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Molecular biology of thermosensory transduction in *C. elegans*

Ichiro Aoki and Ikue Mori

As the environmental temperature prominently influences diverse biological aspects of the animals, thermosensation and the subsequent information processing in the nervous system has attracted much attention in biology. Thermotaxis in the nematode *Caenorhabditis elegans* is an ideal behavioral paradigm by which to address the molecular mechanism underlying thermosensory transduction. Molecular genetic analysis in combination with other physiological and behavioral studies revealed that sensation of ambient temperature is mediated mainly by cyclic guanosine monophosphate (cGMP) signaling in thermosensory neurons. The information of the previously perceived temperature is also stored within the thermosensory neurons, and the consequence of the comparison between the past and the present temperature is conveyed to the downstream interneurons to further regulate the motor-circuits that encode the locomotion.

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Introduction

Using a compact nervous system consisting of only 302 neurons, *Caenorhabditis elegans* exhibits a large repertoire of behavioral outputs in response to external stimuli [1]. The complete knowledge of connectivity of the neural circuits and the accessibility to powerful genetic techniques make *C. elegans* the sole model animal in which to understand how the nervous system regulates the behaviors in a single-cell resolution.

C. elegans senses ambient temperature, associates it with the existence of food, and migrates toward the previous cultivation temperature when placed on a thermal gradient without food [2]. Laser ablation studies identified neurons and the neural circuit required for this behavioral response called thermotaxis (Figure 1a) [3], and molecular genetic analysis revealed neurons and molecules that are particularly important for thermosensation to induce thermotactic behavior (Figure 1a).

Thermotransduction in the major thermosensory neuron AFD

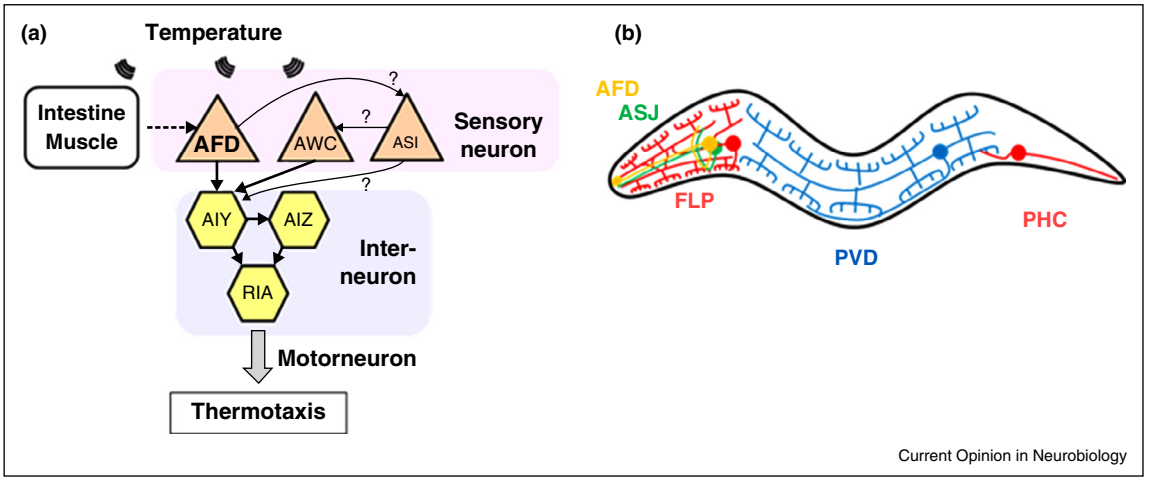
Only limited numbers of neurons are known to have ability to sense temperature in *C. elegans*. Through the analysis of thermotaxis, the AFD neuron is considered to be the major thermosensory neuron. It was later shown by calcium imaging [4–10] and electrophysiology [11,12] that AFD responds to temperature increment only when the temperature stimulus is above a threshold temperature that is around 2° lower than the previous cultivation temperature (Figure 2).

The sensory ending of AFD *per se* appears to sense temperature, since it can still respond to temperature change even after separated from the cell body by the femtosecond laser surgery, although the cell body no longer responds to temperature after the surgery [6]. Further, the structure of the sensory ending of AFD is important for thermosensation, since *ttx-1* mutants that have abnormal structure in AFD sensory ending show aberrant cryophilic phenotype, in which the mutant animals migrate to colder temperature than the cultivation temperature on a temperature gradient [13]. In addition, the ablation of amphid sheath glia cells resulted in the abnormal structure of sensory ending, which made the animals thermophilic, in which the animals migrate toward warmer temperature than the cultivation temperature [14,15].

