

Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species

Arif N. Nathoo, Rachael A. Moeller*, Beth A. Westlund†, and Anne C. Hart*

Massachusetts General Hospital Cancer Center and Harvard Medical School Department of Pathology, 149-7262 13th Street, Charlestown, MA 02129

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Neuropeptides play critical roles in synaptic signaling in all nervous systems. Unlike classical neurotransmitters, peptidergic neurotransmitters are encoded as preproproteins that are posttranslationally processed to yield bioactive neuropeptides. To identify novel peptidergic neurotransmitters, the *Caenorhabditis elegans* genome was searched for predicted proteins with the structural hallmarks of neuropeptide preproproteins. Thirty-two *C. elegans* neuropeptide-like protein (nlp) genes were identified. The nlp genes define at least 11 families of putative neuropeptides with unique motifs; similar expressed sequence tags were identified in other invertebrate species for all 11 families. Six of these families are defined by putative bioactive motifs (FAFA, GGxYamide, MRxamide, LQFamide, LxDuamide, and GGARAF); the remaining five families are related to allatostatin, myomodulin, buccalin/drosulfakinin, orcoxinin, and APGWamide neuropeptides (MGLFamide, PRFamide, MGFamide, GFaGF, and YGGWamide families, respectively). Most *C. elegans* nlp gene expression is in neurons. The *C. elegans* nlp genes and similar genes encoding putative neuropeptides in other species are likely to play diverse roles in nervous system function.

Chemical signaling via neurotransmitters is critical for synaptic transmission of information between neurons. Neuropeptides are the most varied and numerous type of neurotransmitters. Invertebrate neuropeptides are thought primarily to modulate synaptic function of classical small-molecule neurotransmitters by means of seven transmembrane domain receptors. However, the recent identification of a FMRFamide-gated sodium channel from *Helix lucorum* suggests that they may also act as fast transmitters (1). In mammals, neuropeptides and their receptors are implicated in behaviors including feeding and sleep (2–5). Despite their clear roles in synaptic signaling and behavior, neuropeptide functions are still not understood.

Biochemical isolation of neuropeptides has been relatively successful in several invertebrate systems, including *Lymnaea stagnalis*, *Drosophila melanogaster*, and *Aplysia californica* (6–8), and has led to the identification of several invertebrate neuropeptide families. In the nematode *Caenorhabditis elegans*, 23 FMRFamide-related proteins (FaRP) neuropeptide genes, designated *nlp-1* to *nlp-23* (FMRFamide-like proteins), have been identified (9). Only *nlp-1* has been characterized at the functional level. Animals lacking *nlp-1* have abnormal behavior, including uncoordinated movement and hyperactivity (10). The only other *C. elegans* non-*nlp* neuropeptide genes that have been identified are the 37 insulin-like genes (11, 12).

Dense-core synaptic vesicles are prevalent in presynaptic terminals of *C. elegans* neurons that are neither FaRP immunoreactive nor catecholaminergic (13), suggesting that non-FaRP neuropeptides are present. Additionally, about 130 genes encoding putative neuropeptide receptors were identified in the *C. elegans* genome (14). This large number of receptors is much higher than the number of *nlp*-encoded FaRPs (9) and is reminiscent of the large number of putative “orphan” neuropeptide receptors in vertebrates. This finding suggests that several more families of neuropeptides are as yet undiscovered.

The relative paucity of non-*nlp* neuropeptide genes previously identified in *C. elegans* (by either genetic or biochemical techniques) led to the suggestion that FaRPs could be responsible for the majority of neuropeptide signaling in this animal (10). Clearly, neuropeptide signaling is implicated in multiple *C. elegans* behaviors, including defecation and social behavior (15, 16). Described herein are 32 non-*nlp* putative neuropeptide genes that are expressed primarily in *C. elegans* neurons. These genes encode 134 unique and 151 total putative neuropeptides. Related candidate neuropeptides are found in other species, suggesting that these genes are functionally conserved.

Materials and Methods

Structural searches used the PATTERNFIND program (http://www.isac.isb-sfb.ch/software/PATFIND_form.html). BLAST searches (17, 18) yielded neuropeptide-like protein (nlp) homologs in other species. Signal peptide cleavage sites were defined by using SIGSALP 1.0 (<http://www.cbs.dtu.dk/services/SignalP/>) (19). Predicted NLPs and peptides were aligned by using CLUSTALW 1.8 (<http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html>) (20). nlp gene families were defined by using (primarily) motifs, phylogenetic tree construction (MEGALION, Dnastar, Madison, WI), and overall predicted peptide homology. A motif is defined as at least three of five identical amino acids at the N or C terminus of at least two predicted peptides within a nlp gene and/or family. Motifs also were used for additional searching with PATTERNFIND.

A mixed cDNA library (M. Vidal, Dana-Farber Cancer Institute, Boston) was screened for *nlp-1* through *nlp-21* by PCR amplification. Primers overlapped nlp gene predicted start and termination sites. Amplified products were directly cloned (pCR2.1-TOPO, Invitrogen). Twelve nlp::gfp reporter constructs were made by insertion of the nlp promoter into a promoterless green fluorescent protein (GFP)-expressing vector, pPD95.67. The sequences and subcloning sites are: *nlp-1*: *NsiI/XbaI* into *PstI/XbaI*; *nlp-3*: *NheI/NsiI* into *XbaI/PstI*; *nlp-16*: *BamHI/NsiI* into *BamHI/PstI*; *nlp-2*, *nlp-25* through *nlp-32*: *BamHI/XbaI* into *SacI/BamHI*. For the remaining nlp genes, the putative regulatory region was amplified by PCR (reaction 1). The gfp coding regions from pPD95.67 (21) were amplified by using a nlp-gene specific primer that also contained GFP vector 5' sequence and by using a 3' GFP vector primer (GFP-C, AAGGGCGCGTACGGCCGACTAGTAGG) in an indepen-

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Abbreviations: FaRP, FMRFamide-related protein; nlp, neuropeptide-like protein; *nlp*, FMRFamide-like protein; GFP, green fluorescent protein; EST, expressed sequence tag.

*Present address: Department of Environmental Studies, Brown University, Providence, RI 02912.

†Present address: Cambria Biosciences, 2 Preston Court, Bedford, MA 01765.

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Neuropeptide Gene Expression

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Neuropeptide Technology P. Michael Conn, 2013-10-22 *Methods in Neurosciences Volume 5* Neuropeptide Technology Gene Expression and Neuropeptide Receptors discusses procedures and tools of assay useful for the identification purification and quantification of neuropeptides and their receptors This volume is divided into two sections neuropeptide gene expression and neuropeptide receptors In these sections this book specifically discusses the assays for peptide products of somatostatin gene expression molecular assays for rat thyrotropin releasing hormone gene and structure expression and chromosomal localization of human gene The gastrin releasing peptide or mammalian bombesin semiquantitative analysis of cellular somatostatin mRNA Levels and atrial natriuretic factor receptor assays are also elaborated This text likewise covers the receptors for opioid peptides in brain and autoradiography of somatostatin receptors in rat cerebellum This publication is beneficial to neuroscientists and students intending to acquire knowledge of gene expression and neuropeptide receptors

Testosterone and Developmental Regulation of Neuropeptide Gene Expression in the Rat Brain Julie Ann Chowen, 1990

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