Video Article

Determining Genetic Expression Profiles in *C. elegans* Using Microarray and Real-time PCR

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Abstract

Synapses are composed of a presynaptic active zone in the signaling cell and a postsynaptic terminal in the target cell. In the case of chemical synapses, messages are carried by neurotransmitters released from presynaptic terminals and received by receptors on postsynaptic cells. Our previous research in *Caenorhabditis elegans* has shown that VSM-1 negatively regulates exocytosis. Additionally, analysis of synapses in *vsm-1* mutants showed that animals lacking a fully functional VSM-1 have increased synaptic connectivity. Based on these preliminary findings, we hypothesized that *C. elegans* VSM-1 may play a crucial role in synaptogenesis. To test this hypothesis, double-labeled microarray analysis was performed, and gene expression profiles were determined. First, total RNA was isolated, reversely transcribed to cDNA, and hybridized to the DNA microarrays. Then, in-silico analysis of fluorescent probe hybridization revealed significant induction of many genes coding for members of the major sperm protein family (MSP) in mutants with enhanced synaptogenesis. MSPs are the major component of sperm in *C. elegans* and appear to signal nematode oocyte maturation and ovulation . In fruit flies, Chai and colleagues [1] demonstrated that MSP-like molecules regulate presynaptic bouton number and size at the neuromuscular junction. Moreover, analysis performed by Tsuda and coworkers [2] suggested that MSPs may act as ligands for Eph receptors and trigger receptor tyrosine kinase signaling cascades. Lastly, real time PCR analysis corroborated that the gene coding for MSP-32 is induced in *vsm-1(ok1468)* mutants. Taken together, research performed by our laboratory has shown that *vsm-1* mutants have a significant increase in synaptic density, which could be mediated by MSP-32 signaling.

Video Link

The video component of this article can be found at https://www.jove.com/video/2777/

Protocol

1. Isolation of Total RNA

1. Healthy synchronized nematodes were harvested by washing plates with room-temperature M9 medium, using 2-3 ml per plate. Resuspended nematodes were transferred to 15 ml conical tubes and pelleted down by centrifugation, 3200 g at 4° C for 4 minutes. Harvested nematodes were cleaned out from floated bacteria by resuspending the pellet with 7 ml of 0.1M NaCl and 7 ml of ice cold 60% w/v sucrose. The resuspended mixture was incubated on ice for 15 minutes and clean nematodes, which swim-up through the sucrose solution, were collected after centrifuging the mixture at 3200 g at 4° C for 4 minutes. Bacteria-free worms were transferred to a new conical tube using sterile pipettes and washed two times with up to 15 ml RNase-free water followed by a 3200 g centrifugation at 4° C for 2 minutes. The clean loosely compacted pellet was transferred to 1.5 ml microcentrifuge tube and centrifuged at maximum speed (about 10,000 g) for 30 seconds. Lastly, most RNase-free water was removed and 10 volumes of RNAlater was added to the pellet, and stored at 4°C until ready to proceed.
2. To prepare total RNA samples, harvested nematodes were centrifuged at maximum speed (about 10,000 g) for 4 minutes and RNAlater was discarded. Then, a pinch of Molecular Grinding Resin (G bioscience) was added to the pellet and mixture was frozen using liquid nitrogen. Frozen worm suspension was grinded into a fine powder with pestle and liquid nitrogen was added as necessary to keep the powder cold. After grinding, the extract was kept on ice for 5 minutes and then mixed with 700 µl RLT/BME (ratio 100 volume of RLT: 1 volume of BME) (Qiagen) and 472 µl 100% ethanol.
3. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and following the manufacturer recommended protocol. Final volume of isolated RNA was 60 µl per biological sample.

2. DNA Digestion and RNA Cleanup

1. 50 µg of total RNA potentially contaminated with genomic DNA was digested with DNase I (Qiagen). Digest reactions containing 0.1 U DNase/1 µg RNA and 1X Buffer RDP were incubated at room temperature for 10 minutes.
2. RNA samples treated with DNase I were cleaned-up using the RNeasy MinElute Clean-Up Kit (Qiagen). Protocols recommended by the manufacturer were followed and 60 µl final eluted volume was obtained.

