Chapter 4

Neuropeptides in Helminths:
Occurrence and Distribution

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Abstract

Nematode neuropeptide systems comprise an exceptionally complex array of ~250 peptidic signaling molecules that operate within a structurally simple nervous system of ~300 neurons. A relatively complete picture of the neuropeptide complement is available for *Caenorhabditis elegans*, with 30 *flp*, 38 *ins* and 43 *nlp* genes having been documented; accumulating evidence indicates similar complexity in parasitic nematodes from clades I, III, IV and V. In contrast, the picture for parasitic platyhelminths is less clear, with the limited peptide sequence data available providing concrete evidence for only FMRFamide-like peptide (FLP) and neuropeptide F (NPF) signaling systems, each of which only comprises one or two peptides. With the completion of the *Schmidtea mediterranea* and *Schistosoma mansoni* genome projects and expressed sequence tag datasets for other flatworm parasites becoming available, the time is ripe for a detailed reanalysis of neuropeptide signaling in flatworms. Although the actual neuropeptides provide limited obvious value as targets for chemotherapeutic-based control strategies, they do highlight the signaling systems present in these helminths and provide tools for the discovery of more amenable targets such as neuropeptide receptors or neuropeptide processing enzymes. Also, they offer opportunities to evaluate the potential of their associated signaling pathways as targets through RNA interference (RNAi)-based, target validation strategies. Currently, within both helminth phyla, the *flp* signaling systems appear to merit further investigation as they are intrinsically linked with motor function, a proven target for successful anti-parasitics; it is clear that some nematode NLPs also play a role in motor function and could have similar appeal. At this time, it is unclear if flatworm NPF and nematode INS peptides operate in pathways that have utility for parasite control. Clearly, RNAi-based validation could be a starting point for scoring potential target pathways within neuropeptide signaling for parasiticide discovery programs. Also, recent successes in the application of *in planta*-based RNAi control strategies for plant parasitic nematodes reveal a strategy whereby neuropeptide encoding genes could become targets for parasite control. The possibility of developing these approaches for the control of animal and human parasites is intriguing, but will require significant advances in the delivery of RNAi-triggers.

Introduction

Twenty years ago almost nothing was known about the occurrence and distribution of peptide signaling molecules in helminth nervous systems. This was despite the publication of a physical map of the nervous system of the free-living nematode, *Caenorhabditis elegans* by White and coworkers,[1] the first ultrastructural reconstruction of an entire metazoan nervous system and one of the foundation stones to the subsequent exploitation of *C. elegans* as a model

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organism for laboratory-based research. Although several authors had reported the occurrence of neurosecretory vesicles in parasitic nematodes in the 1960s,[2,3] it was not until indirect immunocytochemical methods were applied to helminths in the mid-late 1980s, that data started to accumulate on the occurrence and distribution of neuropeptides in nematode and platyhelminth nervous systems.[4-13] These and subsequent studies provided information that revolutionized our view of the chemical complexity of helminth nervous systems and provided strong evidence that neuropeptides played a major role in neuronal signaling processes in worms.

Although invaluable in demonstrating the widespread occurrence of neuropeptides in worms and indeed generating data on the complexity and organization of nervous systems in numerous helminth parasites, these studies did not provide information on the primary structures of these peptides. This breakthrough followed the sterling efforts of Tony Stretton and colleagues who managed to extract, purify and sequence small quantities of neuropeptides from large-scale tissue extracts of the gastrointestinal parasite of pigs, *Ascaris suum*.[14] Subsequent studies on this and other nematodes generated a constant flow of new peptide sequences that served to expand our knowledge of their peptide complements.[15-29] One noticeable feature at this time was that almost all the primary sequence data were for multiple members of one neuropeptide family, the FMRFamide-related peptides (FaRPs) or FMRFamide-like peptides (FLPs). During the same period, the structures of a relatively small number of flatworm neuropeptides were determined.[30-35] Again, FLPs featured amongst the flatworm neuropeptide sequence data, but a second family, designated neuropeptide F (NPF) was also much in evidence.

In the 1990s, the molecular biological and genomics revolutions were making a significant impact on nematode neuropeptide discovery. The first reported nematode *flp* gene was significant in that it encoded multiple copies of distinct FLPs and was alternatively spliced to facilitate variation in the resultant peptide products.[36] However, even then, the extent of neuropeptide diversity in nematodes was not realized. In the late 1990s, several groups reported the occurrence of numerous neuropeptide genes, including insulin-like peptide encoding *ins* and multiple *flp* genes in *C. elegans*.[37-40] The completion of the *C. elegans* genome (at time of writing, still the only complete [to the last base] metazoan genome sequence) provided the opportunity for more comprehensive neuropeptide gene discovery efforts and allowed the identification of a wide range of non-*ins* and non-*flp* neuropeptide genes that were grouped together and designated *nlp* (neuropeptide-like protein) genes.[41,42] Taken together, these findings resulted in a broad reevaluation of the importance and complexity of neuropeptides to neuronal signaling processes in nematodes and presented *C. elegans* neurobiologists with a bewilderingly diverse catalog of peptide signaling molecules for which there was very little known about the associated biology. Further, this knowledge base provided a tool for the interrogation of expressed sequence tag (EST) datasets which have been accumulating for numerous, important pathogenic nematodes since the turn of the century. Recently, this has facilitated the discovery of *flp* genes in a wide range of parasitic nematodes revealing significant conservation in the *flp* complements across phylum Nematoda.[43,44]

In direct contrast to the situation in nematodes, progress in flatworm peptide discovery stalled through the late 1990s and early years of this century, largely because the laborious biochemical methods used to generate individual peptide sequences were now out of vogue and could not compete with the rapidly evolving genomics approaches. Although our knowledge of flatworm neuropeptides is still trailing far behind that for nematodes, significant EST deposits for a relatively small number of flatworms and genome sequence data for the blood fluke *S. mansoni* and the turbellarian *S. mediterranea*, at least threaten to rectify this situation and provide much needed data on neuropeptide complements in platyhelminths.

For those working in helminth parasite control, interest in neuropeptide signaling systems stems from several observations. Firstly, unlike the situation in mammals, where neuropeptides are most commonly involved in the modulation of synaptic function, invertebrate neuropeptides act as both modulators and fully fledged neurotransmitter molecules. This latter function has much appeal as a target system for chemotherapies aimed at disrupting normal parasite behaviors. Secondly, there are stark differences in the neuropeptides found in invertebrates and vertebrates such that target proteins

ensconced within the associated signaling pathways are likely to be distinct from host proteins. Thirdly and most importantly, many of the leading anthelmintics (especially those used to treat nematode parasites) disrupt neuromuscular function, primarily by acting at receptors within classical transmitter signaling pathways.[45,46] Currently, none of these drugs act on the neuropeptide signaling processes which impact neuromuscular function, exposing the potential of these systems as target sources. Several recent reviews provide comprehensive coverage of neuropeptides in flatworms and nematodes.[45,47-50] This chapter aims to provide an overview of current knowledge on neuropeptides and their expression in helminths and how this might relate to targets for parasite control.

Nematode Neuropeptides

All nematodes have around 300 neurons (the *C. elegans* hermaphrodite has 302 neurons), making their nervous system the most structurally simple amongst the triploblastic metazoans. Nematodes appear to compensate for these structural limitations by employing a huge number of intercellular signaling molecules, of which the largest structural class is the neuropeptides. In nematodes, these neuropeptides currently fall within three distinct groupings, the insulin-like peptide family encoded on *ins* genes, the FMRFamide-like peptide family encoded on *flp* genes and a group encompassing all other neuropeptide families designated the neuropeptide-like proteins and encoded on *nlp* genes. Presently, these groupings encompass 102 distinct genes that encode in excess of 250 different neuropeptides—a quite staggering complement for relatively simple animals with ~300 nerve cells.

Nematode FMR Famide-Like Peptides (FLPs)

Nematodes show exceptional diversity with respect to the complement of FLPs they express. The number of different FLPs identified (so far) in *C. elegans* stands at >70 and these are encoded on a battery of 30 distinct genes (designated *flp-1* to *flp-28*, *flp-32* and *flp-33*); note that *flp-32* has only been predicted such that its transcript awaits identification.[43,44,50,51] Bioinformatic and PCR/DNA sequencing studies have provided the bulk of the available data on *C. elegans* FLPs,[36,40,43,51,52] and until recently, only a small number of studies contributed peptide sequence information.[18,23-28] More recently, the powerful peptidomic approaches adopted by the Schoofs laboratory have provided peptide structural data that support many of the earlier predictions on peptide sequences and which have uncovered some additional peptides, e.g., a *flp-33* encoded peptide.[50,53]

C. elegans has been the most important tool in our understanding of the nematode FLP complement, although studies on other species have contributed to this knowledge base. Multiple FLPs have been structurally characterized from the large gastrointestinal parasite of swine and Clade III nematode, *A. suum*.[14,16,17,29,54] Small numbers of FLPs have been structurally isolated from *Panagrellus redivivus*,[15,19-21] the free-living, Clade III nematode (*C. elegans* belongs to Clade V) and the sheep parasite from Clade V, *Haemonchus contortus*.[22,27] A screen of the growing expressed sequence tag (EST) datasets for parasitic nematodes revealed that there was much similarity in the *flp* genes across phylum Nematoda.[43] Indeed, all but one (*flp-20*) of the *C. elegans flp* genes were identified in the EST dataset of at least one parasitic nematode. All in all, this effort uncovered a total of 33 *flp* genes encoding over 90 distinct FLPs in 33 different nematode species from Clades I, III, IV and V; there was no evidence of significant inter-Clade or inter-lifestyle variation in FLP complement. The range of FLP signature sequences that have been identified in nematodes are outlined in Table 1. One feature that stands out is the large number of species in which most *flp* genes have been identified. Only *flp-29*, *-30* and *31* do not appear to be expressed in *C. elegans*. Indeed, *flp-30* and *31* have only been identified in plant parasitic nematodes of Clade IV and could play a role specific to plant-nematode interactions. *flp-29* has been identified in parasites of Clades III and V and yet appears to be absent from *C. elegans* (also Clade V) such that it could play a role in parasite-specific activities.

Most FLPs appear to be associated with intercellular communication in nematodes and they have been shown to have wide-ranging functions. In particular, many are associated with the modulation of motor activities or sensory modalities such that the appeal of proteins associated with these signaling pathways as drug targets is strong. The specific functions of nematode FLPs are discussed in detail in Chapter 5 and will not be discussed further here.

Table 1. Nematode FMRFamide-like peptide (FLPs) signature sequences and their occurrence in nematode genera

Gene Name	Amino Acid Sequence/ C-Terminal Signature	Length of N-Terminal Extension	Genera in Which Transcript/Peptide/Peptide with Corresponding Mass Has Been Identified
flp-1	x PNFLRF.NH_2 *PNFMRY.*NH_2	0-15 0-1	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Heterodera, Meloidogyne, Necator, Onchocerca, Parastrongyloides, Strongyloides, Wuchereria*
flp-2	2x EPIRF.NH_2	3	*Ancylostoma, Caenorhabditis, Necator, Ostertagia*
flp-3	x SPX_oGTMRF.NH_2	0-15	*Ascaris, Caenorhabditis, Heterodera, Meloidogyne*
flp-4	2x PX_iFIRF.NH_2	2-6	*Ancylostoma, Ascaris, Caenorhabditis, Heterodera*
flp-5	3x KFIRF.NH_2	2-7	*Ancylostoma, Caenorhabditis, Globodera, Heterodera, Meloidogyne, Necator, Pratylenchus*
flp-6	x KSAYMRF.NH_2	0	*Ancylostoma, Ascaris, Brugia, Caenorhabditis, Globodera, Heterodera, Meloidogyne, Necator, Ostertagia, Onchocerca, Strongyloides, Teladorsagia*
flp-7	x P$X_o X_i$RSX$X_o X_o$RF.NH_2	1	*Ancylostoma, Caenorhabditis, Globodera, Heterodera, Meloidogyne, Ostertagia, Strongyloides*
flp-8	x KNEFX_oRF.NH_2	0	*Ancylostoma, Ascaris, Caenorhabditis, Necator, Onchocerca, Xiphinema*
flp-9	2x KPSFVRF.NH_2	0	*Ancylostoma, Ascaris, Caenorhabditis, Necator, Ostertagia*
flp-10	YX_oRF.NH_2	4	*Ancylostoma, Caenorhabditis, Xiphinema*
flp-11	x RNXLVRF.NH_2 NGAPQPFVRF.NH_2	2-7 0	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Haemonchus, Heterodera, Meloidogyne, Necator, Ostertagia, Pratylenchus, Radopholus, Strongyloides, Teladorsagia, Wuchereria*

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Table 1. Continued

Gene Name	Amino Acid Sequence/ C-Terminal Signature	Length of N-Terminal Extension	Genera in Which Transcript/Peptide/Peptide with Corresponding Mass Has Been Identified
flp-12	x (R/K)NKFEFIRF.NH_2	1	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Heterodera, Meloidogyne, Necator, Onchocerca*
flp-13	x PLIRF.NH_2 $PX_oX_oRF.NH_2$	4-5 4-5	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Haemonchus, Heterodera, Meloidogyne, Necator, Onchocerca, Ostertagia, Pratylenchus, Pristionchus, Strongyloides*
flp-14	x KHEYLRF.NH_2 *KHEFVRF.NH_2*	0 0	*Ancylostoma, Ascaris, Brugia, Caenorhabditis, Globodera, Meloidogyne, Necator, Onchocerca, Parastrongyloides, Pratylenchus, Radopholus, Strongyloides, Teladorsagia, Trichinella*
flp-15	GPQGPLRF.NH_2 GP(S/T)GPLRF.NH_2	1-2	*Ancylostoma, Caenorhabditis, Necator, Nippostrongylus, Ostertagia, Teladorsagia*
flp-16	x AQTFVRF.NH_2 *GQTFVRF.NH_2*	0 0	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Haemonchus, Heterodera, Meloidogyne, Necator, Onchocerca, Ostertagia, Parastrongyloides, Pratylenchus, Radopholus*
flp-17	KSAFVRF.NH_2 KSQYIRF.NH_2	0 0	*Ancylostoma, Caenorhabditis, Haemonchus, Necator, Ostertagia, Strongyloides, Xiphinema*
flp-18	x PGVLRF.NH_2 *$PGX_oX_oRF.NH_2$*	2-9 2-9	*Ancylostoma, Ascaris, Caenorhabditis, Globodera, Haemonchus, Meloidogyne, Necator, Onchocerca, Ostertagia, Pristionchus, Strongyloides, Trichinella*
flp-19	x $WXX_iQX_oRF.NH_2$	0-3	*Caenorhabditis, Dirofilaria, Heterodera, Meloidogyne, Necator, Pratylenchus, Strongyloides, Teladorsagia*
flp-20	$X_oX_oMRF.NH_2$	0	*Ancylostoma, Ascaris, Caenorhabditis, Haemonchus, Ostertagia, Parastrongyloides, Pratylenchus*

