The ever-expanding neuropeptide gene families in the nematode *Caenorhabditis elegans*

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SUMMARY

Neuropeptides act as chemical signals in the nervous system to modulate behaviour. With the ongoing EST projects and DNA sequence determination of different genomes, the identification of neuropeptide genes has been made easier. Despite the relatively 'simple' repertoire of behaviours in the nematode *Caenorhabditis elegans*, this worm contains a surprisingly large and diverse set of neuropeptide genes. At least 109 genes encoding over 250 potential neuropeptides have been identified in *C. elegans*; all genes are likely to be expressed and many, if not all, of the predicted peptides are produced. The predicted peptides include: 38 insulin-like peptides, several of which are involved in development and reproductive growth, and over 70 FMRFamide-related peptides, some of which are involved in locomotion, reproduction, and social behaviour. Many of the *C. elegans* peptides are identical or highly similar to those isolated or predicted in parasitic nematodes, such as *Ascaris suum*, *Haemonchus contortus*, *Ancylostoma caninum*, *Heterodera glycines* and *Meloidogyne arenaria*, suggesting that the function of these peptides is similar across species. The challenge for the future is to determine the function of all the genes and individual peptides and to identify the receptors through which the peptides signal.

Key words: Neuropeptides, gene families, insulin, FMRFamide, *flp*, *nlp*.

INTRODUCTION

The sequence determination of numerous genomes has given us a glimpse into the diversity of neuropeptides present in the animal kingdom. This class of neurotransmitters has been implicated in a multitude of behaviours in both vertebrates as well as invertebrates. In the mammalian nervous system, neuropeptides act predominantly as modulators of synaptic activity, whereby they modulate the action of a primary transmitter, such as the small molecule, classical transmitters. By contrast, neuropeptides function not only as neuromodulators, but also as primary transmitters in the invertebrate nervous system.

The free-living soil nematode *Caenorhabditis elegans* presents a tractable genetic model for the nervous system of many parasitic nematodes. *C. elegans* has a total of 302 neurons (Sulston & Horvitz, 1977; Sulston *et al*. 1983), and the structure of its nervous system is similar to that of parasitic nematodes, such as that of *Ascaris lumbricoides* (Stretton *et al*. 1978). To date, 109 neuropeptide genes have been identified in *C. elegans*. These genes have been divided into three main categories: the *ins* genes, which encode most of the insulin-like peptides, the *flp* genes, which encode the FMRFamide-related peptides, and the *nlp* genes, which encode non-insulin, non-FMRFamide-related peptides. With the ongoing EST genome and proteome projects of many parasitic nematodes, it is striking that many, if not all, of the *C. elegans* neuropeptide genes have counterparts in parasitic nematodes, and the isolated and encoded neuropeptide sequences are highly similar, if not identical to the *C. elegans* peptides (McVeigh *et al*. 2005; Yew *et al*. 2005). This review serves to summarize the current state of the neuropeptide field in *C. elegans*.

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FORMATION OF MATURE NEUROPEPTIDES

Neuropeptides are derived from larger precursor molecules, which must be cleaved to yield individual, active neuropeptides. Precursor molecules may contain multiple neuropeptides that are distinct from each other, a single neuropeptide, multiple copies of the same neuropeptide or a combination of these possibilities. Precursor molecules that encode multiple, distinct neuropeptides may undergo differential cleavage patterns among different cell types, thereby changing the composition of neuropeptides available in a specific cell.

The typical cleavage site in *C. elegans* is C-terminal to dibasic residues, which flank the peptide sequence (Rosoff *et al*. 1993; Marks *et al*. 1995, 1997, 1998, 1999*a*, 2001); however, cleavage after mono- or tribasic residues also occurs (Rosoff *et al*. 1993; Marks *et al*. 1997, 2001; Husson *et al*. 2005). These

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basic site cleavages are performed by kex2/subtilisin-like proprotein convertases (PC). *C. elegans* contains four PCs, but whether all four of the PCs cleave neuropeptide precursors is still unclear. Loss of *kpc-1* and *egl-3/kpc-2* results in behavioural deficits, such as defects in locomotion (Thacker & Rose, 2000; Kass *et al*. 2001; Jacob & Kaplan, 2003), egg-laying (Kass *et al*. 2001; Jacob & Kaplan, 2003), mechanosensation (Kass *et al*. 2001; Jacob & Kaplan, 2003), and growth (Thacker & Rose, 2000), suggesting that these two PCs are involved in neuropeptide processing. *egl-3/kpc-2* is expressed in many, but not all of the neurons in the nervous system (Kass *et al*. 2001). Because neuropeptide immunoreactivity is decreased but not eliminated in *egl-3/kpc-2* mutants, multiple PCs are likely to function in single neurons (Kass *et al*. 2001). Complete loss of function alleles in *bli-4/kpc-4* result in lethality (Thacker *et al*. 1995), so the role of *bli-4/kpc-4* in the nervous system has been difficult to assess. However, like *egl-3/kpc-2* (Kass *et al*. 2001), *bli-4/kpc-4* is expressed in the nervous system (Thacker *et al*. 1995; Thacker & Rose, 2000), suggesting that *bli-4/kpc-4* may also function as a PC cleavage enzyme.

Further processing of precursor molecules by carboxypeptidases E (CPEs) removes the flanking basic residues from the neuropeptide sequences (Steiner, 1998). Three CPEs are present in *C. elegans* (Jacob & Kaplan, 2003). *egl-21* CPE is expressed in roughly 60% of the neurons. Loss of *egl-21* shows similar, but more severe phenotypes than loss of the PCs. The level of FLP neuropeptide immunoreactivity is also greatly diminished in *egl-21* CPE mutants, suggesting that the remaining two CPEs are responsible for cleaving the remaining FLP precursors, as well as other neuropeptides (Jacob & Kaplan, 2003).

As with the mammalian neuropeptides, many *C. elegans* neuropeptides are modified at their N- or C-termini, presumably to protect against degradation and/or to generate an active form of the peptide (Schinkmann & Li, 1992; Steiner, 1998; Husson *et al*. 2005). The most common known modification in *C. elegans* is amidation. Peptides are presumably amidated if they contain a C-terminal glycine, which donates an amino group in the amidation process. By definition, all predicted FLPs are amidated, and the presence of a C-terminal glycine indicates that many of the NLPs are also likely to be amidated. The enzyme(s) involved in *C. elegans* amidation is(are) unknown. Based on homology to the mammalian amidation enzymes, peptidylglycine-alpha-hydroxylating monooxygenase (PHM), peptidyl-alpha-hydroxyglycine alpha-amidating lyase and the bifunctional peptidylglycine α-amidating monooxygenase (PAM) (Eipper *et al*. 1993), *C. elegans* contains at least one PAM-like and one PHM molecule (Han *et al*. 2004).

