CREB regulates expression of neuropeptide FLP-18 and controls backward movement

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Certificate of Examination

This is to certify that the dissertation titled "CREB regulates expression of neuropeptide FLP-18 and controls backward movement" submitted by Mr. Saurabh Thapliyal (Reg. No. MS11053) for the partial ful-filment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Dated: April 19, 2016

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Kavita Babu at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

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In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Kavita Babu
(Supervisor)

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Saurabh Thapliyal

List of Figures

List of Photographs

List of Tables

Contents

Abstract

CREB is a transcription factor. CREB is known to regulate expression of various genes involved in different functions. We found that CREB regulates expression of neuropeptide FLP-18 in *C.elegans*. FLP-18 is known to effect backward movement in *C.elegans*. Further we go on to show that CREB controls length of backward movement in *C.elegans.* Mutation in CREB homolog *crh-1* resulted in decreased length of backward movement. This phenotype was rescued by expressing repressor isoform of CREB, CRH-1f in *crh-1* mutants. These results suggests that CREB regulate FLP-18 expression and length of backward movement during exploratory behavior in *C.elegans*.

Chapter 1

Introduction

1.1 Basic Theory

1.1.1 Behavioral responses in *C.elegans*

For the survival of an organism, it's critical to modulate its behavior in response to various environmental conditions. The nervous system of higher mammalian organisms is very complex. They can have a wide variety of behavioral response in different conditions. We human beings continuously sense our environment, process information and execute a behavioral response. In response to some visual, auditory and other sensory cues we can have positive, negative or neutral behavioral responses. We express emotions, sleep, dance, play, walk, run and think in different situations of our life. We have the ability to perform cognitive tasks which simple vertebrates can't perform. *C.elegans* is a very simple model organism, which is widely used in different scientific studies as development, aging, cellular processes, metabolic pathways. One of the most important features of *C.elegans* is its well-defined nervous system which is made up of 302 neurons having identified connections with each other[1]. It has been studied widely to understand functions of various genes involved in various nervous system functions.

To understand the nervous system, it's crucial to correlate functions of various molecular and cellular targets with the behavioral response.

Different types of behavioral studies have been performed in *C.elegans*. Some of the most studied behaviors in *C.elegans* are:

1. **Mechanosensation:**

   This behavior worms show in response to mechanical stimuli. The response of worm varies for different types of mechanosensory cues like gentle touch[2,3], harsh touch to the midbody[4] and nose touch[5].

2. **Osmotic avoidance:**

   This behavioral output helps the worms to avoid high concentration of a solution[6].

3. **Chemosensation:**

   This behavior involves the movement of worms toward or away from a chemical cue in response to change in its concentration gradient. Various chemicals are sensed by different sensory neurons which can act as an attractant or repellent[7,8]. Worms can sense different volatile and gustatory cues[9,10].

4. **Learning, adaptation and habituation:**

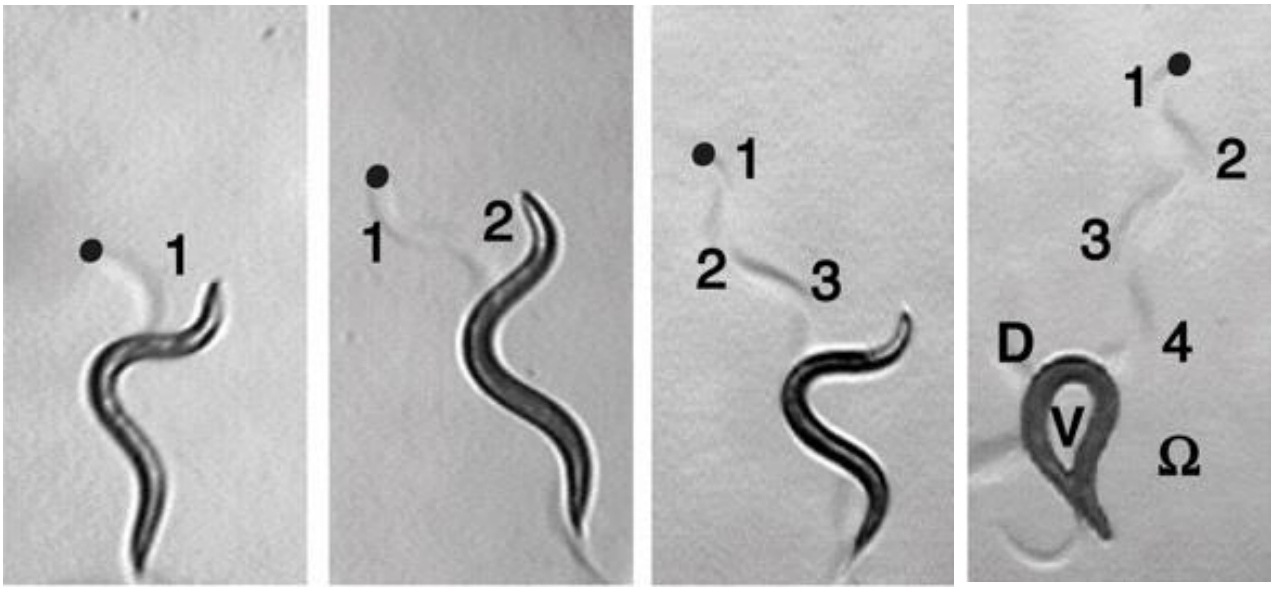
   Worms can learn to suppress behavioral response when continuously exposed to a sensory cue[11]. This type of learning is called habituation.

5. **Thermal response:**

   Worms can sense the temperature associated with food and avoid temperature of starvation[12]. This type of behavior is known as thermotaxis.

6. **Mating behavior**

Most important and shared feature underlying all the behaviors is change in locomotion. Worm can either move forward, backward movement (reversal) or take omega turn. Change in frequency[13,14] and length of reversals[14] and omega turns[15] can create bias in movement which can ultimately change behavior in response to various sensory cues.



Picture1: Spontaneous reversal followed by Omega turn[16].

Reversals and omega turns are widely studied in various behaviors. So the significance of frequency of reversals is:

1. Increased reversal frequency increases local search behavior but suppresses long distance search.
2. Decreased reversal frequency can suppress local search which is important in conditions like starvation.

