

File S1

Supporting Materials and Methods

Strain List

N2	Wild type	Figure 4A, B
RM2256	<i>pha-1(e2123)III</i>	Recipient for transgenic injections
RM523	<i>unc-17(cn355)IV</i>	Figure 4C, D
RM3288	<i>pha-1(e2123)III; mdEx833[Punc-17::dual-reporter(wt) pBX pBS]</i>	Figure 3; 4E, F; 5A
RM3302	<i>pha-1(e2123)III; mdEx847[Punc-17::dual-reporter(cn355) pBX pBS]</i>	Figure 4G, H
RM3376	<i>pha-1(e2123)III; mdEx909[dual-reporter(wt) pBX pBS]</i>	No promoter (Control)
RM3317	<i>pha-1(e2123)III; mdEx857[Punc-17::dual-reporter(R1u:scr)* pBX pBS]</i>	Figure 5B
RM3323	<i>pha-1(e2123)III; mdEx863[Punc-17::dual-reporter(R1ud:scr) pBX pBS]</i>	Figure 5C
RM3319	<i>pha-1(e2123)III; mdEx859[Punc-17::dual-reporter(R2d:scr) pBX pBS]</i>	Figure 5D
RM3321	<i>pha-1(e2123)III; mdEx861[Punc-17::dual-reporter(R2ud:scr) pBX pBS]</i>	Figure 5E
RM3347	<i>pha-1(e2123)III; mdEx885[Punc-17::dual-reporter(R1uR2d:scr) pBX pBS]</i>	Figure 5F

*Note: R1u:scr = R1 upstream element scrambled (see Table S1);

R1ud:scr = R1 upstream and downstream elements scrambled; etc.

Details for the *unc-17(cn355)* allele (see Figure 4)

Nature of mutation: A>G transition

Flanking Sequences: AAATTTAGAAAAATAAAATATTCC/ **A>G** /GGGGGAGAGAGAGAGATGGGCTTCA

(in direction of transcription)

Sources and accession numbers of genomic CGL sequences

Genomic sequences for all *Caenorhabditis* species were downloaded from WormBase, Release WS240 (www.wormbase.org).

Downloaded from the Sanger Center (www.sanger.ac.uk/resources/downloads/helminths/globodera-pallida.html):

Globodera pallida pathogens_Gpal_scaffold_214.1
pathogens_Gpal_scaffold_214.2
pathogens_Gpal_scaffold_146

Downloaded from NCBI (www.ncbi.nlm.nih.gov):

<i>Ciona intestinalis</i>	NW_001955240 REGION: 1..30000
<i>Drosophila melanogaster</i>	NT_033777 REGION: 14525001..14560000
<i>Meloidogyne hapla</i>	ABLG01001183 and ABLG01002582
<i>Mus musculus</i>	AC167565
<i>Rattus norvegicus</i>	NW_047469 REGION: complement(7900000..8006000)
<i>Saccoglossus kowalevskii</i>	NW_003151267 REGION: 100001..150000
<i>Schistosoma mansoni</i>	NS_000200 REGION: 148400001..148540000
<i>Takifugu rubripes</i> *	CGL a (chr 1): NC_018890 REGION: complement(3185000..3200000) CGL b (chr 4): NC_018893 REGION: 10170000..10190000

* Note: The genomes of *T. rubripes* and other teleosts reflect a whole genome duplication, and therefore have 2 (somewhat diverged) copies of many genes, including the CGL. Official *T. rubripes* gene names for the CGLs are still unassigned, but we provisionally refer to the CGL on Chromosome 1 as CGL a, and the locus on Chromosome 4 as CGL b.

DNA/RNA sequence analysis

Sequence analysis utilized Vector NTI® software (Life Technologies Corporation, Carlsbad, CA) or the Lasergene® Suite (DNASTAR, Inc., Madison, WI). For several species, genomic annotation of the CGL was incomplete or incorrect; in such cases, the genomic organization of the *unc-17* and *cha-1* homologs was deduced through analysis with TBlastN, identification of splice-site consensus sequences, and direct determination of homology. RNA structures were analyzed with Mfold (Zuker 2003) or Sfold (Ding and Lawrence 2003; Ding *et al.* 2005). The criteria for R1-like elements (in addition to complementarity) were that they flank the complete VAcHT coding sequence but do not include any part of the ChAT coding sequence; the criterion for R2-like elements was that they flank or overlap the splice site of the first VAcHT coding exon.

Construction of dual-reporter plasmid RM#942p from 7 fragments cloned into pCRII-TOPO

Starting Plasmids:

pBS: pBlueScript (Stratagene)

TOPO: pCRII-TOPO (Invitrogen)

pAA64: mCherry plasmid with 3 artificial introns (McNally *et al.* 2006; Green *et al.* 2008).

RM#651p: Fire-type modular plasmid with an empty cloning site 1 and GFP in cloning site 2.

RM#691p: Fire-type plasmid with CFP driven by 4.4 kb of genomic sequence upstream of the *unc-17* start codon.