IDENTIFICATION OF PUTATIVE NEUROPEPTIDE GENES

The complete DNA sequence of the *C. elegans* genome (*C. elegans* Sequencing Consortium, 1998) allowed scanning of the genome for candidate neuropeptide genes. The sheer number of identified neuropeptide genes, particularly within certain families, was surprising. For instance, thirty-eight genes that encode insulin-like molecules were identified (Table 1; Duret *et al*. 1998; Gregoire *et al*. 1998; Kawano *et al*. 2000; Pierce *et al*. 2001; Li, Kennedy & Ruvkun, 2003), but at the time, only one insulin-like receptor had been identified (Kimura *et al*. 1997). Our laboratory identified twenty-four *flp* genes (*flp-1* to *flp-23* and *flp-28*) that encode peptides sharing a common C-terminal RF-amide moiety (Li, Kim & Nelson, 1998; Kim & Li, 2004; C. Li, unpublished observations); recently, McVeigh and co-workers (2005) reported the identification of five other *flp* genes, *flp-24* to *flp-27* and *flp-32*, by EST data mining and BLAST searches (Table 2). Hart and co-workers used similarity and pattern-based scans in more general BLAST screens to identify other neuropeptide genes. Using the characteristics of neuropeptide precursor processing, pattern-based scans designed to search for peptide sequences that were flanked by mono- or dibasic sequences revealed 34 non-insulin-like, non-FLP-like *nlp* genes (Table 3; Li *et al*. 1999; Nathoo *et al*. 2001; A. Hart, personal communication); *nlp-33* was recently identified by Couillault and co-workers (2004; Table 3) and *nlp-36* to *nlp-42* by Husson and co-workers (2005; Table 3). A total of 109 neuropeptide genes encoding over 250 putative neuropeptides have now been identified (Tables 1–3), but this number is likely to be an underestimate of the total number of *C. elegans* neuropeptide genes (see below).

CONFIRMATION OF NEUROPEPTIDE GENES

The large number of neuropeptide genes and predicted peptides was surprising, particularly because so many of the predicted neuropeptides have similar sequences. This multitude of genes immediately raised two questions: (1) are all the genes actually transcribed and (2) if the genes are transcribed, are all the predicted peptides produced? To determine whether the genes are transcribed, two basic approaches have been used. The first is to take advantage of the ongoing *C. elegans* EST and ORFeome projects and the second is to isolate cDNAs by reverse transcription/polymerase chain reaction. Based on the isolated cDNAs, 36 of the 38 insulin-encoding genes (Table 1; Gregoire *et al*. 1998; Kawano *et al*. 2000; Pierce *et al*. 2001; Li *et al*. 2003), 28 of the 29 *flp* genes (Table 2; Rosoff, Burglin & Li, 1992; Nelson *et al*. 1998; Kim & Li, 2004; McVeigh *et al*. 2005; I. Miskelly, N. J. Marks and A. G. Maule, personal communication; unpublished

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Table 1. Insulin-like peptide encoding genes

Gene[#]	Encoded peptides	Expression pattern	Function	Receptor
daf-28	VPGVAVRACGRRLVPYVWSVCGDACEPQ EGIDIATQCCTYQCTAEYIQTACCPRLL	ASI, ASJ, PQR, other neurons, hindgut, pharyngeal muscle, hypodermis	promotes reproductive growth	DAF-2
ins-1	SIRLCGSRLTTTLLAVCRNQLCTGLTAF GGIATECCEKRCSFAYLKTFCCNQDDN	ASI, ASJ, ASH, NSM, other neurons, intestine, vulval muscles	DAF-2 antagonist?	
ins-2	VQKRLCGRRLILFMLATCGECDTD SSEDLSHICCIKQCDVQDIIRVCCPNSFRK	amphidial, labial, ventral cord and tail neurons, pharynx, vulva		
ins-3	GDKVKICGTKVLKMVMVMCGGECSS TNENIATECCEKMCTMEDITTKCCPSR	amphidial, labial, lateral, ventral cord and dorsal projecting neurons		
ins-4	VPAGEVRACGRRLLLFVWSTCGEPCTPQ EDMDIATVCCTTQCTPSYIKQACCPEK	amphidial, labial, ventral cord, dorsal projecting and tail neurons, hypodermis		DAF-2
ins-5	ADRHTNYRSCALRLIPHVWSVCGDACQPQ NGIDVAQKCCSTDCSSDYIKETCCPFD	amphidial, labial, ventral cord, lateral projecting and tail neurons, vulva		
ins-6	VPAPGETRACGRKLISLVMAVCGDLCNPQ EGKDIATECCGNQCSDDYIRSACCP	amphidial, labial, ventral cord and tail neurons		DAF-2
ins-7	VPDEKKIYRCGRRIHSYVFAVCGKACESN TEVNIASKCCREECTDDFIRKQCCP	amphidial, labial, ventral cord and tail neurons		
ins-8	VPEQKNKLCGKQVLSYVMALCEKACDSN TKVDIATKCCRDACSDEFIRHQCCP	amphidial, labial, ventral cord and tail neurons, vulva		
ins-9	TLETEKIYRCGRKLYTDVLSACNGPCEPG TEQDLSKICCGNQCTFVIRKACCADKL	ASI, ASJ	over-expression causes embryonic and larval arrest	
ins-10	AFPFQICVKKMEKMCRIINPEQCAQVNKITEI GALTDCCTGLCSWEEIRISCCSVL			
ins-11	APHHDKRHTACVLKIFKALNVMCNHEGDAD VLRRTASDCCRESCSLTEMLASCTLTSSEESTRDI	labial, ventral cord and tail neurons		
ins-12	APSHEKTHKKCSDKLYLAMKSLCSYRGYSE FLRNSATKCCQDNCEISEMMALCVVAPNFDDDLLH			
ins-13	NKCOYSKKKYKICGVRALKHMKVYCTRGMTRD YGKLLVTCCSKGCNAIDIQRICL			
ins-14	SEDIKCDAKFISRITKLCIHGITED KLVRLLTRCCTSHCSKAHLKMFCTLKPHEEEPHHEI			
ins-15	GNDFQPRDNKHHSYRSCGESLSRRVAFLCNGGAIQT EILRALDCCSTGCTDKQIFSWCDFQI			
ins-16	RELKRCSVKLFDILSVICGTESDAE ILQKVAVKCCQEQCGFEEMCQHANLKIDKI			

Table 1. (*Cont.*)

Gene[#]	Encoded peptides	Expression pattern	Function	Receptor
ins-17	GSLKLCPPGGASFLDAFNLICPMRRRRR SVSENYNDGGGSLLGRTMNMCCETGCEFTDIFAICNPFG			
ins-18	ISLQQADGRMKMCPPGGSTFTMAWSMSCSMRR KRALIAPSIRQLQTICCQVGCNVEDLLAYCAPI	amphidial, ventral cord, tail and pharyngeal neurons	DAF-2 antagonist?	
ins-19	YIIDSSESYEVLMLFGYKRTCGRRLMNRINRVCVKDID PADIDPKIKLSEHCCIKGCTDGWIKKHICSEEVLNFGFFEN		over-expression causes larval arrest	
ins-20	KEPKHHHHHHHRHKGYCGVKAVKKLKQICPDLCSNVDD NLLMEMCSKNLTDDDILQRCCPE			
ins-21	SKSHSKKHVRFLCATKAVKHIRKVCPDMCLTGE EVEVNEFCRMGYSDSQIKYICCPE	amphidial, ventral cord and tail neurons		
ins-22	MDAHTDKYVRTLCGKTAIRNIANLCPPKPEMKGICSTGE YPSITEYCSMGFSDSQIKFMCCDNQ	amphidial, labial, ventral cord, lateral process projecting and tail neurons		
ins-23	QVTDAHSELHVRRVCGTAIIKNIMRLCPGVPACENGE VPSPTEYCSMGYSDSQVKYLCCPTSQ	amphidial, labial, & ventral cord neurons		
ins-24	MGLIRANQGPQKACGRSMMMKVQKLCAGGCTIQNDD LTIKSCSTGYTDAGFISACCPSGFVF			
ins-25	KPEAQRRCGRYLIRFLGELCNGPCSGVSSVD IATIACATAVPIEDLKNMCCPNL			
ins-26	IGNHHHGTKAGLTCGMNIIERVDQLCNGQCTRNYDA LVIKSCHRGVSDMEFMVACCPTMKLFIH			
ins-27	FLAPSTAAKRRCGRRLIPYVYSICGGPCENGD IIIEHCFSGTTPTIAEVQKACCPELSEDPTFSS			
ins-28	ASPTCGRALLHRIQSVCGLCTIDAHHE LIAIACSRGLGDKEIIEMCCPI			
ins-29	DFGAQRRCGRHLVNFLEGLCGGPCSEAPTVE LASWACSSAVSIQDLEKLCCPSNLA			
ins-30	REPVVAAQGAKKTCGRSLLIKIQQLCHGICTVHADD LHETACMKGLTDSQLINSCCPPIPQTPFVF			
ins-31 a	FVHHFDHSMFARPEKTCGGLLIRRVDRICPNLNY TYKIEWELMDNCCEVVCEDQWIKETFCRAPRFNFFGPSF		over-expression causes larval arrest[$]	
ins-31 b	KALERSCGPKLFTRVKTVCGE DINVDNKVKISDHCCTPEGGCTDDWIKENVCKQTRFNFFRQFL			
ins-31 c	DSPQRSCGPQLFKRVNTLCNE NINVENNVSVSKSCCESAAGCTDDWIKKNVCTQHKPFVFRPGFY			
ins-32	RSRRELICGRRLSKTVTNLCVEMN PQKEEDIATKCCKNKGCSREYIKSIMCPDE			