3. Qualitative and Quantitative Analysis of RNA

1. RNA concentration was determined using the UV spectrophotometer Nanodrop (Thermo Scientific).
2. Integrity of RNA samples was evaluated using agarose electrophoresis. Briefly, 1% RNase-free agarose gels were prepared using 1X Northern Max Gly Gel Prep/Running Buffer (Ambion), 1% RNase-free Agarose LE (Ambion) and 0.5 µg/ml ethidium bromide. While the gel was polymerizing, RNA samples were glyoxylated by adding 5 µl of Glyoxyl Sample Loading Dye/sample and incubating samples at 50°C for 30 minutes. RNA ladder was also glyoxylated and loaded for size comparison.

4. cDNA Synthesis

1. For each synthesis reaction, 8.4 µg RNA sample was mixed with 1 µl RT primer (1 pmole/µl). One sample (WT or mutant) received the Cy5 capture RT primer; the other sample received the Cy3 capture RT primer (provided with the Array 350 Labeling -Detection Kit, Genisphere). Sample mixtures were heated at 75-80°C for 10 minutes and transferred to ice for 2-3 minutes.
2. While the RNA samples incubate, the MasterMix (Genisphere) was prepared as follows: for each RT reaction, we combined 4 µl 5X Reaction Buffer for RT, 1 µl dNTP (10 mM each dATP, dCTP, dGTP, dTTP), 1 µl Superase-In RNase Inhibitor (provided with the Array 350 Labeling-Detection Kit, Genisphere), 1 µl RT Enzyme (200 u/µl). Last, 7 µl of MasterMix was added to each RNA sample, gently mixed (do not use vortex) and incubated 2 hours at 42° C.

5. RNA Degradation and Purification of cDNA Samples

1. cDNA synthesis was stopped by adding 3.5 µl 0.5M NaOH/50mM EDTA (final concentration) and incubated at 65°C for 15 minutes. Then, reactions were neutralized using 1M Tris-HCl, pH 8.0 to reach a final concentration of 850 mM Tris-HCl. Lastly, WT and mutant cDNAs were combined into one microcentrifuge tube. The empty tube was rinsed with 73 µl 1X TE buffer and added to the cDNA containing tube; the final volume of the combined cDNAs tube was 130 µl.
2. Mixed cDNA was purified following manufacturer's protocol and the QIAquick PCR Purification Kit (Qiagen). The resultant eluted volume of purified cDNA was 60 µl.

6. First Microarray Hybridization

1. *C. elegans* microarray slides obtained through GCAT (Genome Consortium for Active Teaching) were blocked at room temperature for 1 hour using 0.1 mg/ml sonicated salmon sperm DNA in 3X SSC, 0.1 % SDS. Blocked slides were rinsed by dipping in ddH2O and spin-dried by centrifuging for 1 minute at 2,000 g in 50 ml conical tubes with KimWipe in bottom.
2. Then, 2X formamide-based hybridization buffer (Genisphere) was incubated at 55°C for 10 minutes, mixed well to dissolve crystals and centrifuged for 1 minute at 10,000 g. Next, 25 µl cDNA sample was gently mixed by flicking with 25 µl of the 2X formamide-based hybridization buffer. Diluted cDNA sample was collected by flash spin for 15 seconds and incubated at 80°C for 10 minutes.
3. Last, cDNA was hybridized to microarray slide by carefully pipetting the entire heated cDNA sample without touching the slide. The sample was uniformly spread onto the microarray by gently lowering a cover slip using a syringe needle. Before the cover slip was completely lowered, the cover slip was pulled back up, then lowered with the needle again, and allowed to fall gently into place, minimizing the formation of air bubbles. First hybridization set up was culminated by putting the slide horizontally in a 50 ml conical tube with 50 µl of ddH_2O below the slide and incubated overnight at 37°C.

