Neuropeptides[*]

Chris Li[1][§], and Kyuhyung Kim[2], [1]Department of Biology, City College of New York [2]Department of Biology, Brandeis University, New York, NY 10031 USAWaltham, MA 02454 USA

Table of Contents

Abstract

The role of neuropeptides in modulating behavior is slowly being elucidated. With the sequencing of the *C. elegans* genome, the extent of the neuropeptide genes in *C. elegans* can be determined. To date, 113 neuropeptide genes encoding over 250 distinct neuropeptides have been identified. Of these, 40 genes encode insulin-like peptides, 31 genes encode FMRFamide-related peptides, and 42 genes encode non-insulin, non-FMRFamide-related neuropeptides. As in other systems, *C. elegans* neuropeptides are derived from precursor molecules that must be post-translationally processed to yield the active peptides. These precursor molecules contain a single peptide, multiple copies of a single peptide, multiple distinct peptides, or any combination thereof. The neuropeptide genes are expressed extensively throughout the nervous system, including in sensory, motor, and interneurons. In addition, some of the genes are also expressed in

---

[§]To whom correspondence should be addressed. Tel. (212) 650-8533, Fax: (212) 650-8585. E-mail: cli@sci.ccny.cuny.edu

non-neuronal tissues, such as the somatic gonad, intestine, and vulval hypodermis. To address the effects of neuropeptides on *C. elegans* behavior, animals in which the different neuropeptide genes are inactivated or overexpressed are being isolated. In a complementary approach the receptors to which the neuropeptides bind are also being identified and examined. Among the knockout animals analyzed thus far, defects in locomotion, dauer formation, egg laying, ethanol response, and social behavior have been reported. These data suggest that neuropeptides have a modulatory role in many, if not all, behaviors in *C. elegans.*

1. Introduction

Neuropeptides are short sequences of amino acids that function either directly or indirectly to modulate synaptic activity. In addition, neuropeptides may also function as primary neurotransmitters. As in mammalian systems, the number of predicted neuropeptides in *C. elegans* is well over one hundred (Li et al., 1999; Pierce et al., 2001); however, most of the neuropeptides fall into two large families: the insulin-like peptides (Pierce et al., 2001; Li et al., 2003) and the FMRFamide (Phe-Met-Arg-Phe-NH$_2$)-related peptides or FaRPs, which are referred to as FLPs in *C. elegans* (Li et al., 1998; Li, 2005). The remaining non-insulin, non-FLP peptides are classified as the neuropeptide-like proteins or NLPs. The NLPs are a diverse group of neuropeptides that have little similarity among each other (Nathoo et al., 2001). With a few striking exceptions, the functions of the different neuropeptides remain largely unknown in *C. elegans*. This overview serves to summarize the current state of the neuropeptide field.

2. Formation of mature neuropeptides

Neuropeptides are typically derived from larger precursor molecules, which undergo posttranslational processing and sometimes modifications to yield mature peptides (see Figure 1). A single neuropeptide precursor molecule can give rise to a single neuropeptide, multiple distinct neuropeptides, multiple copies of a single neuropeptide, or any combination thereof. As an additional mechanism to increase neuropeptide complexity in mammals, a single precursor molecule can be differentially cleaved to yield different sets of peptides in different cell types (Strand, 1999; Salio et al., 2006). Furthermore, in the mollusk *Aplysia* peptides from a single precursor molecule can be sorted into different nerve terminals (Sossin et al., 1990). Whether such differential processing and trafficking occurs in *C. elegans* is unclear.

2.1. Processing of neuropeptide precursor molecules

Like mammalian precursor molecules (Steiner, 1998), the initial cleavages in *C. elegans* occur C-terminal to dibasic residues flanking the peptide sequence (Rosoff et al., 1993; Marks et al., 1995, 1997, 1998, 1999a, 2001; Husson et al., 2005, 2006); however, cleavages C-terminal to mono- and tribasic residues have also been reported (Rosoff et al., 1993; Marks et al., 1997, 2001). The enzymes responsible for the initial endoproteolytic cleavage are kex2/subtilisin-like proprotein convertases, which must themselves be cleaved to become active. Cleavage of proprotein convertase is dependent on a chaperonin protein SBT-1 7B2 (Lindberg et al., 1998; Sieburth et al., 2005). Four *C. elegans* proprotein convertases, *kpc-1, egl-3/kpc-2*, *aex-5/kpc-3*, and *bli-4/kpc-4*, are present in *C. elegans* (Thacker and Rose, 2000). *egl-3/kpc-2* is expressed in many, but not all neurons in the nervous system (Kass et al., 2001). Loss of *egl-3/kpc-2* results in defects in egg-laying, mechanosensation, and locomotion (Kass et al., 2001; Jacob and Kaplan, 2003), indicating that EGL-3/KPC-2 cleaves precursors whose peptides have diverse functions. Moreover, using a polyclonal anti-FMRFamide antibody that recognizes the Arg-Phe-amide but not the non-amidated Arg-Phe-OH moiety (Marder et al., 1987), Kass and co-workers (2001) found decreased FMRFamide-like immunoreactivity in *egl-3/kpc-2* mutants, suggesting that *egl-3/kpc-2* cleaves some, but not all FLP precursors and that other proprotein convertases are active in the same cells. Among the other proprotein convertases, a *kpc-1* deletion mutant shows mild locomotory defects and slow growth, suggesting that KPC-1 cleaves precursors of peptides involved in movement and growth (Thacker and Rose, 2000). Mutations in *aex-5/kpc-3* cause defecation defects (Thomas, 1990). *aex-5/kpc-3* is expressed in muscle (Thacker and Rose, 2000), and has been proposed to cleave a precursor molecule in muscle to produce a peptide that serves as a retrograde signal to regulate exocytosis (Doi and Iwasaki, 2002). A major function of BLI-4/KPC-4 is to cleave procollagen into collagen so that it can be deposited in the cuticle to give the cuticle structural integrity. Hence, null alleles of *bli-4/kpc-4* cause lethality (Thacker et al., 1995). However, transcripts of *bli-4/kpc-4* are also expressed in the nervous system (Thacker et al., 1995; Thacker and Rose, 2000), although phenotypes associated with loss of *bli-4/kpc-4* neural transcripts are unknown. Collectively, these data indicate that multiple proprotein convertases are active in neurons.
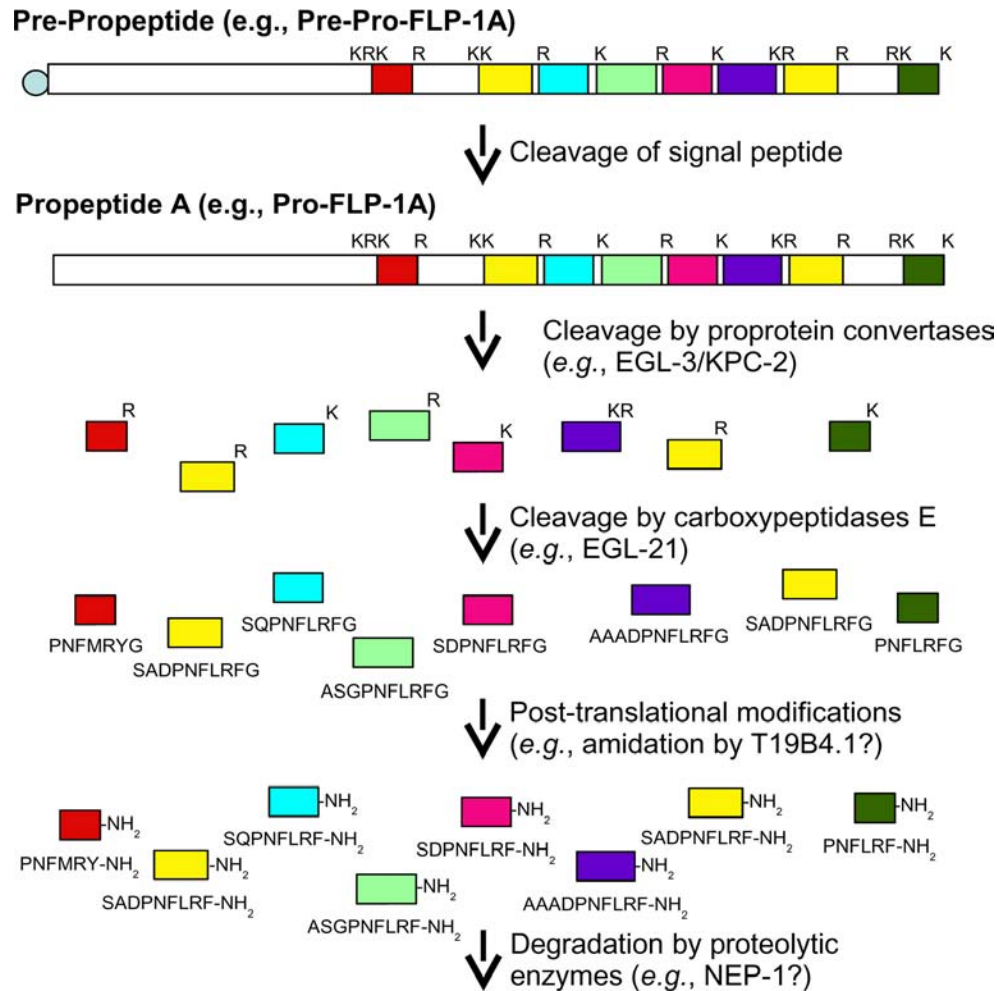
Figure 1. Processing of a neuropeptide gene product: *flp-1* **as an example.** After translation of the *flp-1A* transcript, pre-pro-FLP-1A is cleaved by signal peptidase in the endoplasmic reticulum to release the signal peptide. The propeptide pro-FLP-1A is further cleaved C-terminal to mono-, di-, or tribasic residues (indicated by K and R) by proprotein convertases, such as EGL-3/KPC-2. The basic amino acids are removed by carboxypeptidases E, such as EGL-21, to yield the basic neuropeptides. The FLP-1A peptides are further modified by the addition of an amide group donated from the C-terminal glycine, a reaction which may be catalyzed by T19B4.1, to yield the active peptides. After release, peptides are removed from the synaptic cleft by proteolytic degradation, which may be mediated by NEP-1. With the exception of PNFMRFYamide, all *flp-1A* encoded peptides have been biochemically isolated (Li, 2005).

A peptidomic approach was also taken to determine the relative contribution of each proprotein convertase in neuropeptide processing (Husson et al., 2006). FLP and NLP peptides were isolated from wild type and different proprotein convertase mutants. *kpc-1(gk8)* and *bli-4(e937)/kpc-4* mutants showed a similar peptide profile as wild type, suggesting that their contribution to peptide processing is minor or that the alleles examined, such as *bli-4/kpc-4(e937)*, do not completely remove gene function. For two alleles of *egl-3*(*n729* and *gk238*)/*kpc-2* no peptides were isolated (Husson et al., 2006), confirming previous work that EGL-3/KPC-2 is the major active proprotein convertase in neurons (Kass et al., 2001). However, as previously reported (Kass et al., 2001), a residual level of FMRFamide-like immunoreactivity remained. Surprisingly, a much decreased peptide profile was seen in *aex-5(sa23)/kpc-3* mutants (Husson et al., 2006), providing strong evidence that *aex-5/kpc-3* is also responsible for neuropeptide precursor processing in neurons.

Once the precursor molecules are cleaved by the proprotein convertases, the basic residues themselves are removed from the peptide sequences by the activity of carboxypeptidase E. *egl-21* encodes a neural-specific carboxypeptidase E that is expressed in about 60% of the neurons (Jacob and Kaplan, 2003). Loss of *egl-21* carboxypeptidase E causes more severe phenotypes than those seen in the *egl-3/kpc-2* proprotein convertase mutants. *egl-21* null alleles show defects in egg laying, locomotion, mechanosensation, and defecation as well as extremely low levels of FMRFamide-like immunoreactivity (Jacob and Kaplan, 2003). These data suggest that cleavage by EGL-21, like EGL-3/KPC-2, yields peptides that function in multiple behaviors and that EGL-21 is one

of the carboxypeptidases E that cleaves FLP precursor molecules. Two other carboxypeptidases are present in the *C. elegans* genome, but their roles in neuropeptide processing have not been investigated (Jacob and Kaplan, 2003).

To protect themselves from degradation, neuropeptides are commonly modified at the N- or C-terminus. In many circumstances, the modification also confers biological activity to the neuropeptide, including in *C. elegans* (Schinkmann and Li, 1992). The most common known modification in *C. elegans* is amidation. Based on the presence of a C-terminal glycine, which donates an amino group in the amidation process, all of the FLPs and many of the NLPs are likely to be amidated. In mammals two enzymes, peptidylglycine-alpha-hydroxylating monooxygenase (PHM) and peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL) act sequentially to catalyze amidation; the enzymes are synthesized on the same molecule as adjacent domains on a bifunctional protein, peptidyl-α-hydroxyglycine α-amidating lyase (PAM; Eipper et al., 1993). *C. elegans* contains at least one PAM-like and one PHM molecule (Han et al., 2004). Decreased activity of T19B4.1, which encodes a monooxygenase, leads to resistance to the acetylcholinesterase inhibitor aldicarb, suggesting that T19B4.1 may be a PAM-like molecule that processes neuropeptides (Sieburth et al., 2005). Whether T19B4.1 and/or other enzymes are involved in neuropeptide amidation has not been determined.

2.2. Neuropeptides are released from dense core vesicles

Bioactive neuropeptides are located in dense core vesicles derived from the trans-Golgi network. By contrast, many of the classical small molecule transmitters are located in small, clear vesicles that are clustered at the synaptic zones. The processing of the neuropeptide precursor molecules starts in the endoplasmic reticulum with the removal of the signal peptide and continues in the Golgi complex and the dense core vesicles themselves as the vesicles are transported to the nerve terminal (Strand, 1999). UNC-104 kinesin is necessary for the transport of small, clear vesicles in *C. elegans* (Hall and Hedgecock, 1991) and can also function as the motor for dense core vesicles. Mutations in *unc-104*, for instance, cause an increase of FMRFamide-like immunoreactivity in neuronal cell bodies (Schinkmann, 1994; Jacob and Kaplan, 2003). Furthermore, fast, but not slow, anterograde transport of IDA-1, a transmembrane protein localized to dense core vesicles, is lost in *unc-104* mutants, suggesting that at least two distinct motors can transport dense core vesicles (Zahn et al., 2004). Disruption of *unc-116*, which encodes a kinesin molecule distinct from UNC-104, decreases overall FMRFamide-like immunoreactivity (Schinkmann, 1994), suggesting that UNC-116 kinesin also plays a role in dense core vesicle trafficking.

