Supporting Information

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SI Materials and Methods

Microarray Fabrication. The construction of the cDNA microarray was essentially the same as described previously (1). Randomly selected EST clones from libraries were amplified by PCR using the following primer set: 5′-GTGGATGTTGCCTTTACTTC-3′ and 5′-GTTCAGGGGGAGGTGTGG-3′ for the clones from the full-length-enriched and 5′-end-enriched library; and 5′-GCGTGAATGTAAGCGTGAC-3′ and 5′-CAGCCTCTTGCTGAGTG-3′ for the clones from the random-primed library. PCR products were purified by ethanol precipitation and resuspended in 50% DMSO. cDNAs were transferred as single spots on glass slides (CMT-GAPS II, Corning) by using the pin array printing system (Cartesian Technologies) and spots were immobilized by baking at 80°C for 2 h. The microarrays also contained probes for 5-HT-regulated (ApC/EBP) as well as consistently expressed genes (e.g., ribosomal protein S4, Hsc70, and actin). cDNAs for GFP and luciferase were spotted as exogenous negative controls.

***In vivo* 5-HT treatment to *Aplysia*.** To screen the 5-HT-regulated genes in pleural ganglia, we treated animals *in vivo* with 250 μM 5-HT. Two hours after onset of 5-HT treatment, animals were anesthetized and pleural ganglia were dissected for analysis. The same number of control animals which were not exposed to 5-HT were handled and dissected in a manner similar to the treated animals.

Microarray Hybridization, Scanning, and Analysis. Pleural ganglia from three *in vivo* 5-HT treated and from the same number of control animals were isolated 2 h after the onset of the 5-HT. To obtain a sufficient amount of RNA for the microarray hybridization, aRNAs were prepared from 2 μg of the total RNA purified from pleural ganglia (RiboAmp RNA amplification kit, Arcturus). Six micrograms of aRNAs were labeled with Cy3 (control group) or Cy5 (5-HT group) by reverse transcription (SuperScript III reverse transcriptase, Invitrogen) and purified using Qiagen PCR purification columns, followed by hybridization to the *Aplysia* cDNA microarray which was prehybridized with 0.1 mg/ml BSA. The slides were then washed, scanned (GenePix 4000B, Axon Instruments), and analyzed using GenePix Pro and Acuity software (Axon Instruments). The results from two independent 5-HT treatments and hybridization analyses were averaged. Only high-quality spots (circularity >80, % saturation <2 for each channel, signal to noise ratio >3 for each channel, Rgn $R^2(635/532) > 0.6$) were used for further analysis. 93% and 88% of spots were found as high quality spots from each microarray, respectively. After filtering out low quality spots, the ratios of medians (5-HT/control) of total 3,813 spots (55% of total spots) which show the expression changes in the same directions were averaged. Correlation coefficient of this replicate was 0.747 (Pearson correlation, $P < 0.0001$).

Real-Time PCR. The cDNAs prepared as described above were used for quantitative real-time PCR. Reactions were performed in the Thermal Cycler Dice Real Time System, TP800 (Takara) using SYBR Premix Ex Taq (Takara) and gene specific primer sets [Primer sequences are shown in Table S4]. Amplification reaction consisted of one cycle of 95°C for 5 min, followed by 60 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 30 s. Data were collected during the extension phase at 72°C. S4 was used as an internal control. For the relative comparison of each mRNA, we analyzed C_T value using the $2^{-\Delta\Delta CT}$ method (2). Data collected by real-time PCR were analyzed using a student's *t* test.

1. Hegde P, *et al.* (2000) A concise guide to cDNA microarray analysis. *Biotechniques* 29:548–556.

2. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.