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Table 1. Continued

Gene Name	Amino Acid Sequence/ C-Terminal Signature	Length of N-Terminal Extension	Genera in Which Transcript/Peptide/Peptide with Corresponding Mass Has Been Identified
flp-21	GPRPLRF.NH_2	2-3	*Ancylostoma, Ascaris, Brugia, Caenorhabditis, Haemonchus, Meloidogyne, Necator, Ostertagia, Pratylenchus, Pristionchus, Radopholus, Strongyloides, Teladorsagia*
flp-22	x KWMRF.NH_2	4-7	*Ancylostoma, Caenorhabditis, Globodera, Heterodera, Ostertagia, Parastrongyloides, Pratylenchus, Pristionchus, Radopholus, Strongyloides, Teladorsagia*
flp-23	QDFLRF.NH_2	4	*Caenorhabditis, Teladorsagia*
flp-24	VPSA(G/A)DMMX_oRF.NH_2	0	*Ancylostoma, Ascaris, Brugia, Caenorhabditis, Necator, Ostertagia, Onchocerca*
flp-25	2x YD(F/Y)(V/I)RF.NH_2	1-8	*Caenorhabditis, Globodera, Meloidogyne, Necator, Strongyloides*
flp-26	EFNADDLTLRFG.NH_2 AFX_iPX_iXLX_iLRF.NH_2	0 4-7	*Ancylostoma, Caenorhabditis, Necator*
flp-27	G(G/S)RMRF.NH_2	2	*Ancylostoma, Caenorhabditis, Heterodera, Meloidogyne, Necator, Radopholus*
flp-28	VLMRF.NH_2	0	*Caenorhabditis, Pristionchus*
flp-29	IX_oMRF.NH_2	0	*Ancylostoma, Ascaris, Haemonchus, Ostertagia*
flp-30	QMREPLRF.NH_2	0	*Meloidogyne*
flp-31	LYRPRGPPRF.NH_2	0	*Meloidogyne, Pratylenchus*
flp-32	AMRNSLVRF.NH_2	0	*Caenorhabditis, Teladorsagia*

*Single letter amino acid annotations are used throughout. Amino acid signature sequences: X_i = hydrophilic residue; X_o = hydrophobic residue; X = variable residue; amino acids in parentheses represent alternatives. Signatures shown are the most commonly encoded peptide(s) on the associated transcripts. Sequences in italics appear on some transcripts. Note that, where possible (i.e., when ESTs from multiple species were available) single amino acid variations in one EST were ignored as the chances of these representing single nucleotide errors in the EST sequences appeared high. Note that the delineation between the *flp-11* and *flp-32* transcripts is unclear from the available EST data for most species; a number of the transcripts tentatively identified as *flp-11s* could actually be *flp-32*. A *flp-33* encoded peptide (APLEGFEDMSGFLRTIDGIQKPRF.NH_2) has been identified in *C. elegans* (Husson et al 2007)[50] but it's occurrence in other nematodes is unknown.

Nematode FLP Distribution/Expression

Much evidence relating to the expression of FLPs in nematodes has accumulated from immunocytochemical studies using polyclonal antisera that cannot distinguish between the multiple similar epitopes the FLP family presents. Although most of these studies do not inform on the distribution of individual FLPs in nematodes, they have provided an overview of FLP distribution in a variety of species.[55-61] The take-home message from these efforts has been that FLPs are widely expressed in nematode nervous systems (reports range from 50 to 75% of neurons) and that they occur in all neuronal subtypes (inter-neurons, motor neurons, pharyngeal neurons and sensory neurons). FLP-immunopositive neurons are common in the brain (circumpharyngeal nerve ring) and associated ganglia as well as in the innervation of muscular organs such as the ovijector/vulva and pharynx.

Most is known about *flp* expression in *C. elegans* which has been monitored using upstream promoter regions of *flp* genes fused to green fluorescent protein (GFP) coding regions in transgenic animals following germ-line transformations.[51,52,62] The expression of *flp-1* to *flp-23* has been examined, although no expression was observed for *flp-9*, *flp-14*, *flp-16* or *flp-23*. While the vast majority of *flps* were expressed exclusively in neurons, some were also expressed in non-neuronal cells: *flp-2* and *flp-11* were expressed in head muscles; *flp-5* and *flp-15* were expressed in pharyngeal muscles; *flp-11* and *flp-15* were expressed in socket and/or sheath cells which form the amphidial channels; *flp-10* was expressed in vulval muscles; *flp-11* and *flp-2* were expressed in uterine cells. Multiple neuronal cells expressed more than one *flp* gene, but no two *flp* genes have identical expression patterns providing a mosaic of *flp* expression. The extent of expression varied considerably with between 2 and 44 neurons expressing individual *flps*. In total, the 19 *flp* genes for which robust expression was recorded (faint GFP expression was ignored) occurred in 53% of *C. elegans* neurons; since the expression of 4 *flp* genes was not determined and another 10 *flp* genes remain to be examined, this is likely to be an underestimate of overall *flp* expression in the worm. Expression onset for most *flps* is during embryogenesis with the earliest expression, during gastrulation, being noted for *flp-15*; most *flps* display expression onset at the comma stage, a mid-stage in gastrulation where the embryo appears slightly folded within the egg. It is unclear why some *flps* display such early expression, but they may play roles in aspects of neuronal development. In some neurons, selected *flp* genes are coexpressed with classical neurotransmitters. However, no consistent relationship between the expression of any individual *flp* and any classical transmitter was reported.

A gene expression fingerprint of *C. elegans* embryonic motor neurons revealed the expression of multiple *flp* genes (*flp-2, 4, 5, 8, 9, 12, 13, 14, 15, 16, 18* and *19*) using GFP fused to the UNC-4 transcription factor that encodes a paired-class homeodomain protein that is expressed in thirteen embryonic motor neurons.[63] Subsequent work used microarray-based methods to monitor gene expression in *C. elegans* neurons.[64] Findings revealed that 20 of the 23 *flp* genes examined displayed enriched expression in a larval pan-neural dataset; those not showing enrichment included *flp-14*, *flp-20* and *flp-23*. Furthermore, a subset of five *flp* genes (*flp-2, 4, 5, 12* and *13*) were enriched in the A-class cholinergic neuron subset, with *flp-13* being the most-highly enriched. These efforts offer a powerful approach to understanding the relationships between gene expression and neural function in *C. elegans* and have clearly emphasized the association between *flps* and motor function in nematodes.

In situ hybridization (ISH) has been employed to examine the expression of 5 *flp* genes in the larval (J2 stage) potato cyst nematode (PCN).[65] As with the pattern of expression observed in *C. elegans* using GFP reporter constructs, ISH indicated neuronal expression with variable patterns for each gene examined. Although the absence of a neuronal map for *G. pallida* and the inability to identify the neuronal axons using ISH made cell identification difficult, comparisons with the expression patterns for the homologous *flps* in *C. elegans* revealed positional differences in the cells expressing some genes. Although *Gp-flp-1* expression was confined to the retrovesicular ganglion (RVG; comprises cell bodies of interneurons and motorneurons situated just posterior to the ventral ganglion), *flp-1* expression was reported in the RVG as well as ventral cord interneurons,

amphidial neurons and a pharyngeal motorneuron. *Gp-flp-6* staining was identified in phasmid-like (chemosensory) cells as well as in the lumbar ganglion of PCN J2s; diffuse *Gp-flp-6* staining occurred around the circumpharyngeal nerve ring and pharynx (metacorpal bulb). In contrast, *flp-6* expression in *C. elegans* was not reported in phasmids or the lumbar ganglion but was confined to amphidial cells and a pharyngeal interneuron. Similarly, differences were apparent in the expression data for *flp-12* and *Gp-flp-12*. The former occurred in a variety of ring neurons, interneurons and motor neurons associated with the head whereas the latter was identified in the RVG and neurons associated with the preanal area and lumbar ganglion. *Gp-flp-14* expression was detected in head motorneurons and nerve ring interneurons but the expression of *flp-14* has not been determined for *C. elegans*. Clearly, a snapshot of *flp* gene expression in PCN J2s suggests that *flp* expression in this plant parasite differs from that seen in *C. elegans*. While the benefits of work on *C. elegans* are unquestionable, these data highlight the importance of performing research on target parasite species.

Another approach to unraveling FLP signaling networks in nematodes has involved the application of mass spectrometric methods to *A. suum*.[54,66,67] This approach has relied on mass spectrometric identification interfaced with chemical derivatization of individual FLPs from neuronal structures including the circumpharyngeal nerve ring (CNR), ventral ganglion, RVG, dorsal ganglion, lateral line ganglia (LLG), ventro-dorsal commissures and segments of the dorsal and ventral nerve cords. These efforts mapped the expression of ~40 neuropeptides and revealed that there were similarities and differences in the FLP peptide complements of each neural structure. FLPs were the most abundant neuropeptides identified and, not surprisingly, the CNR expressed the most diverse range of FLPs (peptides with signatures common to products from the following *C. elegans* genes: *flp-1, 3, 4, 6, 8, 9, 11, 12, 13, 14, 16, 18* and *21*). All but one (*flp-3*) of the peptides identified in the CNR was also identified in the ventral ganglion; in addition, the ventral ganglion also expressed ILMRFamide (*As-flp-29*). Not surprisingly, the RVG appeared to express a less diverse complement of FLPs than the CNR and ventral ganglion with no signals being detected for *flp-6, 13, 21* or *29*. The dorsal ganglion expressed peptides matching those expressed on *flp-3, 4, 6, 13, 14* and *18* whereas the LLG expressed peptides predicted to be encoded on genes homologous to *flp-4, 6, 8, 11, 12, 13, 14, 16, 18* and *21*. Although the peptide complement of the dorsal cord appeared less complex than that of the ventral cord, both expressed peptides corresponding to those encoded on *flp-1, 4, 8, 11, 12, 13, 14, 18* and *21*; a mass corresponding to a *flp-16* product was only detected in the ventral cord. The ventro-dorsal commissures yielded little in the way of strong peptide signals although peaks matching *flp-9* and *flp-21* products were detected; the authors voiced caution as this tissue is likely to contain nonneural hypodermal tissue. It is evident from this work that many *flp* genes are expressed across the main neural processes in *A. suum*. Immunocytochemical methods which employed a monoclonal antibody have been used to investigate the distribution of the *As-flp-8* gene products (AF1; KNEFIRFamide) in *A. suum*.[29,57] A small subset of neurons in the head (including pharyngeal neurons) and neurons in the dorsal and ventral nerve cords were immunopositive. It is noteworthy that *flp-8* was not reported to be expressed in pharyngeal or nerve cord neurons in *C. elegans*.

The available literature provides data on *flp* expression that were derived using different techniques from only a few nematode species and reveals a rather complex and incomplete picture which indicates both similarities and differences in inter-species *flp* expression patterns. For example, the RVG of *A. suum* expresses peptides corresponding to predicted products of *flp-1, 3, 4, 8, 9, 11, 12, 14, 16* and *18*. In contrast, using GFP-reporter data, *flp-1, 2, 7, 10, 11, 13* and *21* were detected in the *C. elegans* RVG; the only matched expression being for genes *flp-1* and *flp-11*. The small amount of ISH data from *G. pallida* indicated the expression of *Gp-flp-1* and *Gp-flp-12* in the RVG, corresponding to the *A. suum* data. However, *Gp-flp-14* was not detected in the *G. pallida* RVG but was reported in the *A. suum* RVG. This difference could be due to the distinct life stages being compared as larval *C. elegans* have 16 RVG neural cells whereas adults have 20 and the data for *A. suum* was derived from adults whereas that for *G. pallida* was derived from J2 larvae. Clearly, too little is known about the cellular complement and organization of these structures to make unequivocal statements regarding *flp* expression.

A recent study on the *afp-6* gene (= *As-flp-11*) employed mass spectrometry, ICC and ISH to determine the expression of *As-flp-11* in *A. suum*.[54] Both ICC (using affinity purified antisera to both AMRNALVRFamide and NGAPQPFVRFamide) and ISH (using an *As-flp-11* specific riboprobe) localized expression to a single RIS-like cell in the ventral ganglion; in *C. elegans* RIS is a GABAergic interneuron which is known to express glutamate, dopamine and serotonin receptors.[68-70] This contrasts markedly with the expression reported for *flp-11* in *C. elegans* which was widespread and did not include the RIS neuron. The accumulation of data on *flp* gene expression across different nematodes is providing an extremely complex picture where highly diverse and species specific peptide expression patterns are superimposed upon an anatomically simple and structurally rigid nervous system.

Nematode Insulin-Like Peptides (INSs)

INSs play key roles in development and metabolism across the metazoa (for more details, see Chapter 5). Forty genes encoding peptides which belong to this family (*daf-28* and all the known *ins* genes) have been reported in the literature (see Table 2).[37,38,50,71-74] In vertebrates, insulin is composed of two polypeptide chains (A and B chains) which are linked by two disulfide bonds; an additional disulfide bond occurs within the A chain. Proinsulin, formed when the signal peptide is removed from preproinsulin, comprises the A and B chains and an interconnecting C peptide which is removed by endopeptidases during maturation. In contrast to the highly complex situation in *C. elegans*, only 10 insulin-related peptides are known from humans. Also, unlike the structural similarity of the human insulin-related peptides, there is much variation in the organization of *C. elegans* INS peptides. The *C. elegans* peptides possess A and B chains, but most commonly lack the intervening C peptide (although it is present in INS-1 and INS-18). Variations in the arrangement of the disulfide bonds have enabled the delineation of three distinct classes of *C. elegans* INS peptides, the α, β and γ (see Fig. 1). At time of writing, virtually nothing is known about INS peptides in parasitic nematodes. However, even a cursory glance at the EST datasets for parasitic nematodes reveals a bountiful supply of INSs and much scope for work in this area. Although their involvement in developmental processes, aging regulation and the control of dauer formation in *C. elegans* does not immediately strengthen their candidature as targets for parasite control, there is an obvious need to know more about their role in parasites and the potential of their signaling pathways as drug targets.