In contrast to small clear vesicles, dense core vesicles are not localized at synaptic zones, but are more diffusely scattered around the nerve terminal (Strand, 1999; Salio et al., 2006). Whereas contents in small clear vesicles can be released by focal increases of calcium at the synaptic zone, release of neuropeptides from dense core vesicles appears to be dependent on a general increase of calcium throughout the nerve terminal, which can occur after high levels of stimulation (Strand, 1999; Salio et al., 2006). The exact mechanism for dense core vesicle movement to the cell membrane is unknown. Some mechanisms may be conserved for small clear and dense core vesicles. For instance, in *C. elegans* UNC-13 is necessary to prime both types of vesicles for release (Richmond et al., 1999; Sieburth et al., 2007). A cytoplasmic protein that promotes vesicle release by bridging between dense core vesicles and the plasma membrane is calcium-dependent activator protein (CAPS; Renden et al., 2001; Grishanin et al., 2002). Similarly, *C. elegans* UNC-31 CAPS also promotes dense core vesicle release (Sieburth et al., 2007); its activity appears to be modulated by IDA-1 (Cai et al., 2004). Mutations in *pkc-1* protein kinase I cause increased punta fluorescence of neuropeptide precursor molecules, but not of GFP-SNB-1 puncta associated with synaptic vesicles, suggesting that PKC-1 is specifically necessary for dense core vesicle release (Sieburth et al., 2007). PKC-1 is expressed in the cholinergic ventral cord motor neurons and not the GABAergic motor neurons (Sieburth et al., 2007), indicating that other protein kinases function to promote dense core vesicle release in the GABAergic motor neurons. After release of the vesicle's contents, neuropeptides are cleared from the cleft by the action of proteolytic enzymes, one of which may be NEP-1 neprilysin (Sieburth et al., 2005). Hence, unlike small molecule transmitters, which can be recycled and re-loaded into synaptic vesicles, neuropeptides must be synthesized *de novo* in the cell body and transported down to the axon terminal.

In addition to their release at synapses, neuropeptides also act as hormones, i.e., as long range signaling molecules. Some of the first isolated mammalian neuropeptides, for instance, were hormones released from the pituitary, adrenal glands, and the gut (Strand, 1999). Similarly, neuropeptides in *C. elegans* released from neurons or non-neuronal cells (see below) may act as hormones (see below). To monitor expression of neuropeptides into the pseudocoelom, Sieburth et al. (2007) took advantage of the scavenger activity of the coelomocytes, which continuously endocytose fluid from the pseudocoelom (Fares and Grant, 2002), and monitored release of GFP-tagged neuropeptide precursors into the pseudocoelom by the appearance of GFP in the coelomocytes.

3. Immunocytochemical localization of neuropeptides

Before the sequencing of the *C. elegans* genome, one common method to identify neuropeptide candidates was to use different antibodies from the mammalian field to stain the *C. elegans* nervous system. Initial work reported immunoreactivity for cholecystokinin, Substance P, melanocyte-stimulating hormone, met-enkephalin, beta-endorphin, and possibly adrenocorticotopic hormone (S. McIntire, pers. comm.). Immunoreactivity was also detected for FMRFamide (Schinkmann and Li, 1992), which was initially isolated from invertebrates (Price and Greenberg, 1977) but for which related peptides were later found in mammals (Dockray, 2004). Subsequent sequencing of the *C. elegans* genome (*C. elegans* Sequencing Consortium, 1998) indicated that with the exception of Substance P- and FMRFamide-related peptides, none of these mammalian peptide families have been identified in *C. elegans* (Nathoo et al., 2001). Because of the similarity to the FLP neuropeptides, anti-cholecystokinin antibodies are likely to have cross-reacted with FLPs. The antigens to which the other mammalian antisera cross-reacted are unknown.

4. Identification of putative neuropeptide genes

With the completion of the *C. elegans* genome, researchers were able to scan the genome for candidate genes encoding neuropeptides. Certain groups focused on specific neuropeptide families. For instance, several groups collectively identified forty genes that encode insulin-like molecules (see Table 1; Duret et al., 1998; Gregoire et al., 1998; Kawano et al., 2000; Pierce et al., 2001; Li et al., 2003). Our lab group identified twenty-four *flp* genes (*flp-1* to *flp-23* and *flp-28*) encoding peptides with a C-terminal RFamide moiety by cDNA isolation and BLAST searches (see Table 2; Li et al., 1998; Kim and Li, 2004; unpubl. obs.), while McVeigh and co-workers (2005; A. Maule, pers. comm.) used EST data mining to identify five additional *flp* genes, *flp-24* to *flp-27* and *flp-32* (see Table 2). Husson and Schoofs (2007) identified *flp-33* by isolating a FLP not encoded by any previously identified *flp* gene (see Table 2). Some of the *flp* genes also encode non-FLP peptides (see Table 2). Hart and co-workers used similarity and pattern-based scans in more general BLAST screens to identify other neuropeptide genes (Nathoo et al., 2001). Using the characteristics of neuropeptide precursor processing, the pattern-based scans were designed to search for peptide sequences that were flanked by mono- or dibasic sequences. 34 non-insulin-like, non-FLP-like genes were identified and are referred to collectively as the *neuropeptide-like protein* or *nlp* genes (Li et al., 1999; Nathoo et al., 2001; A. Hart, pers. comm.); an additional eight *nlp* genes were recently identified in a different screen and using peptidomic analysis (see Table 3; Couillault et al., 2004; Husson et al., 2005). A total of 113 neuropeptide genes encoding over 250 putative neuropeptides have now been identified in *C. elegans* (see Tables 1, 2 and 3).

What is striking about the neuropeptide genes is how many of them are clustered on a chromosome, suggesting that they arise from tandem gene duplications. In some cases, the neuropeptide genes are only a few hundred or thousand base pairs apart. For instance, within about 25,000 bp on chromosome I there are seven *ins* genes, *ins-24* through *ins-30*, which do not appear to be part of an operon. Similarly, many of the *nlp* genes are also physically close (Nathoo et al., 2001). While the *flp* genes are clustered on certain chromosomes, particularly the X chromosome, only a few (e.g., *flp-2, 3*, and *28*) are as physically close as some of the *ins* genes.

Because many of the *flp* neuropeptide genes encode multiple, distinct peptides, a nomenclature was developed to designate the distinct peptides produced by one gene. Each peptide is now designated by the gene name and a number. For instance, the *flp-1* gene encodes eight distinct peptides designated as FLP-1-1, FLP-1-2, etc. (see Table 4). As more of the NLP and INS peptides are isolated and their sequences confirmed, these peptides may also be given specific designations.

Table 1. Neuropeptide genes encoding insulin-like peptides in *C. elegans*

Gene[#] Cosmid LG	Putative peptides	Expression Pattern	Function or Phenotype	Receptor	Refer-ences[##]
daf-28 Y116F11B.1 V	VPGVAVRACGRRLVPYVWSVCGDACEPQ EGIDIATQCCTYQCTAEYIQTACCPRLL	ASI, ASJ, PQR, other neurons, hindgut, pharyngeal muscle, hypodermis	promotes repro-ductive growth	DAF-2	1
ins-1 F13B12.5 IV	SIRLCGSRLTTTLLAVCRNQLCTGLTAF GGIATECCEKRCSFAYLKTFCCNQDDN	ASI, ASJ, ASH, ADF, AIA, AIM, ASE, ASG, AWA, BAG, NSM, intestine, vulval muscles	DAF-2 antagonist?	DAF-2?	2, 3, 4
ins-2 ZK75.2 II	VQKRLCGRRLILFMLATCGECDTD SSEDLSHICCIKQCDVQDIIRVCCPNSFRK	amphidial, labial, ventral cord, & tail neurons, pharynx, vulva			2
ins-3 ZK75.3 II	GDKVKICGTKVLKMVMVMCGGECSS TNENIATECCEKMCTMEDITTKCCPSR	amphidial, labial, lateral, ventral cord, & dorsal projecting neurons			2
ins-4 ZK75.1 II	VPAGEVRACGRRLLLFVWSTCGEPCTPQ EDMDIATVCCTTQCTPSYIKQACCPEK	amphidial, labial, ventral cord, dorsal projecting, & tail neurons, hypodermis		DAF-2	2, 5
ins-5 ZK84.3 II	ADRHTNYRSCALRLIPHVWSVCGDACQPQ NGIDVAQKCCSTDCSSDYIKETCCPFD	amphidial, labial, ventral cord, lateral projecting, & tail neurons, vulva			2
ins-6 ZK84.6 II	VPAPGETRACGRKLISLVMAVCGDLCNPQ EGKDIATECCGNQCSDDYIRSACCP	amphidial, labial, ventral cord, & tail neurons		DAF-2	2
ins-7 ZK1251.2 IV	VPDEKKIYRCGRRIHSYVFAVCGKACESN TEVNIASKCCREECTDDFIRKQCCP	amphidial, labial, ventral cord, & tail neurons			2

Gene[#] Cosmid LG	Putative peptides	Expression Pattern	Function or Phenotype	Receptor	References[##]
ins-8 ZK1251.11 IV	VPEQKNKLCGKQVLSYVMALCEKACDSN TKVDIATKCCRDACSDEFIRHQCCP	amphidial, labial, ventral cord, & tail neurons, vulva			2
ins-9 C06E2.8 X	TLETEKIYRCGRKLYTDVLSACNGPCEPG TEQDLSKICCGNQCTFVIRKACCADKL	ASI, ASJ	Overexpression causes embryonic and larval arrest	DAF-2?	2
ins-10 T08G5.12 V	AFPFQICVKKMEKMCRIINPEQCAQVNKITEI GALTDCCTGLCSWEEIRISCCSVL				2
ins-11 C17C3.4 II	APHHDKRHTACVLKIFKALNVMCNHEGDAD VLRRTASDCCRESCSLTEMLASCTLTSSEESTRDI	labial, ventral cord, & tail neurons			2
ins-12 C17C3.19 II	APSHEKTHKKCSDKLYLAMKSLCSYRGYSE FLRNSATKCCQDNCEISEMMALCVVAPNFDDDLLH				2
ins-13 C17C3.18 II	NKCOYSKKKYKICGVRALKHMKVYCTRGMTRD YGKLLVTCCSKGCNAIDIQRICL				2
ins-14 F41G3.16 II	SEDIKCDAKFISRITKLCIHGITED KLVRLLTRCCTSHCSKAHLKMFCTLKPHEEEPHHEI				2
ins-15 F41G3.17 II	GNDFQPRDNKHHSYRSCGESLSRRVAFLCNGGAIQT EILRALDCCSTGCTDKQIFSWCDFQI				2
ins-16 Y39A3A.5 III	RELKRCSVKLFDILSVICGTESDAE ILQKVAVKCCQEQCGFEEMCQHANLKIDKI				2
ins-17 F56F3.6 III	GSLKLCPPGGASFLDAFNLICPMRRRRR SVSENYNDGGGSLLGRTMNMCCETGCEFTDIFAICNPFG				2
ins-18 T28B8.2 I	ISLQQADGRMKMCPPGGSTFTMAWSMSCSMRR KRALIAPSIRQLQTICCQVGCNVEDLLAYCAPI	amphidial, ventral cord, tail, & pharyngeal neurons	DAF-2 antagonist?	DAF-2?	2

Gene[#] Cosmid LG	Putative peptides	Expression Pattern	Function or Phenotype	Receptor	References[##]
ins-19 T10D4.13 II	YIIDSSESYEVLMLFGYKRTCGRRLMNRINRVCVKDID PADIDPKIKLSEHCCIKGCTDGWIKKHICSEEVLNFGFFEN		Overexpression causes larval arrest	DAF-2?	2
ins-20 ZK84.7 II	KEPKHHHHHHRHKGYCGVKAVKKLKQICPDLCSNVDD NLLMEMCSKNLTDDDILQRCCPE				2
ins-21 M04D8.1 III	SKSHSKKHVRFLCATKAVKHIRKVCPDMCLTGE EVEVNEFCRMGYSDSQIKYICCPE	amphidial, ventral cord, & tail neurons			2
ins-22 M04D8.2 III	MDAHTDKYVRTLCGKTAIRNIANLCPPKPEMKGICSTGE YPSITEYCSMGFSDSQIKFMCCDNQ	amphidial, labial, ventral cord, lateral process projecting, & tail neurons	Modulates acetyl-choline signaling		2, 6
ins-23 M04D8.3 III	QVTDAHSELHVRRVCGTAIIKNIMRLCPGVPACENGE VPSPTEYCSMGYSDSQVKYLCCPTSQ	amphidial, labial, & ventral cord neurons			2
ins-24 ZC334.3 I	MGLIRANQGPQKACGRSMMMKVQKLCAGGCTIQNDD LTIKSCSTGYTDAGFISACCPSGFVF				2
ins-25 ZC334.8 I	KPEAQRRCGRYLIRFLGELCNGPCSGVSSVD IATIACATAVPIEDLKNMCCPNL				2
ins-26 ZC334.1 I	IGNHHHGTKAGLTCGMNIIERVDQLCNGQCTRNYDA LVIKSCHRGVSDMEFMVACCPTMKLFIH				2
ins-27 ZC334.11 I	FLAPSTAAKRRCGRRLIPYVYSICGGPCENGD IIIEHCFSGTTPTIAEVQKACCPELSEDPTFSS				2
ins-28 ZC334.9 I	ASPTCGRALLHRIQSVCGLCTIDAHHE LIAIACSRGLGDKEIIEMCCPI				2
ins-29 ZC334.10 I	DFGAQRRCGRHLVNFLEGLCGGPCSEAPTVE LASWACSSAVSIQDLEKLCCPSNLA				2