7. Second Hybridization

1. Hybridized microarrays were washed with 2X SSC at various temperatures. First, microarray slides were transferred to conical tubes containing room temperature 2X SSC and 0.2% SDS and sloshed gently to remove cover slips. Then, microarray slides were transferred to conical tubes containing 55°C 2X SSC and 0.2% SDS and incubated at 55°C for 15 minutes. Next, slides were transferred to 2X SSC and incubated at room temperature for 15 minutes, shaking gently periodically. Last, slides were transferred to 0.2X SSC and incubated for 15 minutes at room temperature, shaking gently periodically. Washed slides were spin-dried by introducing label-side down slides in 50 ml conical tubes with a KimWipe in the bottom and centrifuged for 1 minute at 2,000 g.
2. Next, second hybridization mixture was prepared using 2X formamide-based hybridization buffer (Genisphere). Hybridization buffer was incubated at 55°C for 10 minutes and centrifuged for 1 minute at 10,000 g. Capture fluorescent reagents (Cy3 and Cy5) and the anti-fade reagent were thawed at room temperature and covered with foil to protect reagents from photobleaching. Following hybridization steps were performed in the dark to minimized light exposure. 150 µl of 2X formamide-based hybridization buffer was combined with 1.5 µl anti-fade reagent to make the anti-fade-treated hybridization mixture.
3. Capture reagents Cy3 and Cy5 were vortexed for 3 seconds and centrifuged for 15 seconds, 10,000 g. Second hybridization mixture was prepared combining 75 µl anti-fade-treated hybridization mix, 60 µl nuclease-free water, 7.5 µl Cy3 capture reagent, and 7.5 µl Cy5 capture reagent. Second hybridization mix was incubated for 10 minutes at 75°C and 50 µl of heated mixture was pipetted very carefully onto the washed/dried microarray slide. To uniformly spread the sample onto the microarray, a cover slip was gently lowered using a syringe needle. Before the cover slip was completely lowered, the cover slip was pulled back up, then lowered with the needle again, and allowed to fall gently into place, minimizing the formation of air bubbles. Hybridizing microarray slide was placed horizontally in 50 ml conical tube covered with foil and 50 µl of ddH_2O was added beneath the slide to create a humid chamber. Second hybridization was incubated at 37°C for 2-5 hours.
4. Second hybridizing microarray was washed in the dark using room temperature 2X SSC, 0.2% SDS, 1 mM DTT and sloshed gently to remove cover slip. Then, microarray was transferred to covered conical tube containing 55°C 2X SSC, 0.2% SDS, 1 mM DTT and incubated for 15 minutes at 55°C. Next, slide was transferred to covered conical tube containing 2X SSC, 1 mM DTT and incubated for 15 minutes at room temperature, shaking gently periodically. Last, slide was transferred to 0.2X SSC, 1 mM DTT and incubated at room temperature for 15

minutes, shaking gently periodically. Microarray slide was dried by centrifuging for 1 minute, 2,000 g, slide label-side down in covered 50 ml conical tube with KimWipe in the bottom. Dry microarray slides were scanned using GenePix Personal Scanner model 4100A at Davidson College.

8. Microarray Analysis

1. Images obtained after scanning were analyzed using the MAGICTool Software developed at Davidson College by Laurie Heyer and her undergraduate students. Briefly, under the "Project" tab, a "New Project" was created and saved. Under the "Build Expression Profile" tab, "Load Image Pair" was selected and the red image file (_635.tif) was uploaded as "Red" and the green image file (_532.tif) was uploaded as "Green". Then, using the "Build Expression Profile" tab and "Load Gene List", a *C. elegans* gene list file obtained from the GCAT website was uploaded.
2. Microarray image was addressed and gridded using the "Build Expression Profile" tab and "Create/Edit Grid" option. The "Grid Setup" dialog box was edited using "48" for the number of grids, "22 rows" and "22 columns" for each grid, spots numbered "Left to Right" and "Top to Bottom". "Percent Contrast Change" was increased and zoomed in on the grid until individual spots were easily distinguishable. Grids were created by first selecting "Set Top Left Spot" on the left-hand panel. Then, the center of the top left spot, top right spot and bottom row were clicked on "Grid 1". This gridding procedure was repeated for each of the 48 grids, proceeding left to right and top to bottom. When gridding was completed, the current grid was saved under "File" "Save Current Grid As". When the grid was successfully saved the "Finished" tab was selected.
3. To eliminate any unrelated spots from the analysis, the "Build Expression Profile" tab was opened. Under the "Addressing Gridding" option "Spot Flagging" was selected. Streaked spots or background fluorescence were flagged and saved by selecting "Save Current Flags As" under the "File Tab".
4. Flagged files were segmented using the "Build Expression Profile" tab and selecting "Fixed Radius" as the segmentation method of choice. The radius was set to the desired size, and "Update Data" was clicked. To make sure that the circle stays within the gridded area (yellow square) we scrolled top to bottom and left to right and once checked "Create Expression Profile" was selected.
5. During segmentation, the software calculated the Red:Green fluorescence ratios for each of the spots that remained after flagging. Using the "Expression" tab, "Manipulate Data" "Transform" was selected. A "Transform Data" dialog box appeared where "logb(x)" option, and b=2 was entered. "Working Expression File" was explored from the "Expression" tab. For an experiment in which the wild type received the Cy3 (green) capture sequence and the mutant received the Cy5 (red) capture sequence, the value of the log transformed ratios were positive for genes that were induced and negative for repressed genes.
6. Last, genes induced or repressed by a specified factor were analyzed selecting "Explore" under the "Expression" tab. In the "Exploring" dialog box, "Find Genes Matching Criteria" was set to genes with a "Max. value > X (positive number for induced genes)" or "Min. value < X (negative number for genes repressed)" in experiments as the one described in #5. Fold-expression was equal to 2x, where ‘x' is the value of the number entered in the selection criteria.