What is the molecular mechanism underlying thermosensation? The experimental evidence indicates that the transient receptor potential (TRP) channels themselves are direct temperature sensors in mammals [16]. However, the involvement of the TRP channels has not yet been clearly shown in thermosensation in the AFD neuron, and instead previous studies showed that cGMP signaling plays an essential role in the AFD thermosensation (Figure 3a) as in phototransduction in vertebrate rods [17,18].

Cyclic nucleotide-gated (CNG) channels, which are permeable for cations such as calcium, sodium and potassium, are crucial for thermosensation. Animals lacking either TAX-4 [19], TAX-2 [20] or CNG-3 [21] channels

Figure 1



(a) A model of neural circuit underlying thermotaxis. Solid and dashed arrows indicate synaptic connections and secretory communications, respectively. Temperature is sensed and thermal information is stored and processed in AFD (and probably in AWC). Stored and processed information is primarily transmitted to AIY interneuron. The model presented here is modified from a previous report [3]. **(b)** Neurons involved in sensation of noxious temperature and cold tolerance. FLP, PHC, and AFD are involved in heat avoidance, PVD in cold avoidance, and ASJ in cold tolerance.

showed athermotactic phenotype, in which the animals move almost randomly on a temperature gradient. Electrophysiological analysis using heterologous cultured cells revealed that TAX-4 homotetramer and TAX-2/4 heterotetramer are far more sensitive to cGMP than to cAMP [19,22], suggesting that TAX-2/4 channel acts as a cGMP-gated cation channel *in vivo*.

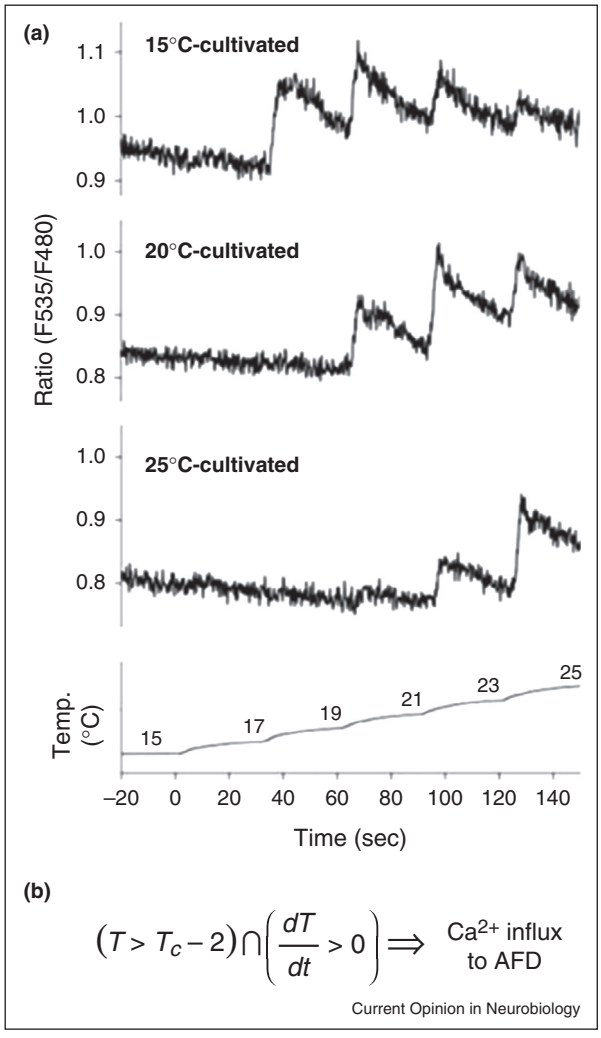
cGMP is synthesized from GTP by guanylate cyclases. Among more than 30 guanylate cyclases in *C. elegans*, three members GCY-8, GCY-18 and GCY-23 are specifically expressed in AFD and are redundantly involved in thermotaxis [23]. Like *tax-2* or *tax-4* mutants, *gcy-8 gcy-18 gcy-23* triple mutants show athermotactic phenotype. Additionally, the whole-cell patch-clamp analysis revealed that AFD response to temperature is completely lost in *gcy-8 gcy-18 gcy-23* triple mutant or *tax-4* mutant animals [11].