ins-33	HGQKHCGTKIVRKLQMLCPKMCTISDD
	TLLTEMCSHSLFDDEIQLRCCPKEDE
ins-34	KTTAAPLAQVNPQCLRRLTLLARGVCRQPCQPSDKPK
	TSAQQLLQLACSARRPTNEQIISYCCCPEKSG
ins-35	KMDENAFGINNRHCQRALKVYSFAICGAICQNYEK
	ILMEGCGSTVMLTMQRTKLICCPEPVDSDELFN
ins-36	IRKRHPEGKLVIRDCKRYLIMYSRTICKEKCEKFD
	ERNDITFSINLQFIFTDLLVEGCHSNQTLSNERTRELCCPNAGSN
ins-37	NPIHPVPNAAFLPYRSCGSHLVHRAFEACSGKKD
	RSSDVDLWKMCCKDECTDLDIKESLCKYASQGYGV

Genes for which ESTs, ORFeomes (OST), cDNAs, or encoded peptides have been isolated are in bold. $ Unclear which *ins-31* construct was used for overexpression and functional data. From Malone & Thomas, 1994; Pierce *et al.* 2001; Li, Kennedy & Ruvkun, 2003.

observations), and 38 of the 42 *nlp* genes (Table 3; Nathoo *et al.* 2001; Couillault *et al.* 2004) are expressed. These data suggest that most, if not all, of the predicted genes are transcribed.

The more difficult problem has been to determine whether the neuropeptides are produced and the actual sequences of the peptides. Most of the biochemical isolations initially centered on the FLPs. Thirteen FLPs encoded by seven *flp* genes (*flp-1*, *6*, *8*, *9*, *13*, *14* and *18*) were isolated by high pressure liquid chromatographic fractionation of *C. elegans* extracts (Table 2; Rosoff *et al.* 1993; Marks *et al.* 1995, 1997, 1998, 1999*a*, 2001). More recently, Husson and co-workers (2005) have used more sophisticated peptidomic analysis involving two-dimensional nanoscale liquid chromatography in tandem with mass spectrometry to isolate a large number of FLP peptides, including many that were previously isolated; in total, 32 FLPs encoded by 12 genes were isolated, bringing the total number of isolated FLPs to 35. A few of the FLPs isolated by Husson and coworkers (2005) are longer peptides than predicted; these peptides may represent either incompletely cleaved peptides or longer peptides that are also functional. Two of the isolated FLPs are shorter than predicted (Husson *et al.* 2005). One of these peptides suggests that the initial prediction (SDRPTRAMSPLIRFamide) was incorrect and a monobasic cleavage site was used (to yield AMDSPLIRFamide). In the second example, a truncated peptide, GAMPGVLRFamide, was isolated in addition to the larger predicted peptide, DFDGAMPGVLRFamide; either the truncated peptide is a degradation product or a novel cleavage site was used in the larger peptide to produce the shorter one (Husson *et al.* 2005). Two FLPs that are not encoded by any of the identified *flp* genes have also been isolated (N. J. Marks and A. O. W. Stretton, personal communication), underscoring the difficulty of identifying small neuropeptide genes with BLAST searches and suggesting that the roughly 70 FLPs encoded by 29 genes is an underestimate.

Concurrent with the biochemical analysis of the FLPs, Husson and co-workers (2005) also isolated the first NLPs from *C. elegans*. Twenty nine NLPs encoded by 19 genes were isolated. As with the FLPs, some of the isolated NLPs are truncated or there are N-terminal extensions of the predicted NLPs, again suggesting that incomplete cleavages, novel cleavages, and/or degradation products are among the isolated peptides. No biochemical isolations have been performed for the insulin-like peptides.

Peptides identical to some of the predicted FLPs and NLPs have also been isolated from related nematodes, such as *Ascaris suum* (Cowden, Stretton & Davis, 1989; Cowden & Stretton, 1993, 1995; Yew *et al.* 2005), *Haemonchus contortus* (Keating *et al.* 1995; Marks *et al.* 1999*b*), and *Panagrellus redivivus*

(Geary *et al*. 1992; Maule *et al*. 1994*a*, *b*, 1995). In particular, of the 16 *C. elegans* FLPs and NLPs isolated from *Ascaris*, five peptides encoded by *flp-3*, *11*, *12*, *18* and *21* and two peptides from *nlp-12* have not been isolated from *C. elegans* thus far (Yew *et al*. 2005), suggesting that these peptides are also produced in *C. elegans*. However, it is the ongoing projects to isolate ESTs from various nematode species that highlight how prevalent the *flp* genes are in Nematoda (McVeigh *et al*. 2005; Yew *et al*. 2005). Indeed, the recent database mining by McVeigh and co-workers (2005) indicates that 33 species of nematodes, including *Ancylostoma caninum*, *Globodera pallida* and *G. rostochiensis*, *Heterodera glycines*, *Meloidogyne arenaria*, *M. chitwoodi*, and *M. incognita* and *Onchocerca volvulus*, have sequelogs to the *C. elegans flp* genes. These data suggest that many of the predicted neuropeptides are indeed produced and highlight the rich diversity of neuropeptides not only in *C. elegans* but in other nematode species as well.

EXPRESSION PATTERN OF NEUROPEPTIDE GENES

The difficulty in generating specific antisera against peptides that have very similar sequences (for instance, the insulin-like peptides share common A and B domains and the FLPs all share a common C-terminal Arg-Phe-NH_2), have led researchers to find other ways to determine the expression pattern of the different neuropeptide genes. Because transgenic animals are relatively easy to generate in *C. elegans*, the most frequently used approach to determine the expression patterns is to construct transcriptional fusions between the promoter region of the neuropeptide gene with the coding region of a reporter gene, of which green fluorescent protein (GFP) is the most commonly used, for microinjection. This relatively simple method has been used to determine the expression pattern of 60 neuropeptide genes (Tables 1–3), including 15 insulin-like genes (Pierce *et al*. 2001; Li *et al*. 2003), 19 *flp* genes (Kim & Li, 2004) and 26 *nlp* genes (Nathoo *et al*. 2001).