Reversal length can also create bias in locomotion along with reversal frequency.

Different underlying neural circuits regulate behaviors in *C.elegans.* Therefore to understand a behavior neural circuitry underlying behavior is studied.

1.1.2. Studying neural circuits in *C.elegans*:

In *C.elegans*, various neurons are associated with different functions. After decades of hard work neural circuitry involved in some behaviors are mapped[17]. With advancement in tools to study circuitry, new circuits are getting mapped every year. Some of the tools used to study neural circuitry in *C.elegans* are:

1. **Ablation:** Neuronal ablation is one of the first method used to examine the function of a particular neuron by killing neuron by laser beam[18]. It's possible to ablate cell body of a neuron or a specific axon[20], which is now used to study neuronal regeneration[19]. Femtosecond lasers are very popular for neuronal ablation studies. New methods have been discovered to ablate neuron by expressing a phototoxic protein in the desired neuron[21]. This protein then generates reactive oxygen species when the green light is shined on the worm and ablates neuron of interest.
2. **Neuronal silencing:** Specific neurons can be silenced during the particular study by using expressing Histamine-gated chloride channels in neurons of interest and exogenously provide histamine during study[22].
3. **Optogenetic activation:** Neurons can be selectively fired by expressing a light activated cation channel Channelrhodopsin in *C.elegans*[23]. This protein is a non-specific cation channel which opens from blue light. This engineered protein have rapid activation and deactivation kinetics. Neurons can be stimulated from this tool with firing frequency of 3-4 Hz.
4. **Optogenetic silencing.** Neurons can be silenced during behavior by expressing a light-driven chloride channel Halorhodopsin[24].
5. **Calcium imaging:** Activity of neuron in behaving can be monitored by expressing genetically engineered calcium indicator GCaMP[25]. This protein fluoresces when calcium binds to it. This protein is a reliable indicator of neuronal activity.

1.1.3. Neural circuits in *C.elegans:*

C.elegans nervous system has very simple architecture. 302 neurons are connected with each other with excitatory, inhibitory chemical synapses and electrical synapses (gap junctions)[17].

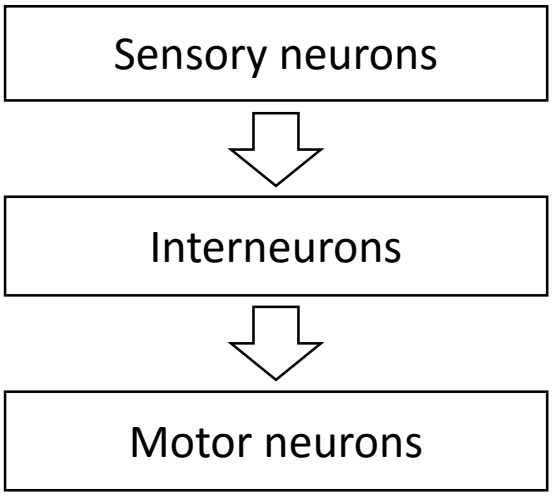


Figure1: Circuit architecture of *C.elegans.*

Sensory neurons sense various cues by different receptors. Three primary sensations in *C.elegans* are:

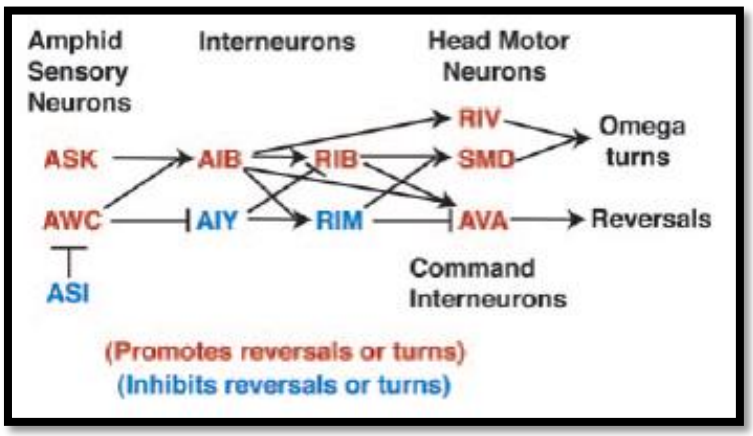
1. Chemosensation
2. Mechanosensation
3. Thermosensation

This sensed information is received by interneurons. Different sensory cues are integrated and processed in two different layers of interneurons. The first layer of interneurons directly receives information from sensory neurons. This information after processing transmitted to command interneurons which final transmit signal to motor neurons. There are three different types of motor neurons in *C.elegans* nervous system. A type motor neurons control backward movement, B-type motor neurons controls forward movement and D-type motor neurons receives a signal from A and B type neurons to control muscle contraction.

1.1.4 Exploratory behavior:

In the absence of food, the worm tries to explore its environment to locate food. For this, they move forward, backward (reversal) and take occasional Omega turns[16]. This type of behavior is known as exploratory behavior. Reversals made during exploratory behavior are known as spontaneous reversals.

1.1.4.1 Neural circuitry involved in exploratory behavior:



Picture2: Neural circuitry involved in exploratory behavior[16]

In the neural circuitry involved in exploratory behavior, AWC is the sensory neuron which is responsible for sensing food in *C.elegans*. AWC is an off type sensory neuron which is off in the presence of sensory cue (food). AWC is switched on in the absence of food. AWC controls reversals from the bidirectional switch of AIY and AIB. AWC inhibits AIY and excites AIB. Inhibition of AIY leads to leads to increase in reversals due to inhibition of RIM, which is an inhibitor of AVA. AIB excites AVA directly and through RIB. AVA is known as the master controller of reversal movement. The firing of AVA promotes backward movement by activating DA and VA motor neurons. Activation of AIB through also promotes omega turns

RIV and SMD. ASK sensory neuron promotes reversals while ASI inhibits reversals. Other than AVA two other command interneurons AVD and AVE are also responsible for controlling backward movement. But it was shown in neuronal ablation studies that worms can initiate reversals in the absence of AVA, AVD, and AVE through an unknown parallel pathway.