Nematode INS Distribution/Expression

Data relating to *ins* gene expression have been examined for 15 *ins* genes (*ins-1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 18, 20, 21, 22, 23*) in *C. elegans* using upstream promoter regions fused to GFP coding regions;[73] currently there are no immunocytochemical or *in situ* hybridization data for *ins* genes or their products. Most *ins* genes examined were found to be expressed in a variety of neurons across numerous life stages (embryos, larvae and adult worms), although no expression was identified for *ins-20*. Excluding *ins-20*, expression was detected for all the *ins* genes examined in all larval stages (L1 to L4) and adults except *ins-2*, which was not detected in L4s and adults. All except *ins-9* were detected in the embryos. Although expression of most *ins* genes was particularly prevalent in sensory neurons (all were detected in some sensory neurons), especially the amphidial and labial neurons, thirteen *ins* genes (all except *ins-9*) were also expressed in other (nonsensory) neurons including dorsal and ventral nerve cord neurons, tail neurons and pharyngeal neurons. An elevated expression in *C. elegans* embryonic and larval stage motor neurons was also reported for some *ins* gene mRNAs using microarray profiling techniques.[63,64] For example, a larval pan-neural dataset revealed enhanced expression of *ins-1*, *3*, *17*, *18*, *21*, *22*, *23*, *24*, *26*, *30* and *daf-28*.[64] Also, six *ins* genes are expressed in nonneuronal tissues, including the hypodermis (*ins-4*), intestine (*ins-1*, *ins-18*), pharynx (*ins-2*), vulva (*ins-2*, *ins-5*, *ins-8*) and vulval muscle (*ins-1*).[73] Unfortunately, there are no data on the structure and expression of INSs in parasitic nematodes. However, it would be interesting to compare INS expression between different nematodes to see if the complexities and differences seen with the FLPs are also apparent in this neuropeptide family.

Table 2. **Caenorhabditis elegans *prepro-insulin like precursor sequences***

Gene Name	Prepro Insulin-Like Protein Amino Acid Sequences*
daf-28	MNCKLIAIFAVLVLSVSAHLGAQAAAANFKAEGPLSRAVRVPGVAVRACGRRLVPYVWSVCGDACEPQEGIDIATQCCTYQCTAEYIQTACCPRLLL
ins-1	MYWFRQVYRPSFFFGFLAILLLSSPTPSDASIRLCGSRLTTTLLAVCRNQLCTGLTAFKRSADQSYAPTTRDLFHIHHQQKRGGIATECCEKRCSFAYLKTFCCNQDDN
ins-2	MNAIIFCLLFTTVTATYEVFGKGIEHRNEHLIINQLDIIPVESTPTPNRASRVQKRLCGRRLILFMLATCGECDTDSSEDLSHICCIKQCDVQDIIRVCCPNSFRK
ins-3	MKLSVVLALFIIFQLGAASLMRNWMFDFEKELEHDYDDSEIGFHNIHSLMARSRRGDKVKICGTKVLKMVMVMCGGECSSTNENIATECCEKMCTME DITTKCCPSR
ins-4	MFSFFTYFLLSALLLSASCRQPSMDTSKADRILREIEMETELENQLSRARRVPAGEVRACGRRLLLFVWSTCGEPCTPQEDMDIATVCCTTQCTPSYIKQACCPEK
ins-5	MHSIVALMLIGTILPIAALHQKHQGFILSSSDSTGNQPMDAISRADRHTNYRSCALRLIPHVWSVCGDAANHKTESMSLKNVAPLIAAPITSKKSAAHLTNWIPE
ins-6	MNSVFTIIFVLCALQVAASFRQSFGPSMSEESASMQLLRELQHNMMESAHRPMPRARRVPAPGETRACGRKLISLVMAVCGDLCNPQEGKDIATECCG NQCSDDYIRSACCP
ins-7	MPPIILVFFLVLIPASQQYPFSLESLNDQIINEEVIEYMLENSIRSSRTRRVPDEKKIYRCGRRIHSYVFAVCGKACESNTEVNIASKCCREECTDDFIRKQCCP
ins-8	MSPIILIFFLVFIPFSQQHTSLEESLNDRIISEEVVEMLSEKEIRPSRVRRVPEQKNKLCGKQVLSYVMALCEKACDSNTKVDIATKCCRDACSDEFIRHQCCP
ins-9	MIVTLIVFLVIGLQMAHLSQVSGNNENGFLNPFDLSQWSEEILHRQYHHHHHHHHGNRARRTLETEKIYRCGRKLYTDVLSACNGPCEPGTEQDLSK LCCGNQCTFVEIRKACCADKL
ins-10	MSLHFSTIQKTILLISFLLLVTLAPRTSAAFPFQICVKKMEKMCRIINPEQCAQVNKITEIGALTDCCTGLCSWEEIRISCCSVL
ins-11	MSSYRQTLFILIILIVIILFVNEGQGAPHHDKRHTACVLKIFKALNVMCNHEGDADVLRRTASDCCRESCSLTEMLASCTLTSSEESTRDI
ins-12	MQSNITASLFIALLIFGVISAAPSHEKTHKKCSDKLYLAMKSLCSYRGYSEFLRNSATKCCQDNCEISEMMALCVVAPNFDDDLLH
ins-13	MKLLHIFIIFLLFQSCSNKMCQYSKKKYKICGVRALKHMKVYCTRGMTRDYGKLLVTCCSKGCNAIDIQRICL
ins-14	MLTHLKFLLLVSLFINFAVSSEDIKCDAKFISRITKLCIHGITEDKLVRLLTRCCTSHCSKAHLKMFCTLKPHEEEPHHEI
ins-15	MKLLPLIVVFALLAVISESYSGNDFQPRDNKHHSYRSCGESLSRRVAFLCNGGAIQTEILRALDCCSTGCTDKQIFSWCDFRKLTRKEKQNYSGLIFRNLNRTQIVSFLLCHVE
ins-16	MQSLPILACLLTLSVFAPEIHGRELKRCSVKLFDILSVICGTESDAEILQKVAVKCCQEQCGFEEMCQHANLKIDKI
ins-17	MFSTRGVLLLLSLMAAVAAFGLFSRPAPITRDTIRPPRAKHGSLKLCPPGGASFLDAFNLICPMRRRRRSVSENYNDGGGSLLGRTMNMCCETGCEFTDIFAICNPFG
ins-18	MVHRLFIVLIAIILVAKSTAISLQQADGRMKMCPPGGSTFTMAWSMSCSMRRRKRDVGRYFEKRALIAPSIRQLQTICCQVGCNVEDLLAYCAPI
ins-19	MIFYLTTYLVTMSPLFLILLLLVSTTYPYIIDSSESYEVLMLFGYKRTCGRRLMNRINRVCVKDIDPADIDPKIKLSEHCCIKGCTDGWIKKHICSEEVLNF GFFEN
ins-20	MDKPSYLSSKEAWKMLNELLKEPKHHHHHHHRHKGYCGVKAVKKLKQICPDLCSNVDDNLLMEMCSKNLTDDDILQRCCPE
ins-21	MKTYSFFVLFIVFIFFISSSKSHSKKHVRFLCATKAVKHIRKVCPDMCLTGEEVEVNEFCKMGYSDSQIKYICCPE

continued on next page

Table 2. Continued

Gene Name	Prepro Insulin-Like Protein Amino Acid Sequences*
ins-22	MHTTTILICFFIFLVQVSTMDAHTDKYVRTLCGKTAIRNIANLCPPKPEMKGICSTGEYPSITEYCSMGFSDSQIKFMCCDNQ
ins-23	MFVLLIILSIILAQVTDAHSELHVRRVCGTAIIKNIMRLCPGVPACENGEVPSPTEYCSMGYSDSQVKYLCCPTSQ
ins-24	MRSPTLFLLLLLVPLALCHVFSEPADLELKSYQALEKSLKEMGLIRANQGPQKACGRSMMMKVQKLCAGGCTIQNDDLTIKSCSTGYTDAGFISACC PSGFVF
ins-25	MLFKIIILFFLLLQLSEAKPEAQRRCGRYLIRFLGELCNGPCSGVSSVDIATIACATAVPIEDLKNMCCPNL
ins-26	MRALVAILCLMALCHAAMLDELEMQKEVQEFHHMNGMLQEFMNKGLIGNHHHGTKAGLTCGMNIIERVDKLCNGQCTRNYDALVIKSCHRGVS DMEFMVACCPTMKLFIH
ins-27	MKFFRLILLCALVLTTMAFLAPSTAAKRRCGRRLIPYVYSICGGPCENGDIIIEHCFSGTTPTIAEVQKACCPELSEDPTFSS
ins-28	MMRSFFVLLALLAIVTSTASPTCGRALLHRIQSVCGLCTIDAHHELIAIACSRGLGDKEIIEMCCPI
ins-29	MFCKFVFLIFLLISLSVATADFGAQRRCGRHLVNFLEGLCGGPCSEAPTVELASWACSSAVSIQDLEKLCCPSNLA
ins-30	MSSHALVLFLLLFLLPVALGHFLSKPAPDPRITFNRKLAETLKELQDMGLIQAPREPVVAAQGAKKTCGRSLLIKIQQLCHGICTVHADDLHETACMKGLTDSQLINSCCPPIPQTPFVF
ins-31	MKMPLILLLLVAAASAFVHHFDHSMFARPEKTCGGLLIRRVDRICPNLNYTYKIEWELMDNCCEVVCEDQWIKETFCRAPRFNFFGPSFKALERSCGPKLFTRVKTVCGEDINVDNKVKISDHCCTEGGCTDDW-IKENVCKQTRFNFFRQFLDSPQRSCGPQLFKRVNTLCNENINVENNVSVSKSCCESAAGCTDDWIKKNVCTQHKPFVFRPGFY
ins-32	MTSILLILLLVITVTGMFQELSDLQNLHRFLEGLQGSSSLAVKSRSRRELICGRRLSKTVTNLCVEMNPQKEEDIATKCCKNKGCSREYIKSIMCPDE
ins-33	MANTCLILLLLLVIFVTVGFSMPRIFRASENGVNSSDEVSEELSYSPEEAMDLVKQVIKVREQRRHRRHRHHGQKHCGTKIVRKLQMLCPKMCTISDDTLLTEMCSHSLFDDEIQLRCCPKEDE
ins-34	MLHHKTLIIALLLTLFISGIDSLPFRKHNNHRHLKNQKAQQLKEEATEAPTPAPTTTKAPSGSATTTTTVKTTAAPLAQVNPQCLRRLTLLARGVCRQPCQPSDKPKTSAQQLLQLACSARRPTNEIISYCCPEKSG
ins-35	MKQIFLVILAACLLAIILASPTGKHHKMDENAFGINNRHCQRALKVYSFAICGAICQNYEKILMEGCGSTVMLTMQRTKLICCPEPVDSDELFN
ins-36	MNIGKCSIIFLLFCVFGSILSRAIRKRHPEGKLVIRDCKRYLIMYSRTICKEKCEKFDERNDITFSINLQFIFTDLLVEGCHSNQTLSNERTRELCCPNAGSN
ins-37	MAAFLPIALSIAMLTVLTNANPIHPVPNAAFLPYRSCGSHLVHRAFEACSGKKDRSSDVDLWKMCCKDECTDLDIKESLCKYASQGYGVKFEEEAEEIDMVSFAAEGFKKSCGHDIVVKTVNVPTKSLKQCARTR-GWRRRPRRIPDKFNRVQNYACINCVKIK
ins-38	MNLFLLVCIAFAIITVTSFTPDEKSQRSHVFSYKKHCGRRIVSLVQACDRIDHDLSIDCCTQNCSSEFVKKIMCPSKL
ins-39	MNTFFFLAVLLVFCSAEQMTAKKFSTKTSSPIPELQEVFATVAADEFPFHKANTQPLAIYLNISTPQDCIHKIFRMTISFCSQVECQNMEAMQKICNTTTPTIKHVGELCCPEFFEQVKDDFVTLL

*Single letter amino acid annotations are used throughout. Underlined sequences are predicted peptide products (see Pierce et al 2001)[73].

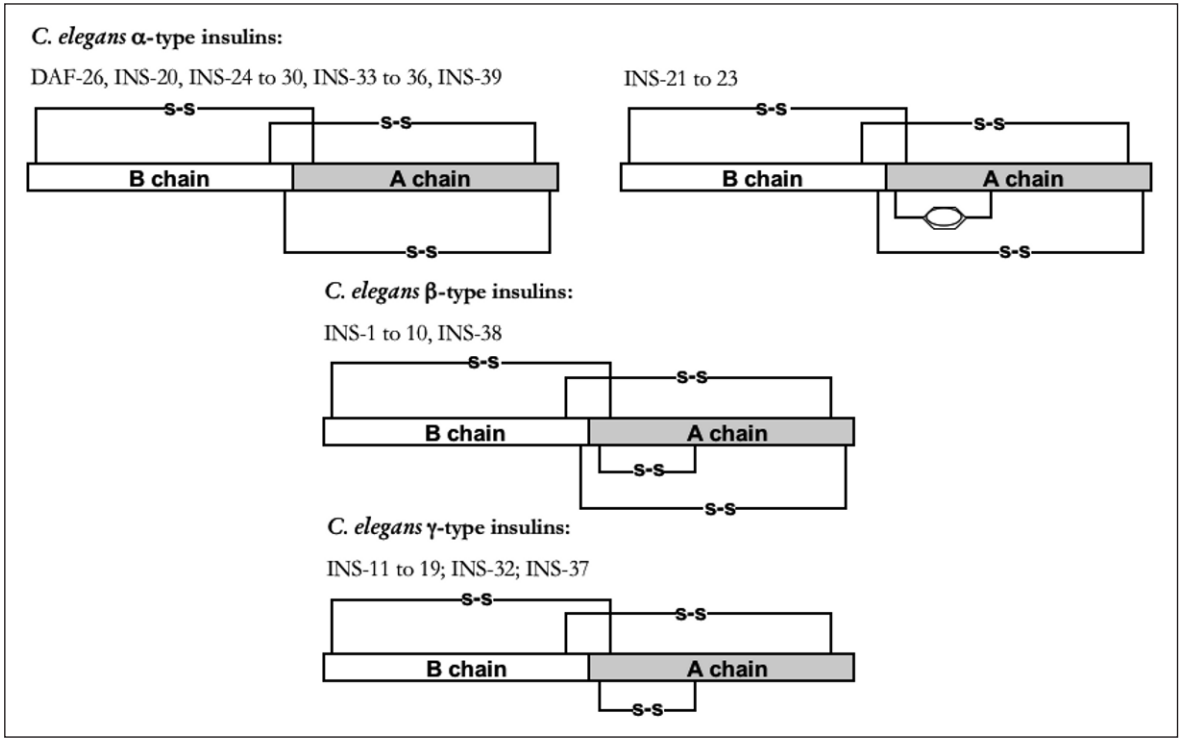

Figure 1. Schematic showing the predicted mature insulin-like peptide (INS) structures from *Caenorhabditis elegans*. The A-chain appears grey and the B-chain appears white. Three groups of INSs have been identified based on the predicted arrangement of the disulfide bonds. *C. elegans* γ-type INSs possess three disulfide bonds which is typical of vertebrate insulins. Both the α- and β-type INSs possess an additional inter-chain disulfide bond. In the α-type INSs the internal A-chain disulfide is either missing or has been replaced by aromatic amino acid interactions. Note that INS-31 has three repeats of each peptide chain and may constitute an additional class. Figure adapted from Duret et al[37] (1998), Pierce et al[73] (2001) and Husson et al[50] (2007).