The activity of guanylate cyclases and the consequent cGMP level are suggested to gate the dynamic range of AFD responses [5]. Consistently, animals lacking phosphodiesterase PDE-2, which hydrolyses cGMP, showed abnormal AFD responses to temperature stimulus [12]. Expression of GCYs is positively regulated by CMK-1, one of the *C. elegans* homologues of calcium/calmodulin kinase (CaMK) [8•,24]. It was recently reported that upshift of cultivation temperature gradually increases the expression levels of GCYs in a manner that was correlated with nuclear localization of CMK-1 [8•]. Although CREB transcription factor CRH-1 is necessary in AFD for thermotaxis [25] and CREB works downstream of CaMKs in many biological processes [26], the expression level of GCY-8 was unaltered in *crh-1* mutant [24], suggesting that the regulation of GCY expressions by CMK-1 is CREB-independent.

What is the downstream signaling of calcium influx through CNG channels? One candidate is calcium/calmodulin-dependent serine/threonine protein phosphatase, calcineurin. Indeed, calcineurin TAX-6 is also involved in thermosensory transduction in the AFD neuron. Loss and gain of TAX-6 activity caused thermophilic and cryophilic phenotypes, respectively [27]. *tax-6* loss-of-function mutants showed diminished calcium influx to AFD in response to thermal stimuli [15]. Thus, TAX-6 might be necessary for amplifying or maintaining calcium influx. Identification of the downstream signaling of TAX-6 will be informative to further reveal the molecular mechanism of thermosensation.

It should be noted that themosensation in *C. elegans* could be influenced by sensations from other environmental stimuli, and an experimentally observable behavior could possibly be a final consequence of the information processing in the neural circuits upon sensation of multiple stimuli. For example, sensation of humidity in *C. elegans* was recently reported to be dependent on both thermosensation in AFD and mechanosensation in multi-dendritic head neuron FLP [28•], suggesting that humidity influences thermosensation in AFD and thermotaxis behavior.

Figure 2



The AFD neuron responds to warming above a threshold temperature dependent on past cultivation temperature. **(a)** Calcium imaging of AFD thermosensory neuron. Relative increase or decrease in the intracellular calcium concentration are measured as increase or decrease in the YFP/CFP fluorescence ratio of yellow chameleon YC2.12, a genetically encoded calcium indicator, respectively. This panel is modified from a previous report [4]. **(b)** Calcium influx to AFD occurs when the present temperature (T) is higher than the threshold that is 2° lower than the past cultivation temperature (T_c) and the time derivative of the temperature is positive.

The thermoreceptor in AFD is still unknown. However, it is tempting to hypothesize that any of guanylate cyclases GCY-8, GCY-18 or GCY-23 act as thermoreceptors based on the following results: GCY-4 and GCY-22 collaborate to sense iodide in ASER chemosensory neuron [21], and GCY-14 is a receptor for alkaline pH in ASEL neuron [29]. Alternatively, one or several of TRP channels may be involved in thermosensing in *C. elegans* as in the case of mammals. Likewise, GPCR could be a thermoreceptor as shown in *Drosophila* [30].

Other neurons sensitive to temperature change

Although AFD seems to be a major thermosensory neuron, additional thermosensory neurons were predicted to exist, since AFD-ablated animals can still migrate to cold region in thermotaxis [3]. AWC neurons also respond to temperature changes [31,32]. Through the analysis of cryophilic *eat-16* mutants, in which G protein signaling is up-regulated due to the loss of regulator of G protein signaling (RGS), activation of the AWC neuron by enhanced G protein signaling was shown to consequently down-regulate the post-synaptic interneuron AIY (Figure 3b) [31]. An urgent question is whether AWC neurons primarily sense temperature even when communication with other neurons is shut down.

ASI chemosensory neurons are also involved in thermotaxis under some specific conditions [33]. Response of ASI to temperature change is dependent on AFD (Figure 1a).

