Circadian regulation of olfaction and an evolutionarily conserved, nontranscriptional marker in Caenorhabditis elegans

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Published in:
Proceedings of the National Academy of Sciences of the United States of America

DOI:
10.1073/pnas.1211705109

Supporting Information

Olmedo et al. 10.1073/pnas.1211705109

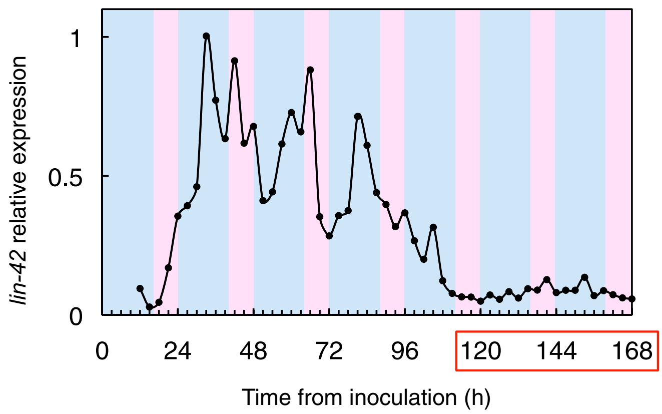


Fig. S1. Expression of the heterochronic gene *lin-42* through development in our temperature entrainment protocol. Experiments represented in all other figures in this article were performed within the time window of 120–168 h after inoculation of the eggs, when *lin-42* is expressed at low levels despite an ongoing temperature cycle. Blue panels represent 13 °C, and pink panels represent 16 °C.

Fig. S2. Multiple sequence alignment showing PRX amino acid sequences. The highly conserved active site is underlined. The sequences analyzed correspond to *At* (*Arabidopsis thaliana*; NP_187769.1), *Se* (*Synechococcus elongatus* PCC 7942; YP_401326.1), *Hm* (*Homo sapiens*; NP_005800.3), *Mm* (*Mus musculus*; NP_035693.3), *Ce2* (*C. elegans*; NP_001122604.1; *prdx-2*), *Dm* (*Drosophila melanogaster*; NP_477510.1), *Ce3* (*C. elegans*; NP_497892.1; *prdx-3*), and *Nc* (*Neurospora crassa*; XP_959621.1).

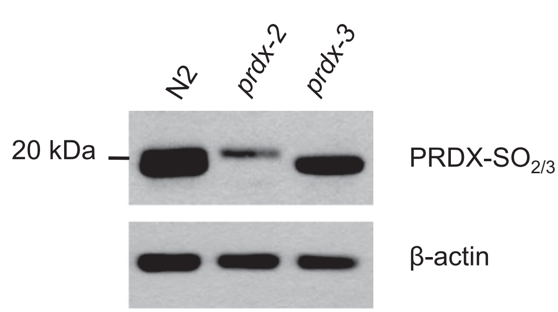


Fig. S3. Antiserum against PRX-$SO_{2/3}$ recognizes the oxidized form of PRDX-2. Wild-type (N2) and mutant *prdx-2* (VC289) and *prdx-3* (VC1151) worms were treated with 1 mM H_2O_2 for 30 min (to induce expression of the PRX proteins) and then lysed for immnoblotting. The immunoblot was probed for PRX-$SO_{2/3}$. The antiserum raised against the oxidized peptide DFTFVCPTEI detects both PRDX-2 and PRDX-3 in wild-type *C. elegans* (*N2*), indicated by a doublet with H_2O_2 treatment. The lower band detected by the antiserum is absent in the mutant *prdx-2* and present in the *prdx-3* mutant, indicating that PRDX-2 is the dominant *C. elegans* ortholog of PRX that is detected in the time-course assays.

