RESEARCH COMMUNICATION

Insulin-like signaling and the neural circuit for integrative behavior in *C. elegans*

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Caenorhabditis elegans exhibits a food-associated behavior that is modulated by the past cultivation temperature. Mutations in *INS-1*, the homolog of human insulin, caused the defect in this integrative behavior. Mutations in *DAF-2*/insulin receptor and *AGE-1*/phosphatidylinositol 3 (PI-3)-kinase partially suppressed the defect of *ins-1* mutants, and a mutation in *DAF-16*, a forkhead-type transcriptional factor, caused a weak defect. In addition, mutations in the secretory protein *HEN-1* showed synergistic effects with *INS-1*. Expression of *AGE-1* in any of the three interneurons, *AIY*, *AIZ*, or *RIA*, rescued the defect characteristic of *age-1* mutants. Calcium imaging revealed that starvation induced *INS-1*-mediated down-regulation of *AIZ* activity. Our results suggest that *INS-1*, in cooperation with *HEN-1*, antagonizes the *DAF-2* insulin-like signaling pathway to modulate interneuron activity required for food-associated integrative behavior.

Supplemental material is available at <http://www.genesdev.org>.

Received August 9, 2006; revised version accepted September 18, 2006.

The secreted peptide hormone insulin modulates neural plasticity. Insulin and insulin receptors are expressed in several regions of the rat brain (Havrankova et al. 1978a,b), insulin receptors localize to post-synapses (Abbott et al. 1999), and insulin can produce long-term depression (LTD) of synaptic transmission through endocytosis of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in rat hippocampal CA1 neurons (Man et al. 2000). In addition, Phosphatidylinositol 3 (PI-3)-kinase that functions in the insulin signaling pathway is thought to induce long-term potentiation (LTP) of synaptic transmission in the dentate gyrus of rat

(Kelly and Lynch 2000). These alterations by proteins of the insulin signaling pathway may be involved in learning and memory, but what kind of behavior the insulin signaling pathway modulates has been largely unknown.

The nematode *Caenorhabditis elegans* is well suited for the analysis of the molecular and cellular mechanisms underlying neural plasticity because of its accessible genetics, stereotyped behavioral responses, and its simple nervous system consisting of 302 neurons whose connections are entirely known (White et al. 1986). Recently, physiological analysis of the neural circuit in live worms has become possible by the use of *cameleon*, a genetically encodable calcium indicator, to measure Ca^{2+} concentration changes (Miyawaki et al. 1997; Kimura et al. 2004).

C. elegans exhibits thermotaxis, an integrative behavior in which well-fed animals in a thermal gradient are attracted to their cultivation temperature, whereas starved animals avoid it (Hedgecock and Russell 1975; Mohri et al. 2005; Rankin 2005). This food-associated behavioral plasticity, regarded the most complex behavior in *C. elegans*, is an ideal behavioral paradigm for comprehensive study of neural plasticity at the molecular, physiological, and behavioral levels. In this study, we show that in cooperation with a secreted protein *HEN-1*, an insulin homolog *INS-1*, and insulin-like signaling pathway modulate neuronal activity of interneurons to execute thermotaxis behavior in *C. elegans*. We suggest that a neuroendocrine system is important for modulating the neural circuit that underlies neural plasticity.

Results and Discussion

Insulin homolog INS-1 is required for food-associated temperature responsive behavior

Wild-type animals cultivated at 17°C in well-fed conditions migrated to the center of the agar plate, which corresponded to their cultivation temperature (Fig. 1A,C,K). By contrast, most of the wild-type animals cultivated at 17°C for 3 h in food-deprived conditions avoided their cultivation temperature (Fig. 1D,K). Similarly, wild-type animals cultivated at 25°C with food migrated to their cultivation temperature (Fig. 1G,L), whereas most avoided their cultivation temperature after being cultivated at 25°C without food for 1 h (Fig. 1H,L). These results indicate that *C. elegans* can associate cultivation temperature with feeding state and memorize this information (Hedgecock and Russell 1975; Mohri et al. 2005).

