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REVIEW

Antimicrobial peptides in annelids

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Abstract

Gene encoded antimicrobial peptides (AMPs) are widely distributed among living organisms including plants, invertebrates and vertebrates. They constitute important effectors of the innate immune response by exerting multiple roles as mediators of inflammation with impact on epithelial and inflammatory cells influencing diverse processes such as cytokine release, cell proliferation, angiogenesis, wound healing, chemotaxis and immune induction. In invertebrates, most of the data describe the characterization and/or the function of AMPs in the numerically and economically most representative group which are arthropods. Annelids are among the first coelomates and are therefore of special phylogenetic interest. Compared to other invertebrate groups, data on annelid's immunity reveal heavier emphasis on the cellular than on the humoral response suggesting that immune defense of annelids seems to be principally developed as cellular immunity.This paper gives an overview of the variety of AMPs identified in the three classes of annelids, i.e. polychaetes, oligochaetes and achaetes. Their functions, when they have been studied, in the humoral or cellular response of annelids are also mentioned.

Key words: antimicrobial peptides; annelids; lophotrochozoan; immunity

Introduction

Numerous studies on the effectors of the innate immune system have demonstrated the contribution of AMP to the host defense (Zasloff, 2002). Antibiotic peptides are small molecules. Basically on their structural features, five major classes were defined (Bulet *et al.*, 2004): 1) linear α helical peptides without cysteines (the prototype of this family are the cecropins); 2) loop forming peptides containing a unique disulfide bond (mainly isolated from amphibian skin); 3) open-ended cyclic cysteine-rich peptides (among which defensins are the most widespread); 4) linear peptides containing a high proportion of one or two amino acids, e.g. indolicidin; 5) peptides derived from larger molecules known to exert multiple functions. In spite of great primary structure diversity, the majority of documented AMPs are characterized by a preponderance of cationic and hydrophobic amino acids. This amphipathic structure allows them to interact with bacterial membrane. However, Brogden *et al.* (1998) have evidenced in ovines that some peptides with anionic properties can also exert antimicrobial activities.

AMPs appear to be essential anti-infectious factors that have been conserved during evolution. Meanwhile, their implications in immune processes are different according to species, cells and tissues. The involvement of AMPs in natural resistance to infection is sustained by their strategic location in phagocytes, in body fluids and at epithelial level, i.e. at interfaces between organisms and its environment. This action is strengthened by the rapid induction of such AMP genes in bacteria-challenged plants or animals (Zasloff, 1992).

Annelids belonging to the group of lophotrochozoans are primitive coelomates that possess specially developed cellular immunity against pathogens including phagocytosis, encapsulation and spontaneous cytotoxicity of coelomocytes against allogenic or xenogenic cells (Salzet *et al.*, 2006). They have also developed an important humoral immunity that is based on antimicrobial, hemolytic and clotting properties of their body fluid. The present review summarizes the function of different AMPs that adaptation has taken during the course of evolution of the three

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classes of annelids, i.e. polychaetes, oligochaetes and achaetes.

Polychaetes

The large majority of polychaetes is restricted to the marine environment. They are considered as the most primitive annelids, based on morphology, physiology and development. To date, AMPs have been studied in three species of polychaetes, *Arenicola marina* (Ovchinnikova *et al.*, 2004), *Nereis diversicolor* (Tasiemski *et al.*, 2007) *and Perinereis aibuhitensis* (Pan *et al.,* 2004). They all live in estuary sediments which are rich in microorganisms but also of toxic agents resulting from pollution. Their abundance in this type of environment suggests these worms have developed efficient detoxification and immunodefense strategies.

In the Asian clamworm *P. aibuhitensis*, a cationic AMP named perinerin was isolated and partially characterized by Pan *et al.*(2004) from the homogenate of adults. This peptide does not show any similarities with other described AMPs. Perinerin consists of 51 amino acids, including 4 cysteine residues presumably implicated in two disulfide bridges. No cDNA cloning was performed so no information is available about the precursor sequence of the mature form. Antimicrobial assays performed with the native perinerin evidenced an activity directed against a large spectrum of microorganisms including fungi, Gram positive and Gram negative bacteria at physiological concentrations. A rapid bactericidal activity was evidenced towards Gram positive bacteria: indeed, when incubated with a culture of *Bacillus megaterium* during exponential phase, perinerin killed all the bacteria in less than three minutes, suggesting a pore forming activity. The cellular localization as well as the physiological role of perinerin in the anti-infectious response of *P. aibuhitensis* has not been yet elucidated. The authors suggest that the peptide should be constitutively expressed since perinerin was purified from unchallenged worms. However, there are no indications that immunization of *P. aibuhitensis* by bacterial challenge has modified the synthesis site and/or the concentration of native perinerin.