9. cDNA Synthesis and Real-Time PCR

1. Total RNA was isolated as previously described. Once the quality and quantity of isolated RNA was determined, cDNA was synthesized using the iScript cDNA Synthesis Kit (BioRad), 500 ng/µl total RNA isolated from the wild type and mutant, and manufacturer recommended protocols.
2. Real time PCR reactions were set-up using the iQ SYBR Green Supermix (BioRad), 100-500 nmoles gene specific primers (table 1) and 4 µl cDNAs produced by the previous step. 10 µl reactions were run using the thermocycler CFX96 Real-Time System (BioRad).
3. The thermocycler was first heated to 95°C for 3 min. This was followed by a step at 95°C for 5 seconds and 58°C for 30 seconds. This loop was completed a total of forty times, and a 65-95°C melt curve with a gradient of 0.5 degrees was also inserted. ΔΔCT values were calculated using three housekeeping genes (act-1, cdc-42, and pmp-3) as controls.

GENE	FORWARD PRIMER	REVERSE PRIMER
gst-5	CTGCTCCATTCGGACAACTT	TCCGTTGAGCTTGAACTCAC
gst-9	GGAAGACAACTGGCACAATC	ACCGAGAGCATCAACTTGAG
kcnl-1	TACGCTCGGCATGTGTTGTATC	CCAATCATCGGCTCCACTATCT
ksr-2	CGAACTGCTGCTGGAATGCT	GTGCTGCGTCTCTTCTGCTT
lim-9	CTCATCGAGTGGCTCAATCT	CACCTTGCTCCATCTTCAAC
lpr-6	CAGCAGTCACTTCTGTTCAC	TCTCCAATAGCGGTACTCC
mes-6	TTGTTCCGTTGGCTCACGTACAG	CGACAGACGCAGATACACGATCA
msp-32	GCCGCACAGGTATGATCCAG	ATCCAATACGGCGAGCCGAG
msp-142	GATCCACCATGTGGAGTTCT	GATTCTTGCGACGGACCATA
msp-38	GCCTTCGGACAAGAGGATACC	CCATACCGTCTCCTTGGAACC
msp-45	TCACCGTTGAGTGGACCAAT	ACGAACCAACCGTCTCCTT
msp-49	CGACGACAAGCACACCTACCACATCAA	TCGAGGACTCCACATGGTGGATCAACT
msp-56	CGAAGATCGTCTTCAATGCGCCATAC	TCCACATGGTGGATCAACTCCAAGTC

Table 1. Forward and Reverse Primer Sequences for Real-Time PCR

10. Representative Results:

RNA isolation and analysis

Fusion of active zone precursor vesicles at presynaptic sites is an obligatory step during synaptogenesis (3). VSM-1, a recently identified v-SNARE master protein, was proposed to inhibit vesicle fusion in yeast and synaptogenesis in nematodes (see Figure 1) by an undetermined mechanism (4, and our unpublished work). To better understand the molecular pathway underlying VSM-1 regulation in nematodes and bring some light into the synaptogenesis field, we began a genome-wide screen and determined that major sperm protein genes (msp) are induced in the absence of fully functional VSM-1.