Gene[#] Cosmid LG	Putative peptides	Expression Pattern	Function or Phenotype	Receptor	Refer-ences[##]
ins-30 ZC334.2 I	REPVVAAQGAKKTCGRSLLIKIQQLCHGICTVHADD LHETACMKGLTDSQLINSCCPPIPQTPFVF				2
ins-31 a T10D4.4 II	FVHHFDHSMFARPEKTCGGLLIRRVDRICPNLNY TYKIEWELMDNCCEVVCEDQWIKETFCRAPRFNFFGPSF		Overexpres-sion causes larval arrest[$]; Modulates acetyl-choline signaling	DAF-2?	2[$]; 6
ins-31 b	KALERSCGPKLFTRVKTVCGE DINVDNKVKISDHCCTPEGGCTDDWIKENVCKQTRFNFFRQFL				2[$]
ins-31 c	DSPQRSCGPQLFKRVNTLCNE NINVENNVSVSKSCCESAAGCTDDWIKKNVCTQHKPFVFRPGFY				2[$]
ins-32 Y8A9A.6 II	RSRRELICGRRLSKTVTNLCVEMN PQKEEDIATKCCKNKGCSREYIKSIMCPDE				2, OST
ins-33 W09C5.4 I	HGQKHCGTKIVRKLQMLCPKMCTISDD TLLTEMCSHSLFDDEIQLRCCPKEDE				2, OST
ins-34 F52B11.6 IV	KTTAAPLAQVNPQCLRRLTLLARGVCRQPCQPSDKPK TSAQQLLQLACSARRPTNEQIISYCCPEKSG				2
ins-35 K02E2.4 V	KMDENAFGINNRHCQRALKVYSFAICGAICQNYEK ILMEGCGSTVMLTMQRTKLICCPEPVDSDELFN				2, OST
ins-36 Y53H1A.4 I	IRKRHPEGKLVIRDCKRYLIMYSRTICKEKCEKFD ERNDITFSINLQFIFTDLLVEGCHSNQTLSNERTRELCCPNAGSN				2
ins-37 F08G2.6 II	NPIHPVPNAAFLPYRSCGSHLVHRAFEACSGKKD RSSDVDLWKMCCKDECTDLDIKESLCKYASQGYGV				2, OST
ins-38 C17C3.20 II	SQRSHVFSYKKHCGRRIVSLVQACD IDHDLSIDCCTQNCSSEFVSQRSHVFSY				
ins-39 F21E9.4 X	IFRMTISFCSQVECQNMEAMQKICNTTTPTI HVGELCCPEFFEQVKDDFVTLL				EST

#Genes for which ESTs, ORFeomes (OST), or cDNAs have been isolated are in bold; encoded peptides are based on sequence homologies to other insulin-like peptides. Cosmid and LG data only indicated for *ins-31 a*; *ins-31 b* and *ins-31 c* are the same. $ Unclear which *ins-31* construct was used for overexpression and functional data. See References. ##References are as follows: 1, Li et al., 2003; 2, Pierce et al., 2001; 3, Kodama et al., 2006; 4, Tomioka et al., 2006; 5, Kao et al., 2007; 6, Sieburth et al., 2005. EST or OST in EST or ORFeome databases indicated only if not identified in canonical reference and sequence spans at least one intron. Modified from Li (2005).

Table 2. Neuropeptide genes encoding FMRFamide-related peptides (FLPs) in *C. elegans*

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Receptor[‡]	References[##]
flp-1 F23B2.5 IV	*SADPNFLRFG *SQPNFLRFG *ASGDPNFLRFG *SDPNFLRFG *AAADPNFLRFG **(K)PNFLRFG AGSDPNFLRFG (K)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; modulates acetylcholine signaling	(C25G6.5, Y58G8A.4, C16D6.2, Y59H11AL.1)	1-11, KA
flp-2 W07E11.3 X	*SPREPIRFG LRGEPIRFG	AIA, RID, PVW, I5, MC (ASI, M4, head muscles, an extra pair of cells in the head)		T19F4.1a/b	2, 9, 10, 12
flp-3 W07E11.2 X	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.4, C16D6.2)	2,6-9, 12

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Receptor[‡]	Refer-ences[##]
flp-4 C18D1.3 II	PTFIRFG ASPSFIRFG	ADL, ASEL, AVM, AWC, FLP, PHA, PHB, PVD, I5, I6, NSM		C16D6.2	2,7,12
flp-5 C03G5.7 X	*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)	2,6-8,12
flp-6 F07D3.2 V	x6 *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		2,6,8, 12,13
flp-7 F49E10.3 X	x3 *SPMQRSSMVRFG x2 *TPMQRSSMVRFG SPMERSAMVRFG SPMDRSKMVRFG	ALA, AVG, PHB, PDA, PVW, RIC, SAA (RMDV/ SMDV, PHA)		Y59H11AL.1, C26F1.6	2, 9, 10, 12, 15
flp-8 F31F6.4 X	x3 *KNEFIRFG	AUA, PVM, URX (RMG/ADA an extra pair of cells in the head); CP9	increases frequency of pharyngeal action potentials; overexpression causes defecation defects		2,6, 12, 16, AS, UP
flp-9 C36H8.3 IV	x2 *KPSFVRFG		inhibits frequency of pharyngeal action potentials; knockout shows slight sluggishness	(Y59H11AL.1)	6, 8, 9, 12, 16, 17, UP

11

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Receptor[‡]	Refer-ences[##]
flp-10 T06C10.4 IV	QPKARSGYIRFG	AIM, ASI, AUA, BAG, BDU, DVB, PQR, PVR, URX, vulD			2,12
flp-11 K02G10.4 X	*AMRNALVRFG *ASGGMRNALVRFG *NGAPQPFVRFG *SPLDEEDFAPESPLQG	AUA, BAG, DA, DD, DVB, LUA, PHC, PVC, SAB, URX, VD, uv1, head muscle (socket cells); ray 4		Y59H11AL.1, C26F1.6 (C16D6.2)	2,6-9, 12, 15
flp-12 C05E11.8 X	*RNKFEFIRFG	AVH/AVJ, BAG, PDA, PVR, SAA, SDQ, SMB (BDU); rays 1, 4, 5, 7, CP9			2, 12, 16
flp-13 F33D4.3 IV	*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	(Y59H11AL.1)	2, 6, 8, 9, 12, 16, 18, 19
flp-14 Y37D8A.15 III	x4 *KHEYLRFG		increases frequency of pharyngeal action potentials	(C25G6.5, C16D6.2)	6, 7, 9, 16, 20, 21
flp-15 ZK525.1 III	*GGPQGPLRFG *RGPSGPLRFG	PHA, I2, socket/ sheath cells (pharyngeal muscle, several cells in the head)		C10C6.2 C16D6.2	2,7, 9, 20, 22
flp-16 F15D4.8 II	x2 *AQTFVRFG *GQTFVRFG		AQTFVRF-NH$_2$ inhibits frequency of pharyngeal action potentials		6, 8, 20

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Receptor[‡]	Refer-ences[##]
flp-17 C52D10.11 IV	x2 KSAFVRFG KSQYIRFG	BAG, M5 (an extra pair of cells in the head); rays 1, 5, 7			2, 20
flp-18 Y48D7A.2 X	**(DFD)GAMPGVLRFG *EMPGVLRFG x3 **(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.4 C53C7.1a NPR-1 (C25G6.5, F41E7.3)	2, 6, 7, 9, 15, 19, 20, 23-26, UP
flp-19 M79.4 X	*WANQVRFG *ASWASSVRFG	AIN, AWA, BAG, HSN, URX (an extra pair of cells in the tail); rays 5, 7, 9, CEM			2, 6, 8, 9, 20
flp-20 E01H11.3 X	x2 AMMRFG	ALM, ASEL, AVM, LUA, PLM, PVC, PVM, PVR, RIB/AIB (PVT)			2, 20, OH
flp-21 C26F1.10 V	GLGPRPLRFG	ADL, ASI, ASH, ASJ, ASK, FLP, URA, MC, M4, M2; CP6–9, SP, DVF	mutation causes mild aggregation behavior	NPR-1 C25G6.5 Y58G8a.4	2,7, 23-25
flp-22 F39H2.1 I	x3 *SPSAKWMRFG	AIM, ASG, AVA, AVG, AVL, CEP, PVD, PVW, RIC/AIZ, RIV, SMD, URA, uv1; 6 out of 9 CP		(Y59H11AL.1)	2, 6, 8, 9, 20

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Receptor[‡]	Refer-ences[##]
flp-23 F22B7.2 III	VVGQQDFLRG (TKFQDFLRFG)				2, AM
flp-24 C24A1.1 III	*VPSAGDMMVRFG				9, 27
flp-25 K04H4.7 III	DYDFVRFG *ASYDYIRFG				9, 27
flp-26 R173.4 X	**(E)FNADDLTLRFG *GGAGEPLAFSPDMLSLRFG *FRLPFQFFGANEDFNSGLT *NYYESKPY				9, 27
flp-27 C25H3.5 II	(EASAFGDIIGELKGK)GLGGRMRFG *pQQPIDEERPIFME				9, 27
flp-28 W07E11.4 X	*APNRVLMRFG				9, UP
flp-32 R03A10.2 X	AMRNSLVRFG				AM
flp-33 T07D10.6 I	*APLEGFEDMSGFLRTIDGIQKPRFG				28
flp-34 R09A1.5 I	*ALNRDSLVASLNNAERLRFG				SH, LS

[#]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 indicated in blue. No. of copies of peptide encoded by gene indicated. A C-terminal glycine donates an amide group during amidation. [†] Based on co-localization with new markers, some expression patterns have been revised from published data (Kim and Li, 2004). Using an ASE-expressed red fluorescent protein, O. Hobert (pers. comm.) found that ASE did not express *flp-5* or *flp-21* and that only ASEL expressed *flp-20*. C. Bargmann (pers. comm.) confirmed that ASE did not express *flp-21*, and found that *flp-21* was also expressed in ASK and ADL. Cells in parentheses are variably expressed and/or tentative identifications. Cells after semi-colons are male-specific. [‡] Receptors with an EC_{50} ≤1.5 μM are indicated; receptors with an EC_{50} >1.5 μM are in parentheses. * Peptides have been biochemically isolated; * peptides including residues in parentheses have been isolated; non-FLP peptides are indicated in green. [##]References are as follows: 1, Rosoff et al., 1992; 2, Kim and Li, 2004; 3, Rosoff et al., 1993; 4, Nelson et al., 1998b; 5, Waggoner et al., 2000; 6, Rogers et al., 2001; 7, Lowery et al., 2003; 8, Husson et al., 2005; 9, Husson et al., 2006; 10, Mertens et al., 2006; 11, Sieburth et al., 2005; 12, Nelson et al., 1998a; 13, Mertens et al., 2005; 14, Marks et al., 1998; 15, Mertens et al., 2004; 16, Davis and Stretton, 1996; 17, Marks et al., 1999a; 18, Marks et al., 1997; 19, Marks et al., 2001; 20, Li et

al., 1999; 21, Marks et al., 1995; 22, Kubiak et al., 2003b; 23, Rogers et al., 2003; 24, Kubiak et al., 2003a; 25, de Bono and Bargmann, 1998; 26, Kubiak et al., 2008; 27, McVeigh et al., 2005; 28, Husson and Schoofs, 2007; pers. comm.: KA, K. Ashrafi; SH, Steven Husson; AM, A. Maule; LS, Lilianne Schoofs; UP, unpublished results; EST or OST in EST or ORFeome databases indicated only if not identified in canonical reference and sequence spans at least one intron. AF, *Ascaris suum*; PF, *Panagrellus redivius*. Modified from Li (2005).

Table 3. Neuropeptide Genes Encoding non-Insulin, non-FLP Peptides in *C. elegans*

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	References[##]
nlp-1 C01C4.1 X	x3 MDANAFRMSFG* MDPNAFRMSFG* VNLDPNSFRMSFG	ASI, AWC, PHB, BDU, 4 head neurons, intestine		1, 2, 2, 3 4
nlp-2 T24D8.5 X	SIALGRSG FRPG SMAMGRLGLRPG x3 SMAYGRQGFRPG	1 head neuron, secretory cells near vulva, intestine		2, OST
nlp-3 F48C11.3 X	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		2
nlp-4 F59C6.6 I	SLILFVILLVAFAAARPVSEEVDRV DYDPRTEAPRRLPADDDEVDGEDRV DYDPRTDAPIRVPVDPEAEGEDRV			2
nlp-5 F35C11.1 II	SVSQLNQYAGFDTLGGMGLG ALSTFDSLGGMGLG ALQHFSSLDTLGGMGFG	ASI, 2 head neurons, spermatheca; 1 male tail neuron		2
nlp-6 T23E7.4 X	(MA)APKQMVFGFG* YKPRSFAMGFG* AAMRSFNMGFG* LIMGLG	ASI, IL1, 2 head neurons, 1 tail neuron, intestine		1, 2, 3, 4
nlp-7 F18E9.2 X	LYLKQADFDDPRMFTSSFG* SMDDLDDPRLMTMSFG* MILPSLADLHRYTMYD*	ADL, AFD, ASE, ASI, PHA, VNC, 4 head neurons, 2 RVG neurons		1, 2, 3, 4

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Refer- ences[##]
nlp-8 D2005.2 I	*AFDRFDNSGVFSFGA *AFDRMDNSDFFGA *SFDRMGGTEFGLM *YPYLIFPASPSSGDSRRLV	ASK, ADL, 6 head neurons, 2 tail neurons, I2, g1D, pm5L, pm5R, 2 RVG, processes in pharynx, intestine; HOB		1, 2, 3, 4
nlp-9 E03D2.2 V	GGARAFYGFYNAGNS GGGRAFNHNANLFRFD GGGRAFAGSWSPYLE *TPIAEAQGAPEDVDDRRELE	ASI, AWB, 4 head neurons, 1 tail neuron, VNC, spermatheca, vulval muscles, intestine		2, 3, 4
nlp-10 F37A8.4 III	AIPFNGGMYG STMPFSGGMYG AAIPFSGGMYG GAMPFSGGMYG	ASK, ADL, CAN, 2 lateral neurons, 1 tail neuron, 2 ant. pharyngeal neurons; 1 male tail neuron		2
nlp-11 ZK1320.10 II	*HISPSYDVEIDAGNMRNLLDIG *SAPMASDYGNQFQMYNRLIDAG *SPAISPAYQFENAFGLSEALERAG	IL1, 2 head neurons, VNC, PVD, 3 tail neurons, precomma embryos		1, 2, 3, 4, EST, OST
nlp-12 M01D7.5 I	x2 *DYRPLQFG *DGYRPLQFG	1 tail neuron	Modulates acetylcholine signaling	2, 4, 5
nlp-13 E03D2.1 V	*NDFSRDIMSFG *SGNTADLYDRRIMAFG QPSYDRDIMSFG *SAPSDFSRDIMSFG *SSSMYDRDIMSFG *SPVDYDRPIMAFG *AEDYERQIMAFG	3 head neurons, NSM, M2, I4, spermatheca, LUA, 1 tail cell, dorsal and ventral hypoderm, intestine		2, 3, 4
nlp-14 D1009.4 X	x2 ALDGLDGSGFGFD x5 ALNSLDGAGFGFE x3 ALDGLDGAGFGFD *ALNSLDGQGFGFE x3 ALNSLDGNGFGFD	ASI, ASK, and another amphidial neuron, PHA, VNC, 2 RVG neurons, intestine		1, 2, 3, EST
nlp-15 CC4.2 I	*AFDSLAGSGFDNGFN x2 AFDSLAGSGFGAFN AFDSLAGSGFSGFD AFDSLAGQGFTGFE AFDTVSTSGFDDFKL	ASH, CAN, HSN, BDU, 5 head neurons, VNC, 3 RVG neurons, 1 tail neuron, intestine		1, 2, 3