Still in 2004, two novel 21 amino acids AMPs, namely arenicin-1 and arenicin-2, were isolated and fully characterized from the coelomocytes of the lugworm, *A. marina* (Ovchinnikova *et al.*, 2004). Each isoform possesses two cysteine residues implicated in one disulfide bond. AMPs containing only one disulfide bond have been isolated from frog skin earlier but their cysteine bridged loop is smaller than the one of arenicin (Bulet *et al.*, 2004). Recent publications showed that arenicin folds into a two stranded antiparallel β-sheet which are twisted to expose an amphipatic surface (Ovchinnikova *et al.*, 2007; Andra *et al.*, 2008). Other AMPs such as the tachyplesin found in the horseshoe crab adopt this conformation although the β-sheet of arenicin is not caged into two disulfide bonds (Tamamura *et al.*, 1993). Both isoforms were shown to be active against fungi, Gram positive and Gram negative bacteria. Antimicrobial activities of arenicin-1 and 2 were absolutely equal. As arenicin is amphipatic and rich in hydrophobic and arginine, Andra *et al.* (2008) hypothesized that the killing of the bacteria implicated a disruption of the membrane. By applying an elegant approach, they demonstrated that incubation of Gram negative bacteria with arenicin leads to a rapid membrane permeabilization accompanied by the intercalation of the AMP peptide into the lipid bilayer and the release of cytoplasmic material. These active forms are processed from a larger precursor containing a predicted signal peptide followed by a long prodomain. The presence of a signal peptide suggests that the peptides can be secreted thought a conventional pathway (Ovchinnikova *et al.*, 2004). Data related to arenicin have been more focused on its structure and mode of action than on its immune function in *A. marina.* The isolation of arenicins from coelomocytes lets presume that arenicin may play function in the cellular immunity of *L. rubellus* in a way comparable to what has been described for hedistin in *N. diversicolor* (Tasiemski *et al.*, 2007).

Hedistin was identified from the coelomocytes of the sandworm *N. diversicolor*. Like perinerin and arenicin, this AMP showed no obvious similarities with other known peptides. Hedistin is active against a large spectrum of Gram positive bacteria. Interestingly, when tested with different Gram negative microorganisms, hedistin is active especially on marine bacteria *V. alginolyticus* which is a causative agent of episodes of mass mortality of larvae of bivalves in commercial hatcheries. This could be attributable to the capacity of *Vibrio* to extensively degrade native cuticle collagen of *Nereis.* Vibrial collagenase would help bacteria entrance into the worm body, making the mechanical defense barrier of the cuticle inefficient against *Vibrio* invasion. Thus, hedistin synthesis would follow from the adaptation of *Nereis* immune defense towards bacteria of its environment. No cytotoxicity of either hedistin forms was observed against *Nereis* cœlomocytes.

Hedistin is a linear peptide containing bromotryptophan residues in its amino acid sequence. Although many antimicrobial peptides have been characterized in marine metazoans, very few are reported to contain bromotryptophan residues (Taylor *et al.*, 2000). Bromination of tryptophan which is described as a result of post translational modifications seems to be typical of organisms living in sea water like tunicates (Craig *et al.*, 1997; Jimenez *et al.*, 1997). For example, mammalian cathelicidins, a family of AMPs, do not contain bromotryptophan while hagfish cathelicidins do (Shinnar, Butler *et al.*, 2003; Uzzell *et al.*, 2003). Bromination of tryptophan residues does not seem to play a role in the antibacterial activity of hedistin.

In addition to bromotryptophan, the hedistin primary structure includes a C-terminal (Ct) amidation. The presence of a Ct amide increases the net cationic charge and consequently the electrostatic attraction to target membrane like the negative charged bacteria membrane. This suggests that C-terminal amidation of hedistin might be implicated in its bactericidal properties. Moreover, as for the hagfish cathelicidins, the C-terminal amidation and the unusual amino acid bromotryptophan could make hedistin a poorer substrate for endogenous proteolytic enzyme by

providing resistance to C-terminal exopeptidase and to proteolysis for steric reasons respectively (Uzzell *et al.*, 2003). Such protease resistance could extend the lifetimes of hedistin *in vivo* sustaining antimicrobial activity.