Nematode Neuropeptide-Like Proteins (NLPs)

In contrast to the FLP and INS family groups, the NLP category has developed as a repository for all the neuropeptides in nematodes which do not qualify as INSs or FLPs. The consequence of this is that the NLPs comprise a wide range of peptide families that display quite disparate structures (and presumably functions). Regardless of this fact, the NLPs have been referred to as a peptide family when they actually represent an amalgamation of multiple peptide families who's only common feature is neuronal expression; note that some NLPs were designated as such and do not display neuronal expression (*nlp-23, 26, 29, 30, 31*) such that they may not be *bona fide* NLPs, unless neuronal expression is subsequently proven. Indeed, this has been further confirmed for peptides encoded by *nlp-27*, *29* and *31* as they were identified as infection-inducible anti-microbial peptides, although the modulation of expression by exposure to bacterial or fungal pathogens was only established for *nlp-29* and *31*; this role was proposed for *nlp-27* encoded peptides based on their structural similarities to *nlp-31* encoded peptides.[75]

The NLPs were originally described as comprising 32 genes that were reported to encode neuropeptides belonging to 11 distinct families.[41,42] These included novel neuropeptide families that were not previously identified in other species as well as peptides that displayed structural similarities with neuropeptide families from other invertebrate phyla. The former included: peptides with DRV C-terminal signatures encoded by *nlp-4*; GGxYamides encoded by *nlp-10*; LxDxamides encoded by *nlp-11*; LQFamides encoded by *nlp-12*; MRxamides encoded by *nlp-17*; histidine rich peptides encoded by *nlp-16*; peptides with a nonterminal GxRLPN motif encoded by *nlp-19*; and, a range of glycine-rich, FG containing peptides with variable sequences encoded on *nlp-26*. The latter included: those with a GFxGF motif, described as orcokinin-like peptides (orcokinin is a myotropic neuropeptide from the crayfish, *Orconectes limosus*) and encoded by *nlp-3, 8, 14* and *15*; those with a FRPamide signature, designated myomodulin-like and encoded by *nlp-2, 22* and *23*; those with MSFamide signatures, designated buccalin-like (buccalin is a neuropeptide from the mollusk *Aplysia californica* that modulates acetylcholine-induced myoexcitation) and encoded by *nlp-1, 7* and *13*; those with a MGL/Famide signature, assigned allatostatin-like and encoded by *nlp-5* and *6*; those with a YGGWamide signature were identified as similar to the APGWamide from *A. californica* and were encoded by *nlp-24, 25, 27, 28, 29, 30, 31* and *32*; those with the N-terminal signature GGARAFamide were identified in other nematode ESTs and in predicted products from *nlp-9* and *21*; and, C-terminal FAFA signatures, identified in other nematode species and encoded by *nlp-18* and *20*.

The original NLP identification was based largely on genome searches that relied on pattern finding approaches that exploited the nature of invertebrate neuropeptide genes to encode multiple copies of similar peptides. However, the authors of this approach stated that singly encoded neuropeptides that did not display homology with other identified neuropeptides would not be identified using these methods.[42] It seems likely therefore that other NLPs await discovery and some evidence for this evolved from two dimensional nanoscale liquid chromatography and tandem mass spectrometry approaches.[53] These efforts identified the occurrence of 21 peptides predicted from known *nlp* genes and peptide products from an additional seven (*nlp-35* to *nlp-41*) 'probable' *nlp* genes.[53] In addition, other putative *nlp* genes (*nlp-33*, *34* and *42*) have been annotated on WormBase (http://www.wormbase.org/db/gene/gene_class?name=nlp; class=Gene_class) (see Table 3). The genes *nlp-33* and *34* both encode peptides with YGGY sequences similar to those of *nlp-24*, *25* and *27* to *32*. The peptides identified by tandem mass spectrometry and encoded by *nlp-35*, *36*, *37*, *39*, *40* and *41* all appear to encode novel peptide families. The peptide products of *nlp-38* include three putative peptides with LWamide C-termini, including two GLWamides and one SLWamide. Interestingly, all three of these peptides encompass a signature sequence (WxxxxxxWamide) that is reminiscent of the arthropod B-type allatostatins (also designated myoinhibiting peptides).

Many of the NLPs reported in *C. elegans* have also been predicted in the closely related *C. briggsae*.[53] The free-living bacterivore, *Pristionchus pacificus* was reported to have ESTs encoding FAFA and YGGWamide peptides.[42] Examination of EST data for parasitic nematodes revealed

Table 3. **Caenorhabditis elegans *prepro-neuropeptide-like protein precursor sequences***

Gene Name	Prepro Neuropeptide-Like Protein Amino Acid Sequences*
nlp-1	MKATFVLACLLVIAAVSHADLLPKR**MDANAFRMSFG**KRSVSNPAEAKRMDPNAFRMSFGKRSAEQNEQANKEDKATSDKLYDDTKFEEMKR **MDANAFRMSFG**KRSDAHQAADDQVEYVNDDFSLPEQKRMDANAFRMSFGKR**VNLDPNSFRMSFG**KRSTVGYNLDARNYFVGLGRR
nlp-2	MRATLVLFALLCAVYSEAVPLQVYRPDESSAVDVVVLENSPELYDSEDEDEWKQEEEFTEGAMGKRSIALGRSGFRPGKRSMDNFHTVDVSDLIMKRSMAMGRLGLRPGKRSMAYGRQGFRPGKR-SMAYGRQGFRPGKRSMAYGRQGFRPGKRSNDMKEVFPQHVPEIYII
nlp-3	MSKIVACLVLLALSVMCVYSAPYEFRAKRAINPFLDSMGKRAVNPFLDSIGKRSFRPDMITEEKRYFDSLAGQSLGKRSNNRYEMLENYY
nlp-4	MPMDIFSEAQMFRYEDFMDRWTNEKGEFDFKAYVTTDAYMITPYVVIIHRCWESKEVPHRCDRKIVQPIPPLNVTVNSKTPDEKKVYNFGKVDL RFKNPAEVTSKMRSLILFVILLVAFAAARPVSEEVDRVKRDYDPRTEAPRRLPADDDEVDGEDRVKRDYDPRTDAPIRVPVDPEAEGEDRVKRDLSFSPNTEAPQPPPKHHPVPIESAN
nlp-5	MLMKIMVLMGIANIAASFVVSSVRFQSAPMRALIEINRELAKRSVSQLNQYAGFDTLGGMGLGKRSEPDQAGEKRALSTFDSLGGMGLGKRSSSSSRVFVYDKRALQHFSSLDTLGGMGFGRK
nlp-6	MLSFSRLAFVLLVSACVMAMA**APKQMVFGFG**KRSMADISDMPEDVPMKRYKPRSFAMGFGKRAAMRSFNMGFGKRSAEPQIDVDSF
nlp-7	MYIKAALLIVVLFGVASQITSA**LYLKQADFDDPRMFTSSFG**KRSAIESEPQAYPKSYRAIRIQRRSMDDLDDPRLMTMSFGKR**MILPSLADLHRYTMYD**KRGSDIDDPRYFLFSNRLTCRC
nlp-8	MSQKLLPISPLQLLFLQCLLIGFTAA**YPYLIFPASPSSGDSRRLV**KRAFDRFDNSGVFSFGAKRFDRYDDETAYGYGFDNHIFKRSADPYRFMSVPTKKAFDRMDNSDFFGAKRKR**SFDRMGGTEFGLM-**KRSAPESREQLINNLAESIITLRRAREAESSPESQRTIITYDD
nlp-9a	MDRFATRFIALLLVLLQIGSIFA**TPIAEAQGAPEDVDDRRELE**KRGGARAFYGFYNAGNSKRDQAAALPYYLYEKRGGGRAFNHNANLFRFDKRGGGRAFAGSWSPYLERFYDYKRSSYPVYFSDNSYY
nlp-9b	MDRFATRFIALLLVLLQIGSIFA**TPIAEAQGAPEDVDDRRELE**KRGGARAFYGFYNAGNSKRDQAAALPYYLYEKRGGGRAFNHNANLFRFDKRGGGRAFAGSWSPYLERDNSYY
nlp-10	MWYIALLLAVIATSVTAQKADDEPIVFLVRVPIDEMDDDSSLLESYYHPRDILSKRAIPFNGGMYGKRSTMPFSGGMYGKRSGQIFAQRRAAIPFSGGMYGKRSLVPQSYSNNENQIKRGAMPFSGGMYGR
nlp-11	MMSTLALVSLAIFGIAVVCAAPKPATVPVANEEDYLAALYGFEAPGSQFKGAPLQSKRHISPSYDVEIDAGNMRNLLDIGKRSAPMASDYGNQFQMY NRLIDAGKKKR**SPAISPAYQFENAFGLSEALERAG**RR
nlp-12	MLRHHSCALLMLILVFVEVFATQSPTFDRQDRDYRPLQFGKRDGYRPLQFGKRDYRPLQFGKRSSGSSGPVVLEPIWEWQ
nlp-13	MQRSLQIFCIMSAIAMAYSQGSRDDNQSAKRNDFSRDIMSFGKRSGNTADLYDRRIMAFGKRQPSYDRDIMSFGKR**SAPSDFSRDIMSFG**KR **SSSMYDRDIMSFG**KR**SPVDYDRPIMAFG-**KRAEDYERQIMAFGRRK
nlp-14	MLHLIVLLVALSSAVTAGRPRRALDGLDGSGFGFDKR**ALNSLDGAGFGFE**KRALNSLDGQGFGFEKRALDGLDGAGFGFDKR**ALNSLDGAGFGFE**KR ALDGLDGSGFGFDKR**ALNSLDGAGFGFE**KR**ALNSLDGAGFGFE**KRALDGLDGAGFGFDKR**ALNSLDGAGFGFE**KRALDGLDGAGFGFDKR ALNSLDGNGFGFDKRTFKHSSNKLRSVFRNLKGFKQH
nlp-15	MPSSSSSSSFFAAVLLVIVMMSTVESAAVRLRPVGSLFFLNRPHEKR**AFDSLAGSGFDNGFN**KRAFDSLAGSGFGAFNKRAFDSLAGSGFSGFDKRAFDSLAGQGFTGFEKR-AFDTVSTSGFDDFKL
nlp-16	MSFRFLILLLLASTLLVNSFPIGKDNESDESEVEVDTTTEAENIEVESQEASLDEQLNEVSEDPTREKRGLYSSERTEEEVEISHGMHHREKRHSEHLPHPDPPSHTAKRSTEHHRVKRSEGHPHEKK-ATHSPEGHIVAKDDHHGHEKRSDSHHGHQKRSVDEHHGHQKKNAEDHHEHQKRSEHVEHQAEMHEHQKRSTQEVSGHPEHHLVKRSGGEGGHRHHRSTDQGLDEDEPEDEIQTDENDEVTEEPGSRKRRNTDTPM-PSFPSDHDSEHSNSVAIRVKRVSRAGSSHKVRTLNKNRGNSKAGETTQNDSLTPNSGGVFNS

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Table 3. Continued

Gene Name	Prepro Neuropeptide-Like Protein Amino Acid Sequences*
nlp-17	MFSKSILFCLLVLVFNVFGANFENDQDVMRPPFQALKR**GSLSNMMRIG**KRQMSRQQEYVQFPNEGVVPCESCNLGTLMRIGRR
nlp-18	MNANVYSIVYFLSFLVLCISAQLHADSGATEVDGIVDKR**SPYRAFAFA**KR**SDEENLDFLE**KRARYGFAKR**SPYRTFAFA**KRASPYGFAFAKRGQFSSFA
nlp-19	MLLRGVCLALLILVTIVQCQNDNDLKEEKRRIGLRLPNFLRFKDPDALMIHKRRIGLRLPNMLKFKDSSNMYHLEKRRMGMRLPNIIFLRNEKKNVLEY
nlp-20	MQVTLLALLLLIVPFFAFAASQYSDDDSELMSNNERFARDLELRKKFAFAFAKRSAGDADVVIEARSGPQAHEGAGMRFAFAKRRAPKEFARFARASFA
nlp-21a	MRNSLFTTLFFGLAALVMVLNA**QYTSELEEDE**KRGGARAMLHKRGGARAFSADVGDDYKRGGARAFYDEKR**GGARAFLTEM**KR**GGARVFQGFEDE** KRGGARAFMMDKRGGGRAFGDMMKRGGARAFVENS-KRDEDWVIRPFEDDRLEKR**GGGRSFPVKPGRLDD**
nlp-21b	MRNSLFTTLFFGLAALVMVLNAQYTSELEEDEKRGGARAMLHKRGGARAFSADVGDDYKRGGARAFYDEKRGGARAFLTEMKRGGARVFQGFEDE KRGGARAFMMDKRGGGRAFGDMMKRGGARAFVENS-KRDEDWVIRPFEDDRLGVF
nlp-22	MRSIIVFIGLTIFALDILLVQTSALGLQGGIDVFRGLGVVDQVDFNQILHRANYLRNTREGRLRYWRLRTLPIMKKSIAIGRAGFRPGKRTTDELTGFPIGV
nlp-23 X	MAAHLVIWMALLGVSAHALELGFYSEHPTSKDDSYESQLLDSNDLDQDDAVDLATPMIVIPNDEDEIYADEEPEPLTMEKRLYISRQGFRPAKRSMAIGRAGMRPGKRAFAAGWNRG-KRSMPFAESYYPLYRNDFSE
nlp-24	MNKLLLLFVLVAVIAMASAQWGGGPYGGYGPRGYGGGYGGGRYGGYGGRGPYGGYGGRGPYGYGGRGPYGGGGLVGALLG
nlp-25	MQSLIALLLLLVCFLHLAQCQWGGGWNNGGGYGNPYGGYGRGGGYGGGYGGGFGAQQAYNVQNAARIGTEVAEGVLVAEEVSEAIGK
nlp-26 X	MNFFVIFSLLLVGLVSAQFGFGGQQSFGGRGGQFGGMQRGGFNGNRGGFGQQSQFGGRGGNQFGGRGGSQFNGRGGNQFGGRGGFGFGKK
nlp-27 x	MISTSSLLILVVLLACFMAASAQWGYGGMPYGGYGGMGGYGMGGYGMGYRRRMWGSPYGGYGGYGGYGGWGK
nlp-28	MISTSSILILVFLLACFMATSAQWGYGGYGRGYGGYGGYGRGMYGGYGRGMYGGYGRGMYGGWGK
nlp-29 X	MISTSSILVLVVLLACFMAASAQWGYGGYGRGYGGYGGYGRGMYGGYGRGMYGGYGRGMYGGYGRGMYGGWGK
nlp-30 X	MISTSSILILVVLLACFMAASAQWGYGGYGRGYGGYGGYGRGYGGYGRGYGGYGRGMWGRPYGGYGWGK
nlp-31 X	MISTSSILVLVVLLACFMAANAQWGYGGYGRGYGGYGGYGRGYGGYGGYGRGYGGYGRGMYGGYGRPYGGYGWGK
nlp-32	MRQFNLLLVFCLIALTALPVFSFPNGLTMDSIDMEPMGAFDENGAADESPRVKRYGGWGGRGGWGRGGGRGYGGRGGGWGGRGGGWGRGGGG RGFYGGGRRGWGK
nlp-33	MISTSLLLVVLLFAILAIVDAQWGYGGPYGGYGGGYGGGPWGYGGGWRRRHWGGYGGGPWGGYGGGPWGGYYGK
nlp-34	MISAKLFFIVLLLIACLLAVAEAQWGYGYRPYGYGGYGGWRRPYYRPWGYRPYGYGWGK
nlp-35	MPRVSSFIVFLTFMVALLAVSHA**AVVSGYDNIYQVLAPRF**RRARMMSFDAEAPQYLQHLLQNLKPRFRRSV