Previous studies have identified the neural circuit and several genes required for thermotaxis (Mori and Ohshima 1995; Komatsu et al. 1996; Ishihara et al. 2002; Kuhara et al. 2002; Murakami et al. 2005; Okochi et al. 2005; Inada et al. 2006). However, how *C. elegans* executes thermotaxis remains to be understood at the molecular and physiological levels. The *aho-2(nj32)* mutant was isolated in our genetic screen for mutants defective in this integrative behavior (Mohri et al. 2005). *aho-2(nj32)* mutants always migrated to their cultivation temperature after starvation as well as after feeding (Fig. 1E,F,I-L); we designated this phenotype as an Abnormal hunger orientation (Aho) phenotype. The Aho phenotype of *aho-2(nj32)* mutants could result from a defect either

[Keywords: *C. elegans*; thermotaxis; learning and memory; integrative behavior; insulin; neural circuit]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.1479906>.

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Figure 1. *aho-2(nj32)* shows defects in food-associated thermotactic behavioral plasticity, normal response to starvation, and normal integration of two different chemosensory inputs. (A, top) Thermotaxis assay system. Single animals were placed on the agar at ~22°C, as indicated by the cross. (Bottom) A thermograph shows the stable radial temperature gradient (17°C–25°C) of the dish surface. (B) Interaction assay system. (C–J) Tracks on a radial temperature gradient of individual animals cultivated at 17°C or 25°C with or without food. (K) Results of the thermotaxis assay for well-fed or starved animals cultivated at 17°C. Thermotaxis was evaluated using “Fraction of 17,” which includes the class “17.” The asterisks indicate $p < 0.005$, as identified by t -tests in a comparison of starved *aho-2(nj32)* mutants with starved wild-type animals. Fed animals, $n \geq 30$; starved animals, $n \geq 60$. Error bars indicate SEM. (L) Results of the thermotaxis assay for animals cultivated at 25°C. Thermotaxis was evaluated using “Fraction of 25,” which includes the class “25.” The asterisks indicate $p < 0.005$ in a comparison of *aho-2(nj32)* mutants with starved wild-type animals ($n \geq 68$ animals). Error bars indicate SEM. (M) Modulation of the locomotory rate. The number of body bends in 20 sec on the assay plates was scored. Gray and black bars indicate the results for well-fed animals transferred to assay plates without food (F > S) and with food (F > F), respectively. Hatched and white bars indicate the results for starved animals transferred to assay plates without food (S > S) and with food (S > F), respectively ($n = 45$ animals). Error bars indicate SD. (N) Wild-type and *aho-2(nj32)* animals were tested in the interaction assay, and the results are represented as Index $[B/(A + B)]$.

in association between cultivation temperature and starvation or in recognition of starvation per se. To address these possibilities, we tested the responses of *aho-2(nj32)* animals to changes in feeding state using a locomotory activity assay (Sawin et al. 2000). Well-fed wild-type animals move more slowly in plates with food than

without food, and starved wild-type animals move even more slowly in plates with food than do well-fed animals (Fig. 1M). *aho-2(nj32)* and wild-type animals exhibited nearly the same responses to changes in feeding state (Fig. 1M), suggesting that *aho-2(nj32)* animals can respond to starvation and exhibit a defect in association between cultivation temperature and starvation (Mohri et al. 2005).

We investigated whether *aho-2(nj32)* mutants had a defect in integration of different chemosensory inputs using an interaction assay (Fig. 1B) that is a behavioral test for the integration of two opposing signals, a signal from an attractive odorant, diacetyl, and from a repulsive metal, Cu^{2+} ion (Ishihara et al. 2002). HEN-1 is a secretory protein with an LDL receptor motif and is required for the behavioral task tested in this interaction assay (Ishihara et al. 2002). In contrast to *hen-1(tm501)*-null mutants, *aho-2(nj32)* mutants responded normally (Fig. 1N). *aho-2(nj32)* mutants exhibited normal responses to diacetyl and Cu^{2+} ions separately (data not shown), implying that the integrative process of the two chemical compounds is normal in *aho-2(nj32)* mutants.

The gene *aho-2* was mapped to the 0.08 map unit region in the center of chromosome IV (Mohri et al. 2005; data not shown), which is covered by the three cosmids. We found that only F13B12 rescued the defect of *aho-2(nj32)* (Fig. 2A). Among six predicted genes in the region covered by F13B12 (data not shown), a PCR product containing *ins-1*, the *C. elegans* gene most closely related to human insulin among 38 insulin-related genes (Pierce et al. 2001; Li et al. 2003), rescued the defect of the *aho-2(nj32)* mutant (Fig. 2A). The *ins-1(nr2091)* mutant, a previously isolated putative null mutant (Pierce et al. 2001), also showed an Aho phenotype (Fig. 2C). We found a 130-base-pair (bp) deletion from the first exon to the second exon in *ins-1* of *aho-2(nj32)* mutants (data not shown). These results led us to conclude that *aho-2* is identical to *ins-1*.