To investigate the induced genes in the *vsm-1 (ok1468)* mutant strain of *C. elegans*, we conducted a microarray gene analysis. This was done by testing transcripts isolated from wild type and the *vsm-1* mutant strain and determining their enrichment. Specifically, we first isolated total RNA from synchronous L4 and L3 wild type and *vsm-1* mutant larvae nematodes. Then, we treated the total RNA obtained with DNase I and examined the quality of extracted RNA using agarose gel electrophoresis. In the experiment shown in figure 2, a ladder was used in the first lane to represent the number of base pairs for the standards. Wild type samples pre-treated and post-treated with DNase I were loaded in lanes 2 and 4, and *vsm-1* mutant samples pre-treated and post-treated with DNase I were loaded in lanes 3 and 5, respectively. Analysis of RNA gel electrophoresis showed that wild type and *vsm-1* mutant RNA samples were intact and of good quality. This was determined by observing two bands which represented the two subunits of ribosomal RNA.

Microarray hybridization

Once the quality of RNA was determined, we proceeded with the cDNA synthesis. To do so, we reverse transcribed the mRNA and added a capture sequence to the tail. The produced cDNAs containing capture sequences for Cy3 and Cy5 were hybridized to microarray slides and sent off for scanning. Microarray images were then entered into an open source computer software program called MAGICTool developed at Davidson College by Laurie Heyer and her undergraduate students. Using this software we overlaid Cy3 and Cy5 images, gridded microarrays, and analyzed fluorescent profiles (see Figure 3). Once gridding was complete we then segmented each individual square making sure the whole printed oligonucleotide was being examined. Then we analyzed the induced ratios and created genetic profile expressions showing that genes coding for the MSP family are induced in nematodes with enhanced synaptogenesis. (Table 3).

Validation and quantification of gene expression using real time PCR.

To further understand the genetic profile in the *vsm-1* mutant we analyzed a number of genes that code for members of the MSP family using real time PCR. First, total RNA was isolated from wild type and *vsm-1(ok1468)* mutant strains. RNA samples were then reversely transcribed into cDNAs and used for real time PCR analysis. Evaluations of real time PCR expression profiles demonstrated that one member of the MSP family seems to be induced in *vsm-1(ok1468)* mutants. Moreover, real time PCR data validates findings from microarrays and narrowed down the search to one *msp* gene candidate (Figure 4).

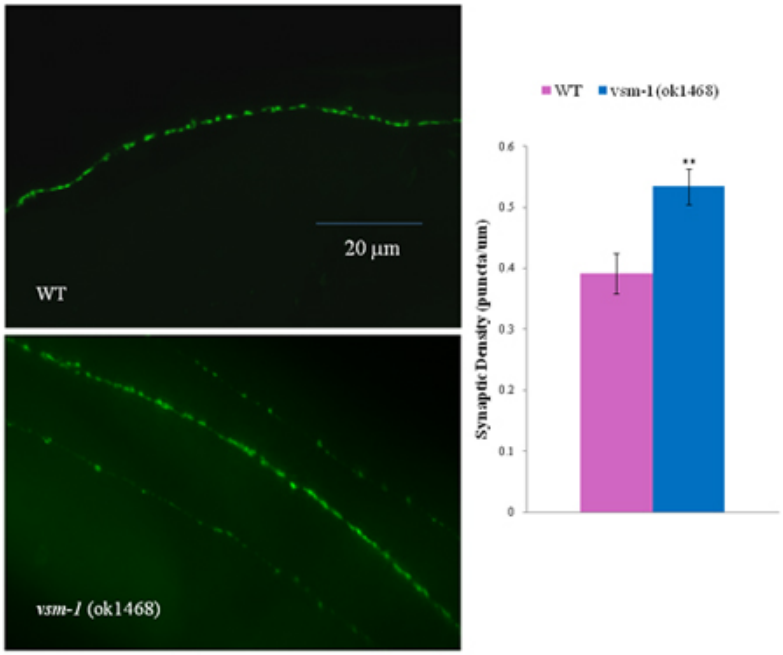

Figure 1. A. UNC-17 Immunostaining revealed that *vsm-1* mutants exhibit higher synaptic density along the dorsal nerve cord. Images were analyzed at 63x magnification, posterior (right) to the vulva. B. Analysis of WT and *vsm-1* (ok1468) mutant synapses denoted statistically significant enhanced synaptic density in the *vsm-1* mutant (**p<0.01). Twenty nematodes were images for each genotype.

