

**A NOVEL HEME RESPONSE ELEMENT MEDIATES TRANSCRIPTIONAL REGULATION  
IN CAENORHABDITIS ELEGANS**  
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**Supplementary Data**

Supplemental Fig. S1. Genomic structure of *hrg-1*. RACE PCR analysis of *hrg-1* revealed a SL1 *trans*-spliced transcript of ~ 0.9 kb nucleotides, and two additional transcripts of ~ 2.89 kb and ~8 kb. Transcriptional start sites are indicated by right angle arrows, exons by open boxes, introns by lines, 5' UTRs by gray boxes, 3' UTRs by gray block arrows, SL1 leader sequence by a black box.

Supplemental Fig. S2. Alignment of the *hrg-1* promoter in five *Caenorhabditis* species. Reversed characters indicate conserved nucleotides. Black outline indicates putative regulatory elements. Arrows show the orientation of the GATA sites. The ATG start codon is underlined.

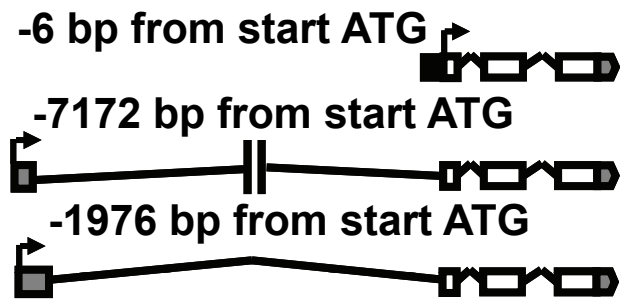
Supplemental Fig. S3. The HEme-Responsive Element (HERE) is within a 67 bp region. Transgenic worms expressing the *hrg-1(92-16)::gfp* construct (Fig. 4C) are heme responsive albeit the GFP levels are weak. \* denotes autofluorescent intestinal gut granules, which are observed when microscopy images are taken with longer shutter exposures.

Supplemental Fig. S4. The 23 bp element is insufficient to activate the *myo-2* basal promoter. The concatamer of six direct repeats of the 23 bp element from Fig. 6A was fused to the *myo-2* basal promoter. This chimeric promoter was placed upstream of *gfp* and the *unc-54* 3' UTR. Transgenic *C. elegans* expressing either *myo-2::gfp* or *myo-2(+23)::gfp* were placed in media supplemented with 2  $\mu$ M heme for 120 h and GFP expression in the worms was analyzed by microscopy. Scale bar = 50  $\mu$ m.

Supplemental Fig. S5. The E-box of the HERE is conserved in *hrg-1*, C15C8.3, and *mrp-5*. Reversed characters indicate E-box. Shaded characters indicate GATA sites.

Supplemental Video 1. Gravid IQ6011 hermaphrodites maintained on NGM plates seeded with *E. coli* strain OP50 were cut open to release embryos. DIC (left panel) and Fluorescence (right panel) images were collected at five-minute intervals.

Supplemental Figure S1



## Supplemental Figure S2

GATA-E → GATA-D →

*C. elegans* T-GAAAACAATGAGAAAAGTCCGATAACTT-GATACGGGAGTCAGCCATATGGTGCA  
*C. japonica* TTAARAACAATAGTGGAGAGCGATAACTT-GATACGAG-GCAAGCCATATGGTGCA  
*C. briggsae* T-AARAACAATAGGGGGAAGCGATAACTT-GATACGAG--AAGCCATATGGTGCA  
*C. remanei* T-GAAAACAATGGGAAA--CGATAACTT-GATACGGGAGTCAGCCATATGGTGCA  
*C. brenneri* T-AARAACAATGAAAGGTGCGATAACTT-GATACGGGAGTCAGCCATATGGTGCA