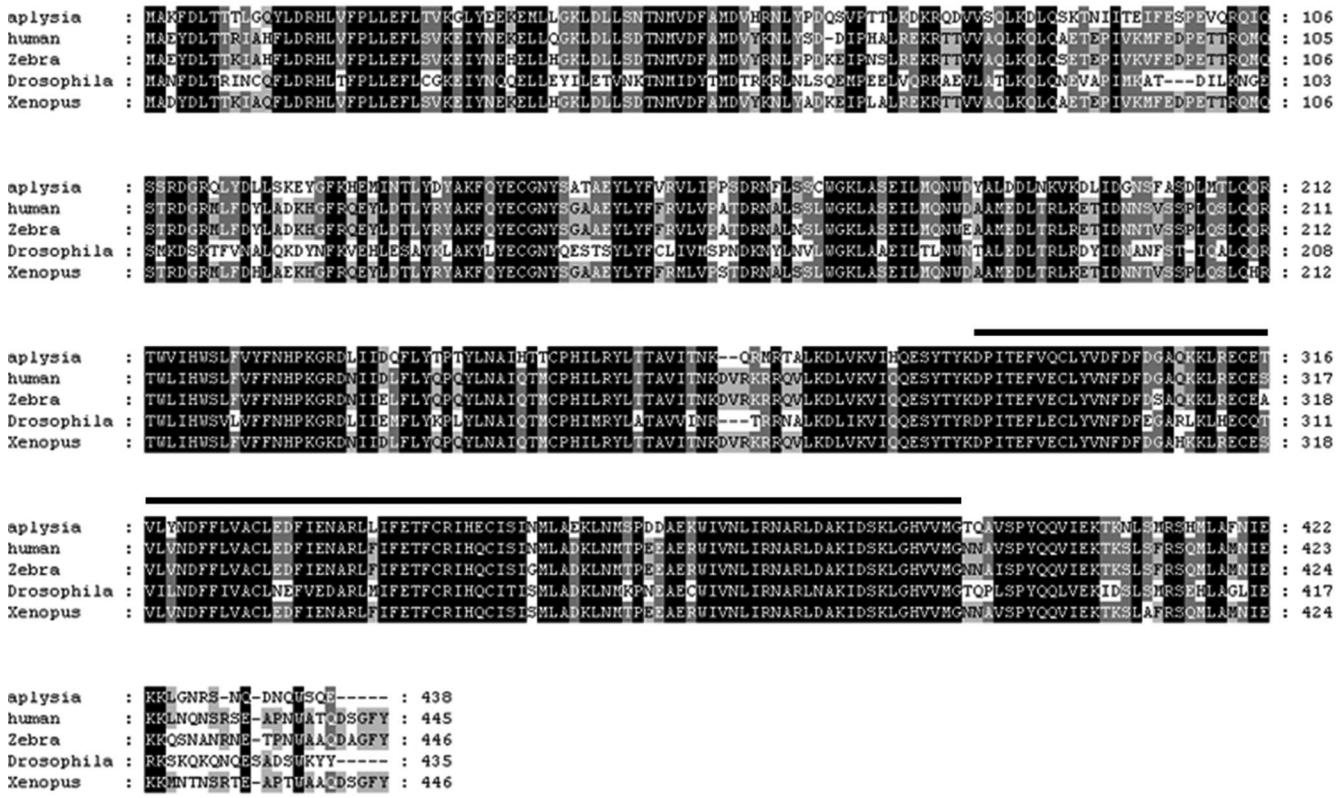

Fig. S1. Predicted amino acid sequence alignments of Ap-eIF3e with eIF3e from other species (human, NP_001559; *Xenopus*, AAF80474; Zebrafish, NP_957133; Drosophila, NP_477385). The conserved PCI domain was marked by line above the sequences.

Table S1. Summary of *Aplysia kurodai* EST clones

Total sequences	11755
Full-length enriched library	5190
5′-enriched library	1675
Random-primed library	3386
5-HT-treated library	750
Buccal muscle library	754
Total high-quality sequences	11493

Table S2. GO classification of unique transcripts: Biological processes

Gene ontology term	Number of putative genes
Total	1052
Cell communication	189
Cell adhesion	22
Cell-cell signaling	8
Transmission of nerve impulse	9
Response to external stimulus	32
Perception of external stimulus	15
Response to biotic stimulus	15
Signal transduction	83
Cell growth and/or maintenance	803
Cell cycle	31
Cell growth	15
Cell motility	15
Cell organization and biogenesis	60
Cell proliferation	3
Metabolism	565
Regulation of cell shape and cell size	6
Transport	94
Death	7
Development	29
Organogenesis	10
Gametogenesis	4
Obsolete	10
Embryogenesis and morphogenesis	6
Histogenesis and organogenesis	4
Physiological processes	14

Hierarchical classification was performed and children terms are indented.

Table S3. GO classification of unique transcripts: Molecular functions

Gene ontology term	Number of putative genes
Total	1015
Binding	462
Neurotransmitter receptor	5
Nucleic acid binding	234
DNA binding	65
Nuclease	4
RNA binding	119
Translation factor	25
Nucleotide binding	65
Protein binding	70
Receptor binding	12
Sterol carrier	5
Cell adhesion molecule	5
Chaperone	23
Defense/immunity protein	5
Enzyme	314
Hydrolase	95
Omerase	11
cis-trans isomerase	4
Kinase	35
Ligase	21
Lyase	16
Oxidoreductase	72
Transferase	45
Enzyme activator	11
Enzyme inhibitor	16
Motor	7
Protein degradation tagging	4
Signal transducer	30
Receptor	15
Receptor signaling protein	8
Structural molecule	81
Structural constituent of cytoskeleton	25
Structural constituent of muscle	11
Structural constituent of ribosome	29
Transporter	84

Hierarchical classification was performed and children terms are indented.