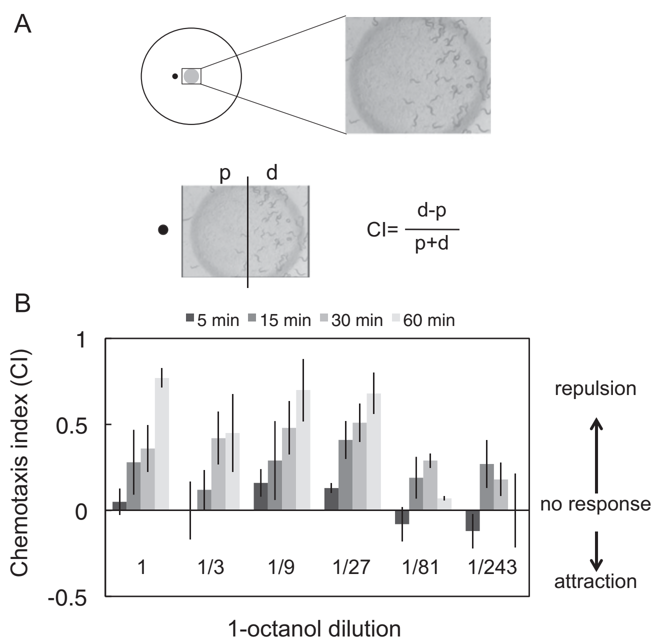


Fig. S4. (*A*) An in situ assay for chemotaxis: population assay in olfaction using 1-octanol. Approximately 100 nematodes were inoculated onto a drop of *E. coli* as eggs. They were then subjected to the development protocol with temperature cycles (Fig. 1*A*). On day 6, olfaction assays were started by placing a 1 μL drop of 1-octanol (shown as a black drop) to one side of the *E. coli*. After a given amount of time, a picture was taken and the CI was determined by counting the animals on the proximal (p) and distal (d) sides of the drop of bacteria. The difference (d – p) was divided by the total number of animals. (*B*) Optimization of the chemotaxis assay. The assay was optimized by comparing different time points after addition of 1-octanol (between 5 and 60 min) and dilutions of the chemorepellant (none to 1/243 in ethanol).

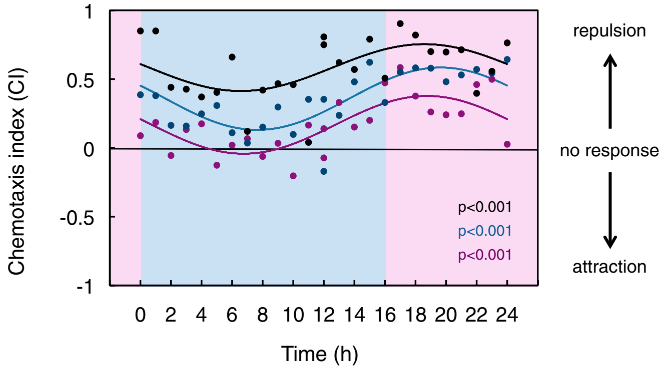
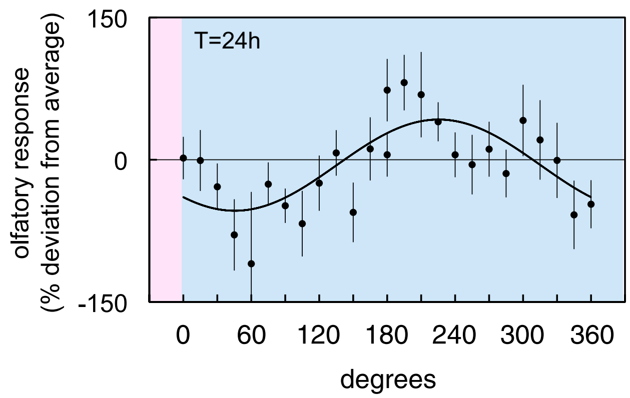


Fig. S5. CI from the three experiments in Fig. 3*B* are plotted independently. A sinewave was fitted to each time series using Circwave.



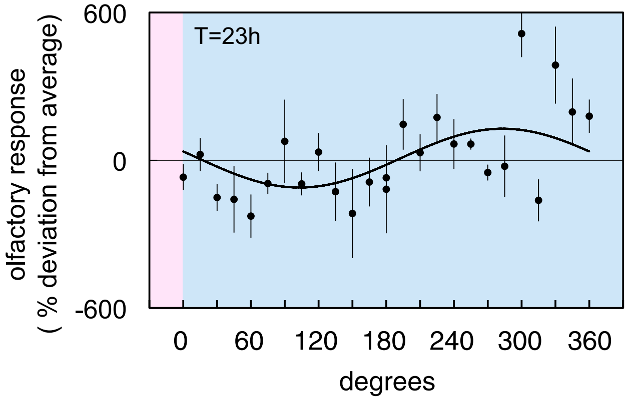


Fig. S6. Response to 1-octanol in constant conditions after entrainment to either a 24 h cycle (*Upper*) or a 23 h cycle (*Lower*). One complete cycle is in both cases represented as 360°. A sinewave was fitted to each series using Circwave. The acrophase of the sinewave adjusted to the T = 24 series (with a $P < 0.001$) is 225.45°, and the acrophase of the sinewave adjusted to the T = 23 series (with a $P = 0.0019$) is 283.35°.