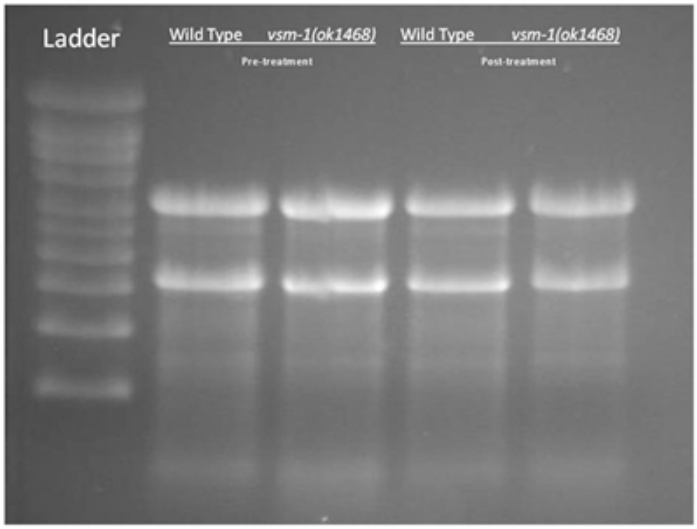

Figure 2. The quality of extracted RNA was determined using agarose gel electrophoresis. The presence of two intact ribosomal subunits before and after DNase I treatment was apparent in RNA extracted from Wild Type and *vsm-1*(*ok1468*) samples.

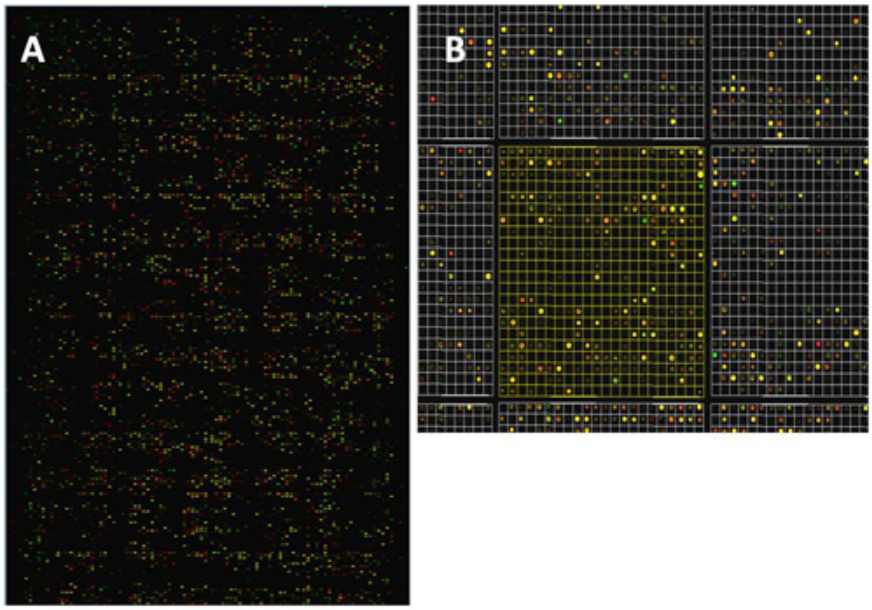

Figure 3. A. Microarray image for an experiment where the control was labeled red (Cy5) and the *vsm-1* mutant was labeled green (Cy3). B. Representative image showing 1 out of 48 grids analyzed per microarray. Each small square on a grid is representative of a single printed oligonucleotide, and each grid is composed by 22 rows and 22 columns.

A.

Genes Induced	WT cy3, *vsm-1* cy5	WT cy5, *vsm-1* cy3	Gene info
cea2.d.32314	2.39	-2.4	msp-63—(Major Sperm Protein)
cea2.d.32749	2.62	-2.11	msp-81
cea2.d.32754	2.17	-2	msp-56
cea2.d.33043	2.65	-2.3	msp-38
cea2.d.34563	2.51	-2.7	msp-32
cea2.d.48458	2.7	-2.5	msp-76
cea2.p.21057	2.23	-2.6	unknown

B.