1.1.5 Modulation of circuitry underlying behavior:

Like every organism in *C.elegans* behavior is also regulated by switching on and switching off various neurons in neuronal microcircuits. Different neuromodulator molecules can also modulate behavior[26]. Unlike neurotransmitters which transmit signals from one neuron to other. Neuromodulators can change the activity of neurons. Two common neuromodulators are:

1. Small molecule neurotransmitters: i.e. serotonin
2. Neuropeptides

1.1.5.1 Neuropeptides:

Neuropeptides are signaling molecules used by neurons to communicate with each other. Neuropeptides can act as a neurotransmitter and a neuromodulator[27]. The fundamental difference between a small molecule neurotransmitter and neuropeptide is:

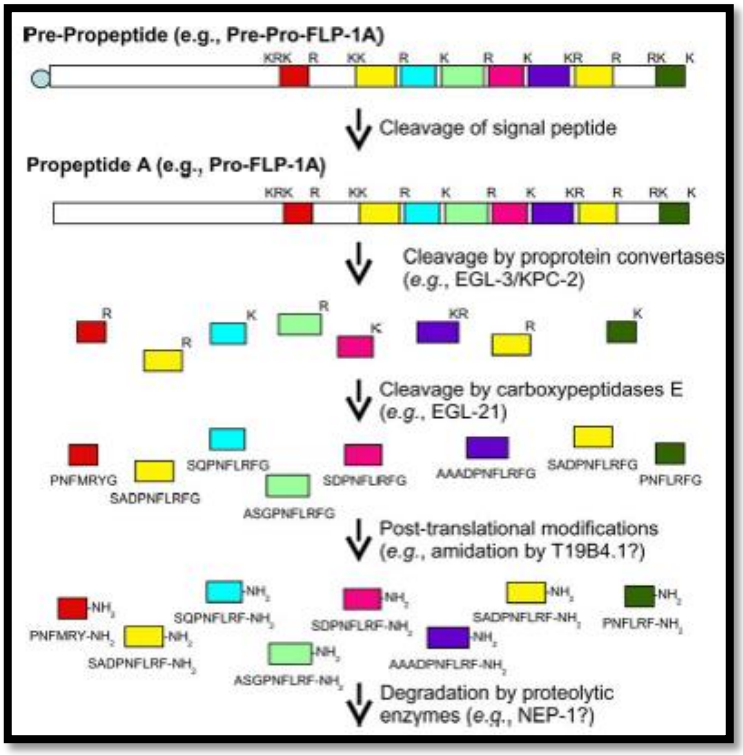
1. **Size:** Neuropeptides are made up of amino acids. The size of a neuropeptide molecule varies from 5-24 amino acids. Small molecule neurotransmitter are very small compared to these neurotransmitter[28].
2. **The time scale of activity:** Neuropeptides can stay in the synapse for much longer time (1.6ms) compared to small molecule neurotransmitter (0.26ms)[29].
3. **No. of released molecules:** A small vesicle neurotransmitter can release 4700 molecules but 18000 neuropeptides can be released from a dense core vesicle[29].
4. **Reuptake:** Small neurotransmitters are reuptake by presynaptic terminals but neuropeptides once released are degraded. So every time cell has to synthesize new neuropeptide molecules[28].
5. **The site of release:** Neurotransmitter are released from the active site of the synaptic terminal, but dense core vesicle (DCV) are released from distinct sites from active zone[30].

1.1.6 Neuropeptides in *C.elegans*:

250 different neuropeptides have been identified to be coded by 113 genes in *C.elegans*. There are three main types of neuropeptides in *C.elegans*[31]:

1. Insulin-like peptides: NLPs
2. FMRFamide related peptides (FaRPs): FLPs
3. Non-insulin, non FLP peptides: Neuropeptide-like proteins NLPs

Mature Neuropeptides are synthesized by posttranslational processing and modification from precursor peptide molecule. A single precursor molecule can lead to the formation of different peptides in different cell types by processing.



Picture3: Processing of Neuropeptide precursor[31]

Various genes like EGL-3, which encodes enzyme pro-protein convertase are involved in the processing of neuropeptides precursor[32].

1.1.6.1 Functions of various neuropeptide genes:

1. Insulin-like gene family:
   Insulin-like peptides play important role decision to enter dauer state in the absence of food[33].
2. The flp family:
   flp gene family is known to effect locomotion[34], egglaying[35].
3. The nlp family:
   nlp gene family is known to have anti-microbial functions[36] and modulating acetylcholine signaling[37].

1.1.7 Neuropeptide FLP-18:

FLP-18 belongs to the family of famide related peptides. *flp-18* gene can form six different types of putative peptides. *flp-18* gene is reported to be expressed in AVA, AIY and RIM neurons in neural circuitry involved in the exploratory behavior of *C.elegans*[39].

Gene	Putative peptide	Peptide name	Expression pattern	Name in other species
flp-18	GAMPGVLRFG	FLP-18-1	AVA, AIY,	*afp-1*
	EMPGVLRFG	FLP-18-2	RIG, RIM, M2,	
	SVPGVLRFG	FLP-18-3	rays 2,6	
	EIPGVLRFG	FLP-18-4		
	SEVPGVLRFG	FLP-18-5		
	DVPGVLRFG	FLP-18-6		

Table1: FLP-18 putative peptides and expression[31].

1.1.7.1 Reported roles of FLP-18

1. Egglaying[40]
2. Fat metabolism[41]

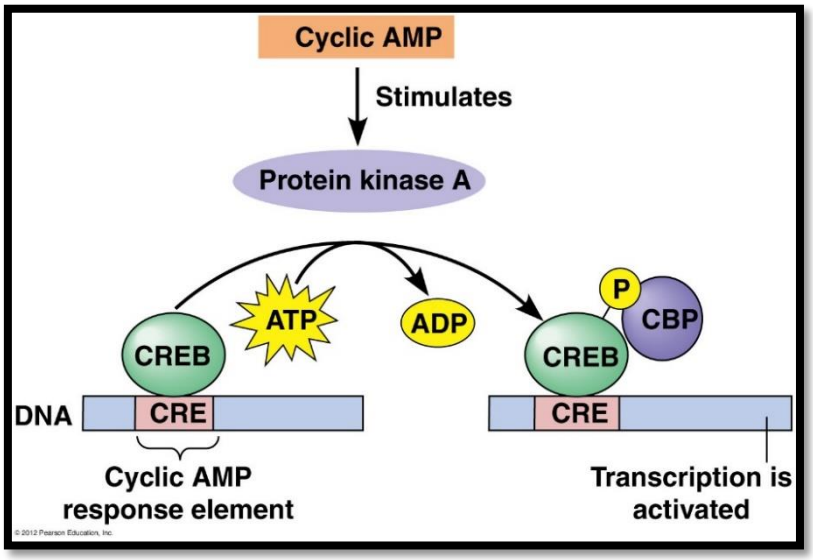
Mario deBono's lab at Cambridge first reported the role of FLP-18 in exploratory behavior. FLP-18 controls reversal frequency during prolonged starvation which helps worm to suppress local search in the absence of food[34].