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Refer-ences[##]
nlp-16 T13A10.5 IV	STEHHRV SEGHPHE ATHSPEGHIVAKDDHHGHE *SSDSHHGHQ *SVDEHHGHQ *NAEDHHEHQ SEHVEHQAEMHEHQ STQEVSGHPEHHLV	7 head neurons, 1 lateral neuron, intestine		2, 4
nlp-17 Y45F10A.5 IV	*GSLSNMMRIG QQEYVQFPNEGVVPCESCNLGTLMRIG			1, 2, 3
nlp-18 F33A8.2 II	*SPYRAFAFA ARYGFA *SPYRTFAFA ASPYGFAFA *SDEENLDFLE	ASI, 4 head neurons, 2 tail neurons, spermatheca, NSM, 2 ant. pharyngeal neurons, rectal gland, intestine		1, 2, 3, 4
nlp-19 K09C8.6 X	IAGLRLPNFL IGLRLPNML MGMRLPNIIFL	4 head neurons, VNC in males, NSM, 4 post. pharyngeal neurons, spermatheca		2, EST, OST
nlp-20 F45E4.8 IV	FAFAFA *SGPQAHEGAGMRFAFA APKEFARFARASFA	4 head neurons, 4 tail neurons, spermatheca, intestine, 1 ant. pharyngeal neuron		2, 4
nlp-21 Y47D3B.2 III	GGARAFSADVGDDY *GGARAFYDE *GGARAFLTEM *GGARVFQGFEDE GGARAFMMD GGGRAFGDMM GGARAFVENS GGGRSFPVKPGRLDD) *pQYTSELEEDE	AFD, 5 head neurons, VNC, 1 ant. pharyngeal neuron, 1 tail neuron, embryo, intestine		1, 2, 3, 4
nlp-22 T24D8.3 X	SIAIGRAGFRPG			2
nlp-23 T24D8.4 X	LYISRQGFRPA SMAIGRAGMRPG AFAAGWNRG	tail, dorsal and ventral hypoderm		2

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	References[##]
nlp-24 F35B12.7 V	QWGGGPYGGYGPRGYGGGYGGG YGGYGGRGPYGGYGGRGPYGYGG GPYGGGGLVGALLG	ASI, spermatheca, 1 pharyngeal neuron	anti-microbial?	2, 6
nlp-25 Y43F8C.1 V	*pQWGGGYGNPYGGYG GGGYGGGYGGGFGAQQAYNVQNAA		anti-microbial?	2, 4, 6
nlp-26 Y43F8C.2 V	QFGFGGQQSFGGRGGQFGGMQRGGFNGN *GGFGQQSQFGG GGSQFNGRGGNQFGG	hypoderm		2, 4
nlp-27 B0213.2 V	QWGYGGMPYGGYGGMGGYGMGGYGMGY MWGSPYGGYGGYGGYGGWG	ASI, 3 head neurons, spermatheca, hypoderm, intestine	anti-microbial?	2, 6
nlp-28 B0213.3 V	QWGYGGYGRGYGGYGGYGRGMYGGYG GMYGGYGRGMYGGWG		anti-microbial?	2, 6, OST
nlp-29 B0213.4 V	QWGYGGYGRGYGGYGGYGRGMYGGYG GMYGGYGRGMYGGYGRGMYGGWG	hypoderm, intestine	anti-microbial?	2, 6
nlp-30 B0213.5 V	QWGYGGYGRGYGGYGGYGRGYGGYG GYGGYGRGMWGRPYGGYGWG	hypoderm	anti-microbial?	2, 6
nlp-31 B0213.6 V	QWGYGGYGRGYGGYGGYGRGYGGYGGYG GYGGYGRGMYGGYGRPYGGYGWG	hypoderm, embryos	anti-microbial	2, 6
nlp-32 F30H5.2 III	YGGWGGRGGWGRGGGRGYGG GGGWGGRGGGWGRGGGRGFYGGG		anti-microbial?	2, 6
nlp-33 T19C4.7 V	QWGYGGPYGGYGGGYGGGPWGYGGGW RHWGGYGGGPWGGYGGGPWGGYY	hypoderm	anti-microbial?	6, EST, OST
nlp-34 B0213.17 V	PYGYGGYGGW PYGYGWG			EST, AH
nlp-35 C33A12.2 IV	*AVVSGYDNIYQVLAPRF			1, 3, 4

Gene[#] Cosmid LG	Putative peptides[§]	Expression pattern[†]	Function or Phenotype	Refer-ences[##]
nlp-36 B0464.3 III	[*]SMVARQIPQTVVADH			1, 3
nlp-37 F48B9.4 X	[*]NNAEVVNHILKNFGALDRLGDVG			1, 3, 4
nlp-38 C01A2.7 I	[**](ASDD[R])VLGWNKAHGLWG [*]TPQNWNKLNSLWG [*]SPAQWQRANGLWG			1, 3, 4, AH, EST
nlp-39 C54C8.9 I	[*]EVPNFQADNVPEAGGRV			1, 3
nlp-40 Y74C9A.2 I	[**]APSAPAGLEEKL(R) [*]pQPAADTFLGFVPQ			1, 3, 4
nlp-41 C04H5.8 II	[*]APGLFELPSRSV			1, 3, 4
nlp-42 Y80D3A.10 V	SALLQPENNPEWNQLGWAWG NPDWQDLGFAWG			AH, EST

[#]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 indicated in blue. No. 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. [†] Based on co-localization with new markers, some expression patterns have been revised from published data. Cells in parentheses are variably expressed and/or tentative identifications. [*] Peptides have been biochemically isolated; [*]peptides including residues in parentheses have been isolated; non-FLP peptides are indicated in green. [##]References are as follows: 1, Li et al., 1999; 2, Nathoo et al., 2001; 3, Husson et al., 2005; 4, Husson et al., 2006; 5, Sieburth et al., 2005; 6, Couillault et al., 2004; pers. comm.: AH, Anne Hart; EST or OST in EST or ORFeome databases indicated only if not identified in canonical reference and sequence spans at least one intron. Modified from Li (2005).

Table 4. Peptides encoded by *flp* neuropeptide genes

Gene[#] Cosmid LG	Putative peptides[§]	Peptide name	Name in other species	References[##]
flp-1 F23B2.5 IV	*SADPNFLRFG *SQPNFLRFG *ASGDPNFLRFG *SDPNFLRFG *AAADPNFLRFG **(K)PNFLRFG AGSDPNFLRFG (K)PNFMRYG	FLP-1-1 FLP-1-2 FLP-1-3 FLP-1-4 FLP-1-5 FLP-1-6 FLP-1-7 FLP-1-8	PF2 PF1 AF26	1-6
flp-2 W07E11.3 X	*SPREPIRFG LRGEPIRFG	FLP-2-1 FLP-2-2		4, 7, 8
flp-3 W07E11.2 X	SPLGTMRFG *TPLGTMRFG *EAEEPLGTMRFG NPLGTMRFG *ASEDALFGTMRFG EDGNAPFGTMRFG *SAEPFGTMRFG *SADDSAPFGTMRFG *NPENDTPFGTMRFG	FLP-3-1 FLP-3-2 FLP-3-3 FLP-3-4 FLP-3-5 FLP-3-6 FLP-3-7 FLP-3-8 FLP-3-9		3, 4, 7, 8
flp-4 C18D1.3 II	PTFIRFG ASPSFIRFG	FLP-4-1 FLP-4-2		7, 8
flp-5 C03G5.7 X	*GAKFIRFG AGAKFIRFG APKPKFIRFG	FLP-5-1 FLP-5-2 FLP-5-3		2, 7, 8
flp-6 F07D3.2 V	x6 *KSAYMRFG *pQQDSEVEREMM	FLP-6-1 FLP-6-2	AF8/PF3	3, 7-11
flp-7 F49E10.3 X	x3 *SPMQRSSMVRFG x2 *TPMQRSSMVRFG SPMERSAMVRFG SPMDRSKMVRFG	FLP-7-1 FLP-7-2 FLP-7-3 FLP-7-4		4, 7, 8
flp-8 F31F6.4 X	x3 *KNEFIRFG	FLP-8	AF1	7, 8, 12, 13
flp-9 C36H8.3 IV	x2 *KPSFVRFG	FLP-9		3, 4, 8, 12, 14

Gene[#] Cosmid LG	Putative peptides[§]	Peptide name	Name in other species	References[##]
flp-10 T06C10.4 IV	QPKARSGYIRFG	FLP-10		7, 8
flp-11 K02G10.4 X	*AMRNALVRFG *ASGGMRNALVRFG *NGAPQPFVRFG *SPLDEEDFAPESPLQG	FLP-11-1￼ FLP-11-2￼ FLP-11-3￼ FLP-11-4	AF21	3, 4, 7, 8, 10
flp-12 C05E11.8 X	*RNKFEFIRFG	FLP-12	AF24	7, 8, 10, 12
flp-13 F33D4.3 IV	*AMDSPFIRFG *AADGAPFIRFG *APEASPFIRFG *ASPSAPFIRFG *SPSAVPFIRFG ASSAPFIRFG *SAAAPLIRFG	FLP-13-1￼ FLP-13-2￼ FLP-13-3￼ FLP-13-4￼ FLP-13-5￼ FLP-13-6￼ FLP-13-7		3, 4, 7, 8, 12, 15, 16
flp-14 Y37D8A.15 III	x4 *KHEYLRFG	FLP-14	AF2/PF5[1]	4, 12, 17-20
flp-15 ZK525.1 III	*GGPQGPLRFG *RGPSGPLRFG	FLP-15-1￼ FLP-15-2		4, 7, 17
flp-16 F15D4.8 II	x2 *AQTFVRFG *GQTFVRFG	FLP-16-1￼ FLP-16-2	AF15	3, 10, 17
flp-17 C52D10.11 IV	x2 KSAFVRFG KSQYIRFG	FLP-17-1￼ FLP-17-2		7, 17
flp-18 Y48D7A.2 X	**(DFD)GAMPGVLRFG *EMPGVLRFG x3 **(SYFDEKK)SVPGVLRFG *EIPGVLRFG *SEVPGVLRFG *DVPGVLRFG	FLP-18-1￼ FLP-18-2￼ FLP-18-3￼ FLP-18-4￼ FLP-18-5￼ FLP-18-6	(*afp-1*)	4, 7, 16, 17, 21
flp-19 M79.4 X	*WANQVRFG *ASWASSVRFG	FLP-19-1￼ GLP-19-2		3, 4, 7, 17

Gene[#] Cosmid LG	Putative peptides[§]	Peptide name	Name in other species	References[##]
flp-20 E01H11.3 X	x2 AMMRFG	FLP-20		7, 17
flp-21 C26F1.10 V	GLGPRPLRFG	FLP-21	AF9	7, 9, 17
flp-22 F39H2.1 I	x3 *SPSAKWMRFG	FLP-22		3, 4, 7, 17
flp-23 F22B7.2 III	VVGQQDFLRG (TKFQDFLRFG)	FLP-23		7, AM
flp-24 C24A1.1 III	*VPSAGDMMVRFG	FLP-24		4, 22, UP
flp-25 K04H4.7 III	DYDFVRFG *ASYDYIRFG	FLP-25-1 FLP-25-2		4
flp-26 R173.4 X	**(E)FNADDLTLRFG *GGAGEPLAFSPDMLSLRFG *FRLPFQFFGANEDFNSGLT *NYYESKPY	FLP-26-1 FLP-26-2 FLP-26-3 FLP-26-4		4, 22
flp-27 C25H3.5 II	(EASAFGDIIGELKGK)GLGGRMRFG *pQPIDEERPIFME	FLP-27-1 FLP-27-2		4, 22
flp-28 W07E11.4 X	*APNRVLMRFG	FLP-28		4, UP
flp-32 R03A10.2 X	AMRNSLVRFG	FLP-32		AM
flp-33 T07D10.6 I	*APLEGFEDMSGFLRTIDGIQKPRFG	FLP-33		23
flp-34 R09A1.5 I	*ALNRDSLVASLNNAERLRFG	FLP-34		SH, LS

[#]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 indicated in blue. No. of copies of peptide encoded by gene indicated. A C-terminal glycine donates an amide group during amidation. [*] Peptides have been biochemically isolated; [*] peptides including residues in parentheses have been isolated; non-FLP peptides are indicated in green. [1]FLP-14/AF2 has also been found in *Haemonchus contortus* and *Panagrellus redivius*. [##]References are as follows: 1, Rosoff et al., 1992; 2, Rosoff et al., 1993; 3, Husson et al., 2005; 4, Husson et al., 2006; 5, Geary et al., 1992; 6, Yew et al., 2005; 7, Kim and Li, 2004; 8, Nelson et al., 1998a; 9, Cowden and Stretton, 1995; 10, Yew et al., 2005; 11, Maule et al., 1994a; 12, Davis and Stretton, 1996; 13, Cowden et al., 1989; 14, Marks et al., 1999a; 15, Marks et al., 1997; 16, Marks et al., 2001; 17, Li et al., 1999; 18, Marks et al., 1995; 19, Cowden and Stretton, 1993; 20, Maule et al., 1994b; 21, Edison et al., 1997; 22, McVeigh et al., 2005; 23, Husson et al., 2007; pers. Comm.: SH, Steven Husson; AM, A. Maule; LS, Lilianne Schoofs; UP unpublished results. Modified from Li (2005).