The *hedistin* gene is strongly and exclusively expressed in cœlomocytes evenly distributed in the whole cœlomic cavity. These are referred as the type 3 granulocytes also called NK-like cells because of their natural cytotoxicity (Porchet-Hennere *et al.*, 1992). Even if the level of transcript does not increase after bacteria challenge, "hedistin containing cœlomocytes" appeared to accumulate around infection sites where the presence of bacterial motifs triggers the release of the active peptide into the local environment. These results are reminiscent of those reported for marine organisms of different taxa like the shrimp *Penaeus vannamei* (Bachere *et al.*, 2004), the mussel *Mytilus galloprovincialis* (Mitta *et al.*, 1999) and the horseshoe crab *Tachyplesus tridentatus* (Iwanaga, 2002). In *M. galloprovincialis*, microbial challenge provokes the release of the antimicrobial peptide MGD1 from the hemocytes into the plasma. In *T. tridentatus*, bacterial stimulation triggers the degranulation and the release of different immune molecules among them AMPs

Oligochaetes

Oligochaetes have a larger repartition area than polychaetes: they can be terrestrial, semi- or fully aquatic in freshwater or, more rarely, in seawater. They live in an environment such as water, soil and manure containing abundant microorganisms that are ingested during feeding. Members of the AMP family have been identified in the following earthworms: *Lumbricus rubellus*, *Pheretima tshiliensis* and *Eisenia foetida.*

The first AMP evidenced in oligochaetes and in annelids in general, was isolated and characterized from a homogenate of *L. rubellus* (Cho *et al.*, 1998). Lumbricin-1 is a proline-rich antimicrobial peptide of 62 amino acids showing antimicrobial activity *in vitro* against fungi, Gram positive and Gram negative bacteria without hemolytic activity. A 29-amino acid peptide, named lumbricin-1 (6-34), which was derived from residues 6-34 of lumbricin-1, showed marginally stronger antimicrobial activity than authentic lumbricin-1. Many proline-rich AMPs were discovered from various vertebrates and invertebrates species (Bulet *et al.*, 2004). Even if they possess the common characteristics of a high content of proline and a highly positive charge, their mode of action and their antimicrobial spectra are quite different from one to another. For example, apidaecin has been shown to act through a non pore forming mechanism involving stereospecificity whereas PR39 blocks protein and DNA synthesis in cells (Casteels and Tempst, 1994; Gaczynska *et al.*, 2003). The mode of action of lumbricin-1 has not been yet described. Concerning its physiological role, lumbricin-1 is processed from a precursor containing a signal peptide directly followed by the active molecule suggesting that this AMP can be found in the extracellular environment.

Northern blot data showed that *lumbricin-1* gene is constitutively expressed and is not inducible upon bacterial infection(Cho *et al.*, 1998). Moreover, lumbricin-1 transcripts were detected in unchallenged six month old adults but not in one week old young adult and in eggs suggesting that the transition to the adult stage might be inducer of lumbricin-1 gene expression. That would be interesting to check whether the expression of the gene encoding lumbricin-1 is not induced in bacterially challenged young or eggs.

In 2003, a cDNA encoding a molecule presenting high percentage homology with lumbricin-1 was described in the Asian worm *P. tschiliensis* (Wang *et al.*, 2003). Despite the great similarity with lumbricin I, this putative antimicrobial peptide, so called PP-1, lacks an obvious signal peptide. However, immunohistochemical studies showed that PP-1 was immunodetected in the mucus covering the animal and not into the epidermis cells. The extracellular presence of peptides devoid of peptide signals was also described for lysenin which was purified from the celomic fluid of *E. foetida* (Sekizawa *et al.*, 1997) and more recently for Hm-lumbricin, an AMP similar to lumbricin-1 that was purified in the medicinal leech *Hirudo medicinalis* (Schikorski *et al.*, 2008). Further investigations on this uncommon mechanism of secretion have been proposed to be performed by Wang *et al.* (2003).