Expression of neuropeptides is regulated by various transcription factors. One important transcription factor which is known to regulate neuropeptide expression is CREB.

1.1.8 Transcription factor CREB

CREB (cAMP response element binding protein) is a transcription factor. CREB binds to cAMP response element (CRE) sites in the regulatory region of a gene[42]. CREB can upregulate or downregulate gene expression by acting as an activator or a repressor. CRE sites contain a highly conserved nucleotide sequence 5'-TGACGTCA-3'. CRE sites are generally found upstream of genes within promoter regions[43].

1.1.8.1 Mechanism of CREB action:



Picture4: CREB activation mechanism[46]

CREB has been known to be involved in long-term memory formation and neuronal plasticity. Activation of CREB is considered a treatment for Alzheimer's disease[44]. CREB regulates expression of various genes which includes *c-fos*, BDNF, tyrosin hydroxylase and numerous neuropeptides[45].

CREB mediated expression of various genes could affect different behaviors. CREB is already known to regulate expression of various neuropeptides. ***We want to test whether CREB controls backward movement during exploratory behavior by regulating neuropeptide expression.***

1.2 Experimental Methods

1.2.1 Strains used:

All strains were cultured and maintained under standard methods at 20^{o}C.(Brenner, 1974). Worms were grown using *Escherichia*strain OP50. The following strains were used: N2, YT17(*crh-1* mutants), BAB719 (*Prab-3*::crh-1f in wild type), BAB750 (*Prab-3*::crh-1f in *crh-1* mutants), SRS85 (*Psra6::ChR2* in wild type), BAB751 (*Psra6::ChR2* in *crh-1* mutants).

1.2.2 Reversal assay

To observe the exploratory behavior of worm worms were picked from a plate with food to a plate without food for 30s to remove food from its body. Then again worm was picked to a plate without food for 5min.

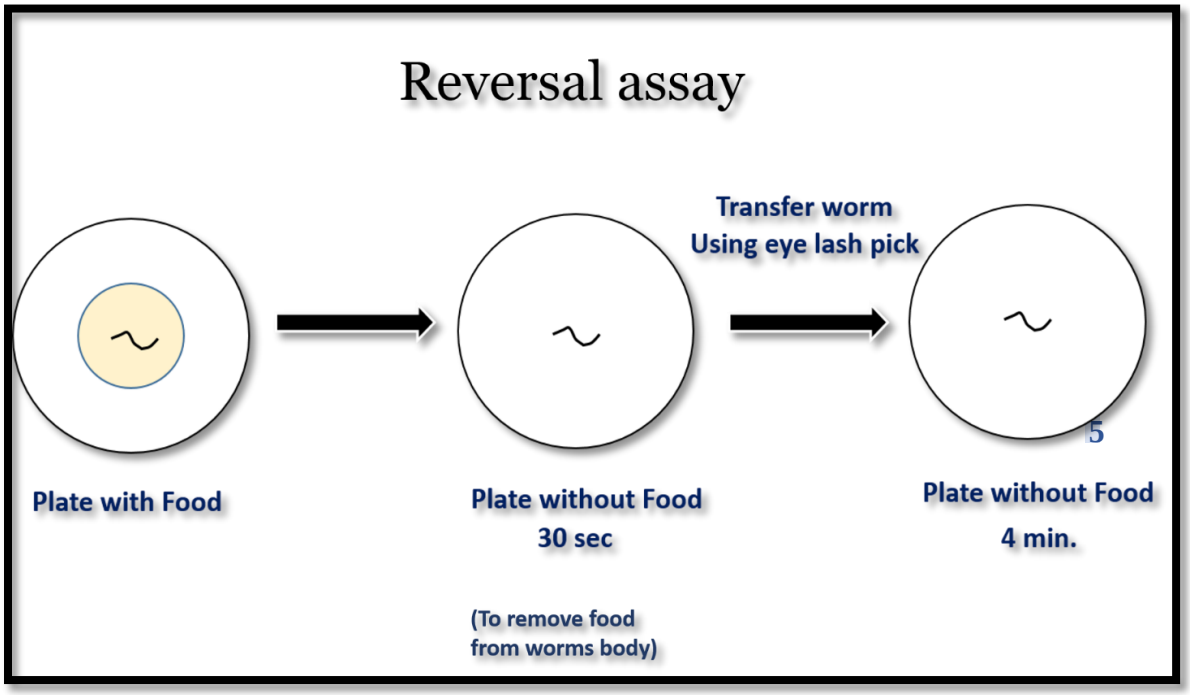


Figure2: Reversal assay

In the absence of food, worm explores its environment to locate food. For this worm moves forward, take occasional reversals and omega turns. Reversal taken during exploratory behavior are called spontaneous reversals. During exploratory behavior I observed:

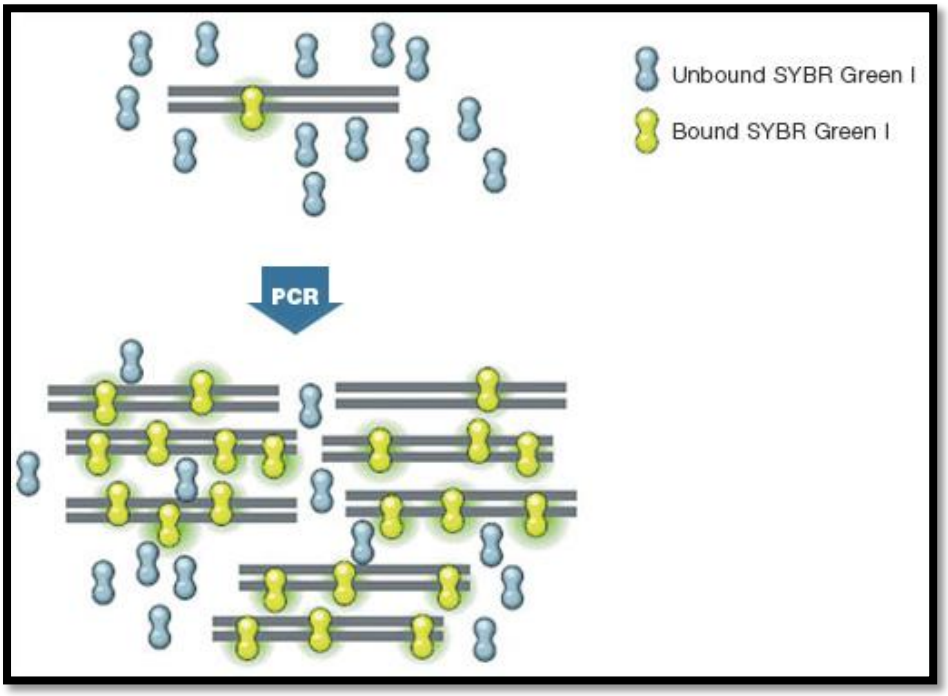
1. The frequency of reversals: No. of reversals taken in 5min.
2. Reversal length: No of body bends taken during reversal.

Experimental conditions: For reversal assay worms were grown at 20ºC. Reversal assay was performed on unseeded 90mm plates at 20ºC. Movement of worms was recorded for 5min. The assay was recorded using a Zeiss AxioCam MRM camera.

1.2.2 Quantitative Real-Time PCR:

Quantitative real-time PCR is a technique used to quantify DNA molecules above or below a certain amount by PCR. Two methods used for detection of PCR products are:

1. Non-specific fluorescent dyes (SYBR Green) cDNA probes.



Picture5: SYBR Green for qRT-PCR[48]

2. Sequence-specific DNA probes consisting of oligonucleotides labeled with a fluorescent reporter.

1.2.2.1 Quantitative real-time PCR to measure gene expression

Gene expression in a cell can be measured by copies of mRNA present in it. This mRNA is first reverse transcribed into cDNA with reverse transcriptase. A fluorophore along with cDNA, primers, deoxy ribonucleotides, buffer and DNA polymerase are added in a PCR reaction to measure generation rate of the specific product.

1.2.3 Optogenetics:

Optogenetics is a method to stimulate/silence a neuron based on genetically engineered light-gated ion channels which allow cations or anions to pass through when light is shined on it. There are two different class of proteins:

1. Channelrhodopsin (Cation channel for neuronal activation).
2. Halorhodopsin and Archaerhodopsin (Anion channels for neuronal silencing).

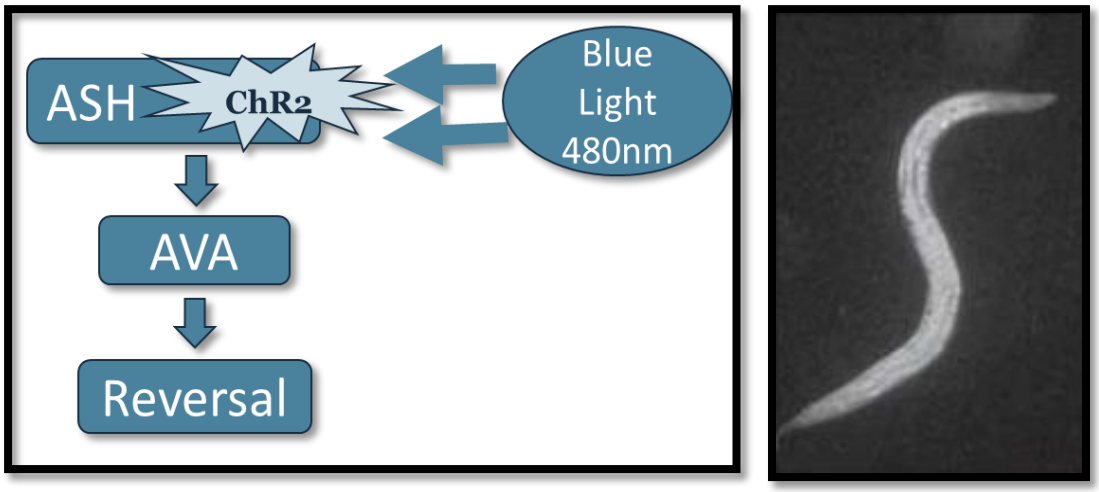


Figure3: Optogenetics in *C.elegans*

AVA is the controller of backward movement in *C.elegans*. The firing of AVA neuron can lead to reversals. During spontaneous reversals stimulation of AVA is not always same but AVA can be stimulated in a controlled way by optogenetic stimulation. In the neural circuitry of exploratory behavior due to unavailability of neuron-specific promotors we chose ASH neuron for AVA stimulation. ASH is located just upstream of AVA and share glutamatergic chemical synapses.

Experimental conditions: For optogenetic assay worms were grown at 20ºC on all-trans-retinal plate. Blue light was shined at fixed magnification on freely moving worms for 3s in a 90mm plate with thin OP50 seeding at 20ºC. This experiment was performed on Zeiss imager.Z2 upright microscope equipped with epifluorescence. Individual animals were exposed to blue light (488 nm), while moving forward. The movement was recorded using a Zeiss AxioCam MRM camera.