5. Expression and localization of neuropeptide genes

To determine whether a candidate neuropeptide gene is transcribed and to confirm the genomic organization of the gene, two basic strategies have been used. One is to isolate cDNAs using reverse transcription (RT)-polymerase chain reaction (PCR); the second is to scan the *C. elegans* EST or ORFeome databases. Through these approaches, several groups have determined that 37 of the 40 insulin-encoding genes (Gregoire et al., 1998; Kawano et al., 2000; Pierce et al., 2001; Li et al., 2003), 28 of the 31 *flp* genes (Rosoff et al., 1992; Nelson et al., 1998a; Kim and Li, 2004; McVeigh et al., 2005; I. Miskelly, N.J. Marks, and A. Maule, pers. comm.; unpublished results), and 39 of the 42 *nlp* genes (Nathoo et al., 2001; Couillault et al., 2004) are expressed. Based on these data, we predict that most, if not all, of the candidate neuropeptide genes are expressed. The neuropeptide genes are relatively small and often have only a few exons. For instance, the coding and intronic regions of sixteen *flp* genes are less than 1 kilobase (Kim and Li, 2004; McVeigh et al., 2005). Among the *nlp* genes, a cluster of six *nlp* genes (*nlp-27, 28, 29, 30, 31*, and *34*) on chromosome V encode very similar transcripts and have coding and intronic regions that range from 190 to 344 bp (Nathoo et al., 2001). The small size of most neuropeptide genes and the small number of exons that comprise the genes contribute to the difficulty of identifying all neuropeptide genes.

Because many of the *C. elegans* peptides have similar structures (for instance, the insulin-like peptides share common A and B domains and the FLPs all share a common C-terminal Arg-Phe-NH$_2$), antibodies are difficult to generate against specific neuropeptides. Most peptide antibodies cross-react with several peptides of similar amino acid sequences. For example, the anti-FMRFamide antibody (Marder et al., 1987) recognizes FLPs encoded by several *flp* genes (Schinkmann and Li, 1992; Kim and Li, 2004). To determine the expression pattern of different neuropeptide genes, most researchers have made gene fusions of the promoter region of the neuropeptide gene to the coding region of a reporter gene, of which green fluorescent protein (GFP) is the most commonly used. The constructs are microinjected to generate transgenic animals. This relatively simple method has been used to determine the expression pattern of 60 neuropeptide genes (see Tables 1, 2 and 3), including 15 insulin-like genes (Pierce et al., 2001; Li et al., 2003), 19 *flp* genes (Kim and Li, 2004), and 27 *nlp* genes (Nathoo et al., 2001). For only one gene thus far, *flp-8*, has the GFP expression pattern been confirmed by immunocytochemistry with a FLP-8-specific monoclonal antibody (Sithigorngul et al., 1991; Kim, 2003).

Although there are inherent caveats to these expression patterns, a number of conclusions can be drawn from the data. First, the expression of neuropeptides is widespread in *C. elegans* and includes expression in the nervous system as well as in non-neuronal tissue (see Tables 1, 2 and 3). For instance, over 160 neurons, which represent over half of the 302 neurons in the *C. elegans* nervous system, express one or more FLPs (Kim and 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. Second, there is considerable overlap in the expression patterns. Although each gene appears to be expressed in a distinct set of neurons, a single neuron can express multiple neuropeptide genes and show a considerable diversity of neuropeptide expression. For instance, the chemosensory neuron 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 and Li, 2004); while some of these expression patterns may be artifactual, nevertheless ASI has the potential to release a plethora of neuropeptides to modulate neuronal activity. Third, neuropeptide expression is not

limited to the nervous system. Neuropeptides, including those predicted by the *ins*, *daf-28*, *flp*, and *nlp* genes, are predicted to be in non-neuronal tissues, including intestine, somatic gonad, muscle, and hypodermis (Nathoo et al., 2001; Pierce et al., 2001; Li et al., 2003; Kim and Li, 2004). Secretion of neuropeptides from these tissues is likely to have an endocrine role.

Initially, the expression patterns of different peptides were compared in *C. elegans* and other nematodes by immunocytochemistry. The *Ascaris* nervous system, for instance, showed an extensive distribution of FMRFamide-like immunoreactivity (Cowden et al., 1993), much more than what was seen in *C. elegans* (Schinkmann and Li, 1992); however, the extent of *flp* expression with the GFP reporters in *C. elegans* now matches that in *Ascaris*. More recently, *in situ* hybridization has been used to examine the gene-specific expression pattern of different neuropeptide genes in the parasitic nematode *Globodera pallida*. For the few *Gp-flp* genes examined, there were several differences in the neurons in which the genes were expressed in *C. elegans* (Kimber et al., 2002). For instance, *flp-6* is not expressed in *C. elegans* tail neurons, whereas *Gp-flp-6* is expressed in several tail neurons (Kimber et al., 2002). The significance of these differences is unknown, but could suggest that the function of the peptides diverged in the related nematodes.

6. Biochemical isolation of neuropeptides

To determine whether the predicted peptides are produced, a few groups have begun the biochemical isolation of the different peptides. Thus far, most of the biochemical isolations have focused on the FLPs. To date, 36 FLPs encoded by seventeen *flp* genes (*flp-1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 16, 18, 19, 22, 24, 26*, and *33*) have been isolated (Rosoff et al., 1993; Marks et al., 1995, 1997, 1998, 1999a, 2001; Davis and Stretton, 1996; Husson et al., 2005; S. Husson and L. Schoofs, pers. comm.; Table 2). Indeed, the identification of *flp-33* was based on the biochemical isolation of the peptide. Two FLPs that are not encoded by any of the identified *flp* genes have also been isolated (Davis and Stretton, 1996; N. Marks and A. Stretton, pers. comm.), underscoring the difficulty of identifying small neuropeptide genes with BLAST searches. In the recent peptidomic analysis by Husson and coworkers (2005), twenty nine NLPs encoded by 19 *nlp* genes were isolated (see Table 3), suggesting that many of the NLPs are also likely to be produced in *C. elegans*. These data also indicate that 113 is likely to be an underestimate of the total number of neuropeptide genes.

Peptides identical to some of the predicted FLPs have been isolated from related nematodes (see Table 4), such as *Ascaris suum* (Cowden et al., 1989; Cowden and Stretton, 1993, 1995; Yew et all., 2005), *Haemonchus contortus* (Keating et al., 1995; Marks et al., 1999b), and *Panagrellus redivius* (Geary et al., 1992; Maule et al., 1994a,b, 1995). To date, most of the isolated peptides in related nematodes belong to the FLP family (Yew et al., 2005). In addition, the FLP-12 (AF24) and FLP-21 (AF9) peptides have been isolated from *Ascaris suum* (Cowden and Stretton, 1995; Yew et al., 2005), suggesting that these peptides are also produced in *C. elegans*. Similarly, several of the predicted NLPs are similar to peptides isolated from other invertebrates (Nathoo et al., 2001). These data suggest that many of the predicted neuropeptides are indeed produced and highlight the rich diversity of neuropeptides in *C. elegans*.

7. Neuropeptide function

Based on the expression pattern of the peptides, the peptides are likely to participate in a multitude of behaviors, including dauer formation, locomotion, egg-laying, and mechano- and chemosensation. To determine the function of the different neuropeptides, the most common strategy has been to inactivate or overexpress specific neuropeptide genes. This strategy has certain drawbacks with large neuropeptide superfamilies because of the possible functional overlap among the family members. Several neuropeptides, for instance, may bind and activate the same receptor. Using RNAi to decrease neuropeptide activity has not been routinely performed because of the inefficiency of RNAi in neurons (Simmer et al., 2002). Nevertheless, recent experiments have shown that several of the neuropeptide genes have unique functions.

7.1. The insulin-like gene family

Newly hatched first larval stage (L1) animals sample their environment to assess its qualities for reproductive growth. In the absence of food, newly hatched L1 animals will arrest reproductive growth and remain in this arrested state until a food source becomes available (Johnson et al., 1984). The decision to enter reproductive growth after hatching is dependent on the activity of DAF-2, an insulin-like receptor, and ASNA-1, an ATPase that acts non-cell autonomously and regulates the insulin pathway (see below; Gems et al., 1998; Kao et al., 2007).

Under continued exposure to harsh environmental conditions, such as overcrowding, high temperatures, or a scarce food supply during L1 or L2, *C. elegans* will undergo an alternative life cycle, referred to as the dauer life cycle (Cassada and Russell, 1975). After L2, animals enter the dauer state rather than L3 and remain in the dauer state until conditions improve, whereupon they exit the dauer state and resume the lifecycle as L4 animals (Cassada and Russell, 1975). The decision to enter reproductive growth or dauer is determined by parallel signaling pathways: the insulin and transforming growth factor β (TGF β) pathways (Riddle and Albert, 1997). Loss of either pathway results in constitutive dauer formation, indicating that the pathways function independently. Many of the insulin and TGFβ pathway mutants are temperature sensitive, and show an incompletely penetrant dauer phenotype at the permissive temperature (Riddle and Albert, 1997). Lowering the activity of an insulin and TGFβ pathway gene has a synergistic effect and causes a stronger dauer phenotype at the permissive temperature than lowering the activity of either gene alone (Thomas et al., 1993). Similarly, lowering the activity of *asna-1* and a TGFβ pathway gene also leads to an enhanced dauer phenotype at the permissive temperature, thereby linking the L1 arrest and dauer decision to the insulin pathway (Kao et al., 2007). Hence, activation of DAF-2 leads to reproductive growth, whereas inactivation of DAF-2 leads to dauer arrest (Riddle and Albert, 1997). DAF-2 also functions to determine lifespan (Kenyon et al., 1993) and to limit body size (McCulloch and Gems, 2003). DAF-2 is the closest homologue to the mammalian insulin-like receptor superfamily (Kimura et al., 1997), but recently a more divergent family of 56 putative insulin-like receptors has been identified in *C. elegans* (Dlakic, 2002).

Members of the insulin superfamily are encoded by the *ins* genes and *daf-28* in *C. elegans*; 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). *ins-1, ins-9*, and *daf-28* are expressed in ASI and ASJ chemosensory neurons, which are critical in the decision for dauer formation (Bargmann and Horvitz, 1991), as well as in other neurons; *ins-1* and *daf-28* are also expressed in non-neuronal tissue, such as intestinal cells (Pierce et al., 2001; Li et al., 2003). Only two insulin-family genes that have been inactivated by mutation have been examined thus far. INS-1 is most similar to mammalian insulin (Pierce et al., 2001). Loss of *ins-1* has no effect on dauer formation or longevity (Pierce et al., 2001). By contrast, the *daf-28(sa191)* mutation causes transient dauer formation (Malone and Thomas, 1994). The mutation is likely to act as a dominant negative whereby the *daf-28(sa191)* gene product antagonizes DAF-2 activity (Li et al., 2003). Overexpression of *daf-28* in a decreased TGFβ signaling background promotes exit from dauer, indicating that increased activity of the insulin pathway can bypass the TGFβ pathway (Kao et al., 2007). These data suggest that DAF-28 normally activates the DAF-2 insulin-like receptor to promote reproductive growth (Li et al., 2003). Consistent with this hypothesis, levels of a P$_{daf-28}$::GFP transgene are decreased during starvation or application of dauer pheromone (Li et al., 2003), suggesting that *daf-28* expression is regulated by environmental cues, as would be expected for a dauer regulator. Expression of the P$_{daf-28}$::GFP transgene is found in an increasing number of cells as the animal ages (Li et al., 2003), suggesting that *daf-28* is also involved in other behaviors, such as aging. ASNA-1 may regulate levels of DAF-28 in the pseudocoelom; *asna-1* mutants show decreased levels of DAF-28::GFP in coelomocytes, indicating that less DAF-28::GFP is being released in the pseudocoelom (Kao et al., 2007).

If other insulin-like peptides also signal through the DAF-2 receptor, then perturbations of other *ins* genes may enhance or suppress the phenotypes of *daf-2* and *daf-28* mutants. The phenotypes caused by overexpression of several *ins* genes, using their endogenous promoters, were examined in different *daf* backgrounds. Overexpression of *ins-1* and *ins-18* caused a low level of dauer arrest and enhanced the dauer phenotype of *daf-2* and/or *daf-7* TGFβ mutants, whereas no dauer effects were seen with overexpression of *ins-9, ins-19, ins-22*, and *ins-31* (Pierce et al., 2001). However, overexpression of *ins-9* or of *ins-31* and *ins-19* in combination in a wild-type or *daf-2* mutant background caused embryonic or larval arrest, a phenotype similar to one shown by some *daf-2* alleles (Pierce et al., 2001). INS-1 and 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-9, INS-31, and INS-19 may signal through DAF-2 to affect other aspects of development. Overexpression of *ins-4* or *ins-6* can suppress or partially suppress, respectively, the *daf-28(sa191)* mutation, suggesting that INS-4 or INS-6 can functionally substitute for DAF-28 and activate the DAF-2 receptor when present at high levels (Li et al., 2003). Furthermore, overexpression of *ins-4* can bypass the effects of a mutation in the TGFβ pathway (Kao et al., 2007). By contrast, overexpression of *ins-7, 9, 17, 21, 22*, and *23* did not suppress the *daf-28(sa191)* mutation (Li et al., 2003). These data indicate that several insulin-like ligands signal through or affect DAF-2 activity to affect developmental growth and dauer formation, while other insulin-like ligands signal through other non-DAF-2 insulin receptors.

What are the roles of the other *ins* genes? Until mutants are isolated, the function of this large class of neuropeptides is largely unknown. Recently, INS-1 was identified as a key neuropeptide in the integration of behavior with the functional state of the animal. When placed on a thermal gradient well-fed animals move towards

the temperature on which they were cultivated, whereas starved animals avoid the temperature at which they were cultivated (Hedgecock and Russell, 1975). This thermotaxis behavior is mediated by the thermosensory neuron AFD, which signals through the AIY interneuron (Mori and Ohshima, 1995). Well-fed *ins-1* mutants exhibit normal thermotaxis, indicating that the basic thermosensory properties of AFD are intact in the mutants. Similar to starved wild-type animals, starved *ins-1* animals slow when encountering food, demonstrating that *ins-1* animals can recognize their starvation state (Kodama et al., 2006). However, starved *ins-1* mutants move towards rather than away from their cultivation temperature, presumably because they cannot integrate cultivation temperature with starvation state (Kodama et al., 2006). This integration defect can be rescued by expression of *ins-1* in different neurons and is partially suppressed by mutations in *daf-2* and *age-1*, suggesting that INS-1 acts non-cell autonomously to antagonize signaling through the DAF-2 receptor (Kodama et al., 2006).