Table S4. Differentially expressed clones by > 2-fold 2 h after 5-HT treatment in microarray

EST name	BLASTX result*	GenBank accession number	Ratio of medians (5-HT/ control)	Sense primer sequence	Antisense primer sequence	Short description of real-time PCR results†
Up-regulated clones						
5CAP092402_H04_760	Matrilin (AAN61407, 1.00E-33)	EY418286	5.377	ATCACCTTCCACCACACCTC	AGCCACATCATTCATCGTCA	Significant
5CAP090501-pMES_D10_142	Antistasin precursor (P38977, 2.00E-25)	EY417467	4.830	AGCATGGAAACGTCTTGGAC	AGAGCGAGGTTTGCATTCAC	Significant
5CAP031402_pMES_B12_1600	Cathepsin L-like cysteine proteinase precursor (AAQ22984, 5.00E-05)	EY417083	4.419	GAGACCAAAGTGGACGAGGA	CTTGAGCGCTTGTTGTGGTA	No change
5HTCNS062702-T3_D01_92	No hits	EY416228	3.582			
R5CAP031403_pMES_E11_756	No hits	EY394288	3.306			
5CAP090504-pMES_E03_435	Alpha tubulin 2 (AAM09674, 6.00E-48)	EY417749	2.984	CTCTCATCTCACGACGGACA	ATACCGTGCTCCAGGCAGTA	No change
5CAP090501-pMES_H12_192	No hits	EY417516	2.942			
5CAP092402_B10_694	LOC443610 protein (AAI28922, 7.00E-55)	EY418226	2.838	TACGCCGCCTATCCTTTATG	TACAGCCCTTGATCCCACTC	No change
C/EBP	ApC/EBP (AAA18286, 0)		2.594	TACTCTCAACCTTCCCTCAAGC	TGACAAATGAACAAAATGGACA	Significant
5CAP031402_pMES_D12_1621	ApCREB2 (AAA92437, 1.00E-25)	EY417103	2.548	AGCGTTTCTCTCCATACTCT	TGCAAGTCATCGACCTTAGTCT	No change
5CAP031402_pMES_B04_1592	No hits	EY417075	2.478			
5HTCNS122105-T3-C10–628	Eukaryotic translation initiation factor 3, subunit 6 (NP_001559, 5.00E-73)	EY416763	2.436	CGAGACATACGCCCAGTGTA	GCGATAGGCGAAAGAAACAG	Significant
5CAP092404_G12_948	No hits	EY418453	2.379			
5CAP090501-pMES_F08_164	hypothetical protein (XP_001176996, 6.00E-33)	EY417489	2.230	CTGGGCCATATTTTCACACC	GAGGCTTCACTCTTGGCAAC	No change
YesTrp090505-Bco3_05_B06_498	No hits	EY422272	2.173			
YesTrp090503-Bco3_03_D10_334	No hits		2.085			
YesTrp090503-Bco3_03_B08_308	No hits	EY422122	2.071			
Down-regulated clones						
5CAP101001_F09_1029	Kazal proteinase inhibitor (ABL74453, 2.00E-22)	EY418526	0.346	GGAAGCTGTGGTGATGGACT	CGGGAAGAGTGAAGGTCTTG	No change
5CAP120302-pMES_C06_1166	unnamed protein product (CAG08644, 2.00E-14)	EY419713	0.356	ATTTTTGCCTCCAACCTGTG	TCTGTCGGACGAATTCAAGA	Reverse trend
5CAP031401_pMES_A06_1493	No hits	EY416984	0.384			
5CAP092402_D03_711	No hits	EY418242	0.387			
5CAP120601_pMES_E03_2269	No hits	EY420078	0.393			
5CAP031403_pMES_B09_1682	No hits	EY417156	0.409			
5CAP092401_E01_625	No hits	EY418164	0.443			
5CAP031405_pMES_G09_1891	hCG1999844 (EAX04582, 0.013)	EY417337	0.460	TGCATTTATTTTCCCCCTCA	CATGCTTGGCATGAAAAAGA	No change
5CAP101001_G01_1033	No hits	EY418530	0.479			
5CAP101001_H07_1051	BAT1 homolog (AAQ13472, 2.00E-26)	EY418548	0.486	GCCGGAGAACTCTGACACAT	CGACCCTCGATGTAAGAGGA	Significant

*BLASTX results are described as: gene description (accession number of BLASTX matched gene, E-value).
†Significant means $P < 0.05$ by student's t test; trend or reverse trend means $0.05 < P \leq 0.10$ by student's t test; no change means $P > 0.10$.