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Role of the flp neuropeptide gene family in C. elegans. Tina Burton, Jenny Chang, Lawrence Ha, Zi Huang, Adewale Olajubelo, **Chris Li**. Dept Biol, City Col New York, New York, NY.

Neuropeptides are short sequences of amino acids that are used in the nervous system to modulate behavior. Because a single neuropeptide can have multiple functions and because multiple neuropeptides can impact the same behaviors, the precise roles of specific neuropeptides in different behaviors have been difficult to assess. We are examining the function, regulation, and signaling pathways of the flp neuropeptide gene family in C. elegans. At least 30 flp genes encode peptides from the FMRFamide (Phe-Met-Arg-Phe-amide) family. To determine the function of the different flp genes, we are isolating deletion mutants for each gene. Thus far, mutations have been identified in 11 flp genes. The mutants are being screened for defects in locomotion, reproduction, and development. We have found that each flp mutant is defective on at least one assay, and several mutants are defective in multiple assays. The number of mutants showing defects on these assays is somewhat surprising, particularly given their expression patterns. To verify that these phenotypes are due to loss of the flp gene, we are performing transgenic rescue. However, we are unable to isolate stable transgenic lines for several flp genes. Because the increased transgenic expression may be toxic to the animals, we are currently decreasing the amount of injected DNA to isolate stable transformants. These data suggest that locomotion, reproduction, and development are finely tuned by levels of the different FLP neuropeptides. In addition, we are characterizing animals carrying mutations in known FLP receptors. Insights into how neuropeptides function in C. elegans may give clues into how neuropeptides are used in higher animals and into the design of antihelminthic drugs.

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FLP-18 Neuropeptide Signaling Can Modulate *C. elegans* Locomotion. **Yin Li**, Niels Ringstad, Bob Horvitz. Biology, MIT, Cambridge, MA.

Neuropeptides are an important class of signaling molecules involved in nervous system function. The *C. elegans* gene *flp-18* is predicted to encode six distinct neuropeptides of the FMRFamide family. We found that a transgene carrying extra copies of *flp-18* causes multiple behavioral abnormalities, including a characteristically coiled posture, uncoordinated reverse locomotion, increased spontaneous reversal frequency, and a Sho (suppression-of-head oscillation) phenotype. We have isolated a *flp-18* deletion allele, *n4766*, in which all the neuropeptide-encoding sequences are absent. In contrast to *flp-18* overexpressors, *flp-18* deletion mutants do not exhibit any obvious defects. We are currently assaying *flp-18* deletion mutants in sensitized backgrounds to detect more subtle behavioral defects.

To characterize the neuronal circuitry underlying the abnormalities in *flp-18* overexpressors, we have generated transgenic animals expressing translational fusions of *flp-18* and GFP. *flp-18::gfp* is expressed in many parts of the *C. elegans* neuromusculature, including head neurons, the ventral and dorsal nerve cords, tail neurons, and (weakly) body-wall muscles. We are currently identifying the *flp-18::gfp*-expressing cells.

To identify other components of the *flp-18* signaling pathway, we screened ~10,000 haploid genomes in *flp-18*-overexpressing backgrounds, looking for suppressors of the posture and locomotion defects. Fifteen independent suppressors were isolated, defining at least four complementation groups. We recovered seven alleles of *Y58G8A.4*, which encodes a G protein-coupled receptor that binds to FLP-18 peptides *in vitro* (Lowery *et al.*, 2003, U.S. Patent 6,632,621). So far, we have identified molecular lesions in the *Y58G8A.4* coding region for five of the seven alleles. *Y58G8A.4* overexpression phenocopies *flp-18* overexpression; the *Y58G8A.4* overexpression phenotype is suppressed by *flp-18* deletion. Of the remaining suppressors, six cause a twitcher phenotype and are probably alleles of *unc-22*, which encodes a structural protein required for proper muscle contraction. Two other suppressors each define one complementation group.

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Determining the mechanism of *daf-7* regulation by serotonin in *C. elegans*. **Edith M. Myers**, Michael R. Koelle. Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

Transforming growth factor (TGF-β) regulates normal cell proliferation, differentiation, and survival. In *C. elegans*, a TGF-β-like gene, *daf-7*, regulates dauer entry. Mutants for *daf-7* are dauer constitutuve (Daf-c). Many compounds including steroids, other growth factors, and the neurotransmitter serotonin regulate the synthesis and/or the secretion of TGF-β in mammals. Serotonin is better known as an important regulator of mood and memory formation, but how it regulates TGF-β expression is generally unknown. Transcription of *daf-7* is also regulated by serotonin, since mutants for the serotonin biosynthetic enzyme TPH-1 have severely reduced *daf-7* expression[1]. Recently it has also been reported that *daf-7* in turn regulates the expression of *tph-1*[2], suggesting a regulatory feedback loop of some kind.

I am using transgenic worm lines that express green fluorescent protein (GFP) under the *daf-7* promoter as a tool to understand how *daf-7* transcription is regulated by serotonin. I have measured changes in GFP fluorescence (*daf-7* transcription) caused by mutations in serotonin receptors. Mutations in any one of the known serotonin G-protein coupled receptors, SER-4, SER-1, or SER-7, do not have significant effects on *daf-7*::GFP expression in L1 larvae. However, mutations in Gα proteins, GOA-1 ($G\alpha_o$) and EGL-30 ($G\alpha_q$) significantly reduce *daf-7*::GFP expression in the ASI neurons of L1 larvae, and have effects on dauer formation as well. I am currently investigating whether combinations of serotonin receptors mediate the serotonergic regulation of *daf-7* transcription. I am also determining how other signaling genes, important for dauer formation, affect *daf-7* transcription. I am using cell-specific promoters to determine whether the G proteins have a cell autonomous effect on *daf-7* expression, and which serotonergic neurons mediate the effect of TPH-1 on *daf-7* transcription.

In addition to looking at the effects of mutations in known serotonin signaling components, I also plan to screen for novel genes that mediate serotonergic regulation of *daf-7* expression.

[1] Sze, JY *et al* (2000) *Nature*: 560-564. [2] Chang, AJ *et al* (2006) *PLoS Biology*:1588-1602.