INS-1, however, can also activate the DAF-2 receptor. Wild-type animals normally chemotax towards sodium chloride (NaCl) (Ward, 1973). However, after pre-exposure to NaCl, starved, but not well-fed animals will avoid NaCl; this behavior is referred to as salt chemotaxis learning (Saeki et al., 2001). Several mutants, including *daf-2, age-1, pdk-1, akt-1*, and *ins-1*, are defective for salt chemotaxis learning (Tomioka et al., 2006), implicating involvement of the DAF-2 pathway in salt chemotaxis learning. Based on transgenic rescues and laser ablations, Tomioka and co-workers (2006) propose that INS-1 is released from AIA interneurons to activate DAF-2 receptors in ASER, thereby initiating the DAF-2 signaling cascade. Hence, INS-1 is involved in multiple integration events and whether it activates or antagonizes DAF-2 signaling is context dependent.

Acetylcholine is the primary excitatory transmitter at the neuromuscular junction in *C. elegans*. Aldicarb blocks the effects of acetylcholinesterase, thereby increasing the amount of acetylcholine at the synapse and causing paralysis and lethality (Nguyen et al., 1995). To identify genes that are resistant to aldicarb, a genome-wide RNAi screen was performed on *eri-1;lin-15B* or *eri-1; dgk-1 lin-15B* mutants, which have a sensitized background for RNAi (Sieburth et al., 2005). In addition to the processing enzymes, decreased activity of four neuropeptide genes, two *ins* genes (*ins-22* and *ins-31*), one *flp* gene (*flp-1*), and one *nlp* gene (*nlp-12*), conferred aldicarb-resistance, suggesting that the peptides encoded by these genes modulate acetylcholine signaling (Sieburth et al., 2005).

7.2. The *flp* family

Deletion mutants have been isolated for eleven *flp* genes (Nelson et al., 1998b; unpubl. obs.). Inactivation of *flp-1* causes several defects, including hyperactive movement (Nelson et al., 1998b), defects in the timing of egg laying (Waggoner et al., 2000), thereby causing a decreased number of eggs laid (unpubl. obs.), and decreased fat stores in *flp-1(yn2)* mutants (K. Ashrafi, pers. comm.). FLP-1 peptides are also necessary for down-regulation of egg laying in the absence of food (Waggoner et al., 2000) and modulation of acetylcholine signaling (see above; Sieburth et al., 2005). The remaining *flp* mutants are currently being examined. Because so many *flp* genes have overlapping expression patterns, the function of these genes may also overlap and, therefore, be difficult to tease apart. Hence, deletion mutants are being screened on a large variety of behavioral assays. For instance, the swimming or thrashing assay, which involves placing animals in physiological buffer and counting the number of thrashes per minute, is more sensitive for detecting locomotion defects than examining the animal's movement on a solid surface. By the thrashing assay, *flp-9* was found to thrash significantly less actively than wild-type animals (unpubl. obs.). Hence, to understand how the FLPs function, mutants will be examined for subtle defects, animals carrying multiple knockouts need to be isolated, and the receptor to which the peptides bind must be identified, as was done in the case of *flp-21*. The function of *flp-21* will be discussed in conjunction with the function of its receptor, NPR-1.

7.3. The *nlp* family

Although no *nlp* mutant has been examined thus far, several of the *nlp* genes may function as anti-microbial peptides. In microarray analyses to identify genes whose expression levels are changed in response to fungal or bacterial insults, expression of *nlp-29, 31*, and *33* was induced (Couillault et al., 2004). Furthermore, the peptide encoded by *nlp-31* has anti-microbial activity and protects against fungal infection (Couillault et al., 2004). The peptides encoded by *nlp-24, 25, 27, 28*, and *30* are similar to those encoded by *nlp-29, 31*, and *33*, suggesting that these peptides also have anti-microbial functions (Couillault et al., 2004). *nlp-29* is expressed in the hypoderm and intestine (Nathoo et al., 2001), *nlp-31* in the hypoderm and embryos (Nathoo et al., 2001), and *nlp-33* exclusively in the hypoderm (Couillault et al., 2004), suggesting that the peptides encoded by these genes only function as anti-microbial agents. By contrast, *nlp-24* and *27* are also expressed in neurons (Nathoo et al., 2001) and may function both as anti-microbial agents and neuropeptides. The expression patterns of *nlp-25* and *28* are unknown, so

whether the peptides encoded by these genes also function as anti-microbial agents is unknown. As described above, *nlp-12* is involved in modulating acetylcholine signaling (Sieburth et al., 2005).

8. Neuropeptide receptors

As mentioned above, the function of a specific neuropeptide may be difficult to discern. Not only are multiple neuropeptides expressed in a single cell, but a specific neuropeptide may bind to multiple receptors. As illustrated above with the insulin-like peptides and their receptors, an alternative strategy to determine the function of neuropeptides is to inactivate the receptors to which the peptides bind.

Of the 1000 G-protein-coupled receptors in *C. elegans*, over 50 of them are likely to be neuropeptide receptors (Bargmann, 1998). Sixty G-protein receptors that were predicted to bind either a small molecule transmitter or a neuropeptide were inactivated by RNAi and screened for behavioral deficits (Keating et al., 2003). RNAi of six receptors, C16D6.2, C25G6.5, C26F1.6, F35G8.1, F41E7.3, and F59C12.2, resulted in either an increased or decreased brood size (Keating et al., 2003). Disruption by RNAi of eight receptors, AC7.1 (tachykinin-like), C15B12.5, C10C6.2, C24A8.4, F15A8.5, F59D12.1, T02E9.1, and T05A1.1, causes locomotion defects (Keating et al., 2003). The phenotypes from the RNAi data were confirmed in two cases by the isolation of deletion mutants in T05A1.1 and F35G8.1 (Keating et al., 2003). Several of the ligands for these receptors have now been identified (see below).

For the FLPs, many FaRP receptors have been isolated from other systems. With the exception of a molluscan FMRFamide-gated amiloride-sensitive channel that has homology to the MEC-4 and MEC-6 mechanoreceptors (Lingueglia et al., 1995), other identified FMRFamide receptors are G-protein coupled receptors (Tensen et al., 1998; Bonini et al., 2000; Cazzamali and Grimmelikhuijzen, 2002; Meeusen et al., 2002; Duttlinger et al., 2003). To match FLP ligands to specific G protein-coupled receptor binding partners, several groups have expressed candidate receptors in either heterologous cells or Xenopus oocytes (Kubiak et al., 2003a, b; Lowery et al., 2003; Rogers et al., 2003; Mertens et al., 2004; Table 2). Different FLP ligands were applied and several assays were used as the read out. Interestingly, all FLP receptors examined thus far can be activated by multiple FLPs. Some of the receptors are activated by multiple peptides encoded by one gene, while other receptors can be activated by peptides encoded by different genes. A few examples will be described below.

The Upjohn/Pharmacia group (Kubiak et al., 2003a, b; Lowery et al., 2003) transfected candidate receptors and chimeric G proteins into Chinese hamster ovary (CHO) cells, and used binding to GTPγS, a non-hydrolyzable form of GTP, as the readout. Binding of the cognate ligand to the receptor would presumably activate the receptor, thereby activating G proteins. GTPγS binding to membranes of transfected cells, therefore, indicates ligand binding. The group determined that peptides encoded by *flp-15*, GGPQGPLRFamide and RGPSGPLRFamide, activate C10C6.2 with an EC_{50} (concentration which produces 50% maximal activation) of 250 and 160 nM, respectively (Kubiak et al., 2003b). No other FLP activated the receptor, despite some FLPs, such as FLP-21 GLGPRPLRFamide, with high sequence similarity at the C-terminus (Kubiak et al., 2003b).

Mertens and coworkers (2004) expressed candidate receptors and $G_{\alpha_{16}}$ in HEK or CHO cells and screened for an increased calcium response, as monitored by an increase in fluorescence. The sensitivity of this system may be lower than that when using GTPγS binding as the readout, because the concentration of peptide needed to activate many receptors is generally much higher (usually in the μM rather than nM range). Nevertheless, Mertens and co-workers found that C26F1.6 binds to peptides encoded by two *flp* genes (Mertens et al., 2004). One *flp-7*-encoded peptide, FLP-7-2 TPMQRSSMVRFamide, activates C26F1.6 with an EC_{50} of ~1 μM; by contrast, FLP-7-1 SPMQRSSMVRFamide, which differs from FLP-7-2 by only one amino acid at the N-terminus, did not active the receptor at concentrations up to 10 μM. Interestingly, despite less sequence similarity, FLP-11-1 AMRNALVRFamide activates C26F1.6 with an EC_{50} of ~1.33 μM.

A FLP receptor can also bind to a diverse group of peptides with a range of activities. The Y59H11AL.1 receptor binds to 15 peptides encoded by 6 *flp* genes with EC_{50} values ranging from 25 nM to 5 μM (Mertens et al., 2006). Only three of the peptides, however, FLP-7-3 SPMERSAMVRFamide (25 nM), FLP-1-8 KPNFMRYamide (100 nM), and FLP-11-1 AMRNALVRFamide (750 nM) have EC_{50} values less than 1 μM (Mertens et al., 2006). While FLP-7-3 and FLP-11-1 have C-terminal sequence similarity, the structure of FLP-1-8 appears to be very different. Note that peptides encoded by *flp-7* bind to two receptors, C26F1.6 and Y59H11AL.1. Where these two receptors are expressed may give us some clues as to how FLP-7 peptides affect behavior. Given that a FLP receptor can bind to multiple peptides encoded by different genes and a single peptide can bind to multiple receptors, the potential complexity of peptide actions in *C. elegans* is astounding.

de Bono and coworkers searched specifically for the ligand to the NPR-1 receptor, a homologue to the mammalian neuropeptide Y receptor. Mutations in the NPR-1 receptor affect aggregation behavior (de Bono and Bargmann, 1998) and tolerance to alcohol (Davies et al., 2004). On an agar plate with a bacterial food source, wild-type animals feed alone (referred to as solitary feeding); a mutation 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 behavior; de Bono and Bargmann, 1998). The aggregation behavior 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). GCY-35 guanylate cyclase binds oxygen, and the aggregation behavior of *npr-1* mutants may be related to oxygen levels in the local environment of the animals (Gray et al., 2004). To identify the NPR-1 ligand, the de Bono group injected constructs for NPR-1 as well as inwardly rectifying potassium channels into Xenopus oocytes, and screened for receptor activation of the potassium channels (Rogers et al., 2003). Because no neuropeptide Y is present in *C. elegans*, the assumption was that the NPR-1 ligand was a FLP. Both Rogers et al. (2003) and Kubiak et al. (2003a) determined that FLP-21 binds to NPR-1; in addition, Rogers et al. (2003) found that peptides encoded by *flp-18* also activated NPR-1. Animals carrying a mutation in *flp-21* display only mild aggregation compared to *npr-1* mutants (Rogers et al., 2003). Two explanations may account for the difference in phenotypes between the FLP-21 ligand and the NPR-1 receptor mutants. The first is that the *flp-21* mutation may be a partial loss of function allele instead of a null allele; the second is that FLP-18 ligands, which also bind NPR-1, may functionally substitute for the loss of FLP-21 ligands (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. As with other FLPs, FLP-18 peptides bind multiple receptors, including NPR-1 (Kubiak et al., 2003a; Rogers et al., 2003) and Y58G8A.4 receptors (Kubiak et al., 2008).

9. Pharmacology of FLP neuropeptides

Because many of the FLPs have been also found in parasitic nematodes (Cowden et al., 1989; Cowden and Stretton, 1993, 1995; Keating et al., 1995; Marks et al., 1999b) and, therefore, present possible targets for anti-helminthetic drugs, there has been an interest in examining the physiological effects of FLPs both in *C. elegans* as well as in parasitic nematodes. In *C. elegans* the effects of FLPs have been examined in a pharyngeal preparation (see Table 2; Rogers et al., 2001). Muscles of exposed pharyngeal terminal bulbs were impaled with microelectrodes to record changes of activity and action potential frequency. Surprisingly, many of the tested FLPs modulated the action potential frequency (Rogers et al., 2001). Peptides encoded by *flp-5, 6, 8*, and *14* increased the action potential frequency, while peptides encoded by *flp-1, 3, 9, 13*, and *16* decreased the action potential frequency. These data are consistent with the expression of some of these FLPs in the pharynx (Kim and Li, 2004) and suggest that multiple FLPs are involved in feeding behavior. Whether the different FLPs signal through one or multiple FLP receptors has yet to be determined.

Similarly, application of peptides encoded by 20 *flp* genes had a range of effects on body wall, reproductive, and pharyngeal muscle of *Ascaris suum* (Fellowes et al., 1998; Bowman et al., 2002; Moffett et al., 2003; Trailovic et al., 2005; for reviews see Maule et al., 1996; Brownlee et al., 1996; Brownlee and Walker, 1999; Brownlee et al., 2000), and it is likely that these FLPs have similar effects in *C. elegans*. On *Ascaris* muscle, different FLPs can activate different ion channels. Application of KPNFLRFamide (FLP-1-6) to somatic muscle cells, for instance, opens chloride channels, while application of SDPNFLRFamide (FLP-1-4) opens potassium channels (Bowman et al., 1996, 2002). The effects of KSAYMRFamide (FLP-6) on somatic muscle are context-dependent: application to ventral muscles causes contraction while application to dorsal muscles causes relaxation (Maule et al., 1994a). The response to FLPs can also be more complex. For instance, application of KNEFIRFamide (FLP-8) or KHEYLRFamide (FLP-14) to somatic muscle strips elicits biphasic responses consisting of an initial hyperpolarization, followed by an excitatory phase of rhythmic contractions (Cowden and Stretton, 1993; Bowman et al., 1996). More recently, Stretton and coworkers have begun to characterize the effects of *C. elegans* and *Ascaris* FLPs on the synaptic activity of *Ascaris* motor neurons (Davis and Stretton, 2001). 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.

10. Summary

The diversity of neuropeptides in *C. elegans* rivals the numbers found in mammals. Although many of the peptides appear to be invertebrate specific, the insulin-like and FMRFamide-related peptides have counterparts in mammals. The daunting task of determining the regulation and functions of the different peptides remains. This task

is complicated by the overlapping expression and redundant functions of the different peptides, as well as by their ability to bind multiple receptors, and, likewise, by the ability of the receptors to bind multiple ligands. While most, if not all, FLPs and NLPs are likely to signal through G protein-coupled receptors and the insulin-like ligands to signal through receptor tyrosine kinases, the identities of these receptors are still largely unknown. Despite the limited information available thus far, we envision that neuropeptides are involved in all behaviors in *C. elegans*.