Although there are inherent caveats to using reporter constructs, the expression data illuminate several points about neuropeptides. First, the expression of neuropeptides is widespread in *C. elegans*; not only is expression seen throughout the nervous system, but expression is also seen in non-neuronal tissues (Nathoo *et al*. 2001; Pierce *et al*. 2001; Li *et al*. 2003; Kim & Li, 2004; Tables 1–3). The FLPs alone are expressed in over 160 neurons, greater than half the number of cells in the *C. elegans* nervous system (Kim & Li, 2004). Fifteen of the *ins* genes and *daf-28* are expressed in neurons, including some of the amphidial chemosensory neurons (Pierce *et al*. 2001; Li *et al*. 2003). In addition, several *ins*, *flp* and *nlp* genes are expressed outside the nervous system, such as in intestine, gonad, muscle, and hypodermal cells (Nathoo *et al*. 2001; Pierce *et al*. 2001; Li *et al*. 2003; Kim & Li, 2004), where they presumably function in a more endocrine fashion. Secondly, as in other vertebrate and invertebrate systems, there is considerable overlap in the expression patterns. For instance, each *flp* gene examined thus far is expressed in a unique set of cells, although a specific cell may express several *flp* genes (Kim & Li, 2004). Similarly, expression of the *ins* (Pierce *et al*. 2001), *daf-28* (Li *et al*. 2003), and *nlp* (Nathoo *et al*. 2001) genes is widespread and multiple peptide families can be expressed in the same cell. The chemosensory neuron ASI, for example, has an extremely rich neuropeptide repertoire. ASI expresses *daf-28*, *ins-1* and *9*, *nlp-1*, *5*, *6*, *9*, *14*, *18*, *24* and *27*, and *flp-2*, *10* and *21* (Nathoo *et al*. 2001; Pierce *et al*. 2001; Li *et al*. 2003; Kim & Li, 2004), suggesting that ASI has the potential to release a diverse range of neuropeptides to modulate neuronal activity.

NEUROPEPTIDE FUNCTION

Bioinformatic tools have allowed the identification of the neuropeptide genes, but the more daunting challenge for researchers is to unravel the function of the different genes and the individual peptides. The functional characterization of the genes is complicated by several factors. First, many of the peptides have highly similar sequences, particularly with peptides encoded by the same gene, but even among peptides encoded by different genes. These peptides may bind to the same receptor, making it difficult to tease apart the functions of the individual peptides. Indeed, EMPGVLRFamide encoded by *flp-18* and GLGPRPLRFamide encoded by *flp-21* bind to a common receptor, NPR-1 (Rogers *et al*. 2003). The widespread expression patterns of the neuropeptide genes suggest that the peptides are involved in a multitude of behaviours in *C. elegans*. However, the extensive overlap among the expression patterns of different genes suggests that many of the peptides will also have functional overlap. Despite these caveats, inactivation of several neuropeptide genes indicates that at least some of the genes have unique functions.

The insulin-like gene family

The major role of insulin in mammals is in glucose metabolism, although insulin-like peptides are also involved in the development of the mammalian nervous system (Russo *et al*. 2005). Despite the large number of insulin-like peptides in *C. elegans*, with a few notable exceptions, the role of the insulin-like peptides in *C. elegans* is largely unknown. Several of the insulin-like peptides are involved in reproductive growth (Malone & Thomas, 1994; Li *et al*. 2003). When exposed to harsh environmental conditions,

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Table 2. FMRFamide-like peptide (*flp*) encoding genes

Gene[#]	Encoded peptides[@]	Expression Pattern[+]	Function	Receptor[^]
flp-1	*SADPNFLRFG *SQPNFLRFG *ASGDPNFLRFG *SDPNFLRFG *AAADPNFLRFG *†(K)PNFLRFG AGSDPNFLRFG *PNFMRYG*	AIA, AIY, AVA, AVE, AVK, RIG, RMG, M5	involved in locomotion, egg laying, and fat deposition; SADPNFLRF-NH_2 inhibits frequency of pharyngeal action potentials	(C25G6.5, Y58G8a.1, C16D6.2)
flp-2	SPREPIRFG LRGEPIRFG	AIA, RID, PVW, I5, MC (ASI, M4, head muscles, an extra pair of cells in the head)		T19F4.1a/b
flp-3	SPLGTMRFG *TPLGTMRFG *EAEEPLGTMRFG NPLGTMRFG *ASEDALFGTMRFG EDGNAPFGTMRFG *SAEPFGTMRFG *SADDSAPFGTMRFG *NPENDTPFGTMRFG	IL1, PQR; SP, CP9	SAEPFGTMRF-NH_2 inhibits frequency of pharyngeal action potentials	C53C7.1a (Y58G8a.1 C16D6.2)
flp-4	PTFIRFG ASPSFIRFG	ADL, ASEL, AVM, AWC, FLP, PHA, PHB, PVD, I5, I6, NSM		C16D6.2
flp-5	*GAKFIRFG AGAKFIRFG APKPKFIRFG	PVT, RMG, I4, M4, pharyngeal muscle, amphidial neuron (PB, I2); rays 1, 5, 7, HOB	GAKFIRF-NH_2 increases frequency of pharyngeal action potentials	(C25G6.5)
flp-6	×6 *KSAYMRFG **pQQDSEVEREMM*	ASE, AFD, ASG, PVT, I1 (one or two pairs of head cells); rays 2, 5, 6, 7	increases frequency of pharyngeal action potentials	
flp-7	×3 SPMQRSSMVRFG ×2 TPMQRSSMVRFG SPMERSAMVRFG SPMDRSKMVRFG	ALA, AVG, PHB, PDA, PVW, RIC, SAA (RMDV/SMDV, PHA)		(C26F1.6)
flp-8	×3 *KNEFIRFG	AUA, PVM, URX (RMG/ADA, an extra pair of cells in the head); CP9	increases frequency of pharyngeal action potentials; over-expression causes defaecation defects	
flp-9	×2 *KPSFVRFG		inhibits frequency of pharyngeal action potentials; knockout shows slight sluggishness	
flp-10	QPKARSGYIRFG	AIM, ASI, AUA, BAG, BDU, DVB, PQR, PVR, URX, vulD		

Table 2. (*Cont.*)

