Supplementary information

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Methods & regents

DNA construction

PCR was used to amplify the *crh-1* cDNA fragment that rescues the defect of *crh-1* mutants from *Caenorhabditis elegans* yeast two-hybrid cDNA library (COSMO BIO co., ltd.), flanked by Acc65I or StyI, and EcoRI sites. The *crh-1* cDNA fragments were subcloned into the HincII-digested vector pBluescript SK (-) (Stratagene) to construct pNYU2 and pNYU3.

All promoter constructs were previously generated by PCR to contain only non-coding region. Expression patterns of *promoter::gfp* constructs were previously verified by examining GFP fluorescence (Kuhara et al., 2008). The *ttx-3p::gfp* (pAK62) contains 0.8 kb of DNA upstream of *ttx-3* gene. The *gcy-8p::gfp* (pINA6) contains 0.8 kb of DNA region upstream of *gcy-8* gene. The *ceh-36p::gfp* contains 1.0 kb of DNA upstream of *ceh-36* gene. The *sra-6p::gfp* (pKDK132) contains 3.8 kb of DNA upstream of *sra-6* gene. The *glr-3p::gfp* (pOKU93) contains 3.0 kb of DNA upstream of *glr-3* gene. The *lin-11p::gfp* (pYOS62) contains 1.4 kb of DNA upstream of *lin-11* gene. The *ges-1p::gfp* (pKDK66) contains 3.4 kb of DNA upstream of *ges-1* gene. The *crh-1* cDNA fragment was digested from pNYU2 by Acc65I or StyI, and EcoRI or SpeI, and

exchanged for the *gfp* in each promoter construct to generate specific *promoter::crh-1* plasmids, *ttx-3p::crh-1* cDNA (pNYU12), *gcy-8p::crh-1* cDNA (pNYU10), *ceh-36p::crh-1* cDNA (pNYU11), *sra-6p::crh-1* cDNA (pNYU15), *glr-3p::crh-1* cDNA (pNYU14) and *lin-11p::crh-1* cDNA (pNYU13).

We used pNYU2 as a template for the site-directed PCR mutagenesis (Quickchange mutagenesis kit, Stratagene), to create the cDNA encoding the dominant negative form of CRH-1 (CRH-1DN), which carries point mutation in its phosphorylation site Ser29. The fragment was digested by Acc65I and EcoRI and exchanged for the native *crh-1* cDNA in pNYU10 and pNYU11 to generate *gcy-8p::crh-1dn* cDNA (pNYU20) and *ceh-36p::crh-1dn* cDNA (pNYU21), respectively.

Behavioral assay

A radial temperature gradient assay was carried out using a 9 cm agar plate and a vial containing frozen acetic acid, as described by Mori and Ohshima (1995) and Mohri et al. (2005) with some modification. Thermotaxis assay by using a liner temperature gradient was also performed as previously reported (Ito et al., 2006). Equipment for establishing the linear thermal gradient was used as essentially described by Hedgecock and Russell, (1975). A stable, linear thermal gradient was established on a 60 cm long aluminum platform, one end of which was placed in a water bath at 5°C and the opposite end in a water bath at 35°C. TTX plate (13.5 cm × 6 cm, 1.8 cm height) containing 10 ml of TTX medium (3 g/l NaCl, 20 g/l Bacto Agar, 25 mM KPO_4) with 2% agar was placed on the thin aluminum platform such that temperature gradient could be established along the agar surface of 13.5 cm long. The

center of the 13.5 cm long-agar surface in TTX plate was adjusted at 20°C and the TTX plate was maintained for 15 min before a linear thermal gradient ranging from approximately 17°C to 23°C was established on the agar surface. Uncrowded and well-fed animals were used for the thermotaxis assay. A single adult animal was placed on a 6 cm plate containing 14 ml of NGM with 2% agar, on which *E. coli* OP50 as a food source was seeded; the animal and its progeny were cultured at respective temperature. The animals were collected with 1 ml of NG buffer kept at 20°C and were washed once with autoclaved water at 20°C. These steps were carried out within 7 min in a water bath with constantly maintained temperature of 20°C. Approximately 80–200 animals were placed at the center of the TTX plate. Excess water surrounding the animals was removed with tissue paper within 20 seconds. After 60 min, the animals were killed by chloroform gas, and the animals in each of the eight regions were scored. The thermotaxis (TTX) index was calculated from the formula shown in Fig 1B.