11. Acknowledgments

12. References

Bargmann, C.I. (1998). Neurobiology of the *Caenorhabditis elegans* genome. Science *282*, 2028–2033. Abstract Article

Bargmann, C.I., and Horvitz, H.R. (1991). Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science *251*, 1243–1246. Abstract Article

Bonini, J.A., Jones, K.A., Adham, N., Forray, C., Artymyshyn, R., Durkin, M.M., Smith, K.E., Tamm, J.A., Boteju, L.W., Lakhlani, P.P., et al. (2000). Identification and characterization of two G protein-coupled receptors for neuropeptide FF. J. Biol. Chem. *275*, 39324–39331. Abstract Article

Bowman, J.W., Friedman, A.R., Thompson, D.P., Maule, A.G., Alexander-Bowman, S.J., and Geary, T.G. (2002). Structure-activity relationships of an inhibitory nematode FMRFamide-related peptide, SDPNFLRFamide (PF1), on *Ascaris suum* muscle. Int. J. Parasitol. *32*, 1765–1771. Abstract Article

Bowman, J.W., Friedman, A.R., Thompson, D.P., Ichhpurani, A.K., Kellman, M.F., Marks, N.J., Maule, A.G., and Geary, T.G. (1996). Structure-activity relationships of KNEFIRFamide (AF1), a nematode FMRFamide-related peptide, on *Ascaris suum* muscle. Peptides *17*, 381–387. Abstract Article

Brownlee, D.J., Fairweather, I., Holden-Dye, L., and Walker, R.J. (1996). Nematode neuropeptides: Localization, isolation and functions. Parasitol. Today *12*, 343–351. Abstract Article

Brownlee, D., Holden-Dye, L., and Walker, R. (2000). The range and biological activity of FMRFamide-related peptides and classical neurotransmitters in nematodes. Adv. Parasitol. *45*, 109–80. Abstract

Brownlee, D.J., and Walker, R.J. (1999). Actions of nematode FMRFamide-related peptides on the pharyngeal muscle of the parasitic nematode, *Ascaris suum*. Ann. N. Y. Acad. Sci. *897*, 228–238. Abstract Article

C. elegans Sequencing Consortium (1998). Genome sequence of the nematode *C. elegans*: A platform for investigating biology. Science *282*, 2012–2018. Abstract Article

Cai, T., Fukushige, T., Notkins, A.L., and Krause, M. (2004). Insulinoma-associated protein IA-2, a vesicle transmembrane protein, genetically interacts with UNC-31/CAPS and affects neurosecretion in *C. elegans*. J. Neurosci. *24*, 3115–3124. Abstract Article

Cassada, R.C., and Russell, R.L. (1975). The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. *46*, 326–342. Abstract Article

Cazzamali, G, and Grimmelikhuijzen, C.J. (2002). Molecular cloning and functional expression of the first insect FMRFamide receptor. Proc. Natl. Acad. Sci. U.S.A. *99*, 12073–12078. Abstract Article

Cheung, B.H.H., Arellano-Carbajal, F., Rybicki, I., and de Bono, M. (2004). Soluble guanylate cyclases act in neurons exposed to the body fluid to promote *C. elegans* aggregation behavior. Curr. Biol. *14*, 1105–1111. Abstract Article

Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J.-F., Kohara, Y., and Ewbank, J.J. (2004). TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. Nat. Immunol. *5*, 488–494. Abstract Article

Cowden, C., Sithigorngul, P., Brackley, P., Guastella, J., and Stretton, A.O. (1993). Localization and differential expression of FMRFamide-like immunoreactivity in the nematode *Ascaris suum*. J. Comp. Neurol. *333*, 455–468. Abstract Article

Cowden, C., and Stretton, A.O. (1993). AF2, an *Ascaris* neuropeptide: isolation, sequence, and bioactivity. Peptides *14*, 423–430. Abstract Article

Cowden, C., and Stretton, A.O. (1995). Eight novel FMRFamide-like neuropeptides isolated from the nematode *Ascaris suum*. Peptides *16*, 491–500. Abstract Article

Cowden, C., Stretton, A.O., and Davis, R.E. (1989). AF1, a sequenced bioactive neuropeptide isolated from the nematode *Ascaris suum*. Neuron *2*, 1465–1473. Abstract Article

Davies, A.G., Bettinger, J.C., Thiele, T.R., Judy, M.E., and McIntire, S.L. (2004). Natural variation in the *npr-1* gene modifies ethanol responses of wild strains of *C. elegans*. Neuron *42*, 731–743. Abstract Article

Davis, R.E., and Stretton, A.O. (1996). The motornervous system of *Ascaris*: eletrophysiology and anatomy of the neurons and their control by neuromodulators. Parasitology *113*, S97–S117. Abstract

Davis, R.E., and Stretton, A.O. (2001). Structure-activity relationships of 18 endogenous neuropeptides on the motor nervous system of the nematode *Ascaris suum*. Peptides *22*, 7–23. Abstract Article

de Bono, M., and Bargmann, C.I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell *94*, 679–689. Abstract Article

Dlakic, M. (2002). A new family of putative insulin receptor-like proteins in *C. elegans*. Curr. Biol. *12*, R155–R157. Abstract Article

Dockray, G.J. (2004). The expanding family of -RFamide peptides and their effects on feeding behaviour. Exp. Physiol. *89*, 229–235. Abstract Article

Doi, M. and Iwasaki, K. (2002). Regulation of retrograde signaling at neuromuscular junctions by novel C2 domain protein AEX-1. Neuron *33*, 249–259. Abstract Article

Duret, L., Guex, N., Peitsch, M.C., and Bairoch, A. (1998). New insulin-like proteins with atypical disulfide bond pattern characterized in *Caenorhabditis elegans* by comparative sequence analysis and homology modeling. Genome Res. *8*, 348–353. Abstract

Duttlinger, A., Mispelon, M., and Nichols, R. (2003). The structure of the FMRFamide receptor and activity of the cardioexcitatory neuropeptide are conserved in mosquito. Neuropeptides *37*, 120–126. Abstract Article

Edison, A.S., Messinger, L.A., and Stretton, A.O. (1997). *afp-1*: a gene encoding multiple transcripts of a new class of FMRFamide-like neuropeptides in the nematode *Ascaris suum*. Peptides *18*, 929–935. Abstract Article

Eipper, B.A., Milgram, S.L., Husten, E.J., Yun, H.Y., and Mains, R.E. (1993). Peptidylglycine alpha-amidating monooxygenase: a multifunctional protein with catalytic, processing, and routing domains. Protein Sci. *2*, 489–497. Abstract

Fares, H. and Grant, B. (2002). Deciphering endocytosis in *Caenorhabditis elegans*. Traffic *3*, 11–19. Abstract Article

Fellowes, R.A., Maule, A.G., Marks, N.J., Geary, T.G., Thompson, D.P., Shaw, C., Halton, D.W. (1998). Modulation of the motility of the vagina vera of *Ascaris suum in vitro* by FMRF amide-related peptides. Parasitology *116*, 277–287. Abstract Article

Geary, T.G., Price, D.A., Bowman, J.W., Winterrowd, C.A., Mackenzie, C.D., Garrison, R.D., Williams, J.F., and Friedman, A.R. (1992). Two FMRFamide-like peptides from the free-living nematode *Panagrellus redivivus*. Peptides *13*, 209–214. Abstract Article

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L., and Riddle, D.L. (1998). Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics *150*, 129–155. Abstract

Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marietta, M.A., and Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature *430*, 317–322. Abstract Article

Gregoire, F.M., Chomiki, N., Kachinskas, D., and Warden, C.H. (1998). Cloning and developmental regulation of a novel member of the insulin-like gene family in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *249*, 385–390. Abstract Article

Grishanin, R.N., Klenchin, V.A., Loyet, K.M., Kowalchyk, J.A., Ann, K., and Martin, T.F. (2002). Membrane association domains in Ca^{2+}-dependent activator protein for secretion mediate plasma membrane and dense-core vesicle binding required for Ca^{2+}-dependent exocytosis. J. Biol. Chem. *277*, 22025–22034. Abstract Article

Hall, D.H., and Hedgecock, E.M. (1991). Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C. elegans*. Cell *65*, 837–847. Abstract Article

Han, M., Park, D., Vanderzalm, P.J., Mains, R.E., Eipper, B.A., and Taghert, P.H. (2004). *Drosophila* uses two distinct neuropeptide amidating enzymes, dPAL1 and dPAL2. J. Neurochem. *90*, 129–141. Abstract Article

Hedgecock, E.M. and Russell, R.L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. *72*, 4061–4065. Abstract Article

Husson, S.J., Clynen, E., Baggerman, G., De Loof, A., and Schoofs, L. (2005). Discovering neuropeptides in *Caenorhabditis elegans* by two dimensional liquid chromatography and mass spectrometry. Biochm. Biophys. Res. Commun. *335*, 76–86. Abstract Article

Husson, S.J., Clynen, E., Baggerman, G., Janssen, T., and Schoofs, L. (2006). Defective processing o fneuropeptide precursors in *Caenorhabditis elegans* lacking proprotein converase 2 (KPC-2/ELG-3): mutant analysis by mass spectrometry. J. Neurochem. *98*, 1999–2012. Abstract Article

Husson, S.J., and Schoofs, L. (2007). Altered neuropeptide profile of *Caenorhabditis elegans* lacking the chaperone protein 7B2 as analyzed by mass spectrometry. FEBS Lett. *581*, 4288–4292. Abstract Article

Jacob, T.C., and Kaplan, J.M. (2003). The EGL-21 carboxypeptidase E facilitates acetylcholine release at *Caenorhabditis elegans* neuromuscular junctions. J. Neurosci. *23*, 2122–2130. Abstract

Johnson, T.E., Mitchell, D.H., Kline, S., Kernal, R., and Foy, J. (1984). Arresting development arrests aging in the nematode *Caenorhabditis elegans*. Mech. Ageing Dev. *28*, 23–40. Abstract Article

Kao, G., Nordenson, C., Still, M., Rönnlund, A., Tuck, S., and Naredi, P. (2007). ASNA-1 positively regulates insulin secretion in *C. elegans* and mammalian cells. Cell *128*, 577–587. Abstract Article

Kass, J., Jacob, T.C., Kim, P., and Kaplan, J.M. (2001). The EGL-3 proprotein convertase regulates mechanosensory responses of *Caenorhabditis elegans*. J. Neurosci. *21*, 9265–9272. Abstract Article

Kawano, T., Ito, Y., Ishiguro, M., Takuwa, K., Nakajima, T., and Kimura, Y. (2000). Molecular cloning and characterization of a new insulin/IGF-like peptide of the nematode *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *273*, 431–436. Abstract Article

Keating, C.D., Holden-Dye, L., Thorndyke, M.C., Williams, R.G., Mallett, A., and Walker, R.J. (1995). The FMRFamide-like neuropeptide AF2 is present in the parasitic nematode *Haemonchus contortus*. Parasitology *111*, 515–521. Abstract

Keating, C.D., Kriek, N., Daniels, M., Ashcroft, N.R., Hopper, N.A., Siney, El.J., Holden-Dye, L., and Burke, J.F. (2003). Whole-genome analysis of 60 G protein-coupled receptors in *Caenorhabditis elegans* by gene knockout with RNAi. Curr. Biol. *13*, 1715–1720. Abstract Article

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. Nature *366*, 461–464. Abstract Article

Kim, K. (2003). Function of a FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. Boston University thesis.

Kim, K., and Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. J. Comp. Neurol. *475*, 540–550. Abstract Article

Kimber, M.J., Fleming, C.C., Prior, Al, Jones, J.T., Halton, D.W., and Maule, A. (2002). Localisation of *Globodera pallida* FMRFamide-related peptide encoding genes using in situ hybridization. Int. J. Parasitol. *32*, 1095–1105. Abstract Article

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science *277*, 942–946. Abstract Article

Kodama, E., Kuhara, A., Mohri-Shiomi, A., Kimura, K.D., Okumura, M., Tomioka, M., Iino, Y., and Mori, I. (2007). Insulin-like signaling and the neural circuit for integrative behavior in *C. elegans*. Genes Dev. *20*, 2955–2960. Abstract Article

Kubiak, T.M., Larsen, M.J., Nulf, S.C., Zantello, M.R., Burton, K.J., Bowman, J.W., Modric, T., and Lowery, D.E. (2003a). Differential activation of "social" and "solitary" variants of the *Caenorhabditis elegans* G protein-coupled receptor NPR-1 by its cognate ligand AF9. J. Biol. Chem. *278*, 33724–33729. Abstract Article

Kubiak, T.M., Larsen, M.J., Zantello, M.R., Bowman, J.W., Nulf, S.C., and Lowery, D.E. (2003b). Functional annotation of the putative orphan *Caenorhabditis elegans* G-protein-coupled receptor C10C6.2 as a FLP15 peptide receptor. J. Biol. Chem. *278*, 42115–42120. Abstract Article

Kubiak, T.M., Larsen, M.J., Bowman, J.W., Geary, T.G., and Lowery, D.E. (2008). FMRFamide-like peptides (FLPs) encoded on the *flp-18* precursor gene activate two isoforms of the orphan *Caenorhabditis elegans* G-protein-coupled receptor Y58G8A.4 heterologously expressed in mammalian cells. Biopolymers, in press. Abstract Article

Li, C. (2005). The ever-expanding neuropeptide gene families in the nematode *Caenorhabditis elegans*. Parasitology *131 Suppl.* S109–S127. Abstract Article

Li, W., Kennedy, S.G., and Ruvkun, G. (2003). *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. *17*, 844–858. Abstract Article

Li, C., Kim, K., Nelson, L.S. (1998). FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. Brain Res. *848*, 26–34. Abstract Article

Li, C., Nelson, L.S., Kim, K., Nathoo, A., and Hart, A.C. (1999). Neuropeptide gene families in the nematode *Caenorhabditis elegans*. Ann. N. Y. Acad. Sci. *897*, 239–52. Abstract Article

Lindberg, I, Tu, B., Muller, L., and Dickerson, I.M. (1998). Cloning and functional analysis of *C. elegans* 7B2. DNA Cell Biol. *17*, 727–734. Abstract

Lingueglia, E., Champigny, G., Lazdunski, M., and Barbry, P. (1995). Cloning of the amiloride-sensitive FMRFamide peptide-gated sodium channel. Nature *378*, 730–733. Abstract Article

Lowery, D.E., Geary, T.G., Kubiak, T.M., and Larsen, M.J. (2003). Pharmacia & Upjohn Company, G protein-coupled receptor-like receptors and modulators thereof, United States, Patent 6,632,621.