Results:

1. Quantitative real-time PCR:

CREB is known to regulate expression of various Neuropeptide genes. FLP-18 neuropeptide is expressed in the neural circuitry of exploratory behavior. When we looked at the promoter region of FLP-18, we found multiple half and full CREB binding sites (CRE sites). We wanted to test whether CREB regulates expression of neuropeptide FLP-18 in *C.elegans.* CRH-1 is CREB homolog in *C.elegans*. We used real-time quantitative PCR to check *flp-18* expression levels in wild-type and *crh-1* mutant background

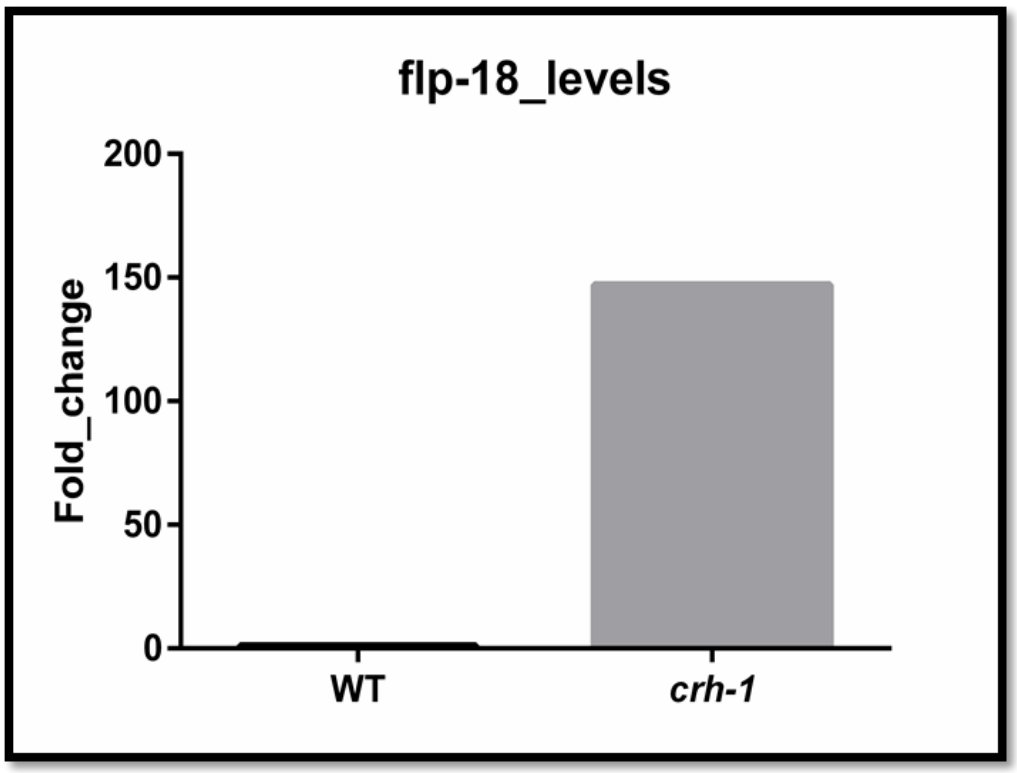


Figure4: *flp-18* expression levels in *crh-1* mutants compared to wild type worms.

We observed 147 fold increase in flp-18 expression levels in crh-1 mutants compared to wild type worms. This data suggests CREB homolog represses flp-18 expression.

2. Reversal assay:

crh-1 mutants showed an increase in *flp-18* levels. FLP-18 is expressed in three neurons AIY, RIM, and AVA in the neural circuitry of exploratory behavior. We wanted to investigate the effect of increased FLP-18 levels in *crh-1* mutants on the backward movement of worms.

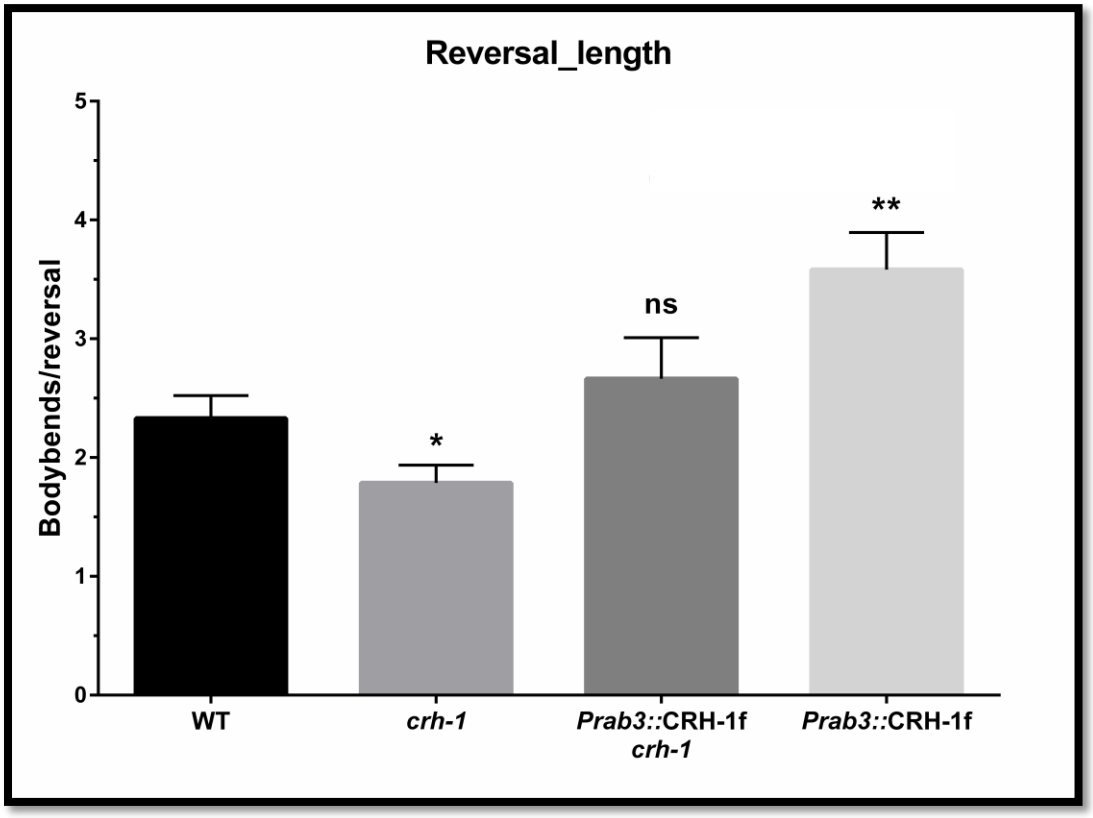


Figure5: Decreased reversal length of *crh-1* mutants

crh-1 mutants showed a decrease in reversal length compared to wild type worms. *crh-1* mutants have increased FLP-18 levels. *We could rescue decreased reversal length of crh-1 mutants by pan-neuronal expressing repressor isoform CRH-1f.* There are six different isoforms of CRH-1 in *C.elegans*. All the other isoforms have DNA binding domain as well as activator domain. CRH-1f has only DNA binding domain therefore, can't recruit CREB binding protein (CBP).