Clone A: RM#691p was digested with *Xba*I and recircularized to remove the 3.2 kb *unc-17* promoter, the common exon, and the first 339 bp of the *unc-17* 1st intron.

Clone D: mCherry fragment (minus the initiation Met) amplified from pAA64 with p2288 and p2206. **Note:** A lower-case "p" followed by a number refers to a primer listed below.

Clone E: *unc-17* 3'-UTR amplified from N2 genomic DNA with p13 and p2301.

Clone F: *cha-1* 3'-UTR amplified from N2 genomic DNA with p2290 and p45.

Clone H: GFP fragment from RM#651p amplified with p2291 and p2292.

Clone I: *unc-17* 5'-UTR amplified from N2 genomic DNA with p62 and p2293.

Clone J: *unc-17* 3'-UTR amplified from N2 genomic DNA with p2294 and p49.

Cloning Steps:

Clone D was transferred from TOPO to pBS with *Xho*I+*Spe*I → Clone D'

Clone F/*Nhe*I+*Spe*I was subcloned into D'/*Spe*I → Clone D'F

Clone E/*Sac*I(blunt) was subcloned into D'F/*Sna*BI → Clone ED'F

Clone ED'F was subcloned into Clone A with *Avr*II+*Spe*I → Clone B

Clone H was transferred from TOPO to pBS with *Apa*I+*Sac*I → Clone H'

Clone I was subcloned into Clone H' with *Bam*HI → Clone IH'(B)

Clone IH'(B) was digested with *Xba*I and recircularized → Clone IH'

Clone J was subcloned into Clone IH' with *Sac*I → Clone IH'J

Clone IH'J was subcloned into Clone B with *Avr*II → RM#942p.

Primers cited:

p13 CCTTCTCTGTTACCTACAA

p45 GTCTGGTGTCTGGGATGA

p49 CATTGGTGGGAAGTTCGTCAAC

p62 TTCCGCATCTCTGTTCAA

p2206 **GAGCTC**TTAGGATCCACTAGTCTTATAC (final amino acids of mCherry with *Sac*I site at 5'-end)

- p2288 **TACGTATCAAAGGGTGAAGAAGATAACA** (adds **SnaBI** site; introduces a silent mutation in Val2 of mCherry)
- p2290 **GCTAGCGGATCCTGAATTTTATTATTATTTTTGAG** (adds the last four amino acids of mCherry with two mutations to alter the *SpeI* site to **NheI** - amino acid sequence from Thr-Ser to Ala-Ser - uses the *cha-1* stop codon TGA)
- p2291 **GGATCCAAAGGAGAAGAAGAACTTTTCACTG** (introduces a **BamHI** site that changes GFP N-terminal amino acid sequence from Met-Ser-Lys to Gly-Ser-Lys)
- p2292 **GAGCTCATCCATGCCATGTGTAATC** (makes two silent mutations; inserts **SacI** site in Glu-Leu near end of GFP)
- p2293 **GGATCCCATCTCTCTCTCC** (creates **BamHI** site - changes GFP amino acid sequence from Met-Ser to Met-Gly)
- p2294 **GAGCTCTACAAATAGTCGTAGATTTGGATCTCTG** (5'-end corresponds to the last four amino acids of GFP with two silent mutations to generate **SacI** site)
- p2301 **GAGCTCATCTGGAACAAAATTTACTTCT** (**SacI** site added at 5'-end)

LITERATURE CITED

- Ding, Y., C. Y. Chan and C. E. Lawrence, 2005 RNA secondary structure prediction by centroids in a Boltzmann weighted ensemble. *RNA* 11: 1157-1166.
- Ding, Y., and C. E. Lawrence, 2003 A statistical sampling algorithm for RNA secondary structure prediction. *Nucleic Acids Res.* 31: 7280-7301.
- Félix, M. A., C. Braendle, and A. D. Cutter, 2014 A streamlined system for species diagnosis in *Caenorhabditis* (Nematoda: Rhabditidae) with name designations for 15 distinct biological species. *PLoS One* 9: e94723.
- Green, R. A., A. Audhya, A. Pozniakovsky, A. Dammermann, H. Pemble *et al.*, 2008 Expression and Imaging of Fluorescent Proteins in the *C. elegans* Gonad and Early Embryo, pp. 179-218 in *Methods in Cell Biology: Fluorescent Proteins*, edited by F. S. Kevin. Academic Press.
- Huang, R. E., X. Ren, Y. Qiu and Z. Zhao, 2014 Description of *Caenorhabditis sinica* sp. n. (Nematoda: Rhabditidae), a Nematode Species Used in Comparative Biology for *C. elegans*. *PLoS One* 9: e110957.
- McNally, K., A. Audhya, K. Oegema and F. J. McNally, 2006 Katanin controls mitotic and meiotic spindle length. *J. Cell Biol.* 175: 881-891.
- Zuker, M., 2003 Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31: 3406-3415.