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Table 3. Continued

Gene Name	Prepro Neuropeptide-Like Protein Amino acid Sequences*
nlp-36	MSVDLKQQLELADYLGALAVWCIFFGVLFILSVIFNFVCIKKDDDVTALERWGYKKNIDMKLGPHRR**SMVARQIPQTVVADH**
nlp-37	MSSRISVSLLLLAVVATMFFTANVVDATPRSQGNMMRYGNSLPAYAPHVLYRFYNSRQFAPINKR**NNAEVVNHILKNFGALDRLGDVG**K
nlp-38	MQLIHFIVGLAMLISLSLAASDDRVLGWNKAHGLWGKRSVQEASQDKR**TPQNWNKLNSLWG**KRSASSFDDDYTTENGDDDVTMLYKR**SPAQWQ RANGLWG**R
nlp-39	MKLLILFSLFAIFFGVIALDSPIPEFYSTGRSTRVPSHRHRIPRLGKR**EVPNFQADNVPEAGGRV**RRYVGPPLKLVILI
nlp-40	MKLVILLSFVATVAVFA**APSAPAGLEEKL**RALQEQLYSLEKENGVDVKQKEQPAAADTFLGFVPQKRMVAWQPMKRSMINEDSRAPLLHAIEARLAEVLRAGERLGVNPEEVLADLRARNQFQ
nlp-41	MLGLVCKILVVLCLSVLCLTVSA**APGLFELPSRSV**RLIRSDPSAYDGYENSFYRGYGSDNQQFRFNSPQNW
nlp-42	MRVQVVTLLAVLLAVLQFTSAAGNYYSGYPSDRTMKRSALLQPENNPEWNQLGWAWGKRSAGMEIPHRAARALHPVKKNPDWQDLGFAWGRK

*Single letter amino acid annotations are used throughout. Underlined sequences are predicted peptide products (see Nathoo et al 2001)[42]; peptides in boldface were identified by mass spectrometry (Husson et al 2005)[53]; a or b after the gene name designate splice variants; the amino acid blocked in grey (*nlp-40*) indicates that peptides with and without the terminal R residues were identified; *X* indicates nonneuronal expression and therefore some doubt that these are neuropeptides; *x* indicates that although neuronal expression has been reported, this peptide has been predicted to be an anti-microbial peptide (Couillault et al 2004); C-terminal G residues represent known or putative amidation sites. Note that homologs of many of these NLPs were identified in *C. briggsae* (Husson et al 2005)[53] and ESTs encoding related peptides were identified in a range of parasitic nematodes (see Nathoo et al 2001)[42].

that peptides structurally similar to many of the NLPs were also present across the nematode clades:[42] *Ancylostoma caninum* ESTs encoded peptides from the FAFA, GFGX, GGxYamide, MSFamide, FRPamide, GGARAF, MGL/Famide and LxDxamide NLP families; *Brugia malayi* ESTs included FRPamide and YGGWamide peptides; *Globodera pallida/rostochiensis* ESTs encoded FAFA, GFGX and GGARAF peptides; *H. contortus* ESTs encoded MSFamide and MRXamide peptides; *Heterodera glycines* ESTs encoded FAFA, GFGFX, GGXYamide and MSFamide peptides; *Meloidogyne incognita/javanica* ESTs encoded GFGFX, GGXYamide, MSFamide, GGARAF, LQFamide and YGGWamides; *Onchocerca volvulus* and *Ostertagia ostertagia* ESTs encoded GFGFX peptides; *Strongyloides stercoralis* ESTs encoded FAFA, GFGFX and GGARAF peptides; *Toxocara canis* ESTs encoded FRPamide peptides.

The *afp-5* gene in *A. suum* encodes seven different peptides with C-terminal (D/S)R(D/N)F(M/L)(N/H/S)Famide signatures, but it is unclear if these are best annotated as FLPs or NLPs. Products from two other *A. suum nlps* have been identified by mass spectrometric methods.[67] These also include peptides identical to and structurally related to *C. elegans* NLP-12s (YRPLQFamides). *As-nlp-12* and *Trichostrongylus colubriformis* (*Tc-nlp-12*) transcripts were characterized and ESTs encoding NLP-12 peptides were identified from *Meloidogyne* spp., *Necator americanus*, *O. ostertagia* and *Wuchereria bancrofti*.[76] An additional and novel NLP signature (RWNamide) was predicted on two peptides, NRRRNAAARWNamide and NRRRNATARWNamide from an *A. suum* EST and a peptide corresponding to the former sequence was identified by mass spectrometry.[67] From the small amount of data available on NLPs from parasitic nematodes it is clear that they are highly divergent peptides that occur across Phylum Nematoda and, due to their dissimilarity to vertebrate peptides their signaling systems could make appealing drug targets. However, much work is needed to unravel the roles of these peptide signaling molecules.

Nematode NLP Distribution/Expression

As with *flp* and *ins* expression, the expression of *nlp-1* to *32* in *C. elegans* has been investigated using promoter sequences from *nlps* to drive GFP expression in transgenic animals.[42] Expression was not identified for *nlp-4*, *17*, *22*, *25*, *28* or *32* and with a small number of exceptions (*nlp-23, 26, 29, 30* and 31), most of the other *nlps* displayed neuronal expression. Those not expressed in neurons could have been wrongly designated as *nlps*. It is noteworthy that all of those not expressed in nerves are expressed to varying degrees in hypodermal tissue and two of these (*nlp-29* and *31*) are the aforementioned anti-microbial peptides. The other *nlps* were expressed in a wide variety of neurons including those of the head and tail, sensory neurons, circumpharyngeal nerve ring and associated ganglia, RVG, nerve cords, vulva and pharynx.[42] Just like the situation with *flps*, *nlps* appear to have distinct but overlapping distribution that presents a complex mosaic of expression across the nervous system of *C. elegans*. Remarkably, 9 different *nlp* genes are expressed in the ASI (amphidial) neurons (*nlp-1, 5, 6, 7, 9, 14, 18, 24* and *27*; note that *flp-10* and *21* are also expressed in these cells) revealing that individual neurons can possess a highly complex array of neuropeptide signaling molecules. The application of a microarray profiling technique to monitor elevated expression of mRNAs in embryonic motorneurons identified multiple *nlp*s, including *nlp-3*, *5*, *7*, *9*, *10*, *11*, *15*, *17*, *18*, *21*, *28*, *29*, *30* and *31*.[63] A pan-neural dataset from larval *C. elegans* revealed enhanced expression of *nlp-1*, *2*, *3*, *5*, *6*, *7*, *8*, *9*, *10*, *11*, *12*, *13*, *14*, *15*, *17*, *18*, *20* and *21*.[64] As well as neuronal expression, multiple *nlps* are additionally expressed in the intestine (*nlp-1, 2, 3, 6, 8, 9, 13, 14, 15, 16, 18, 20, 21, 27* and *29*), spermatheca (*nlp-5, 13, 18, 19, 20, 24* and *27*), embryo (*nlp-9, 11, 21* and *31*), rectal gland (*nlp-18*) and vulval secretory cells (*nlp-2*).[42]

Reverse transcriptase (rt-)PCR indicated that *nlp-12* was expressed throughout the larval and adult stages of *C. elegans*; transcription was also identified in the L3 and adult stages of *T. colubriformis*. Curiously, ISH identified *Tc-nlp-12* expression in a single tail neuron, matching that reported for *C. elegans*.[76] However, rt-PCR indicated the expression of *As-nlp-12* in both head and tail tissue,[76] matching mass spectrometric data which identified masses identical to

NLP-12 predicted peptides in the nerve ring, RVG, ventral and dorsal nerve cords.[67] Furthermore, peptides matching the mass of the RWNamides were detected by mass spectrometry in the nerve ring, ventral ganglion and RVG of *A. suum*.[67] Therefore, it seems that the pattern of neuropeptide distribution/expression in nematodes differs across species and may reflect significant plasticity in the nervous systems of nematodes which could facilitate the various behaviors and life styles they adopt in spite of a rather structurally-simple nervous system. Clearly, the sophistication and huge success (in terms of biomass) displayed by nematodes may be, in part, due to their highly complex networks of peptide signaling molecules that appear to facilitate much diversity and, ultimately, subtlety in intercellular communication.

Platyhelminth Neuropeptides

Unlike the situation in nematodes, flatworms have nervous systems that commonly comprise several thousands of neurons and there appears to be no rigorous conformity in structure across the classes. Having said that, a bilobed anterior brain that supplies longitudinal cords which are connected by commissures to create a ladder like (=orthogonal) arrangement provides the most common structural theme to their central nervous system (reviewed by ref. 77). Disadvantaged by the unavailability of complete and readily accessible genomic information, our knowledge of flatworm neuropeptides is sparse, even though the first report of neuropeptide immunoreactivity in the nervous system of flatworms was in 1981 with a report on vertebrate neurohormone immunoreactivities in the free-living turbellarian, *Dugesia lugubris*.[78] Many subsequent studies have employed immunocytochemistry to map the distribution of immunoreactivities to a vast range of vertebrate and invertebrate neuropeptides in all four classes of flatworms (reviewed by refs. 45, 47, 49, 77, 79-81). This section will focus only on those peptides for which peptide or nucleotide sequence data are available. Taking this approach, two distinct neuropeptide families dominate current understanding of flatworm neuropeptides, the FLPs and NPF.

Platyhelminth Neuropeptide F (NPF)

In the late 1980s/early 1990s, indirect immunocytochemistry and confocal microscopy combined to record the widespread occurrence in flatworm parasites of immunostaining with antisera that were raised against the C-termini of vertebrate neuropeptide Y (NPY) family peptides.[10-13,82,83] Most commonly, these antisera had been raised against pancreatic polypeptide (PP) or peptide YY (PYY); less commonly, immunoreactivity was reported to NPY, a third member of this vertebrate peptide family. Within vertebrates, these three peptides were usually 36 amino acids long and had several structural features in common: a polyprolyl N-terminus; tyrosyl residues situated 10 and 17 amino acids from the C-terminus; and, an RXRYamide C-terminal signature. Note that relatively recent gene duplication events that have led to the rapid evolution of the duplicate genes have generated additional members of the NPY family that have quite distinct structural features and biological functions—these will not be considered further here.[84-86]

In the early 1990s, NPF from the cestode *Moniezia expansa* (mxNPF) became the first flatworm neuropeptide to be structurally characterized following acid ethanol extraction of whole worms and a series of chromatographic purification steps interfaced with radioimmunometric monitoring of the immunoreactive fraction.[30] This peptide comprised 39 amino acid residues and possessed a distinctive RPRFamide C-terminal signature and came to define a new class of peptides, designated NPF. Although this peptide was a little longer than any previously reported NPY superfamily member, it did display key assets that led the discoverers to designate this peptide as an invertebrate NPY. These features included the C-terminal arginyl residues and tyrosyl residues situated 10 and 17 amino acids from the C-terminus; these are invariant amongst vertebrate NPY superfamily peptides (Table 4). Further efforts structurally characterized a 36 amino acid NPF from the land planarian *Artioposthia triangulata* (subsequently redesignated *Arthurdendyus triangulatus*).[33] This peptide had an identical GRPRFamide C-terminus and the signature tyrosyl residues in identical relative positions.