Table S1. Period estimates and statistical parameters calculated by Circwave and JTK_Cycle

	Circwave			JTK_Cycle		
Molecular marker	Tau	*F* statistic	*P*	Tau	Phase	ADJ p
B0507.8	22.6	7.7037	0.0016	24	18	0.0589
PRX-$SO_{2/3}$	24.5	6.008	0.0056	28	26	0.0361
GRK-2	31.3	18.9573	<0.0001	32	30	0.000016

Circwave is an analysis tool for determining circadian profiles and their significance using harmonic regression in combination with the *F*-test statistic. A fundamental sinusoidal wave is fitted through the data, and its significance is tested against a fitted horizontal line through the overall average (www.euclock.org). JTK_CYCLE is a nonparametric statistical algorithm designed to identify and characterize cycling variables. JTK_CYCLE provides optimal phase, amplitude, and period estimates for each variable, and permutation-based *P* values (1).

1. Hughes ME, Hogenesch JB, Kornacker K (2010) JTK_CYCLE: An efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *J Biol Rhythms* 25(5): 372–380.

Table S2. List of volatile odorants tested in the in situ chemotaxis assay

Chemical*	Type of response (ref. 1)	Type of chemical
1-Butanol	Attraction	Alcohol
2-Butanol	Attraction	Alcohol
Isoamyl alcohol	Attraction	Alcohol
Acetone	Attraction	Ketone
Diacetyl	Attraction	Ketone
Ethyl acetate	Attraction	Ester
Propyl acetate	Attraction	Ester
n-Butil acetate	Attraction	Ester
Isoamyl acetate	Attraction	Ester
Aniline	Attraction	Aromatic compound
2-Phenylethanol	Weak attraction	Alcohol
Benzylalcohol	Weak attraction	Alcohol
1-Propanol	Weak attraction	Alcohol
1-Octanol	Repulsion	Alcohol

*All of the chemicals were diluted with ethanol at a ratio of 1:1 except for 1-butanol and the three weak attractants, which were used undiluted.

1. Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* 74(3):515–527.

Table S3. List of primers for quantitative RT-PCR

Gene	Forward primer	Reverse primer
F47F6.1 (*lin-42*)	5′-CCACTGACCCGAGAAGCAC-3′	5′-GAGTTGGTGCCACTTGTCGG-3′
F01D5.5	5′-AACCTGTAACATGTGCCCAGGA-3′	5′-GCCGTTTTTCACCCAGTTGAC-3′
Y110A2AL.9	5′-ACCAAGGATGTTTTTGACCCC-3′	5′-TTGGTGACACTGTAGCCGGTT-3′
T16D1.2 (*pho-4*)	5′-GAAATTGATGATGGTTCAGGCG-3′	5′-ACCACCTCCTCCAAACATCCA-3′
M199.4 (*clec-190*)	5′-ATGATTGTGAACCTGAACGCG-3′	5′-CCAGAAAAATCCGGTTCCGT-3′
F15A4.6	5′-CAATGCAATCGGTCTTCTTGGT-3′	5′-CCATTGGCATTGGTCTTGTCA-3′
C30G12.2	5′-CTGCAGAAGGAGATGAAGCAAG-3′	5′-ACTCATTCGGTATGCGGTCA-3′
F15E6.8 (*dtc-7*)	5′-TCTCCTCGGCCTTATTGCTATG-3′	5′-CGTAGGCTCCTTGGTTTCCAT-3′
B0507.8	5′-AAAGAGAAGCAGCGTCGAGTGA-3′	5′-TCCCATTGACTGCACGTCAAC-3′
ZC308.1 (*gld-2*)	5′-TCACTTCTTGCAATGCGGC-3′	5′-CCATCGTAACATTCAATGTGCG-3′
F09E5.15 (*prdx-2*)	5′-GAGGACGAAGGAATTGCTTTCC-3′	5′-GGAAGGCCTGAACAAGACGAA-3′
W02B3.2 (*grk-2*)	5′-AGGTAGTGAATCAGGATGCGGA-3′	5′-CCCGTGGACTATAACATCGGAA-3′
M03F4.2 (*act-4*)	5′-GGCATCACACCTTCTACAACGA-3′	5′-TGGATTGAGTGGAGCCTCAGT-3′