Malone, E.A., and Thomas, J.H. (1994). A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. Genetics *136*, 879–886. Abstract

Marder, E., Calabrese, R.L., Nusbaum, M.P., and Trimmer, B. (1987). Distribution and partial characterization of FMRFamide-like peptides in the stomatogastric nervous systems of the rock crab, *Cancer borealis*, and the spiny lobster, *Panulirus interruptus*. J. Comp. Neurol. *259*, 150–163. Abstract Article

Marks, N.J., Maule, A.G., Geary, T.G., Thompson, D.P., Li, C., Halton, D.W., and Shaw, C. (1998). KSAYMRFamide (PF3/AF8) is present in the free-living nematode, *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *248*, 422–425. Abstract Article

Marks, N.J., Maule, A.G., Geary, T.G., Thompson, D.P., Davis, J.P., Halton, D.W., Verhaert, P., and Shaw, C. (1997). APEASPFIRFamide, a novel FMRFamide-related decapeptide from *Caenorhabditis elegans*: structure and myoactivity. Biochem. Biophys. Res. Commun. *231*, 591–595. Abstract Article

Marks, N.J., Maule, A.G., Li, C., Nelson, L.S., Thompson, D.P., Alexander-Bowman, S., Geary, T.G., Halton, D.W., Verhaert, P., and Shaw, C. (1999a). Isolation, pharmacology and gene organization of KPSFVRFamide: a neuropeptide from *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *254*, 222–230. Abstract Article

Marks, N.J., Sangster, N.C., Maule, A.G., Halton, D.W., Thompson, D.P., Geary, T.G., and Shaw, C. (1999b). Structural characterisation and pharmacology of KHEYLRFamide (AF2) and KSAYMRFamide (PF3/AF8) from *Haemonchus contortus*. Mol. Biochem. Parasitol. *100*, 185–194. Abstract Article

Marks, N.J., Shaw, C., Halton, D.W., Thompson, D.P., Geary, T.G., Li, C., and Maule, A.G. (2001). Isolation and preliminary biological assessment of AADGAPLIRFamide and SVPGVLRFamide from *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *286*, 1170–1176. Abstract Article

Marks, N.J., Shaw, C., Maule, A.G., Davis, J.P., Halton, D.W., Verhaert, P., Geary, T.G., and Thompson, D.P. (1995). Isolation of AF2 (KHEYLRFamide) from *Caenorhabditis elegans*: evidence for the presence of more than one FMRFamide-related peptide-encoding gene. Biochem. Biophys. Res. Commun. *217*, 845–851. Abstract Article

Maule, A.G., Bowman, J.W., Thompson, D.P., Marks, N.J., Friedman, A.R., and Geary, T.G. (1996). FMRFamide-related peptides (FaRPs) in nematodes: occurrence and neuromuscular physiology. Parasitology *113*, S119–S135. Abstract

Maule, A.G., Shaw, C., Bowman, J.W., Halton, D.W., Thompson, D.P., Geary, T.G., and Thim, L. (1994a). 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. *200*, 973–980. Abstract Article

Maule, A.G., Shaw, C., Bowman, J.W., Halton, D.W., Thompson, D.P., Geary, T.G., and Thim, L. (1994b). The FMRFamide-like neuropeptide AF2 (*Ascaris suum*) is present in the free-living nematode, *Panagrellus redivivus* (Nematoda, Rhabditida). Parasitology *109*, 351–356. Abstract

Maule, A.G., Geary, T.G., Bowman, J.W., Marks, N.J., Blair, K.L., Halton, D.W., Shaw, C., and Thompson, D.P. (1995). Inhibitory effects of nematode FMRFamide-related peptides (FaRPs) on muscle strips from *Ascaris suum*. Invert. Neurosci. *1*, 255–265. Abstract Article

McCulloch, D., and Gems, D. (2003). Body size, insulin/IGF signaling and aging in the nematode *Caenorhabditis elegans*. Exp. Gerontol. *38*, 129–136. Abstract Article

McIntire, Sl.L., Jorgensen, E., Kaplan, J., and Horvitz, H.R. (1993). The GABAergic nervous system of *Caenorhabditis elegans*. Nature *364*, 337–341. Abstract Article

McVeigh, P., Leech, S., Mair, G.R., Marks, N.J.,. Geary, T.G., and Maule, A.G. (2005). Analysis of FMRFamide-like peptide (FLP) diversity in phylum Nematoda. Int. J. Parasitol. *35*, 1043–1060. Abstract Article

Meeusen, T., Mertens, I., Clynen, E., Baggerman, G., Nichols, R., Nachman, R.J., Huybrechts, R., De Loof, A., and Schoofs, L. (2002). Identification in *Drosophila melanogaster* of the invertebrate G protein-coupled FMRFamide receptor. Proc. Natl. Acad. Sci. U.S.A. *99*, 15363–15368. Abstract Article

Mertens, I., Clinckspoor, I., Janssen, T., Nachman, R., and Schoofs, L. (2006). FMRFamide-related peptide ligands activate the *Caenorhabditis elegans* orphan GPCR Y59H11AL.1. Peptides *27*, 1291–1296. Abstract Article

Mertens, I., Meeusen, T., Janssen, T., Nachman, R., and Schoofs, L. (2005). Molecular characterization of two G protein-coupled receptor splice variants as FLP2 receptors in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. *330*, 967–974. Abstract Article

Mertens, I., Vandingenen, A., Meeursen, T., Janssen, T., Luyten, W., Nachman, R.J., De Loof, A., and Schoofs, L. (2004). Functional characterization of the putative orphan neuropeptide G-protein coupled receptor C26F1.6 in *Caenorhabditis elegans*. FEBS Lett. *573*, 55–60. Abstract Article

Moffett, C.L., Beckett, A.M., Mousley, A., Geary, T.G., Marks, N.J., Halton, D.W., Thompson, D.P., and Maule, A.G. (2003). The ovijector of Ascaris suum: multiple response types revealed by *Caenorhabditis elegans* FMRFamide-related peptides. Int. J. Parasitol. *33*, 859–876. Abstract Article

Mori, I. and Ohshima, Y. (1995). Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature *376*, 344–348. Abstract Article

Morton, D.B., Hudson, M.L., Waters, E. and O'Shea, M. (1999). Soluble guanylyl cyclases in *Caenorhabditis elegans*: NO is not the answer. Curr. Biol. *9*, R546–R547. Abstract Article

Nathoo, A.N., Moeller, R.A., Westlund, B.A., and Hart, A.C. (2001). Identification of *neuropeptide-like protein* gene families in *Caenorhabditis elegans* and other species. Proc. Natl. Acad. Sci. U.S.A. *98*, 14000–14005. Abstract Article

Nelson, L., Kim, K., Memmott, J., and Li, C. (1998a). FMRFamide-related gene family in the nematode *Caenorhabditis elegans*. Mol. Br. Res. *58*, 103–111. Abstract Article

Nelson, L.S., Rosoff, M.L., and Li, C. (1998b). Disruption of a neuropeptide gene, *flp-1*, causes multiple behavioral defects in *Caenorhabditis elegans*. Science *281*, 1686–1690. Abstract Article

Nguyen, M., Alfonso, A., Johnson, C.D., and Rand, J.B. (1995). *Caenorhabditis elegans* mutants resistant to inhibitors of acetylcholinesterase. Genetics *140*, 527–535. Abstract

Pierce, S.B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S.A., Buchman, A.R., Ferguson, K.C., Heller, J., Platt, D.M., Pasquinelli, A.A. et al. (2001). 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. *15*, 672–678. Abstract Article

Price, D.A., and Greenberg, M.J. (1977). Structure of a molluscan cardioexcitatory neuropeptide. Science *197*, 670–671. Abstract Article

Renden, R., Berwin, B., Davis, W., Kyoungsook, A., Chin, C.-T., Kreber, R., Ganetzky, B, Martin, T.F.J., and Broadie, K. (2001). *Drosophila* CAPS is an essential gene that regulates dense-cre vesicle release and synaptic vesicle fusion. Neuron *31*, 421–437. Abstract Article

Richmond, J.E., Davis, W.S, and Jorgensen, E.M. (1999). UNC-13 I srequired for synaptic vesicle fusion in *C. elegans*. Nat. Neurosci. *2*, 959–964. Abstract Article

Riddle, D.L., and Albert, P.S. (1997). Genetic and environmental regulation of dauer larva development. In *C. elegans* II, D.L. Riddle, T. Blumenthal, B.J. Meyer, and J.R. Priess, eds. (New York, NY: Cold Spring Harbor Laboratory Press), pp. 739–768.

WormBook.org

Rogers, C.M., Franks, C.J., Walker, R.J., Burke, J.F., and Holden-Dye, L. (2001). Regulation of the pharynx of *Caenorhabditis elegans* by 5-HT, octopamine, and FMRFamide-like neuropeptides. J. Neurobiol. *15*, 235–244. Abstract Article

Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P., and de Bono, M. (2003). Inhibition of *Caenorhabditis elegans* social feeding by FMRFamide-related peptide activation of NPR-1. Nat. Neurosci. *6*, 1178–85. Abstract Article

Rosoff, M.L., Burglin, T.R., and Li, C. (1992). Alternatively spliced transcripts of the *flp-1* gene encode distinct FMRFamide-like peptides in *Caenorhabditis elegans*. J. Neurosci. *12*, 2356–2361. Abstract

Rosoff, M.L., Doble, K.E., Price, D.A., and Li. C. (1993). The *flp-1* propeptide is processed into multiple, highly similar FMRFamide-like peptides in *Caenorhabditis elegans*. Peptides *14*, 331–338. Abstract Article

Saeki, S., Yamamoto, M., and Iino, Y. (2001). Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. J. Exp. Biol. *204*, 1757–1764. Abstract

Salio, C., Lossi, L., Ferrini, F., and Merighi, A. (2006). Neuropeptides as synaptic transmitters. Cell Tissue Res. *326*, 583–598. Abstract Article

Schinkmann, K. (1994). FMRFamide-like peptides in the nematodes *Caenorhabditis elegans* and *Caenorhabditis vulgaris*. Boston University, Boston, MA, Ph.D. thesis.

Schinkmann, K., and Li, C. (1992). Localization of FMRFamide-like peptides in *Caenorhabditis elegans*. J. Comp. Neurol. *316*, 251–260. Abstract Article

Simmer, F., Tijsterman, M., Parrish, S., Koushika, S.P., Nonet, M.L., Fire, A., Ahringer, J., and Plasterk, R.H. (2002). Loss of the putative RNA-directed RNA polymerase RRF-3 makes *C. elegans* hypersensitive to RNAi. Curr. Biol. *12*, 1317–1319. Abstract Article

Sieburth, D., Ch'ng, Q.L., Dybbs, M., Tavazoie, M., Kennedy, S., Want, D., Dupuy, D., Rual, J.-F., Hill, D.E., Vidal, M., Ruvkun, G., and Kaplan, J.M. (2005). Systematic analysis of genes required for synapse structure and function. Nature *436*, 510–517. Abstract Article

Sieburth, D., Madison, J.M., and Kaplan, J.M. (2007). PKC-1 regulates secretion of neuropeptides. Nat. Neurosci. *10*, 49–57. Abstract Article

Sithigorngul, P., Stretton, A.O., and Cowden, C. (1991). A versatile dot-ELISA method with femtomole sensitivity for detecting small peptides. J. Immunol. Methods *141*, 23–32. Abstract Article

Sossin, W.S., Sweet-Cordero, A., and Scheller, R.H. (1990). Dale's hypothesis revisited: different neuropeptides derived from a common prohormone are targeted to different processes. Proc. Natl. Acad. Sci. U.S.A. *87*, 4845–4848. Abstract Article

Steiner, D.F. (1998). The proprotein convertases. Curr. Opin. Chem. Biol. *2*, 31–39. Abstract Article

Strand, F.L. (1999). Neuropeptides. MIT Press, Cambridge, MA.

Tensen, C.P., Cox, K.J., Smit, A.B., van der Schors, R.C., Meyerhof, W., Richter, D., Planta, R.J., Hermann, P.M., van Minnen, J., Geraerts, W.P., et al. (1998). The *Lymnaea* cardioexcitatory peptide (LyCEP) receptor: a G-protein-coupled receptor for a novel member of the RFamide neuropeptide family. J. Neurosci. *18*, 9812–9821. Abstract

Thacker, C., Peters, K., Srayko, M., and Rose, A.M. (1995). The *bli-4* locus of *Caenorhabditis elegans* encodes structurally distinct Kex2/Subtilisin-like endoproteases essential for early development and adult morphology. Genes Dev. *9*, 956–971. Abstract

Thacker, C., and Rose, A.M. (2000). A look at the *Caenorhabditis elegans* Kex2/Subtilisin-like proprotein convertase family. BioEssays *22*, 545–553. Abstract

Thomas, J.H. (1990). Genetic analysis of defecation in *Caenorhabditis elegans*. Genetics *124*, 855–872. Abstract

Thomas, J.H., Birnby, D.A., and Vowels, J.J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics *134*, 1105–1117. Abstract

Tomioka, M., Adachi, T., Suzuki, H., Kunitomo, H., Schafer, W.R., and Iino, Y. (2006). The insulin/PI 3-kinase pathway regulates salt chemotaxis learning in *Caenorhabditis elegans*. Neuron *51*, 613–625. Abstract Article

Trailovic, S.M., Clark, C.L., Robertson, A.P., and Martin, R.J. (2005). Brief application of AF2 produces long lasting potentiation of nAChR responses in *Ascaris suum*. Mol. Biochem. Parasitol. *139*, 51–64. Abstract Article

Waggoner, L.E., Hardaker, L.A., Golik, S., and Schafer, W.R. (2000). Effect of a neuropeptide gene on behavioral states in *Caenorhabditis elegans* egg-laying. Genetics *154*, 1181–1192. Abstract

Ward, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans*: Identification of attractants and analysis of the response by use of mutants. Proc. Natl. Acad. Sci. U.S.A. *70*, 817–821. Abstract Article

Yew, J.Y., Kutz, K.K., Dikler, S., Messinger, L., Li, L., and Stretton, A.O. (2005). Mass spectrometric map of neuropeptide expression in *Ascaris suum*. J. Comp. Neurol. *488*, 396–413. Abstract Article

Zahn, T.R., Angleson, J.K., MacMorris, M.A., Domke, E., Hutton, J.F., Schwartz, C., and Hutton, J.C. (2004). Dense core vesicle dynamics in *Caenorhabditis elegans* neurons and the role of kinesin UNC-104. Traffic *5*, 544–559. Abstract Article

WormBook.org