Gene[#]		Encoded peptides[@]	Expression Pattern[+]	Function	Receptor[^]
flp-11		AMRNALVRFG *ASGGMRNALVRFG *NGAPQPFVRFG **SPLDEEDFAPESPLQG*	AUA, BAG, DA, DD, DVB, LUA, PHC, PVC, SAB, URX, VD, uv1, head muscle (socket cells); ray 4		C26F1.6 (C16D6.2)
flp-12		RNKFEFIRFG	AVH/AVJ, BAG, PDA, PVR, SAA, SDQ, SMB (BDU); rays 1, 4, 5, 7, CP9		
flp-13		*AMDSPFIRFG *AADGAPFIRFG *APEASPFIRFG *ASPSAPFIRFG *SPSAVPFIRFG ASSAPFIRFG *SAAAPLIRFG	ASE, ASG, ASK, BAG, DD, I5, M3, M5 (an extra pair of cells in the head); VSP	APEASPFIRF-NH_2 inhibits frequency of pharyngeal action potentials	
flp-14	×4	*KHEYLRFG		increases frequency of pharyngeal action potentials	(C25G6.5, C16D6.2)
flp-15		GGPQGPLRFG RGPSGPLRFG	PHA, I2, socket/sheath cells (pharyngeal muscle, several cells in the head)		C10D6.2 C16D6.2
flp-16	×2	*AQTFVRFG *GQTFVRFG		AQTFVRF-NH_2 inhibits frequency of pharyngeal action potentials	
flp-17	×2	KSAFVRFG KSQYIRFG	BAG, M5 (an extra pair of cells in the head); rays 1, 5, 7		
flp-18	 ×3	*†(DFD)GAMPGVLRFG EMPGVLRFG *†(SYFDEKK)SVPGVLRFG *EIPGVLRFG *SEVPGVLRFG DVPGVLRFG	AVA, AIY, RIG, RIM, M2 (M3, two extra pairs of cells in the head); rays 2, 6		C16D6.2 Y58G8a.1 C53C7.1a NPR-1 (C25G6.5, F41E7.3)
flp-19		*WANQVRFG ASWASSVRFG	AIN, AWA, BAG, HSN, URX (an extra pair of cells in the tail); rays 5, 7, 9, CEM		
flp-20	×2	AMMRFG	ALM, ASEL, AVM, LUA, PLM, PVC, PVM, PVR, RIB/AIB (PVT)		
flp-21		GLGPRPLRFG	ADL, ASI, ASH, ASJ, FLP, URA, MC, M4, M2; CP6–9, SP, DVF	mutation causes mild aggregation behaviour	NPR-1 C25G6.5 Y58G8a.1
flp-22	×3	*SPSAKWMRFG	AIM, ASG, AVA, AVG, AVL, CEP, PVD, PVW, RIC/AIZ, RIV, SMD, URA, uv1; 6 out of 9 CP		
flp-23		VVGQQDFLRG (TKFQDFLRFG)			
flp-24		*VPSAGDMMVRFG			

flp-25		DYDFVRFG
		ASYDYIRFG
flp-26	*†	(E)FNADDLTLRFG
	*	GGAGEPLAFSPDMLSLRFG
flp-27		GLGGRMRFG
flp-28		VLMRFG
flp-32		AMRNSLVRFG

Genes for which ESTs, ORFeomes (OST), cDNAs, or encoded peptides have been isolated are in bold. @ Common sequences among peptides encoded by the same gene are underlined. Number of copies of peptide encoded by gene indicated. A C-terminal glycine donates an amide group during amidation. All encoded peptides are included; amino acid sequences of non-FLP peptides are indicated in italics. * Peptides have been biochemically isolated; † peptides including residues in parantheses have been isolated. + Based on co-localization with new markers, some expression patterns have been revised from published data. Cells in parentheses are variably expressed. Cells after semi-colons are male-specific. ^ Receptors with an $EC_{50} \leqslant 1\ \mu M$ are indicated; receptors with an $EC_{50} > 1\ \mu M$ are in parentheses. From Rosoff, Burglin & Li, 1992; Rosoff *et al.* 1993; de Bono & Bargmann, 1998; Marks *et al.* 1995, 1997, 1998, 1999*a*, 2001; Li *et al.* 1998; Li *et al.* 1999; Waggoner *et al.* 2000; Rogers *et al.* 2001, 2003; Kubiak *et al.* 2003*a*, *b*; Lowery *et al.* 2003; Kim & Li, 2004; Mertens *et al.* 2004, 2005; Husson *et al.* 2005; McVeigh *et al.* 2005; K. Ashrafi, A. Hart, O. Hobert, A. G. Maule, A. Stretton, personal communications; unpublished results.

such as over-crowding, high temperatures or a scarce food supply, *C. elegans* will undergo an alternative life cycle, referred to as the dauer life cycle. Dauer formation allows animals to survive harsh conditions for long periods of time; when conditions become favourable again, animals will leave the dauer cycle and resume reproductive growth (Cassada & Russell, 1975). The decision to enter the dauer life cycle is mediated by the ASI and ASJ chemosensory neurons (Bargmann & Horvitz, 1991), and is determined by parallel pathways. One pathway is mediated by the DAF-2/insulin-like receptor (Kimura *et al.* 1997; Riddle & Albert, 1997), indicating that insulin-like peptides are involved in the decision between reproductive and dauer growth. The second pathway is mediated by DAF-7/transforming growth factor (TGF) β/DAF-11 guanylate cyclase (Riddle & Albert, 1997). Loss of either pathway results in dauer formation, indicating that the pathways function independently. The DAF-2/insulin-like receptor also functions to determine lifespan (Kenyon *et al.* 1993) and to limit body size (McCulloch & Gems, 2003), suggesting that insulin-like peptides are also involved in these processes.

Three insulin-like peptide encoding genes, *ins-1*, *ins-9* and *daf-28*, are expressed in ASI and ASJ (Pierce *et al.* 2001). However, only mutations in *daf-28* cause transient dauer formation (Malone & Thomas, 1994). The genetic data suggest that the DAF-28/insulin-like peptide normally activates the DAF-2/insulin-like receptor to promote reproductive growth (Li *et al.* 2003). Levels of a *daf-28* transgene changes according to feeding status and levels of dauer pheromone, suggesting that expression of *daf-28* may be regulated by environmental cues, as would be expected for a dauer regulator (Li *et al.* 2003). Over-expression of the *ins-4* or *ins-6* insulin-like peptide encoding genes can partially or fully suppress the *daf-28* mutation, suggesting that at high levels, INS-4 and INS-6 can functionally substitute for DAF-28 and activate DAF-2 (Li *et al.* 2003). Loss of *ins-1*, which encodes a protein most similar to mammalian insulin, and *ins-9* have no affect on dauer formation or longevity. Over-expression of *ins-1*, however, causes a low level of dauer arrest. Enhanced dauer formation is seen when *ins-1* is overexpressed in a *daf-2* and *daf-7* mutant background; this phenotype is similar to that seen in some of the *daf-2* alleles (Pierce *et al.* 2001). Over-expression of *ins-9* has no effect unless in a *daf-2* mutant background, whereby it causes embryonic and/or larval arrest. These data suggest that INS-1 and INS-9 can antagonize DAF-2 activity (Pierce *et al.* 2001).

Over-expression of several insulin-like peptides that are not expressed in ASI and ASJ can also cause dauer formation. For instance, over-expression of *ins-18* causes a low level of dauer arrest, and this dauer arrest is enhanced in a *daf-2* mutant