Genes Induced	WT cy3, *vsm-1* cy5	WT cy5, *vsm-1* cy3	Gene info
cea2.d.32647	1.11	-1.55	col-106—(COLlagen)
cea2.d.32310	1.8	-2.31	msp-142--(Major Sperm Protein)
cea2.d.34563	2.51	-2.7	msp-32
cea2.d.33043	2.65	-2.3	msp-38
cea2.d.15452	1.91	-2.19	msp-49
cea2.d.32754	2.17	-2	msp-56
cea2.d.32314	2.39	-2.4	msp-63
cea2.d.48458	2.7	-2.5	msp-76
cea2.d.38781	1.19	-1.57	msp-78
cea2.d.32749	2.62	-2.11	msp-81

Table 2. Microarray analysis of transcripts isolated from Larvae 4 (A) and Larvae 3 (B) *C. elegans* nematodes showed that genes coding for the major sperm proteins are induced in mutants with enhanced synaptogenesis. Highlighted genes indicates induced genes in both Larvae 3 and Larvae 4.

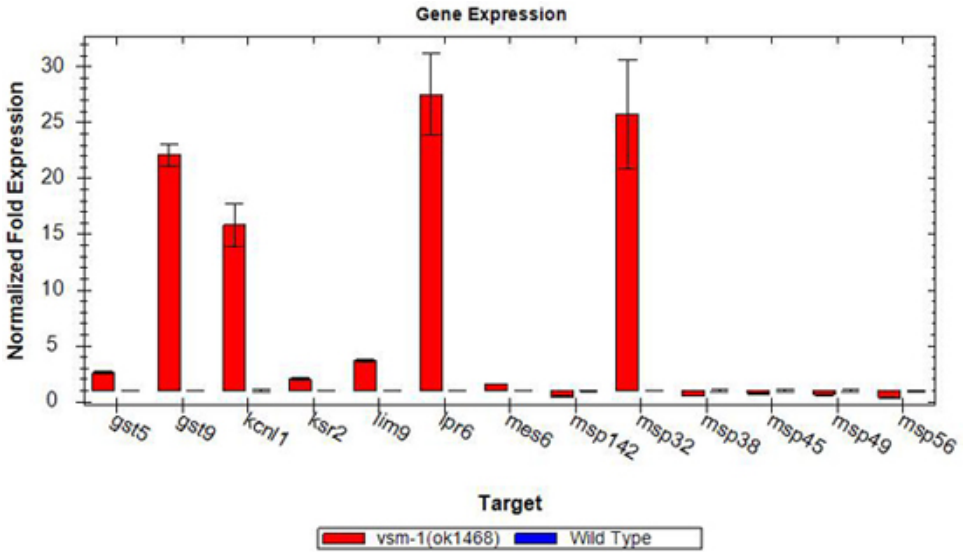

Figure 4. Real-Time PCR analysis demonstrated that one member of the msp gene family, *msp*-32 was induced in *vsm-1(ok1468)* mutants. Representative normalized fold expression graph shows that the *vms-1* (ok1468) ΔΔCT values for *msp-32* are significantly different when compared to wild type (WT) and three housekeeping genes are used for normalization (act-1, cdc-42, and pmp-3). Average of three replicas are plotted +/- standard deviation.

Discussion

In this study we developed an easy, user friendly protocol that can be used to determined gene expression profiles underlying various biological phenomena. In this protocol, total RNA was first isolated and treated using DNase I. Our method of RNA isolation has been dramatically simplified by omitting phenol extractions and using affinity resins for clean up [5,6]. Briefly, *C. elegans* cuticule and cell membranes were cracked-open by freezing nematodes with liquid nitrogen and grinding with molecular resin. Next, extracted RNA was treated with DNase I before cDNA synthesis. The later step was added to minimize unspecific hybridizations; RNA samples contaminated with genomic DNA could introduce interference and produce many false-positives. Then, wild type and *vsm-1(ok1468)* mutant cDNAs were tagged with Cy3 and Cy5 capture sequences and hybridized onto *C. elegans* microarrays obtained through the GCAT program. This system is more sensitive than similar products, and its two-color design decreases the number of arrays needed, resulting in greater time and cost efficiency [7,8]. Additionally, this

system does not require the specialized equipment of other similar systems; therefore, this procedure could be accomplished in any standard laboratory setting [7]. Third, fluorescent hybridized array images were analyzed using the open source software MAGICTool, and gene expression profiles were created. MAGICTool is a user-friendly program that is easily utilized by individuals of all experience levels, from the undergraduate to the post-doctorate. However, optimal performance requires large memory availability. Lastly, candidate genes obtained using microarray analysis were validated with real time PCR.