In the tiger worm *E. foetida,* three very short antibiotic peptides, F-1, F-2 and OEP3121, have been isolated and identified (Zhang *et al.*, 2002; Liu *et al.*, 2004). These are described as exhibiting an activity against Gram positive, Gram negative bacteria and fungi. All of three are composed of only five amino acids that do not present primary structural homology with other known AMPs. To date, there is no information in the literature about the nature of their eventual precursors or their production site.

Achaetes

Achaetes, also called leeches, live in an environment similar to that of their near relatives, oligochaetes. Both are clitellates and in contrast to polychaetes do not present a larval stage during their development. AMPs have been studied in two species of leeches: the rhynchobdellid leech *Theromyzon tessulatum* and the gnathobdellid leech *H. medicinalis*. These are blood suckers of vertebrates suggesting that they could have developed an immune response adapted to microorganisms possibly pathogens of mammals.

T. tessulatum is an ectoparasite of aquatic birds. Its life cycle was arbitrarily subdivided in stages (these are not larval stages) defined by taking, as indicators, the three blood meals. The third stage which corresponds to the gametogenesis phase is characterized by an important water uptake making the collect of the body fluid easy. For this reason, *T. tessulatum* constitutes a convenient model for studying the antimicrobial response which takes place at the systemic level of the leech. Three AMPs were isolated and fully characterized from the body fluid of *T. tessulatum*. These are theromacin, a cysteine rich AMP exhibiting bactericidal activities, theromyzin an anionic peptide with bacteriostatic properties (Tasiemski *et al.*, 2004) and peptide B an

anionic peptide matured from a neuropeptide precursor, proenkephalin A (PEA) (Tasiemski *et al.*, 2000). They all present an activity directed against Gram positive bacteria.

Several types of AMPs containing cysteine residues have been described in multiple organisms. In invertebrates, most of them share the disulfide array of the insect/arthropod defensin (Bulet *et al.*, 2004). In addition to having ten cysteine residues instead of six, theromacin does not harbor this consensus sequence. Besides, it appeared that the peptide has no significant similarity with other known peptides. So theromacin is a novel cysteine rich AMP. Majority of AMPs including members of the cysteine rich family possess a global positive charge allowing their interaction with the negatively charged bacterial membrane.

Theromyzin and peptide B, in contrast to theromacin, are anionic molecules. In vertebrates, AMPs with anionic properties were evidenced in the human and the sheep lung (Brogden *et al.*, 1998). AMPs are anionic because of homopolymeric regions of aspartate, and require zinc as a cofactor for bactericidal activity (Melino *et al.*, 1999). Histatins, a family of histidin rich AMPs found in human saliva, also need the presence of zinc ions for bactericidal activities. Circular dichroism studies showed that the antimicrobial activities of histatin-5 require a conformational change that results from the interaction of the peptide with both zinc ions and negatively charged membranes (Brewer and Lajoie, 2000). The abundance of histidine residues at the N-terminal part of theromyzin could argue in favor of some common structures between the leech antibacterial peptide and histatins. Based on these data, we can hypothesize that the active part of theromyzin might be reduced to the N-terminal part enriched in histidine and aspartate residues and that theromyzin could require a cofactor for bactericidal activity.

Theromacin and theromyzin genes are exclusively expressed in large fat cells (LFC) evenly distributed in the leech. Their transcriptional level is enhanced after bacteria challenge evidencing a regulation of the leech AMP similar to that of the insect antimicrobial peptides genes. Indeed, in the fruit fly, genes encoding antibiotic peptides are rapidly induced following a septic injury (Hoffmann, 2003). The similarity between the antibacterial response of the leech and those of holometabolous insects is also supported by the functional resemblance between the leech LFC and the insect fat body which possess the common capacity to produce egg-yolk proteins (Baert *et al.*, 1991).

Moreover, no difference in gene expression was observed after Gram positive or Gram negative injection suggesting that the antibacterial response of *Theromyzon* is aspecific. This non-specificity has also been assumed in *Drosophila* until the work of Lemaitre *et al.* (1997) demonstrated that the humoral antimicrobial response of the fruit fly discriminates between various classes of microorganisms and mounts a response that is adapted to the infection. This suggested that in a more natural modality of infection, also the leech could adapt its antimicrobial response, what was recently confirmed (Schikorski *et al.*, 2008).