For the temperature shift assay based on the population TTX assay (Mohri et al., 2006), wild-type animals and other animals were cultivated with food. At the first day, two wild-type animals and three mutants were each deposited on the NGM plate and cultivated at 17°C. After 12 h, the deposited P0 worms were removed from each plate to segregate F1 progeny. F1 animals were grown at 17°C for 4.5 days (after depositing P0 on the NGM plate). After animals grew to the adult stage, these animals were shifted to a new temperature 23°C, and the population thermotaxis assay was performed at each time point (Fig 4G).

In vivo calcium imaging analysis

In vivo calcium imaging was performed essentially according to Kimura et al., (2004) and Kuhara et al., (2008). To monitor the temperature-evoked response of the AFD thermosensory neuron, the yellow cameleon 3.6 under the *gcy-8* promoter was expressed in the wild-type animals and *crh-1* mutants. Each animal was cultivated at 23°C, glued onto a 1.5% agar pad on glass, immersed in M9 buffer, and covered by cover glass. The agar pad and M9 buffer were kept at the initial imaging temperature. Sample preparation was completed within 2 min. The sample was then placed onto a peltier-based thermocontroller (Tokai Hit, Japan) on the stage of an Olympus BX61WI at the initial imaging temperature for 2 min, and fluorescence was introduced into a Dual-View (Molecular devices, USA) optics systems. CFP and YFP images were simultaneously captured by an EM-CCD camera C9100-13 ImagEM (Hamamatsu Photonics). Using the pulse generator SG-4115 (IWATSU), images were taken with a 100-ms excitation pulse at a 1 Hz frame rate with 1×1 binning. The temperature on the agar pad was monitored by a thermometer system, DCM-20 (Tokai Hit and Hamamatsu Photonics). For each imaging experiment, fluorescence intensities were measured using the MetaMorph (Molecular Device) imaging analysis system.

Supplementary figure legend

Supplementary Fig 1 List of promoters and transgenic straines

(A) List of cells expressing GFP fluorescence driven by fusion genes encoding the

indicated promoters and *gfp* gene (*promoter::gfp*).

(B) List of transgenic strains.

Supplementary Fig 2 Calcium imaging of the AFD neurons in 23°C-cultivated wild-type animals

An example of the fluorescence ratio change in AFD of wild-type animal. Intensities of YFP (Yellow), CFP (Blue), the ratio (Black) and temperature are shown. During oscillatory temperature warming, the reciprocal changes in the fluorescence intensities of CFP and YFP were observed, indicating that ratio change was due to a change in fluorescence resonance energy transfer.

References

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Nishida, Sugi *et al*, Supplementary Fig 1

A

	Expression pattern (*promoter::gfp*)
gcy-8p	AFD
ceh-36p	AWC
ttx-3p	AIY, ADL, ASI, AIA
lin-11p	ADF, ADL, AIZ, AVG, AVH, AVJ, RIC
glr-3p	RIA, ASE
sra-6p	ASH, ASI, PVQ
ges-1p	intestine

B

Strain name	
IK868	*crh-1(tz2); Ex[gcy-8p::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK869	*crh-1(tz2); Ex[ceh-36p::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK870	*crh-1(tz2); Ex[ttx-3p::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK871	*crh-1(tz2); Ex[lin-11p::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK872	*crh-1(tz2); Ex[glr-3p::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK873	*crh-1(tz2); Ex[sra-6p ::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK874	*crh-1(tz2); Ex[[ceh-36p::crh-1 cDNA (2 ng/µl), ttx-3p::crh-1 cDNA (2 ng/µl), lin-11p::crh-1 cDNA (2 ng/µl), glr-3p::crh-1 cDNA (2 ng/µl), sra-6p ::crh-1 cDNA (2 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK875	*N2; EX[gcy-8p::crh-1-dn (20 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK876	*N2; EX[gcy-8p::crh-1-dn (100 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK877	*N2; EX[ceh-36p::crh-1-dn (20 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*
IK878	*N2; EX[ceh-36p::crh-1-dn (100 ng/µl), ges-1p::NLS-GFP (50 ng/µl)]*

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