Table 3. Neuropeptide-like peptide (*nlp*) encoding genes

Gene[#]		Encoded peptides[@]	Expression pattern	Function
nlp-1	×3	*MDANAFRMSFG MDPNAFRMSFG *VNLDPNSFRMSFG	ASI, AWC, PHB, BDU, 4 head neurons, intestine	
nlp-2		SIALGRSGFRPG SMAMGRLGLRPG	1 head neuron, secretory cells near vulva, intestine	
	×3	SMAYGRQGFRPG		
nlp-3		AINPFLDSMG AVNPFLDSIG YFDSLAGQSLG	ADF, ASE, ASH, AWB, ASJ, BAG, HSN, I1, I2, I3, I4, MI, M3, NSMR, 3 head neurons, VNC, occ. I6, M2, pm1VL, intestine	
nlp-4		SLILFVILLVAFAAARPVSEEVDRV DYDPRTEAPRRLPADDDEVDGEDRV DYDPRTDAPIRVPVDPEAEGEDRV		
nlp-5		SVSQLNQYAGFDTLGGMGLG ALSTFDSLGGMGLG ALQHFSSLDTLGGMGFG	ASI, 2 head neurons, spermatheca; 1 male tail neuron	
nlp-6		*(MA)APKQMVFGFG YKPRSFAMGFG AAMRSFNMGFG LIMGLG	ASI, IL1, 2 head neurons, 1 tail neuron, intestine	
nlp-7		*LYLKQADFDDPRMFTSSFG SMDDLDDPRLMTMSFG MILPSLADLHRYTMYD	ADL, AFD, ASE, ASI, PHA, VNC, 4 head neurons, 2 RVG neurons	
nlp-8		AFDRFDNSGVFSFGA AFDRMDNSDFFGA *SFDRMGGTEFGLM YPYLIFPASPSSGDSRRLV	ASK, ADL, 6 head neurons, 2 tail neurons, I2, g1D, pm5L, pm5R, 2 RVG, processes in pharynx, intestine; HOB	
nlp-9		GGGRAFNHNANLFRFD GGGRAFAGSWSPYLE TPIAEAQGAPEDVDDRRELE	ASI, AWB, 4 head neurons, 1 tail neuron, VNC, spermatheca, vulval muscles, intestine	
nlp-10		AIPFNGGMYG STMPFSGGMYG AAIPFSGGMYG GAMPFSGGMYG	ASK, ADL, CAN, 2 lateral neurons, 1 tail neuron, 2 ant. pharyngeal neurons; 1 male tail neuron	
nlp-11		HISPSYDVEIDAGNMRNLLDIG SAPMASDYGNQFQMYNRLIDAG *SPAISPAYQFENAFGLSEALERAG	IL1, 2 head neurons, VNC, PVD, 3 tail neurons, precomma embryos	

nlp-12	×2	DYRPLQFG DGYRPLQFG	1 tail neuron
nlp-13		NDFSRDIMSFG SGNTADLYDRRIMAFG QPSYDRDIMSFG	3 head neurons, NSM, M2, I4, spermatheca, LUA, 1 tail cell, dorsal and ventral hypoderm, intestine
	*	SAPSDFSRDIMSFG	
	*	SSSMYDRDIMSFG	
	*	SPVDYDRPIMAFG	
		AEDYERQIMAFG	
nlp-14	×2	ALDGLDGSGFGFD	ASI, ASK, and another amphidial neuron, PHA, VNC, 2 RVG neurons, intestine
	×5	ALNSLDGAGFGFE	
	×3	ALDGLDGAGFGFD	
	*	ALNSLDGQGFGFE	
	×3	ALNSLDGNGFGFD	
nlp-15		AFDSLAGSGFDNGFN	ASH, CAN, HSN, BDU, 5 head neurons, VNC, 3 RVG neurons, 1 tail neuron, intestine
	×2	*AFDSLAGSGFGAFN	
		AFDSLAGSGFSGFD	
		AFDSLAGQGFTGFE	
		AFDTVSTSGFDDFKL	
nlp-16		STEHHRV	7 head neurons, 1 lateral neuron, intestine
		SEGHPHE	
		ATHSPEGHIVAKDDHHGHE	
		SSDSHHGHQ	
		SVDEHHGHQ	
		NAEDHHEHQ	
		SEHVEHQAEMHEHQ	
		STQEVSGHPEHHLV	
nlp-17	*	GSLSNMMRIG	
		QQEYVQFPNEGVVPCESCNLGTLMRIG	
nlp-18	*	SPYRAFAFA	ASI, 4 head neurons, 2 tail neurons, spermatheca, NSM, 2 anterior pharyngeal neurons, rectal gland, intestine
		ARYGFA	
	*	SPYRTFAFA	
		ASPYGFAFA	
		SDEENLDFLE	
nlp-19		IAGLRLPNFL	4 head neurons, VNC in males, NSM, 4 posteior pharyngeal neurons, spermatheca
		IGLRLPNML	
		MGMRLPNIIFL	
nlp-20		FAFAFA	4 head neurons, 4 tail neurons, spermatheca, intestine, 1 anterior pharyngeal neuron
		SGPQAHEGAGMRFAFA	
		APKEFARFARASFA	

Table 3. (*Cont.*)

Gene[#]		Encoded peptides[@]	Expression pattern	Function
nlp-21		GGARAMLH GGARAFSADVGDDY GGARAFYDE	AFD, 5 head neurons, VNC, 1 anterior pharyngeal neuron, 1 tail neuron, embryo, intestine	
	*	GGARAFLTEM		
	*	GGARVFQGFEDE		
		GGARAFMMD GGGRAFGDMM GGARAFVENS (GGGRSFPVKPGRLDD) pQYTSELEEDE		
nlp-22		SIAIGRAGFRPG		
nlp-23		LYISRQGFRPA SMAIGRAGMRPG AFAAGWNRG	tail, dorsal and ventral hypoderm	
nlp-24		QWGGGPYGGYGPRGYGGGYGGG YGGYGGRGPYGGYGGRGPYGYGG GPYGGGGLVGALLG	ASI, spermatheca, 1 pharyngeal neuron	anti-microbial?
nlp-25		QWGGGYGNPYGGYG GGGYGGGYGGGFGAQQAYNVQNAA		anti-microbial?
nlp-26		QFGFGGQQSFGGRGGQFGGMQRGGFNGN GGFGQQSQFGGRGGNQFGG GGSQFNGRGGNQFGGRGGFGFG	hypoderm	
nlp-27		QWGYGGMPYGGYGGMGGYGMGGYGMGY MWGSPYGGYGGYGGYGGWG	ASI, 3 head neurons, spermatheca, hypoderm, intestine	anti-microbial?
nlp-28		QWGYGGYGRGYGGYGGYGRGMYGGYG GMYGGYGRGMYGGWG		anti-microbial?
nlp-29		QWGYGGYGRGYGGYGGYGRGMYGGYG GMYGGYGRGMYGGYGRGMYGGWG	hypoderm, intestine	anti-microbial?
nlp-30		QWGYGGYGRGYGGYGGYGRGYGGYG GYGGYGRGMWGRPYGGYGWG	hypoderm	anti-microbial?
nlp-31		QWGYGGYGRGYGGYGGYGRGYGGYGGYG GYGGYGRGMYGGYGRPYGGYGWG	hypoderm, embryos	anti-microbial
nlp-32		YGGWGGRGGWGRGGGRGYGG GGGWGGRGGGWGRGGGGRGFYGGG		anti-microbial?
nlp-33		QWGYGGPYGGYGGGYGGGPWGYGGGW RHWGGYGGGPWGGYGGGPWGGYY	hypoderm	anti-microbial?
nlp-34		PYGYGGYGGW PYGYGWG		
nlp-35	*	AVVSGYDNIYQVLAPRF		

nlp-36	*		SMVARQIPQTVVADH
nlp-37	*	*	NNAEVVNHILKNFGALDRLGDVG
nlp-38			VLGWNKAHGLWG
		*	TPQNWNKLNSLWG
		*	SPAQWQRANGLWG
nlp-39	*		EVPNFQADNVPEAGGRV
nlp-40	*	*	APSAPAGLEEKL(R)
nlp-41	*	*	APGLFELPSRSV
nlp-42			SALLQPENNPEWNQLGWAWG
			NPDWQDLGFAWG

Genes for which ESTs, ORFeomes (OST), cDNAs, or encoded peptides have been isolated are in bold. @ Common sequences among peptides encoded by the same gene are underlined. Number of copies of peptide encoded by gene indicated. A C-terminal glycine donates an amide group during amidation. Some *nlp* peptide predictions have been revised. * Peptides have been biochemically isolated. From Li *et al*. 1999; Nathoo *et al*. 2001; Couillault *et al*. 2004; Husson *et al*. 2005.

background (Pierce *et al*. 2001). Over-expression of *ins-31* and *ins-19* in combination in a *daf-2* mutant background causes embryonic and/or larval arrest, whereas no phenotype is seen when over-expression is performed in a wild-type background (Pierce *et al*. 2001). Like INS-1, INS-18 may function to antagonize the activity of DAF-2 or to down-regulate *daf-2* to promote dauer formation (Pierce *et al*. 2001), while INS-31/INS-19 may signal through DAF-2 to affect other aspects of development. These data indicate that several insulin-like ligands signal through or affect DAF-2 activity to affect developmental growth and dauer formation, while other INS ligands signal through other non-DAF-2 insulin receptors.