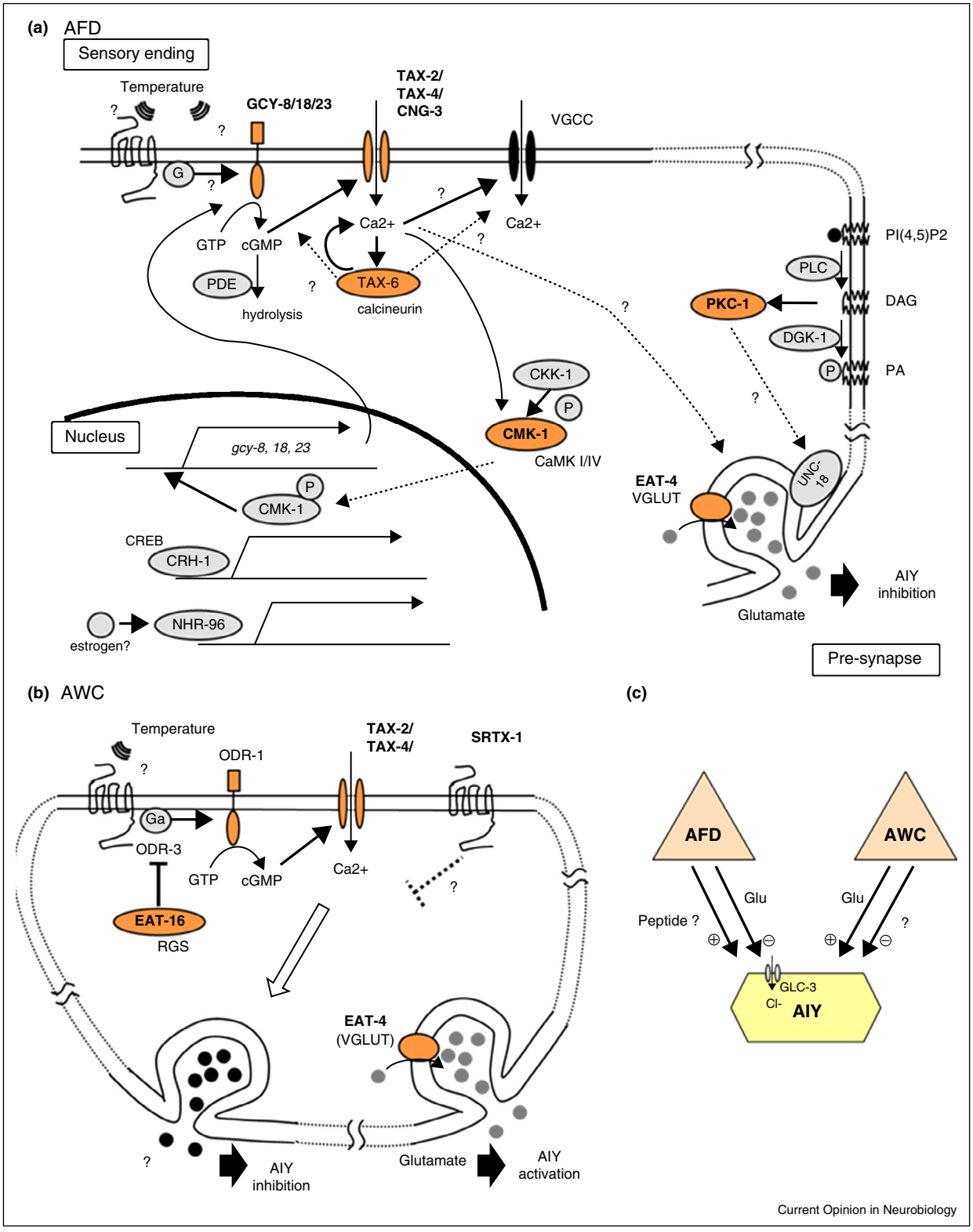
Signal transmission from sensory neurons to AIY interneurons

The temperature sensation and information processing in sensory neurons is transmitted to the downstream interneurons. AFD and AWC sensory neurons both project onto AIY interneurons *via* chemical synapses (Figure 1a) [1]. AIY interneurons are required for promoting thermophilic drive or inhibiting cryophilic drive, since ablation of AIY [3] and *ttx-3* mutation [34] that causes defect in AIY differentiation both results in cryophilic phenotype.

The excitatory transmission pathway from AFD to AIY was implicated by a study in which optogenetic stimulation of AFD with channelrhodopsin-2 caused depolarization of AIY membrane potential [35]. By contrast, optogenetic inhibition of AFD with halorhodopsin caused the increase of calcium influx into AIY, suggesting that inhibitory transmission also occurs from AFD to AIY (Figure 3c) [15]. These reports suggest that both excitatory and inhibitory transmission exist from AFD to AIY.