One feature which was characteristic of vertebrate NPY superfamily peptides and yet absent from these worm NPFs was a polyprolyl N-terminus, leaving their relationship to the vertebrate peptides unclear. However, characterization of *Mx-npf*, the first known flatworm neuropeptide encoding gene, revealed another characteristic that indicated a relationship to NPY superfamily peptides. This was a Phase-2 intron within the penultimate R residue, a feature common to both *npy* superfamily genes in vertebrates and *Mx-npf*.[87] However, this trait was not observed in the *At-npf* gene or in the more recently characterized *S. mansoni* and *S. japonicum npf* genes.[88,89] Alignment of the prepropeptides for all known platyhelminth NPFs and a selection of other invertebrate NPFs and human NPY reveals the diversity in peptide sequence around the conserved C-terminal and fixed tyrosyl residues (see Table 4). Further evidence to support the relationship between helminth NPF and vertebrate NPYs was derived from examination of the solution structure of Mx-NPF using nuclear magnetic resonance. This work revealed a random structure for the N- (Pro^{1} to Asn^{16}) and C- (Gly^{35} to Phe^{39}) termini either side of an alpha helix with a structure described as similar to that of porcine NPY.[90]

Platyhelminth NPF Distribution/Expression

Information on the distribution/expression of NPF in flatworms is restricted to immunocytochemical data generated using C-terminal or whole-molecule directed antisera and fluorescence or confocal scanning laser microscopy. However, it is highly likely that previous staining patterns obtained using antisera to vertebrate NPY superfamily members (pancreatic polypeptide, peptide YY and/or neuropeptide Y) were in fact due to their cross-reactivity with NPF. Studies in the early-mid 1990s began to employ specific NPF antisera to localize expression to the nervous systems of *M. expansa* and other platyhelminth parasites.[91-97] General observations included the fact that immunostaining was confined to neuronal elements and that expression was widespread in the nervous system with both central and peripheral nerves being immunopositive. The widespread distribution of NPF-immunoreactivity was evident in cestodes, monogeneans and trematodes with much staining localizing not only to the cerebral ganglia and associated nerve cords but also to innervation of muscular organs such as suckers and/or holdfasts, egg chambers (ootypes), uteri and pharynges. Comparative studies on the distribution of NPF, serotonin and acetylcholine expression indicated that peptidergic signaling systems more closely following those of the cholinergic systems and were distinct and clearly distinguishable from serotoninergic nerve pathways (for example, see reference 98). Further, immunogold labeling of immunogenic peptides for electron microscopic observation localized staining in dense-cored secretory vesicles in a number of different flatworm parasites and confirmed that these were associated with secretory pathways.[92,93,95,97,99,100] The broad range of immunocytochemical studies on NPF in flatworms confirms the abundance of this peptide in flatworm neuronal tissues, a situation similar to that seen for NPY in the vertebrate brain.

Platyhelminth FMRFamide-Like Peptides (FLPs)

FMRFamide-like peptides (FLPs) in flatworms conform to the most common FLP signature of a C-terminal tetrapeptide comprising an aromatic residue, a hydrophobic residue and an RFamide. The only published data on FLP sequences from flatworms have been derived from biochemical studies that employed ethanolic extraction and chromatographic purification procedures similar to those used for *M. expansa* NPF, except that FLP antisera were employed in radioimmunometric-based peptide monitoring. Again, the first success was seen with the large and widely available tapeworm, *M. expansa* with the structural characterization of the hexapeptide GNFFRFamide.[31] At this time, this peptide remains the only FLP that has been structurally characterized from a parasitic platyhelminth.

Three other FLPs have been structurally characterized from free-living turbellarians including: GYIRFamide (from *Bdelloura candida*, an ecto-commensal of the horseshoe crab; *Girardia tigrina*, a fresh water planarian; *Procerodes littoralis*, a marine planarian); YIRFamide (from *B. candida*); RYIRFamide (from the land planarian, *A. triangulatus*) (see Table 5) (R.N. Johnston,

Table 4. Neuropeptide F (NPF) prepropeptide precursor sequences. Selected sequelogs are shown for comparison

Peptide	Prepropeptide Neuropeptide F Amino Acid Sequences*
At-NPF	MSYLSFLFVSVIYLAIYLINFSSVTVDA**KVVHLRPRSSFSSEDEYQIYLRNVSKYIQLYGRPRFG**KRDLDRGQFEQYEK
Me-NPF	MDLNKEVMDEFSAYVKR**PDQDSIVNPSDLVLDNKAALRDYLRQINEYFAIIGRPRFG**
Sj-NPF	MHCHGYRQFSSKWNVKLMNSMINILLILLIRCV LFQSNIILCNAIELSSNSNLNGPDEQQR**AQALAKLMTLFYTSDAFNKYMENLDAYYMLRGRPRFG**KRNSYHRENNYDGVMQNHLLNSLLRQQLFRKFPTDNLDNDGDENFVI
Sm-NPF	MYSYMYGQYSFNWNVKSMNSMSILLILLLKFILFQ SNSIFINADELNLNTNNNLSNIPNDQQR**AQALAKLMSLFYTSDAFNKYMENLDAYYMLRGRPRFG**KRNYNPIKDNNDLMKNDLINNYLRQKLLQKYLIDNYNNDGDENFLRN
Ac-NPF	MQRVILVVLLLSCMAVLSVRADNSE**MLAPPPRPEEFTSAQQLRQYLAALNEYYSIMGRPRFG**KRGDSFRKREFFRTNGERYPEDAAAWTEFQ
Ls-NPF	MHKLLLVSLLVLSLAVMEVLCTEA**MLTPPQRPEEFKNPNELRKYLKALNEYYAIVGRPRFG**KRNGARVSDMFRPSGDDFGDYSANWGDF
Dm-NPF	MCQTMRCILVACVALALLAAGCRVEA**SNSRPPRKNDVNTMADAYKFLQDLDTYYGDRARVRFG**KRGSLMEILRNHEMDNINLGKNANNGGEFVSIE
Hs-NPY	MLGNKRLGLSGLTLALSLLVCLGALAEA**YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRYG**KRSSPETLISDLLMRESTENVPRTRLEDPAMW

*Single letter annotation for amino acids used throughout. Amino acids that are identical throughout the family members shown are highlighted in bold and common residues are underlined. At, *Arthurdendyus triangulatus* (turbellarian flatworm); Me, *Moniezia expansa* (cestode flatworm); Sj, *Schistosoma japonicum* (trematode flatworm); Sm, *Schistosoma mansoni*; Ac, *Aplysia californica* (mollusk); Ls, *Lymnaea stagnalis* (mollusk); Dm, *Drosophila melanogaster* (arthropod); Hs, *Homo sapiens*; NPY, neuropeptide Y. Note that all, except Me-NPF have dibasic cleavage sites (blocked in grey) following the glycyl amide donor at the C-terminus of the peptide (*M. expansa* possesses a stop codon immediately following the G).

Table 5. FMRFamide-like peptide (FLP) sequences from platyhelminths and a mollusk

Species (platyhelminth unless indicated)	Amino Acid Sequence*
Arthurdendyus triangulatus	R**YIRF.NH$_2$**
Bdelloura candida	G**YIRF.NH$_2$**
	YIRF.NH$_2$
Girardia tigrina	G**YIRF.NH$_2$**
Moniezia expansa	GN**FFRF.NH$_2$**
Procerodes littoralis	G**YIRF.NH$_2$**
Macrocallista nimbosa (mollusk)	**FMRF.NH$_2$**

*Single letter annotation for amino acids used throughout. The FLP signature comprising: aromatic amino acid, hydrophobic amino acid, arginine and phenylalaninamide, is shown in boldface text.

Queen's University Belfast, unpublished observations).[32,34,35] In all cases, only one FLP (and possibly two FLPs in the case of *B. candida*) has been identified in acid ethanol extractions of flatworms, a situation that contrasts markedly with the FLP diversity seen in nematodes. Although no trematode or monogenean FLPs have been structurally characterized, the chromatographic fractionation of acid ethanol extracts have only reported a single immunoreactive peak, consistent with the occurrence of one FLP or a small number of FLPs in these species.[34,95] More recent genomic and EST analyses have identified a raft of novel neuropeptide encoding transcripts, including some encoding FLPs, in *S. mansoni* and other free-living flatworms (P. McVeigh and G.R. Mair, Queen's University Belfast and T.A. Day, Iowa State University, unpublished data). It seems likely that our very limited knowledge of platyhelminth neuropeptides will be radically expanded soon.

Platyhelminth FLP Distribution/Expression

Many immunocytochemical studies document the widespread occurrence of FLP immunoreactivity in platyhelminths and provide a broad picture that is similar to that seen for NPF (reviewed by refs. 45,47,49,77). The first immunocytochemical study reporting FLP immunoreactivity in the nervous system of a parasite demonstrated anti-FMRFamide antisera cross-reaction with central and peripheral nerve elements in the tapeworm, *Diphyllobothrium dendriticum*.[7] Subsequent studies revealed that this observation was common to the other classes of flatworm parasite and confirmed a widespread distribution for this peptide family.[9,13] The data generated on FLP distribution displayed the same key features as that for NPF immunoreactivity in that both central and peripheral nerve elements were immunopositive. As with NPF, FLP distribution patterns are similar to those of acetylcholine but distinct from those for serotonin (for example see refs. 101-107). Furthermore, ultrastructural studies have localized immunoreactivity to dense-cored secretory vesicles in nerves of both the central and peripheral nervous systems of parasitic flatworms (reviewed by refs. 45,47,49,77,79-81,108). Of particular note, is the widespread abundance of FLP-immunoreactivity in the innervation of muscular structures associated with the body wall, attachment organs, reproductive systems and feeding organs such as the pharynx. One particularly interesting observation made using immunocytochemistry was the link between the expression of FLPs in the reproductive system of the monogenean parasite *Polystoma nearcticum* and spawning of the grey treefrog host, *Hyla versicolor*.[109] This work revealed a role for FLPs in the reproductive synchrony between the parasite and its host. All these observations have pointed to a role for flatworm FLPs in muscle modulation, hypotheses which were subsequently confirmed experimentally (see Chapter 5 in this book).

Targets for Parasite Control

Currently, much more is known about parasite neuropeptides than either the receptors they act upon or the associated signaling pathways (see Chapter 5 in this book). Indeed, only a small number of neuropeptides have been receptor-matched in *C. elegans* (see Chapter 5 in this book) and no neuropeptide receptors have been functionally characterized from a parasitic worm. Regardless, the peptides themselves are useful as tools to initiate receptor discovery efforts and also to validate signaling pathways as targets. The application of gene silencing through RNA interference (RNAi) as a mechanism to validate potential targets in parasitic worms has been widely discussed.[110-113] However, complications in the utility of RNAi abound from the lack of genomic data and optimized RNAi protocols for the vast majority of parasites, although the *S. mansoni* and *B. malayi* genome projects and ongoing efforts to address RNAi optimization are beginning to counter these weaknesses.[114-116] Further, the application of RNAi to parasitic nematodes and, indeed, to neuronal targets in *C. elegans*, has had mixed and moderate successes, respectively (for example see refs. 117-122).

Even where RNAi has proven successful, within the confines of target validation studies it can only really inform on the potential utility of antagonistic drugs and does not speak to the potential of agonists that act on the same pathways. Nevertheless, even before receptors and signaling pathways are determined and characterized, the application of RNAi for each neuropeptide-encoding gene could offer a rational approach to the selection of neuropeptide signaling pathways for further study. For example, null phenotypes associated with the silencing of a neuropeptide gene would effectively rule out that pathway from a target discovery program. In contrast, a lethal or incapacitating phenotype associated with silencing a neuropeptide gene would be extremely appealing; at time of writing a lethal phenotype associated with neuropeptide silencing has not been reported. However, the silencing of five different *flp* genes in *G. pallida* each generated worms with profound aberrant phenotypes which appear incompatible with survival in the host and which indicate the importance of neuropeptide signaling pathways to parasite behavior.[123] So even though only five neuropeptide genes have been examined in this way, already several offer appeal as targets for the control of plant parasitic nematodes. Clearly, interrogating the entire peptide complement in this way could provide a large set of validated target systems. Although there appear to be numerous hurdles to the application of RNAi in some animal parasites,[121] the success seen in the application of RNAi to interrogate gene function in plant parasitic nematodes (reviewed by refs. 120,124) and the potential for the application of plant-based RNAi strategies for parasite control are very promising.[125-128] Time will tell if the translation of these successes to animal and human parasites is a real possibility.

Conclusion

This chapter has focused on neuropeptide signaling molecules that play a major role in worm neurobiology and beyond. Due to the success of anthelmintics that have acted to compromise normal motor function in helminth parasites, peptides with associated roles have much appeal as conduits to target systems for parasite control. Although the peptides themselves do not provide useful chemotherapeutic targets, the various enzymes that contribute to generation of the mature peptide products, the receptors (ion channels or G-protein coupled receptors) they interact with, the enzymes that break down the peptides after signal initiation and components of the signaling pathways they trigger, all provide potential targets for parasite control (see Fig. 2).

Unfortunately, at this time, our knowledge of these facets of peptide signaling networks in parasitic worms is at best rudimentary, although data on neuropeptide degradation is starting to accumulate for *C. elegans*,[44,129,130] and an amidating enzyme has been characterized from *S. mansoni*.[131] At least some of these will have potential in mechanism-based drug discovery programs. The recent successes in the application of RNAi-based control measures for plant parasitic

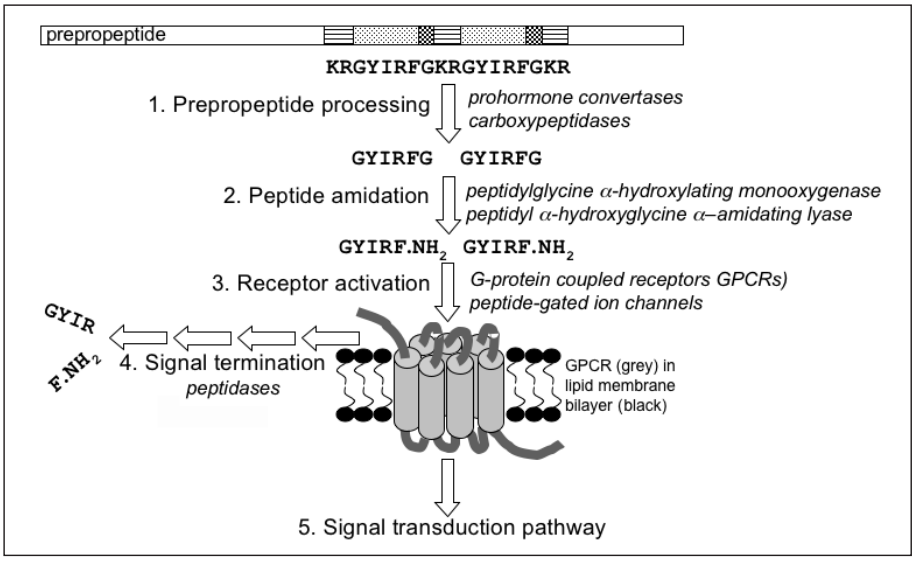

Figure 2. Within neuropeptide signaling systems in parasitic worms there are five obvious groups of targets that could be exploited for parasite control. Here, these are shown for the hypothetical GYIRFamide encoding gene. 1) The enzymes associated with preproprotein processing (prohormone convertases and carboxypeptidases) could provide targets that would compromise multiple neuropeptide signaling pathways by stopping the generation of mature peptide products. 2) A similar scenario would apply to the amidation process whereby peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidyl α-hydroxyglycine α-amidating lyase (PAL) act sequentially to generate C-terminal amide moieties from glycyl residues. Most neuropeptides display C-terminal amidation and this is commonly essential to receptor binding and/or activation. Again, compromising the amidation process would prevent the generation of amidated peptide products and thus would broadly disrupt peptide signaling processes. 3) Receptors often receive the most attention with respect to drug target exploitation, mainly because high throughput screens involving receptors are well established. Critically, data on neuropeptide receptors in *C. elegans* and some parasites are now beginning to accumulate and appear to offer real potential for receptor exploitation. 4) Very little is known about neuropeptide signal termination in parasitic worms, but compromising the signal termination processes would have obvious merits in the disruption of peptide signaling pathways. Again, if the peptidases involved are widely conserved across peptide signaling networks then there is the potential for broad-scale disruption of these signaling pathways. 5) Least is known about the signal transduction pathways associated with neuropeptide action, but often these encompass various enzymatic steps which provide multiple opportunities for disruption of the associated signaling processes. All of these target groups may also be amenable to gene silencing through RNA interference (RNAi) which could provide a valuable tool for the validation of these targets in parasites. If RNAi approaches become feasible for parasite control (and they show promise for the control of plant parasitic nematodes) then the genes encoding neuropeptides could be added to the above-mentioned list of target groups.

nematodes underscore the potential of this approach and could provide a real opportunity to exploit neuropeptides for parasite control.