Though the genomic approach is an efficient method for studying biological phenomena, there are some limitations one should take into consideration. First, despite the specificity of this microarray protocol, transcripts within a family are indistinguishable from one another if the printed oligonucleotide is taken from conserved sequences. Real time PCR compensates for similarities within a gene family and may even distinguish between specific isoforms of the same gene. However, with real time PCR it is a challenge to find true controls that are expressed equally throughout an organism's lifespan. Because of this, results will be relative. A proteomic approach is one alternative to our technique. Individual proteins may be isolated using 2D gel electrophoresis and identified with mass spectrometry.

Our studies specifically show that many members of the Major Sperm Protein (MSP) family are induced in *vsm-1* mutant nematodes with enhanced synaptic density. MSPs are unique to *C. elegans* and are responsible for oocyte maturation and ovulation. Chai [1] has demonstrated that regulation of presynaptic bouton number and size at the neuromuscular junction in fruit flies is likely controlled by molecules containing Major Sperm Protein domains. Studies by Tsuda and colleagues [2] have demonstrated Major Sperm Protein domains may serve as ligands for Ephrine receptors, triggering receptor tyrosine kinase signaling cascades. Moreover, real time PCR analysis validated and narrowed down microarray results to one member of the *msp* family, *msp-32*. Taken together, the work presented here represents a genome-wide analysis of a central neuroscience dilemma as well as the power of undergraduate research in the scientific endeavor.

Disclosures

No conflicts of interest declared.

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References

1. Chai, A., Withers, J., Koh, Y.H., Parry, K., Bao, H., Zhang, B., Budnik, V., & Pennetta, G. hVAPB, the causative gene of a heterogeneous group of motor neuron diseases in humans, is functionally interchangeable with its Drosophila homologue DVAP-33A at the Neuromuscular Junction. *Hum Mol Genet*. **17**, 266-280 (2008).
2. Tsuda, H., Han, S.M., Yang, Y., Tong, C., Lin, Y.Q., Mohan, K., Haueter, C., Zoghbi, A., Harati, Y., Kwan, J., Miller, M., & Bellen, H.J. The Amyotrophic Lateral Sclerosis 8 protein VAPB is cleaved, secreted, and acts as a ligand for Eph receptors. *Cell*. **133** (6), 963-977 (2008).
3. Dresbach, T., Torres, V., Wittenmayer, N., Altrock, W.D., Zamorano, P., Zuschratter, W., Nawrotzki, R., Ziv, N.E., Garner, C.C., & Gundelfinger, E.D. Assembly of Active Zone Precursor Vesicles. *Journal of Biological Chemistry*. **281** (9), 6038-6047 (2005).
4. Lustgarten, V. & Gerst, J.E. Yeast VSM1 Encodes a v-SNARE Binding Protein That May Act as a Negative Regulator of Constitutive Exocytosis. *Molecular and Cellular Biology*. **19** (6), 4480-4494 (1999).
5. Viñuela, A., Snoek, L., Riksen, J., & Kammenga, J. Genome-Wide Gene Expression Analysis in Response to Organophosphorous Pesticide Chlorpyrifos and Diazinon in *C. elegans*. *PloSone*. **5** (8), 1-8 (2010).
6. Niwa, R., Hada, K., Moliyama, K., Ohniwa, R., Tan, Y., Olsson-Carter, K., Chi, W., Reinke, V., & Slack, F. *C. elegans sym-1* is downstream target of the hunchback-like-1 developmental timing transcription factor. *Cell Cycle*. **8** (24), 4147-4154 (2009).
7. Kershner, A. & Kimble, J. Genome-wide analysis of mRNA targets for *Caenorhabditis* elegans FBF, a conserved stem cell regulator. *PNAS* **107** (8), 3963-3941 (2010).
8. Hammock, E., Eagleson, K., Barlow, S., Earls, L., Miller, D., & Levitt, P. Homologs of genes expressed in Caenorhabditis elegans GABAergic neurons are also found in the developing mouse forebrain. *Neural Development*. **5** (32), 1-14 (2010).