Excitatory transmission from AFD to AIY may be mediated by peptides, since this AFD–AIY transmission is reduced in animals lacking UNC-31 (calcium-dependent activator protein for secretion, CAPS) [35] that is necessary for the exocytosis of dense-core vesicles (DCVs) [36]. The AFD–AIY inhibitory transmission pathway is mediated by glutamate release from AFD and subsequent reception of glutamate by AIY *via* GLC-3, a ionotropic glutamate receptor permeable to chloride ion, probably leading to polarization of AIY membrane potential (Figure 3c) [37]. Given that thermophilic mutant *tax-6* [27] shows diminished calcium influx into AFD [15], TAX-6-dependent calcium influx into AFD may well be necessary for migration toward cold temperature, possibly by increasing the exocytosis of synaptic vesicles

Figure 3



Possible mechanisms of thermosensory signal transduction. **(a)** In AFD, thermal stimuli are received directly by GCYs at the sensory ending or by unidentified receptors and then unidentified G proteins activate guanylate cyclases, GCY-8, GCY-18 and GCY-23. Activated GCYs then increase intracellular cGMP concentration, which opens the putative TAX-2/TAX-4/CNG-3 cyclic nucleotide-gated channels, leading to the calcium influx and thereby the depolarization of AFD. In order to maintain the calcium concentration, TAX-6 calcineurin, a calcium/calmodulin-dependent serine/

containing glutamate from AFD, thereby leading to the negative regulation of AIY through GLC-3 (Figure 3a).

It is reasonable to hypothesize that PKC-1, a *C. elegans* ortholog of nPKC-epsilon/eta, also positively regulates glutamate release in pre-synapses of AFD, thereby negatively regulating the AIY activity. The activity of PKC-1 is dependent on diacylglycerol, and similar to *tax-6* mutants, *pkc-1* loss-of-function mutants are thermophilic (Figure 3a) [38]. Consistently, animals lacking diacylglycerol kinase DGK-1, in which PKC-1 should be activated, are cryophilic [38]. Interestingly, although the expression of PKC-1 solely in AFD rescued the thermophilic phenotype of *pkc-1* mutant, the AFD calcium response in *pkc-1* mutants was indistinguishable from that of wild type animals [9]. This result suggests that PKC-1 regulates thermotaxis by acting downstream of calcium influx in AFD. Further, PKCs are reported to enhance exocytosis in many biological contexts [39]. For instance, PKC-1 positively regulates DCV exocytosis in motorneurons in *C. elegans* [40]. PKC-2, another member of protein kinase C family, may regulate AFD function by phosphorylating UNC-18, a chaperone for syntaxin, a SNARE protein [41].

As is the case for the transmission from AFD to AIY, bidirectional excitatory and inhibitory transmission is also proposed from AWC to AIY. Whereas glutamate release from AWC to AIY is excitatory [37], inhibitory transmission from AWC to AIY is also implicated, since in *eat-16* mutants showing the enhanced AWC activity, the AIY activity is down-regulated (Figure 3c).

Non-neuronal thermosensation

Non-neuronal cells are also known to participate in thermosensation. Body wall muscle and intestine transmit temperature signals in a heat shock transcription factor HSF-1-dependent manner [42]. HSF-1 regulates the synthesis of estrogen, which is probably to mediate the temperature signal to the AFD thermosensory neuron *via* nuclear hormone receptor NHR-96 (Figure 3a) [42].

Animals live longer at lower body temperature [43]. Recent study showed that longer life span of *C. elegans* at cold temperature is not only due to the slower rate of chemical reactions but also regulated by a signaling pathway that includes TRPA-1, PKC-2 and the transcription factor DAF-16/FOXO in intestines [44•]. Surprisingly, intestines respond to temperature decrement with calcium influx through TRPA-1 channel [44•].

Heat and cold avoidance

Besides the sensation of the ambient temperature, *C. elegans* avoids noxious heat [45]. The heat avoidance behavior triggered by infrared (IR) irradiation to nose tip was dependent on the functions of AFD and multidendritic head neuron FLP, and the tail avoidance response was dependent on PHC tail sensory neurons (Figure 1b) [46••]. GCY-12 in AFD was important in addition to the functions of GCY-8, GCY-18 and GCY-23, and TRPV channels OSM-9 and OCR-2 in FLP were important in the avoidance response [46••]. TRPV channels were again important in PHC neurons during tail avoidance. In contrast to the role of AIY interneurons as a downstream of AFD in thermotaxis, AIB interneurons that are connected with AFD *via* gap junctions were involved in heat avoidance by head irradiation [46••].