The flp *family*

Although FMRFamide was first isolated as a molluscan cardioactive agent (Price & Greenberg, 1977), a large family of FMRFamide-related peptides have now been isolated from throughout the animal kingdom, where the peptides have various effects, including on muscle activity (Maule *et al*. 1996; Brownlee, Holden-Dye & Walker, 2000; Rogers *et al*. 2001; Moffett *et al*. 2003), pain modulation (Tang, Yang & Costa, 1984; Roumy & Zajac, 1998), and feeding (Dockray, 2004). The strategy that our lab has taken to determine the function of the different *flp* genes is to inactivate each gene genetically. Although RNA-mediated interference (RNAi) is a possible strategy to inactivating genes, RNAi of genes expressed in neurons is inefficient, and many times the RNAi phenotype does not match the phenotype of genetic mutants (Simmer *et al*. 2002). Thus far, deletion mutants have been isolated for eleven *flp* genes (*flp-1*, *3*, *4*, *6*, *8*, *9*, *10*, *12*, *19*, *20* and *21*; Nelson, Rosoff & Li, 1998; C. Li unpublished observations). Analysis of these deletion mutants has indicated that the *flp* genes are involved in locomotion (Nelson, Rosoff & Li, 1998; C. Li unpublished observations), reproductive behaviour (Waggoner *et al*. 2000; M. Barr, personal communication; C. Li unpublished observations), and fat storage (K. Ashrafi, personal communication). For instance, inactivation of *flp-1* causes several defects, including hyperactive movement (Nelson, Rosoff & Li, 1998), defects in the timing of egg laying (Waggoner *et al*. 2000), thereby causing a decreased number of eggs laid (C. Li unpublished observations), and decreased fat stores (K. Ashrafi, personal communication). FLP-1 peptides are also necessary for down-regulation of egg laying in the absence of food (Waggoner *et al*. 2000). *flp-9* mutants were found to swim significantly less than wild-type animals (C. Li unpublished observations). Several of the deletion mutants have defects in male reproductive behaviour (M. Barr, personal communication). The function of *flp-18* and *flp-21* will be discussed in conjunction with the function of their

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receptor, NPR-1. Most of the *flp* mutants, however, do not have any obvious behavioural phenotypes, and are now being re-screened on new assays that test for more subtle phenotypes.

Analysis of genetic mutants is being complemented by pharmacological studies in *C. elegans* and closely related nematodes. Using a *C. elegans* pharyngeal preparation, Rogers and co-workers (2001) applied different FLPs onto pharyngeal muscle to examine their effects on muscle activity. Surprisingly, a large number of FLPs affected the activity of the pharyngeal muscle. Peptides encoded by four genes, *flp-5*, *6*, *8* and *14*, increased the frequency of action potentials, whereas peptides from five genes, *flp-1*, *3*, *9*, *13* and *16*, decreased the frequency (Rogers *et al*. 2001). Several of these *flp* genes are not expressed in the pharyngeal nervous system, suggesting that either some peptides can act hormonally or the expression patterns for the *flp* genes are incomplete.

Because many of the FLPs have been also found in parasitic nematodes (Cowden *et al*. 1989; Cowden & Stretton, 1993, 1995; Keating *et al*. 1995; Marks *et al*. 1999*b*; McVeigh *et al*. 2005), researchers have used a number of parasite muscle systems to examine the physiological effects of the different FLPs. Application of peptides encoded by 20 *flp* genes have a range of effects on body wall, reproductive and pharyngeal muscle of *Ascaris suum* (Bowman *et al*. 1996; Fellowes *et al*. 1998; Davis & Stretton, 2001; Bowman *et al*. 2002; Moffett *et al*. 2003; for reviews see Maule *et al*. 1996; Brownlee *et al*. 1996; Brownlee & Walker, 1999; Brownlee *et al*. 2000). Some of the peptides appear to have parallel functions in *C. elegans* and *Ascaris*. For instance, deletion of *flp-1* in *C. elegans* causes animals to become hyperactive, suggesting that FLP-1 peptides have inhibitory effects on locomotion (Nelson, Rosoff & Li, 1998). Similarly, FLP-1 peptides have inhibitory effects on *Ascaris* body wall muscle (Holden-Dye *et al*. 1995; Maule *et al*. 1995). Some peptides behave slightly differently in the two nematodes. KSAYMRFamide is excitatory on *C. elegans* pharyngeal muscle (Rogers *et al*. 2001), but elicits a transient excitatory, followed by a long-lasting inhibitory response on *Ascaris* pharyngeal muscle (Brownlee *et al*. 1995). This biphasic response may be due to activation of more than one FLP receptor. Clues into the behaviours in which different FLPs are involved in *C. elegans* can be rapidly assessed in related nematodes, particularly *Ascaris*. Overall, the number of FLPs that can elicit physiological effects is striking and highlights the complex and intricate ways that different FLPs can modulate synaptic and muscle activity.

The nlp *family*

Although no *nlp* mutant has been examined thus far, the widespread expression of the *nlp* genes suggests that, like the FLP neuropeptides, the NLPs are involved in multiple behaviours (Nathoo *et al*. 2001). In particular, many of the genes are expressed in chemosensory neurons, which mediate responses to the environment, and the HSN neuron, which regulates egg-laying. The genes have also been implicated as anti-microbial peptides. In microarray analyses to identify genes whose expression levels are changed in response to fungal and/or bacterial insults, expression of three *nlp* genes, *nlp-29*, *31* and *33*, was induced (Couillault *et al*. 2004; Table 3). Furthermore, the peptide encoded by *nlp-31* has anti-microbial activity and protects against fungal infection (Couillault *et al*. 2004). *nlp-29*, *31* and *33* are all expressed in the hypoderm and not in any neuronal tissue (Nathoo *et al*. 2001; Couillault *et al*. 2004), as would be expected for peptides serving only as anti-microbial agents. The peptides encoded by *nlp-24*, *25*, *27*, *28* and *30* are similar to *nlp-29*, *31* and *33*, suggesting that these peptides may also have anti-microbial functions (Couillault *et al*. 2004). Moreover, a few of the genes, namely *nlp-24* and *nlp-27*, are also expressed in neurons (Nathoo *et al*. 2001), suggesting that these peptides may have additional functions.

NEUROPEPTIDE RECEPTORS

A parallel method towards determining the function of the different neuropeptides is to inactivate the receptor(s) through which the peptides signal. This strategy circumvents the problem when multiple peptides bind to the same receptor and have functional overlap. Inactivation of a specific peptide, for instance, may not reveal a phenotype, whereas inactivation of the receptor may give insights into the function of the peptides.