Note Added in Proof

A recent bioinformatic trawl of the available flatworm EST and genomic datasets identified ~60 distinct neuropeptide precursors encompassing 96 neuropeptides from 10 species of

flatworm.[132] Although some of these peptides belong to peptide families previously recognised in flatworms (FLP- and NPF-like families) or other animal phyla (myomodulin-, buccalin- and neuropeptide FF (NPFF)-like peptide families) most are novel and, therefore, flatworm-specific. This is significant as ligand uniqueness underscores the potential for the cognate receptors to provide drug targets that are easily discriminated between parasite and host. For more details, readers are directed to the original manuscript available at http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T7F-4W1BV9T-1-D&_cdi=5057&_user=126523&_orig=search&_coverDate=09%2F30%2F2009&_sk=999609988&view=c&wchp=dGLbVlz-zSkWA&md5=53005d80cea0568efb49434fe9ae1c8c&ie=/sdarticle.pdf.

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References

1. White JG, Southgate E, Thompson JN et al. The structure of the nervous system of the nematode Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci 1986; 314(1165):1-340.
2. Davey KG. Neurosecretion and molting in some parasitic nematodes. Am Zool 1966; 6(2):243-9.
3. Rogers WP. Neurosecretory granules in the infective stage of Haemonchus contortus. Parasitology 1968; 58(3):657-62.
4. Leach L, Trudgill DL, Gahan PB. Immunocytochemical localization of neurosecretory amines and peptides in the free-living nematode, Goodeyus ulmi. Histochem J 1987; 19(9):471-5.
5. Atkinson HJ, Isaac IE, Harris PD et al. FMRFamide-like immunoreactivity within the nervous-system of the nematodes Panagrellus redivius, Caenorhabditis elegans and Heterodera glycines. J Zool 1988; 216:663-71.
6. Davenport TR, Lee DL, Isaac RE. Immunocytochemical demonstration of a neuropeptide in Ascaris suum (Nematoda) using an antiserum to FMRFamide. Parasitology 1988; 97(1):81-8.
7. Gustafsson MK, Wikgren MC, Karhi TJ et al. Immunocytochemical demonstration of neuropeptides and serotonin in the tapeworm Diphyllobothrium dendriticum. Cell Tissue Res 1985; 240(2):255-60.
8. Gustaffson MK, Lehtonen MA, Sundler F. Immunocytochemical evidence for the presence of "mammalian" neurohormonal peptides in neurones of the tapeworm Diphyllobothrium dendriticum. Cell Tissue Res 1986; 243(1):41-9.
9. Gustafsson MK. Immunocytochemical demonstration of neuropeptides and serotonin in the nervous systems of adult Schistosoma mansoni. Parasitol Res 1987; 74(2):168-74.
10. Fairweather I, MaCartney GA, Johnston CF et al. Immunocytochemical demonstration of 5-hydroxytryptamine (serotonin) and vertebrate neuropeptides in the nervous system of excysted cysticercoid larvae of the rat tapeworm, Hymenolepis diminuta (Cestoda, Cyclophyllidea). Parasitol Res 1988; 74(4):371-9.
11. Magee RM, Fairweather I, Johnston CF et al. Immunocytochemical demonstration of neuropeptides in the nervous system of the liver fluke, Fasciola hepatica (Trematoda, Digenea). Parasitology 1989; 98(2):227-38.
12. Maule AG, Shaw C, Halton DW et al. Localization, quantification and characterization of pancreatic polypeptide immunoreactivity in the parasitic flatworm Diclidophora merlangi and its fish host (Merlangius merlangus). Gen Comp Endocrinol 1989a; 74(1):50-6.
13. Maule AG, Halton DW, Johnston CF et al. Immunocytochemical demonstration of neuropeptides in the fish-gill parasite, Diclidophora merlangi (Monogenoidea). Int J Parasitol 1989b; 19(3):307-16.
14. Cowden C, Stretton AO, Davis RE. AF1, a sequenced bioactive neuropeptide isolated from the nematode Ascaris suum. Neuron 1989; 2(5):1465-73.
15. Geary TG, Price DA, Bowman JW et al. Two FMRFamide-like peptides from the free-living nematode Panagrellus redivivus. Peptides 1992; 13(2):209-14.
16. Cowden C, Stretton AO. AF2, an Ascaris neuropeptide: isolation, sequence and bioactivity. Peptides 1993; 14(3):423-30.
17. Cowden C, Stretton AO. Eight novel FMRFamide-like neuropeptides isolated from the nematode Ascaris suum. Peptides 1995; 16(3):491-500.
18. Rosoff ML, Doble KE, Price DA et al. The flp-1 propeptide is processed into multiple, highly similar FMRFamide-like peptides in Caenorhabditis elegans. Peptides 1993; 14(2):331-8.
19. Maule AG, Shaw C, Bowman JW et al. KSAYMRFamide: a novel FMRFamide-related heptapeptide from the free-living nematode, Panagrellus redivivus, which is myoactive in the parasitic nematode, Ascaris suum. Biochem Biophys Res Commun 1994a; 200(2):973-80.

20. Maule AG, Shaw C, Bowman JW et al. The FMRFamide-like neuropeptide AF2 (Ascaris suum) is present in the free-living nematode, Panagrellus redivivus (Nematoda, Rhabditida). Parasitology 1994b; 109(3):351-6.
21. Maule AG, Shaw C, Bowman JW et al. Isolation and preliminary biological characterization of KPNFIR-Famide, a novel FMRFamide-related peptide from the free-living nematode, Panagrellus redivivus. Peptides 1995; 16(1):87-93.
22. Keating CD, Holden-Dye L, Thorndyke MC et al. The FMRFamide-like neuropeptide AF2 is present in the parasitic nematode Haemonchus contortus. Parasitology 1995; 111(4):515-21.
23. Marks NJ, Shaw C, Maule AG et al. Isolation of AF2 (KHEYLRFamide) from Caenorhabditis elegans: evidence for the presence of more than one FMRFamide-related peptide-encoding gene. Biochem Biophys Res Commun 1995a; 217(3):845-51.
24. Marks NJ, Maule AG, Geary TG et al. APEASPFIRFamide, a novel FMRFamide-related decapeptide from Caenorhabditis elegans: structure and myoactivity. Biochem Biophys Res Commun 1997; 231(3):591-5.
25. Marks NJ, Maule AG, Geary TG et al. KSAYMRFamide (PF3/AF8) is present in the free-living nematode, Caenorhabditis elegans. Biochem Biophys Res Commun 1998; 248(2):422-5.
26. Marks NJ, Maule AG, Li C et al. Isolation, pharmacology and gene organization of KPSFVRFamide: a neuropeptide from Caenorhabditis elegans. Biochem Biophys Res Commun 1999a; 254(1):222-30.
27. Marks NJ, Sangster NC, Maule AG et al. Structural characterisation and pharmacology of KHEYLRFamide (AF2) and KSAYMRFamide (PF3/AF8) from Haemonchus contortus. Mol Biochem Parasitol 1999b; 100(2):185-94.
28. Marks NJ, Shaw C, Halton DW et al. Isolation and preliminary biological assessment of AADGAP-LIRFamide and SVPGVLRFamide from Caenorhabditis elegans. Biochem Biophys Res Commun 2001; 286(5):1170-6.
29. Davis RE, Stretton AO. The motornervous system of Ascaris: electrophysiology and anatomy of the neurons and their control by neuromodulators. Parasitology 1996; 113:S97-117.
30. Maule AG, Shaw C, Halton DW et al. Neuropeptide F: a novel parasitic flatworm regulatory peptide from Moniezia expansa (Cestoda: Cyclophyllidea). Parasitol 1991; 102:309-16.
31. Maule AG, Shaw C, Halton D et al. GNFFRFamide: a novel FMRFamide-immunoreactive peptide isolated from the sheep tapeworm, Moniezia expansa. Biochem Biophys Res Commun 1993a; 193(3):1054-60.
32. Maule AG, Shaw C, Halton DW et al. RYIRFamide: a turbellarian FMRFamide-related peptide (FaRP). Regul Pept 1994c; 50(1):37-43.
33. Curry WJ, Shaw C, Johnston CF et al. Neuropeptide F: primary structure from the tubellarian, Artioposthia triangulata. Comp Biochem Physiol C 1992; 101(2):269-74.
34. Johnston RN, Shaw C, Halton DW et al. GYIRFamide: a novel FMRFamide-related peptide (FaRP) from the triclad turbellarian, Dugesia tigrina. Biochem Biophys Res Commun 1995; 209(2):689-97.
35. Johnston RN, Shaw C, Halton DW et al. Isolation, localization and bioactivity of the FMRFamide-related neuropeptides GYIRFamide and YIRFamide from the marine turbellarian Bdelloura candida. J Neurochem 1996; 67(2):814-21.
36. Rosoff ML, Burglin TR, Li C. Alternatively spliced transcripts of the flp-1 gene encode distinct FMRF-amide-like peptides in Caenorhabditis elegans. J Neurosci 1992; 12(6):2356-61.
37. Duret L, Guex N, Peitsch MC et al. New insulin-like proteins with atypical disulfide bond pattern characterized in Caenorhabditis elegans by comparative sequence analysis and homology modeling. Genome Res 1998; 8(4):348-53.
38. Gregoire FM, Chomiki N, Kachinskas D et al. Cloning and developmental regulation of a novel member of the insulin-like gene family in Caenorhabditis elegans. Biochem Biophys Res Commun 1998; 249(2):385-90.
39. Nelson LS, Kim K, Memmott JE et al. FMRFamide-related gene family in the nematode, Caenorhabditis elegans. Mol Brain Res 1998; 58(1-2):103-11.
40. Li C, Kim K, Nelson LS. FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. Brain Res 1999a; 848:26-34.
41. Li C, Nelson LS, Kim K et al. Neuropeptide gene families in the nematode Caenorhabditis elegans. Ann N Y Acad Sci 1999b; 897:239-52.
42. Nathoo AN, Moeller RA, Westlund BA et al. Identification of neuropeptide-like protein gene families in Caenorhabditis elegans and other species. Proc Natl Acad Sci USA 2001; 98:14000-5.
43. McVeigh P, Leech S, Mair GR et al. Analysis of FMRFamide-like peptide (FLP) diversity in phylum Nematoda. Int J Parasitol 2005a; 35(10):1043-60.
44. McVeigh P, Geary TG, Marks NJ et al. The FLP-side of nematodes. Trends Parasitol 2006a; 22(8):385-96.
45. Maule AG, Mousley A, Marks NJ et al. Neuropeptide signaling systems—potential drug targets for parasite and pest control. Curr Top Med Chem 2002; 2(7):733-58.
46. Mousley A, Marks NJ, Maule AG. Neuropeptide signalling: a repository of targets for novel endectocides? Trends Parasitol 2004; 20(10):482-7.

47. Gustaffson MK, Halton DW, Kreshchenko ND et al. Neuropeptides in flatworms. Peptides 2002; 23(11):2053-61.
48. Li C. The ever-expanding neuropeptide gene families in the nematode Caenorhabditis elegans. Parasitol 2005; 131:S109-S27.
49. McVeigh P, Kimber MJ, Novozhilova et al. Neuropeptide signalling systems in flatworms. Parasitology 2005b; 131:S41-S55.
50. Husson SJ, Mertens I, Janssen T et al. Neuropeptidergic signaling in the nematode Caenorhabditis elegans. Prog Neurobiol 2007; 82(1):33-55.
51. Kim K, Li C. Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. J Comp Neurol 2004; 475:540-50.
52. Nelson LS, Rosoff ML, Li C. Disruption of a neuropeptide gene, flp-1, causes multiple behavioral defects in Caenorhabditis elegans. Science 1998; 281:1686-90.
53. Husson SJ, Clynen E, Baggerman G et al. Discovering neuropeptides in Caenorhabditis elegans by two dimensional liquid chromatography and mass spectrometry. Biochem Biophys Res Commun 2005; 335(1):76-86.
54. Yew JY, Davis R, Dikler S et al. Peptide products of the afp-6 gene of the nematode Ascaris suum have different biological actions. J Comp Neurol 2007; 502:872-82.
55. Sithigorngul P, Stretton AO, Cowden C. Neuropeptide diversity in Ascaris: an immunocytochemical study. J Comp Neurol 1990; 294(3):362-76.
56. Schinkmann K, Li C. Localization of FMRFamide-like peptides in Caenorhabditis elegans. J Comp Neurol 1992; 316(2):251-60.
57. Cowden C, Sithigorngul P, Brackley P et al. Localization and differential expression of FMRFamide-like immunoreactivity in the nematode Ascaris suum. J Comp Neurol 1993; 333(3):455-68.
58. Brownlee DJ, Fairweather I, Johnston CF. Immunocytochemical demonstration of neuropeptides in the peripheral nervous system of the roundworm Ascaris suum (Nematoda, Ascaroidea). Parasitol Res 1993; 79(4):302-8.
59. Brownlee DJ, Fairweather I, Johnston CF et al. Immunocytochemical demonstration of neuropeptides in the central nervous system of the roundworm, Ascaris suum (Nematoda: Ascaroidea). Parasitology 1993; 106(3):305-16.
60. Brownlee DJ, Brennan GP, Halton DW et al. Ultrastructural localization of pancreatic polypeptide- and FMRFamide immunoreactivities within the central nervous system of the nematode, Ascaris suum (Nematoda: Ascaroidea). Parasitology 1994; 108(5):587-93.
61. Brownlee DJ, Fairweather I, Johnston CF et al. Immunocytochemical demonstration of peptidergic and serotoninergic components in the enteric nervous system of the roundworm, Ascaris suum (Nematoda, Ascaroidea). Parasitology 1994; 108(1):89-103.
62. Rogers C, Reale V, Kim K et al. Inhibition of Caenorhabditis elegans social feeding by FMRFamide-related peptide activation of NPR-1. Nat Neurosci 2003; 6:1178-85.
63. Fox RM, Von Stetina SE, Barlow SJ et al. A gene expression fingerprint of C. elegans embryonic motor neurons. BMC Genomics 2005; 6(1):42-65.
64. Von Stetina SE, Watson JD, Fox RM et al. Cell-specific microarray profiling experiments reveal a comprehensive picture of gene expression in the C. elegans nervous system. Genome Biol 2007; 8(7):R135 [Epub ahead of print].
65. Kimber MJ, Fleming CC, Prior A et al. Localisation of Globodera pallida FMRFamide-related peptide encoding genes using in situ hybridization. Int J Parasitol 2002; 32:1095-105.
66. Yew JY, Dikler S, Stretton AO. De novo sequencing of novel neuropeptides directly from Ascaris suum tissue using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight. Rapid Commun Mass Spectrom 2003; 17(24):2693-8.
67. Yew JY, Kimberly KK, Dikler S et al. Mass spectrometric map of neuropeptide expression in Ascaris suum. J Comp Neurol 2005; 488:396-413.
68. Eastman C, Horvitz HR, Yin Y. Coordinated transcriptional regulation of the unc-25 glutamic acid decarboxylase and the unc-47 GABA vesicular transporter by the Caenorhabditis elegans UNC-30 homeodomain protein. J Neurosci 1999; 19(15):6225-34.
69. Hart AC, Sims S, Kaplan JM. Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. Nature 1995; 378:82-5.
70. Tsalik EL, Niacaris T, Wenick AS et al. LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. Dev Biol 2003; 263(1):81-102.
71. Wilson R, Ainscough R, Anderson K et al 2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans. Nature 1994; 368:32-8.
72. Kawano T, Ito Y, Ishiguro M et al. Molecular cloning and characterization of a new insulin/IGF-like peptide of the nematode Caenorhabditis elegans. Biochem Biophys Res Commun 2000; 273:431-6.

