression, antiparasitic activity, and functional evolution of the drosomycin family. Mol. Immunol. 45: 3909-3916, 2008.

Ueno S, Kusaka K, Tamada Y, Minaba M, Zhang H, Wang P-C*, et al.* Anionic C-terminal proregion of nematode antimicrobial peptide Cecropin P4 precursor inhibits antimicrobial activity of the mature peptide. Biosci. Biotechnol. Biochem. 72: 3281-3284, 2008.

Varshavsky A. 'Spalog' and 'sequelog': neutral terms for spatial and sequence similarity. Curr. Biol. 14: R181-R183, 2004.

Wang L, Lai C-e, Wu Q, Liu J, Zhou M, Ren Z*, et al.* Production and characterization of a novel antimicrobial peptdie HKABF by *Pichia pastoris.* Process Biochem. 43: 1124-1131, 2008.

Wiens M, Schröder HC, Korzhev M, Wang X-H, Batel R, Müller WEG. Inducible ASABF-type antimicrobial peptide from the sponge *Suberites domuncula:* Microbicidal and hemolytic activity *in vitro* and toxic effect on molluscs *in vivo.* Mar. Drugs 9: 1969-1994, 2011.

Xu W, Faisal M. Defensin of the zebra mussel (*Dreissena polymorpha*): Molecular structure, *in vitro* expression, antimicrobial activity, and potential functions. Mol. Immunol. 47: 2138-2147, 2010.

Yang Y-S, Mitta G, Chavanieu A, Calas B, Sanchez JF, Roch P*, et al.* Solution structure and activity of the synthetic four-disulfide bond Mediterranean mussel defensin (MGD-1). Biochemistry 39: 14436-14447, 2000.

Yeaman MR, Yount NY. Unifying themes in host defence effector polypeptides. Nat. Rev. Microbiol. 5: 727-740, 2007.

Yount NY, Yeaman MR. Multidimensional signatures in antimicrobial peptides. Proc. Natl. Acad. Sci. USA 101: 7363-7368, 2004.

Zhang H, Kato Y. Common structural properties specifically found in the CS$\alpha\beta$-type antimicrobial peptides in nematodes and mollusks: evidence for the same evolutionary origin? Dev. Comp. Immunol. 27: 499-503, 2003.

Zhang H, Yoshida S, Aizawa T, Murakami R, Suzuki M, Koganezawa N*, et al.* In vitro antimicrobial properties of recombinant ASABF, an antimicrobial peptide isolated from the nematode *Ascaris suum*. Antimicrob. Agents Chemother. 44: 2701-2705, 2000.

Zhao C, Liaw L, Lee IH, Lehrer RI. cDNA cloning of three cecropin-like antimicrobial peptides (Styelins) from the tunicate, *Styela clava*. FEBS Lett. 412: 144-148, 1997.

Zhu S. Discovery of six families of fungal defensin-like peptides provides insights into origin and evolution of the CS$\alpha\beta$ defensins. Mol. Immunol. 45: 828-838, 2008.

Zhu S. Evidence for myxobacterial origin of eukaryotic defensins. Immunogenetics 59: 949-954, 2007.

Zhu S, Gao B, Tytgat J. Phylogenetic distribution, functional epitopes and evolution of the CS$\alpha\beta$ superfamily. Cell. Mol. Life Sci. 62: 2257-2269, 2005.