3. Optogenetic stimulation:

We wanted to test whether crh-1 mutants show a decrease in reversal length when AVA is stimulated using optogenetic stimulation. We stimulated AVA in wild-type and crh-1 mutants by shining a blue light (480nm) for 3s in freely moving worms in a plate with thin OP50 seeding. The reversal length was observed in terms of body bends reversed during each reversal.

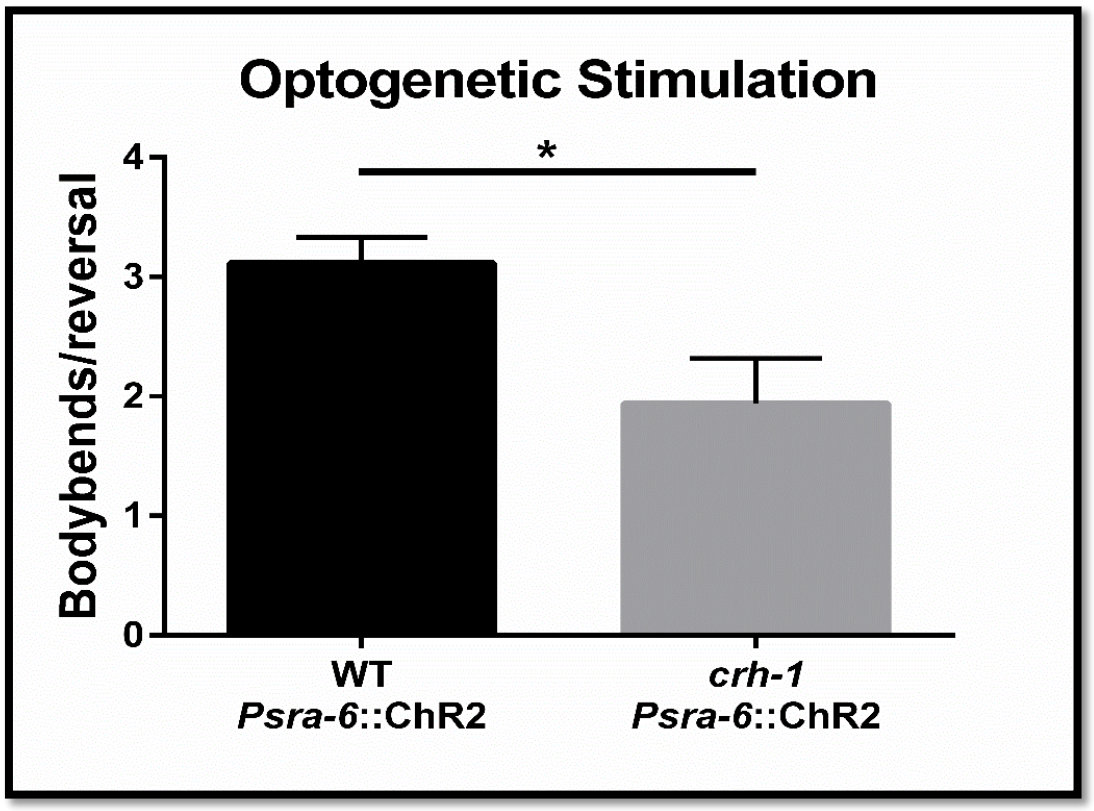


Figure6: Optogenetic stimulation of ASH neuron in wild-type and *crh-1* mutants

crh-1 mutants showed a decrease in reversal length compared to wild type worms which is consistent with our observation of exploratory behavior.

Chapter 2

Summary & Conclusions

2.1 Concluding Remarks

CREB is a transcription factor responsible for regulating expression of many genes involved in various functions. One of a major class of genes regulated by CREB is neuropeptides. Neuropeptides can work as neuromodulators and modulate the activity of a neuron. Neuropeptide FLP-18 belongs family of Famide related peptides. FLP-18 is expressed in the neural circuitry of exploratory behavior. FLP-18 is also known to effect reversal frequency during starvation. There is a 147 fold increase in *flp-18* expression levels in *crh-1* mutants. *crh-1* mutants showed decrease length of reversals compared to wild type worms. This decrease in length can be a due increase in *flp-18* levels which need to confirm by investigating the reversal length of *flp-18* mutant and *flp-18;crh-1* double mutant. Decreased length of *crh-1* mutants is rescued by expressing repressor isoform CRH-1f of CREB. These results suggest that CREB regulates expression of neuropeptide FLP-18, and CREB controls length of backward movement. CREB mediated control of reversals could be due to the regulation of FLP-18 expression which requires further investigation.

2.2 Future Outlook

1. **Rescue quantitative real-time PCR:**

   Quantitative real-time PCR of *Prab-3*::crh-1f in the *crh-1* mutant background to prove FLP-18 expression is regulated by repressor isoform of CRH-1.

2. **Rescue reversal assay:**
   Expression of CRH-1f in neurons expressing FLP-18 [*Pflp18*::crh-1f] should rescue decreased reversal length of *crh-1* mutants.