Heat avoidance was also investigated by scoring animals that could not cross the heat barrier to reach the attractant odor with a different assay system. It was shown that NPR-1 neuropeptide receptor in RMG interneuron contributes to the process whereby animals hesitate to cross the heat barrier [47]. Further, pre-exposure to high temperature such as 28 °C caused acclimation in the same assay system. Similarly to the case of thermotaxis [8•], nuclear localization of CMK-1 in FLP neuron was necessary for this experience-dependent acclimation to heat [48•].

C. elegans also avoids noxious cold temperature. During cold avoidance, TRPA-1 channel functions as a cold sensor in PVD multi-dendritic nociceptor neurons in the body (Figure 1b) [49].

In addition, *C. elegans* shows cold tolerance in a past cultivation temperature-dependent manner [50,51]. Recently, ASJ neurons were found to sense temperature on their own and mediate the information of temperature to other tissues such as intestine by means of insulin secretion, resulting in the change of lipid composition in membranes (Figure 1b) [52•].

threonine protein phosphatase, may be necessary for activating CNG channels or voltage-gated calcium channels (VGCCs). Then, calcium may activate calcium/calmodulin kinase CMK-1, which contributes to the transcription of *gcy* genes. Calcium in addition to PKC-1 may regulate exocytosis. **(b)** In AWC, SRTX-1/G protein-coupled receptor is reported to slow down the responding rate to temperature change [32]. ODR-3/Ga protein activates ODR-1/guanylate cyclase, leading to change of cGMP concentration and regulating the CNG channel, and EAT-16/RGS suppresses the G protein signaling [31]. **(c)** A model for signal transmission to AIY from AFD and AWC. EAT-4-dependent glutamatergic transmission from AFD inhibits AIY activity *via* GLC-3 ionotropic glutamate receptor that is permeable to chloride ion and thereby promotes cryophilic behavior, while EAT-4-dependent glutamatergic transmission from AWC excites AIY activity and thereby promotes thermophilic behavior [37]. Excitatory peptidergic transmission from AFD is suggested by optogenetic stimulation of AFD [35]. Inhibitory transmission from AWC is enhanced by G protein signaling and down-regulated by SRTX-1 [31,32].

Conclusion and perspective

In vertebrates, thermosensation is predominantly governed by TRP channels [53]. In *C. elegans*, on the other hand, TRP channels are involved in the sensation of noxious temperature rather than that of ambient temperature. Instead, the latter is mediated by cGMP and CNG channels, which is reminiscent of the vertebrate visual system (Figure 3a). It is of especial interest that a subtype of mammalian guanylate cyclase was recently reported to respond to cool temperatures [54].

The major thermosensory neuron AFD in *C. elegans* responds to the warming above the threshold according to the calcium imaging. By contrast, exocytosis from AFD, monitored by pHluorin, a fluorescent pH probe, fused to synaptobrevin, is down-regulated when the ambient temperature is equal to past cultivation temperature [55]. It is of great interest whether the discrepancy between calcium influx and synaptic release of AFD results from the information integration between temperature and the existence of food, as is the case in salt chemotaxis [56]. It would also be valuable to specifically monitor exocytosis of synaptic vesicles and that of DCVs in order to distinguish whether each signal molecule contributes to excitation or inhibition of the downstream neurons.

Exploration and exploitation is an important issue for understanding animal behavior. Interestingly, AWC negatively regulates isothermal tracking (IT) behavior. AWC neurons of *srtx-1* loss-of-function mutant animals lacking G protein-coupled receptor SRTX-1 are hyperactive, and the *srtx-1* mutants show greatly reduced IT behavior, which is an opposite behavioral phenotype as compared with the AWC-ablated animals [32]. Since migration to the cultivation temperature is regarded as an exploration strategy and IT behavior at around the cultivation temperature as an exploitation strategy, we propose that the investigation of the molecular mechanism for the thermotaxis neural circuit shed light onto the switching of exploration–exploitation strategy. Appropriate selection of exploration and exploitation strategies should further lead to dissecting the suboptimal behavior, which may contribute to the behavioral plasticity.

Conflicts of interest statement

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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- •• of outstanding interest

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