As for lumbricin-1 in *L. rubellus*, physiological events occurring during gametogenesis phase appeared to be inducers of the theromacin and theromyzin gene expression in *T. tessulatum*. This indicates that several hormonal factors implicated in the sexual maturation may participate in the induction of genes encoded AMPs in annelids as described in *Drosophila* by Meister and Richards (1996).

The peptide sequences deduced from the theromacin and theromyzin genes contain putative signal peptides, indicating that mature peptides correspond to secreted molecules. The massive production of theromacin and theromyzin is immediately followed by a rapid release of the peptides into the celomic fluid after septic injury. Thus theromacin and theromyzin may play their antimicrobial activities through a systemic action. Moreover the presence of these peptides in the intestinal epithelial cells and at the epidermis level suggests that leech antimicrobial peptides could also play a role in epithelial defense. The localization of antibiotic molecules in gastrointestinal tract has also been reported in insects and in vertebrates where they provide a rapid local immune response against exogenous pathogens brought in during feeding (Zasloff, 2002). As for PP-1, theromacin and theromyzin were detected in the mucous that covers the animal. That reminds the local defensive response reported in frogs in which antibacterial peptides secreted in the mucous prevent bacteria colonization and/or subsequent infection (Zasloff, 1992). In addition, since a mucous membrane covers the eggs after laying, we hypothesized a protective role of leech theromacin and theromyzin against bacteria during eggs development.

Peptide B is not produced by the LFC although it was also isolated from the body fluid of the leech. Its precursor, PEA, was immunodetected into circulating coelomocytes suggesting that peptide B could be released from these cells. In contrast to theromacin and theromyzin, the production of peptide B seems to be more regulated at the translational level by the enzymes implicated in the PEA processing than at the transcriptional level.

Consequently, *T. tessulatum* is an original invertebrate model which has developed two modes of fighting infections by AMPs: (i) storage of antibacterial peptide derived from PEA and release of the peptide into the celomic fluid after immune challenge (ii) induction after septic injury of gene coding for more classical AMPs, mainly in LFC, and rapid release into the body fluid of the antibiotic peptides.

Unpublished data collected by our group evidence that the same AMPs participate to the systemic antimicrobial response of the medicinal leech *H. medicinalis*. This leech presents celomic cavities filled by cells constituting the bothryoidal tissue, making the collect of the body fluid impossible. In fact, the medicinal leech has been extensively used as a model organism for cellular analyses of nervous system function (Blackshaw and

Table 1 Summary of the AMPs characterized in anellids

Origin	Organism	Name	Primary structure	Production site
Polychaetes	*Perinereis aibuhitensis*	perinerin	cysteine-rich	undetermined
	Arenicola marina	arenicins-1, 2	loop forming peptides	coelomocytes
	Nereis diversicolor	hedistin	linear peptide containing bromo-tryptophans	coelomocytes (NK cells-like)
Oligochaetes	*Lumbricus rubellus*	lumbricin-1	linear peptide, proline-rich	undetermined
	Pheretima tschiliensis	PP-1	linear peptide, proline-rich	tegument
	Eisenia foetida	OEP3121	short linear peptide	undetermined
		F-1, F-2	short linear peptides	undetermined
Achaetes	*Theromyzon tessulatum,* *Hirudo medinalis*	peptide B	peptide derived from a larger precursor	coelomocytes
		theromacin	cysteine-rich	large fat cells (LFC)
		theromyzin	linear, anionic	LFC
		Hm-lumbricin and *Tt*-lumbricin	linear peptide, proline-rich	LFC, neuron and microglia
		neuromacin	cysteine-rich	neuron and microglia

Nicholls, 1995; Emes *et al.*, 2003). Of particular significance, the medicinal leech has been used to study repair of the nervous system at the level of identified nerve cells, an approach that is currently difficult or not possible with more complex nervous systems. The nerve cord of the leech can be easily removed from the animal and maintained in culture for weeks in the absence of peripheral immune system components and blood cells that might infiltrate the nerve cord after injury. That allows focusing the studies on the intrinsic immune response developed by the leech nervous system. Unlike mammals, the leech central nerovous system (CNS) has a demonstrated capacity to repair itself after injury and to restore function (Blackshaw *et al.*, 1997; Burrell *et al.*, 2003). The process of regeneration is accompanied by a rapid activation of microglial cells leading to their accumulation at the lesion site where they phagocytize damaged tissue. Interestingly, we recently evidenced that the leech nerve cord used a common panel of proteins to initiate an antimicrobial response and regrowth program. Indeed, we have demonstrated that microbial challenge promoted the regenerative process of the injured CNS of the medicinal leech by inducing the synthesis of AMPs in neurons and microglia (Schikorski *et al.*, 2008). Two newly characterized AMPs, Hm-lumbricin and neuromacin have been shown to be produced by microglial cells and by neurons themselves in response to CNS injury.