DAF-2, a tyrosine kinase receptor, is most similar to the mammalian insulin-like receptor and was the only insulin-like receptor identified for several years (Kimura *et al*. 1997). As discussed above, inactivation of *daf-2* leads to constitutive dauer formation (Riddle & Albert, 1997), increased longevity (Kenyon *et al*. 1993), and increased body size (McCulloch & Gems, 2003). The primary ligand of DAF-2 appears to be DAF-28 (Li *et al*. 2003), although INS-4 and INS-6 can also activate DAF-2 if expressed at high levels (Pierce *et al*. 2001). Given the plethora of insulin-like peptides, however, the dearth of insulin-like receptors was somewhat surprising. More recently, Dlakic (2002) used different search paradigms to uncover a family of 56 divergent insulin-like, tyrosine kinase receptors. Many of the receptors are clustered and appear to result from recent gene duplications (Dlakic, 2002). Presumably, some of the insulin-like peptides signal through these receptors to affect dauer formation and other processes.

As with the insulin receptors, many FLP receptors have been isolated from other systems. By contrast

to the insulin receptors, however, the FLP receptors are G protein-coupled receptors (Tensen *et al*. 1998; Bonini *et al*. 2000; Cazzamali & Grimmelikhuijzen, 2002; Meeusen *et al*. 2002; Duttlinger, Mispelon & Nichols, 2003), with the exception of a molluscan FMRFamide-gated amiloride-sensitive channel, which has homology to the MEC-4 and MEC-6 mechanoreceptors (Lingueglia *et al*. 1995). Of the 1000 G protein-coupled receptors in *C. elegans*, over 50 are candidate neuropeptide receptors (Bargmann, 1998). Keating and co-workers (2003) used RNAi to inactivate sixty G protein receptors that were predicted to bind either a small molecule transmitter or a neuropeptide. Inactivation of six receptors, C16D6.2, C25G6.5, C26F1.6, F35G8.1, F41E7.3, and F59C12.2, affected brood size by either increasing or decreasing the number of progeny (Keating *et al*. 2003). Disruption of eight receptors, AC7.1 (tachykinin-like), C15B12.5, C10C6.2, C24A8.4, F15A8.5, F59D12.1, T02E9.1, and T05A1.1, affected the movement of the animals (Keating *et al*. 2003). The phenotypes from the RNAi data were confirmed in two cases by the isolation of deletion mutants for T05A1.1 and F35G8.1 (Keating *et al*. 2003). Several of the ligands for these receptors have now been identified (see below). No NLP receptors have been identified thus far.

Given the large number of G protein-coupled receptors and the even larger set of FLP ligands, several groups have developed high throughput methods to match FLP ligands to specific G protein-coupled receptor binding partners. Specifically, candidate receptors are expressed in either heterologous cells or *Xenopus* oocytes, FLP ligands are applied singly or in combination, and different assays are used as the readout (Kubiak *et al*. 2003*a*, *b*; Lowery *et al*. 2003; Rogers *et al*. 2003; Mertens *et al*. 2004, 2005; Table 2). Using Chinese hamster ovary (CHO) cells, the Upjohn/Pharmacia group (Kubiak *et al*. 2003*a*, *b*; Lowery *et al*. 2003) transfected candidate receptors and chimeric G proteins and screened for ligand-induced GTPγS binding to membranes of transfected cells. By this method, *flp-15* peptides were matched to C10C6.2 (Kubiak *et al*. 2003*b*), *flp-18* peptides to C16D6.2, F41E7.3, Y58G8a.1, C53C7.1, and C25G6.5 (Lowery *et al*. 2003), *flp-3* peptides to C53C7.1 (Lowery *et al*. 2003), and FLP-21 to C25G6.5 (Lowery *et al*. 2003). FLPs encoded by other genes also bind to C16D6.2 and C10C6.2 (Lowery *et al*. 2003). Mertens and coworkers (2004, 2005) expressed candidate receptors and $G_{\alpha 16}$ in human embryonic kidney (HEK) or CHO cells and screened for an increased calcium response, as monitored by an increase in fluorescence. Their group identified that FLPs encoded by *flp-7* and *flp-11* bind to C26F1.6 (Mertens *et al*. 2004), and FLP-2 peptides bind to two isoforms of T19F4.1 (Mertens *et al*. 2005). Given that a receptor can bind to multiple FLPs encoded by distinct genes and a single FLP can bind to multiple receptors, the potential complexity of peptide actions in *C. elegans* is enormous.

The NPR-1 receptor is a G protein-coupled receptor that is homologous to the mammalian neuropeptide Y receptor (de Bono & Bargmann, 1998). Disruption of the NPR-1 receptor affects aggregation behaviour (de Bono & Bargmann, 1998) and tolerance to alcohol (Davies *et al*. 2004). In the presence of ample food, wild-type animals feed alone (referred to as solitary feeding); a specific amino acid change in NPR-1 causes the animals to aggregate during feeding (referred to as social feeding) and accumulate at the edges of the bacteria (referred to as bordering behaviour; de Bono & Bargmann, 1998). The aggregation behaviour of *npr-1* mutants can be suppressed by mutations in *gcy-35* or *gcy-36* (Cheung *et al*. 2004), both of which encode soluble guanylate cyclases (Morton *et al*. 1999). Interestingly, GCY-35 guanylate cyclase binds oxygen, suggesting that the aggregation behaviour of *npr-1* mutants is related to oxygen levels in the local environment of the animals (Gray *et al*. 2004). No neuropeptide Y (NPY) homologue is present in the *C. elegans* genome. Because NPY has some sequence similarity to the FLPs, the de Bono group hypothesized that the NPR-1 ligand was a FLP and injected constructs for NPR-1 and an inwardly rectifying potassium channel into *Xenopus* oocytes; different FLPs were applied and receptor activation of the potassium channels was used as the readout (Rogers *et al*. 2003). Both Rogers *et al*. (2003) and Kubiak *et al*. (2003*a*) determined that NPR-1 binds to FLP-21; in addition, Rogers *et al*. (2003) found that peptides encoded by *flp-18* also activated NPR-1. Animals carrying mutations in *flp-21* display only mild aggregation defects compared to *npr-1* mutants (Rogers *et al*. 2003; M. de Bono, personal communication; C. Li unpublished observations), presumably because FLP-18 ligands can functionally substitute for loss of FLP-21 (Rogers *et al*. 2003). As with the other FLP receptors, NPR-1 is promiscuous in its binding to multiple FLP ligands produced by different *flp* genes.

CONCLUSIONS

The number of potential neuropeptides in *C. elegans* is immense and rivals the numbers found in mammals thus far. Although some of the peptides may be invertebrate specific, the insulin-like and FMRFamide-related peptides have counterparts in mammals. If the current EST databases are any guide, parasitic nematodes are likely to share the enormous neuropeptide diversity of *C. elegans*. In contrast to the mammalian systems, the diversity of the neuropeptides comes from utilizing similar motifs repeatedly. The ~70 FLPs, for instance, all share a common C-terminal Arg-Phe-amide and

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the N-terminal sequences are often also very similar. Many challenging tasks await future studies. Specifically, the problem of determining the functions of individual peptides is as overwhelming as matching the different peptide ligands to specific receptors. Nonetheless, it is apparent that despite the relatively 'simple' behaviours of nematodes, there is a large collection of neuropeptides available to mediate or modulate these behaviours.

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