GATA-C →

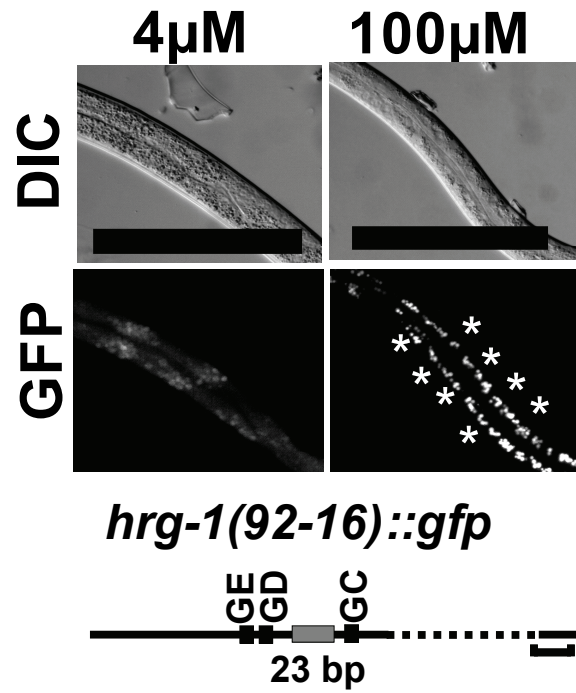
*C. elegans* ACAATGATTTCGATGATAAAGACCGAAACATATAATATGGGGCAGTAGTGTGGT  
*C. japonica* ACAATGATTTTTATGATAAAGACCGAAACATATAATATC----CAATAG---GAA  
*C. briggsae* ACAATGATTTCGTGATAAAGCTTGAACGCATATAAAGT-----GGTCTGAGGAA  
*C. remanei* ACAATGATTTCGATGATAAAGTCTGAACACATATAATATGGGA-CAGAAGTGTAGT  
*C. brenneri* ACAATGATTTCGATGATAAAGACCGAAACATATAA-ATGGG-CAGGTGTGTAGT

GATA-B GATA-A ←

*C. elegans* TTGGGTCGAAAGGTTCTTAATATATCATATCCCAATGCACCTTTCCGACCGTTTCTTT  
*C. japonica* GCGGGTTGGCTGTTCACTTAATTTCTACCAA----CCAATTGACCACTGACTAA  
*C. briggsae* GCGGGTCGAGTCCACATTTGACTCTCTATC-----CCTCCTTAACAGACATATT  
*C. remanei* TTGGGTCGATCTCGTAC--GCATTCCGTA-----CTTTTGGCTTACTTTCTA  
*C. brenneri* TTGGGTCGATCTTGTCC-----CAGT-----TTCACTTCTGTTCTCT

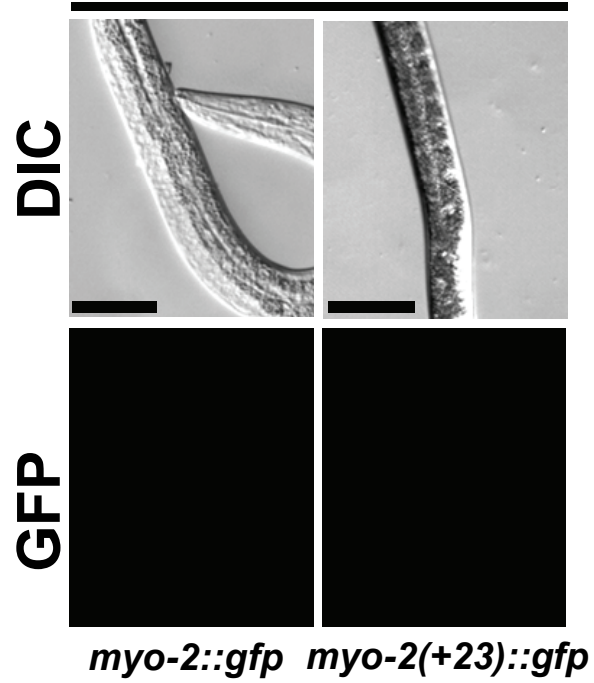
*C. elegans* TTTCTAATTG-TTTTTTTTCAGTCAATG  
*C. japonica* GTTTAACC---ATTAAATTTTCAGTCAATG  
*C. briggsae* TTTCTAATATTCTCATTTTTTCAGTCAATG  
*C. remanei* AATTCAGT--TATTTTTTCAGTCAATG  
*C. brenneri* AATTAATTA-TTTGATTTTCAGTCAATG

Supplemental Figure S3



Supplemental Figure S4

**2  $\mu$ M heme**



## Supplemental Figure S5

*hrg-1* TTATCATGTCACAATCATTGTTCCATCATATGCTCGACTTCCCACTATCAACTTATCG  
C15C8.3 CATAATGATAGTCATTAGTTCCATCATATGCTCCTGCTCGCTCCTCCTATCTCATA  
*mrp-5* GAACAGTACCCCTACCCCATTTCCATCATATGCTCGCAAATGACATTATCAGTTGCGA

**Supplemental Video 1**