73. Pierce SB, Costa M, Wisotzkey R et al. Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. Genes Dev 2001; 15:672-86.
74. Li W, Kennedy SG, Ruvkun R. daf-28 encodes a C. elegans insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. Genes Dev 2003; 17(7):844-58.
75. Couillault C, Pujol N, Reboul J et al. TLR-independent control of innate immunity in Caenorhabditis elegans by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. Nat Immunol 2004; 5(5):488-94.
76. McVeigh P, Leech S, Marks NJ et al. Gene expression and pharmacology of nematode NLP-12 neuropeptides. Int J Parasitol 2006b; 36(6):633-40.
77. Halton DW, Maule AG. Flatworm nerve and muscle: structural and functional analysis. Canadian J Zool 2004; 82:316-33.
78. Schilt J, Richoux JP, Dubois MP. Demonstration of peptides immunologically related to vertebrate neurohormones in Dugesia lugubris (Turbellaria, Tricladida). Gen Comp Endocrinol 1981; 43(3):331-5.
79. Halton DW, Fairweather I, Shaw C et al. Regulatory peptides in parasitic platyhelminths. Parasitol Today 1990; 6(9):284-90.
80. Halton DW, Shaw C, Maule AG et al. Regulatory peptides in helminth parasites. Adv Parasitol 1994a; 34:163-227.
81. Halton DW, Maule AG, Mair GR et al. Monogenean neuromusculature: some structural and functional correlates. Int J Parasitol 1998; 28(10):1609-23.
82. Skuce PJ, Johnston CF, Fairweather I et al. Immunoreactivity to the pancreatic polypeptide family in the nervous system of the adult human blood fluke, Schistosoma mansoni. Cell Tissue Res 1990; 261(3):573-81.
83. Skuce PJ, Johnston CF, Fairweather I et al. A confocal scanning laser microscope study of the peptidergic and serotoninergic components of the nervous system in larval Schistosoma mansoni. Parasitology 1990; 101(2):227-34.
84. Herzog H, Hort Y, Schneider R et al. Seminalplasmin: recent evolution of another member of the neuropeptide Y gene family. Proc Natl Acad Sci USA 1995; 92(2):594-8.
85. Couzens M, Liu M, Tüchler C et al. Peptide YY-2 (PYY2) and pancreatic polypeptide-2 (PPY2): species-specific evolution of novel members of the neuropeptide Y gene family. Genomics 2000; 64(3):318-23.
86. Conlon JM, Larhammar D. The evolution of neuroendocrine peptides. Gen Comp Endocrinol 2005; 142(1-2):53-9.
87. Mair GR, Halton DW, Shaw C et al. The neuropeptide F (NPF) encoding gene from the cestode, Moniezia expansa. Parasitology 2000; 120(1):71-7.
88. Dougan PM, Mair GR, Halton DW et al. Gene organization and expression of a neuropeptide Y homolog from the land planarian Arthurdendyus triangulatus. J Comp Neurol 2002; 454(1):58-64.
89. Humphries JE, Kimber MJ, Barton YW et al. Structure and bioactivity of neuropeptide F from the human parasites Schistosoma mansoni and Schistosoma japonicum. J Biol Chem 2004; 279(38):39880-5.
90. Miskolzie M, Kotovych G. The NMR-derived conformation of neuropeptide F from Moniezia expansa. J Biomol Struct Dynamics 2002; 19:991-8.
91. Maule AG, Shaw C, Halton DW et al. Neuropeptide F (Moniezia expansa): localization and characterization using specific antisera. Parasitology 1992a; 105(3):505-12.
92. Maule AG, Brennan GP, Halton DW et al. Neuropeptide F-immunoreactivity in the monogenean parasite Diclidophora merlangi. Parasitol Res 1992b; 78(8):655-60.
93. Hrckova G, Halton DW, Maule AG et al. Neuropeptide F-immunoreactivity in the tetrathyridium of Mesocestoides corti (Cestoda: Cyclophyllidea). Parasitol Res 1993; 79(8):690-5.
94. Marks NJ, Maule AG, Halton DW et al. Distribution and immunochemical characteristics of neuropeptide F (NPF) (Moniezia expansa)—immunoreactivity in Proteocephalus pollanicola (Cestoda: Proteocephalidea). Comp Biochem Physiol C 1993; 104(3):381-6.
95. Marks NJ, Halton DW, Maule AG et al. Comparative analyses of the neuropeptide F (NPF)- and FMRFamide-related peptide (FaRP)-immunoreactivities in Fasciola hepatica and Schistosoma spp. Parasitology 1995b; 110(4):371-81.
96. Gustafsson MK, Fagerholm HP, Halton DW et al. Neuropeptides and serotonin in the cestode, Proteocephalus exiguus: an immunocytochemical study. Int J Parasitol 1995; 25(6):673-82.
97. Brennan GP, Ramasamy P. Ultrastructure of the surface structures and electron immunogold labeling of peptide immunoreactivity in the nervous system of Pseudothoracocotyla indica (Polyopisthocotylea: Monogenea). Parasitol Res 1996; 82(7):638-46.
98. Maule AG, Halton DW, Shaw C et al. The cholinergic, serotoninergic and peptidergic components of the nervous system of Moniezia expansa (Cestoda, Cyclophyllidea). Parasitology 1993b; 106(4):429-40.

99. Brennan GP, Halton DW, Maule AG et al. Electron immunogold labeling of regulatory peptide immunoreactivity in the nervous system of Moniezia expansa (Cestoda: Cyclophyllidea). Parasitol Res 1993; 79(5):409-15.
100. Halton DW, Maule AG, Brennan GP et al. Grillotia erinaceus (Cestoda, Trypanorhyncha): localization of neuroactive substances in the plerocercoid, using confocal and electron-microscopic immunocytochemistry. Exp Parasitol 1994b; 79(3):410-23.
101. Maule AG, Halton DW, Johnston CF et al. The serotoninergic, cholinergic and peptidergic components of the nervous system in the monogenean parasite, Diclidophora merlangi: a cytochemical study. Parasitology 1990; 100(2):255-73.
102. Biserova NM, Dudicheva VA, Terenina NB et al. The nervous system of Amphilina foliacea (Platyhelminthes, Amphilinidea) an immunocytochemical, ultrastructural and spectrofluorometrical study. Parasitology 2000; 121(4):441-53.
103. Zurawski T, Mousley A, Mair GR et al. Immunomicroscopical observations on the nervous system of adult Eudiplozoon nipponicum (Monogenea: Diplozoidae). Int J Parasitol 2001; 31(8):783-92.
104. Kotikova EA, Raikova OI, Reuter M et al. The nervous and muscular systems in the free-living flatworm Castrella truncata (Rhabdocoela): an immunocytochemical and phalloidin fluorescence study. Tissue Cell 2002; 34(5):365-74.
105. Stewart MT, Marks NJ, Halton DW. Neuroactive substances and associated major muscle systems in Bucephaloides gracilescens (Trematoda: Digenea) metacercaria and adult. Parasitol Res 2003; 91(1):12-21.
106. Stewart MT, Mousley A, Koubkova B et al. Development in vitro of the neuromusculature of two strigeid trematodes, Apatemon cobitidis proterorhini and Cotylurus erraticus. Int J Parasitol 2003; 33(4):413-24.
107. Stewart MT, Mousley A, Koubkova B et al. Gross anatomy of the muscle systems and associated innervation of Apatemon cobitidis proterorhini metacercaria (Trematoda: Strigeidea), as visualized by confocal microscopy. Parasitology 2003; 126(3):273-82.
108. Shaw C, Maule AG, Halton DW. Platyhelminth FMRFamide-related peptides. Int J Parasitol 1996; 26(4):335-45.
109. Armstrong EP, Halton DW, Tinsley RC et al. Immunocytochemical evidence for the involvement of an FMRFamide-related peptide in egg production in the flatworm parasite Polystoma nearcticum. J Comp Neurol 1997; 377(1):41-8.
110. Behm CA, Bendig MM, McCarter JP et al. RNAi-based discovery and validation of new drug targets in filarial nematodes. Trends Parasitol 2005; 21(3):97-100.
111. Jones AK, Buckingham SD, Sattelle DB. Chemistry-to-gene screens in Caenorhabditis elegans. Nat Rev Drug Discov 2005; 4(4):321-30.
112. Lochnit G, Bongaarts R, Geyer R. Searching new targets for anthelminthic strategies: Interference with glycosphingolipid biosynthesis and phosphorylcholine metabolism affects development of Caenorhabditis elegans. Int J Parasitol 2005; 35(8):911-23.
113. Zawadzki JL, Presidente PJ, Meeusen EN et al. RNAi in Haemonchus contortus: a potential method for target validation. Trends Parasitol 2006; 22(11):495-9.
114. Aboobaker AA, Blaxter M. Use of RNA interference to investigate gene function in the human filarial nematode parasite Brugia malayi. Mol Biochem Parasitol 2003; 129(1):41-51.
115. Issa Z, Grant WN, Stasiuk S et al. Development of methods for RNA interference in the sheep gastrointestinal parasite, Trichostrongylus colubriformis. Int J Parasitol 2005; 35(9):935-40.
116. Krautz-Peterson G, Radwanska M, Ndegwa D et al. Optimizing gene suppression in schistosomes using RNA interference. Mol Biochem Parasitol 2007; 153(2):194-202.
117. Simmer F, Tijsterman M, Parrish S et al. Loss of the putative RNA-directed RNA polymerase RRF-3 makes C. elegans hypersensitive to RNAi. Curr Biol 2002; 12(15):1317-9.
118. Kennedy S, Wang D, Ruvkun G. A conserved siRNA-degrading RNase negatively regulates RNA interference in C. elegans. Nature 2004; 427(6975):645-9.
119. Asikainen S, Vartiainen S, Lakso M et al. Selective sensitivity of Caenorhabditis elegans neurons to RNA interference. Neuroreport 2005; 16(18):1995-9.
120. Fleming CC, McKinney S, McMaster S et al. Getting to the root of neuronal signalling in plant parasitic nematodes using RNA interference. Nematology 2007; [in press].
121. Geldhof P, Visser A, Clark D et al. RNA interference in parasitic helminths: current situation, potential pitfalls and future prospects. Parasitology 2007; 134(5):609-19.
122. Knox DP, Geldhof P, Visser A et al. RNA interference in parasitic nematodes of animals: a reality check? Trends Parasitol 2007; 23(3):105-7.
123. Kimber MJ, McKinney S, McMaster S et al. flp gene disruption in a parasitic nematode reveals motor dysfunction and unusual neuronal sensitivity to RNA interference. FASEB J 2007; 21(4):1233-43.
124. Bakhetia M, Charlton WL, Urwin PE et al. RNA interference and plant parasitic nematodes. Trends Plant Sci 2005; 10(8):362-7.

125. Huang G, Allen R, Davis EL et al. Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 2006; 103(39):14302-6.
126. Yadav BC, Veluthambi K, Subramaniam K. Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol 2006; 148(2):219-22.
127. Steeves RM, Todd TC, Essig JS et al. Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress Heterodera glycines reproduction. Funct Plant Biol 2006; 33:991-999.
128. Fairbairn DJ, Cavallaro AS, Bernard M et al. Host-delivered RNAi: an effective strategy to silence genes in plant parasitic nematodes. Planta 2007; [Epub ahead of print].
129. Husson SJ, Clynen E, Baggerman G et al. Defective processing of neuropeptide precursors in Caenorhabditis elegans lacking proprotein convertase 2 (KPC-2/EGL-3): mutant analysis by mass spectrometry. J Neurochem 2006; 98(6):1999-2012.
130. Husson SJ, Janssen T, Baggerman G et al. Impaired processing of FLP and NLP peptides in carboxypeptidase E (EGL-21)-deficient Caenorhabditis elegans as analyzed by mass spectrometry. J Neurochem 2007; 102(1):246-60.
131. Mair GR, Niciu MJ, Stewart MT et al. A functionally atypical amidating enzyme from the human parasite Schistosoma mansoni. FASEB J 2004; 18(1):114-21.
132. McVeigh P, Mair GR, Atkinson L et al. Discovery of multiple neuropeptide families in the phylum Platyhelminthes. Int J Parasitol 2009; 39(11):1243-52.