Neuromacin is a relative of theromacin. Theromacin possesses a longer C-terminal domain than neuromacin, which probably results in two different conformations, that could determine different biological activities for the two peptides. Theromacin and neuromacin present a differential tissue expression.

Theromacin is expressed in the peripheral LFC, whereas neuromacin expression is restricted to the nervous system. Curiously, only neuromacin-like molecules have been found in other invertebrates, such as molluscs and other annelids (Mitta *et al.*, 2005; Moroz *et al.*, 2006). Thus far, the larger theromacin appears to be unique to leeches.

Neuromacin, like theromacin, displayed bactericidal activity against Gram-positive bacteria. The importance of neuromacin and Hm-lumbricin in the antiinfectious immunity of the leech CNS is emphasized by the presence of their transcripts in neuronal cells and by the fact that their gene expression is upregulated by some microbial components. A difference in neuromacin and Hm-lumbricin gene expression was observed after infection with different microbial components, suggesting that the antibacterial response of the medicinal leech CNS is specific to the antigens presented. This was not expected, given that in the leech *T. tessulatum* our previous work demonstrated the non-specificity of the humoral antimicrobial response to infection. This ability to discriminate pathogens might be relevant to the use, in the experiments presented here, of bacteria naturally living in the environment of the leech. Intriguingly, a neuromacin-like peptide reported as theromacin-like has recently been detected by sequencing cDNA libraries from the CNS of the mollusc *Aplysia californica*, but the roles and the production sites of the peptide were not detailed (Moroz *et al.*, 2006). In *Caenorhabditis elegans*, several genes encoding neuropeptide-like proteins named NLP-29, NLP-31 and NLP-33, the sequences of which were deduced from an *in silico* analysis of an EST library, have been shown to be induced in the hypodermis by fungal infection.

Interestingly, the chemically synthesized NLP-31 exhibited antifungal activity but in contrast to most of the nlp family members, NLP-29, NLP-31 and NLP-33 were not detected in neurons using GFP reporter genes (Couillault *et al.*, 2004).

In addition to manifesting antibacterial properties, neuromacin and Hm-lumbricin exert impressive regenerative effects on the leech CNS. In vertebrates, one study provided evidence for the positive effects of an antimicrobial peptide on the restoration of the functions of a lesioned peripheric nerve. Indeed, the addition of neutrophil defensin NP-1 on the lesioned sciatic nerve in rats lead to increase the rate of growth of regenerative nerve fibers by 30 % (Nozdrachev *et al.*, 2006). So these data are the first evidencing the participation of an AMPs produced by the nervous system itself in the regeneration process of the CNS.

Conclusions

This review presents the large variety of AMPs that can be found in annelids since the five groups of AMPs evoked in introduction are all represented (Table 1). AMPs produced by polychaetes seem to be distinct from those produced by leeches and oligochaetes. For examples, *H. medicinalis* and *L. rubellus* produce common AMPs, such as lumbricin and theromacin (found in the *Lumbricus* EST database) which are different from those synthesized by polychaetes. Neither hedistin nor arenicin was found in the leech. This suggests that for the selection of AMPs retained by an organism, the environment where it lives might be relevant. It is interesting to remark that, at present, no defensins have been isolated from annelids although more than 70 different invertebrate defensins have been identified in molluscs, nematods and arthropods. Defensins which are considered as the most widespread family of invertebrate AMPs have not been found neither in the genome of the leech *Helobdella robusta* (http://genome.jgi-psf.org/Helro1/Helro1.home.html) nor in the Expressed Sequence Tag (EST) libraries of *Lumbricus terrestris*, *E. foetida* (http://www.earthworms.org) and *H. medicinalis*. (http://genome.uiowa.edu/projects/leech/). Reciprocally, most AMPs described in this review have not been found in the genomes of ecdysozoan invertebrates such as *C. elegans* and *Drosophila melanogaster*. This underlines the importance of enlarging the number of invertebrate models dedicated to study AMPs and their functions in innate immunity.

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