3. The reversal length of *flp-18* and *flp-18;crh-1* double mutants.

4. Calcium imaging of AVA in wild-type and *crh-1* mutants.

Bibliography

1. White, J. G., E. Southgate, J. N. Thomson, and S. Brenner, (1986) The structure of the nervous system of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. 314: 1-340.
2. Sulston, J., Dew, M., and Brenner, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neurol.*163*, 215–226
3. Chalfie, M., and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev. Biol. *82*, 358–370.
4. Way, J.C., and Chalfie, M. (1989). The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. Genes Dev. *3*, 1823–1833.
5. Kaplan, J.M., and Horvitz, H.R. (1993). A dual mechanosensory and chemosensory neuron in *C. elegans*. Proc. Natl. Acad. Sci. USA *90*, 2227–2231
6. Culotti, J.G., and Russell, R.L. (1978). Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics *90*, 243–256
7. Ward, S., Thomson, N., White, J.G., and Brenner, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. *160*, 313–337.
8. Ware, R.W., Clark, D., Crossland, K., and Russell, R.L. (1975). The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. J. Comp. Neurol. *162*, 71–110.
9. Grewal, P.S., and Wright, D.J. (1992). Migration of *Caenorhabditis elegans* larvae towards bacteria and the nature of the bacterial stimulus. Fundam. Appl. Nematol
10. Bargmann, C.I., Hartwieg, E., and Horvitz, H.R. (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell*74*, 515–527.
11. Rose, J.K., and Rankin, C.H. (2001). Analyses of habituation in*Caenorhabditis elegans*. Learn. Mem.
12. Hedgecock, E.M., and Russell, R.L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *72*, 4061–4065.
13. Croll, N.A. (1975). Components and patterns in the behavior of the nematode *Caenorhabditis elegans*. J. Zool. *176*, 159–176
14. Chiba, C.M., and Rankin, C.H. (1990). A developmental analysis of spontaneous and reflexive reversals in the nematode *Caenorhabditis elegans*. J. Neurobiol. *21*, 543–554.
15. Pierce-Shimomura, J.T., Morse, T.M., and Lockery, S.R. (1999). The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. J. Neurosci. *19*, 9557–9569.
16. Jesse M. Gray, Joseph J. Hill, and Cornelia Bargmann. (2005) A Circuit for navigation in Caenorhabditis elegans PNAS 102, 3184-3191.
17. Oshio et al. (2003) Database of Synaptic Connectivity of C. elegans for Computation. Technical Report of CCeP, Keio Future, No.3,
18. Avery L, Horvitz HR (1989) Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron. 3(4):473-85.

19. Yan D, Wu ZL, Chisholm AD, Jin YS. (2009) The DLK-1 kinase promotes mRNA stability and local translation in C. elegans synapses and axon regeneration. Cell. 138:1005–1018.
20. Yanik MF et al. 2004 Neurosurgery –functional regeneration after laser axotomy. Nature. 432:822.
21. Kobayashi et al. (2013) A method for selective ablation of neurons in *C.elegans* using the phototoxic fluorescent protein, KillerRed. Neuroscience Lett. 548:261-4.
22. Navin Pokala, Qiang Liu, Andrew Gordus, Cornelia Bargmann (2013) Inducible and titratable silencing of Caenorhabditis elegans neurons in vivo with histamine-gated chloride channels (2013) PNAS 2770-2775.
23. Nagel, G.; Brauner, M.; Liewald, J. F.; Adeishvili, N.; Bamberg, E.; Gottschalk, A. (2005). "Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses". Curr. Biol. 15 (24): 2279–84.
24. Zhao, S. et al. (2008). "Improved expression of halorhodopsin for light-induced silencing of neuronal activity". Brain Cell Biology 36 (1–4): 141–154.
25. Ji, Guangju et al. (2004-05). "Ca2+-sensing transgenic mice: postsynaptic signaling in smooth muscle". The Journal of Biological Chemistry 279 (20): 21461–21468.
26. Conlay, Sabounjian, & Wurtman (1992). Exercise and neuromodulators: choline and acetylcholine in marathon runners. International Journal of Sports Medicine(13), pp 141-2
27. Neuropeptides and Other Bioactive Peptides: From Discovery to Function, L.D.Fricker, Morgan & Claypool Publishers, 2012
28. Chris Li, Kyuhyung Kim (2014) Family of FLP Peptides in Caenorhabditis elegans and Related Nematodes Front Endocrinol 5: 150.
29. Bruns D, Jahn R. (1995) Real-time measurement of transmitter release from single synaptic vesicles. Nature 377:62–510.
30. Hammarlund M, Watanabe S, Schuske K, Jorgensen EM. CAPS and syntaxin dock dense core vesicles to the plasma membrane in neurons. J Cell Biol (2008) 180:483–9110.
31. Chris Li, and Kyuhyung Kim, (2008) Neuropeptides, WormBook, ed. The C. elegans Research Community.
32. Thacker, C., and Rose, A.M. (2000). A look at the Caenorhabditis elegans Kex2/Subtilisin-like proprotein convertase family. BioEssays 22, 545–553.
33. Bargmann, C.I., and Horvitz, H.R. (1991). Control of larval development by chemosensory neurons in Caenorhabditis elegans. Science 251, 1243–1246.
34. deBono et al. (2009) Coordinated Regulation of Foraging and Metabolism in C. elegans by RFamide Neuropeptide Signaling Cell Metabolism 9: 375-385
35. 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.
36. Couillault, C. et al. 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.
37. Sieburth, D., Ch'ng, Q.L., Dybbs, M., Tavazoie, M., Kennedy, S., Want, D., Dupuy, D., Rual, J.-F., Hill, D.E.,

38. Vidal, M., Ruvkun, G., and Kaplan, J.M. (2005). Systematic analysis of genes required for synapse structure and function. Nature 436, 510–517.
39. Kim, K., & Li, C. (2004). Expression and regulation of an FMRFamide-related neuropeptide gene family in Caenorhabditis elegans. J Comp Neurol, 475, 540-50
40. Waggoner, L. E., Zhou, G. T., Schafer, R. W., & Schafer, W. R. (1998). Control of alternative behavioral states by serotonin in Caenorhabditis elegans. Neuron, 21, 203-14.
41. Greer, E. R., Perez, C. L., van, M. R. G., Lee, B. H., & Ashrafi, K. (2008). Neural and molecular dissection of a C. elegans sensory circuit that regulates fat and feeding. Cell Metab, 8, 118-31.
42. Montminy MR and Bilezikjian LM. (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature. 328(6126):175-8
43. "The many faces of CREB". linkinghub.elsevier.com.
44. Pugazhenthi S1, Wang M, Pham S, Sze CI, Eckman CB. Downregulation of CREB expression in Alzheimer's brain and in Aβ-treated rat hippocampal neurons.
45. Purves, Dale (2008). Neuroscience. 4th ed. Sinauer Associates. pp. 170–6.
46. Pearson education Inc. (www.mnu.ca)
47. www.sciencemag.org
48. www.bio-rad.com