Neuronal expression of INS-1 is important for integrative thermotaxis behavior

We observed *ins-1*-expressing cells using an *ins-1* promoter::GFP fusion gene. As previously reported, fluorescence was observed in many head neurons, including ADF, AIA, AIM, ASE, ASG, ASH, ASI, ASJ, AWA, BAG, and NSM, and was also observed in the intestine, hypodermis, and vulval muscle (Fig. 2E; Pierce et al. 2001; data not shown).

Expressing *ins-1* cDNA from its own promoter or in all neurons using the *unc-14* promoter almost fully rescued the Aho phenotype of *ins-1(nr2091)* mutants, whereas no rescue occurred when expressing the *ins-1* cDNA in intestine using the *ges-1* promoter (Fig. 2C). These results indicate that neuronal expression of INS-1 is sufficient to rescue the food-associated thermotactic behavior defect of *ins-1(nr2091)* mutants. We conducted cell-specific rescue experiments to determine if the expression of INS-1 in any particular neuron is required for association between temperature and feeding state. The expression of *ins-1* cDNA using the *ins-1*, *ncs-1*, *lin-11*, *unc-86*, and *ceh-14* promoters effectively rescued the defect of *ins-1(nr2091)* mutants, and weak rescue occurred upon *ins-1* cDNA expression from *osm-6* and *gpa-2* promoters (Fig. 2D). In contrast, no rescue occurred upon expression of *ins-1* cDNA using the *unc-42*, *tph-1*, *gcy-8*, *glr-3*, or

Figure 2. INS-1 is required in neurons for integrative thermotactic behavior. (A) Rescue experiments for the food-associated thermotactic behavior defect in *aho-2(nj32)* mutants. The asterisks indicate $p < 0.05$ for a comparison with starved *aho-2(nj32)* mutants. Fed animals, $n \geq 30$; starved animals, $n \geq 60$. Error bars indicate SEM. (B) The thermotaxis behavior of animals cultivated at 20°C with food. The phenotypic categories are described in the Supplemental Material. The data, presented as horizontal stacked bar graphs, represent the average of three independent thermotaxis assays using 20 animals per assay. Three independent transgenic lines were tested for the INS-1 overexpression (O/E) line (containing 100 ng/μL *ins-1* genomic DNA); $n = 60$ for wild type, *ins-1(nr2091)*, and each INS-1 O/E line. (C,D) Rescue experiments of the food-associated thermotactic behavior defect in the *ins-1(nr2091)* mutant. Single and double asterisks indicate $p < 0.05$ and $p < 0.01$, respectively, for a comparison with starved *ins-1(nr2091)* mutants. Fed animals, $n \geq 30$; starved animals, $n \geq 60$. Error bars indicate SEM. (E) Summary of results of the rescue experiments for *ins-1(nr2091)* and neuronal expression pattern of INS-1 with different promoters.

AIY promoter (Fig. 2D). Essentially, we did not identify any single neuron where the expression of INS-1 was required for the rescue, suggesting that INS-1 acts cell nonautonomously. Close examination of the rescue results and *ins-1p::GFP* expression in neurons revealed that the defect of the *ins-1* mutant was rescued when INS-1 was expressed in at least one of the neurons that appeared to express INS-1 normally (Fig. 2E).

To address the possibility that the lack of rescue by several promoters was caused by either too low or too high expression of the *ins-1* cDNA, we constructed *ins-1* mutant strains transgenic with different concentrations of *ges-1p::ins-1* cDNA or *gcy-8p::ins-1* cDNA, and tested those strains for food-associated thermotactic responses. *ges-1-* or *gcy-8-*-driven INS-1 expression in the *ins-1(nr2091)* strain did not significantly rescue the defect regardless of the concentration used (Supplementary Fig. 1), suggesting that the inability of some of the promoters to rescue the defect was not caused by insufficient or excessive expression of the *ins-1* cDNA.

ins-1 may be regulated transcriptionally, at the hormone processing level, and/or at the level of secretion. We observed whether there was any difference in INS-1

expression between the fed state and the starved state using an *ins-1* promoter::GFP fusion gene and a rescuable *ins-1::GFP* fusion gene. Light microscopic observation failed to find the difference in expression level or localization of the fusion protein (data not shown). These results argue against transcriptional regulation of *ins-1* expression. If INS-1 is regulated at the level of secretion in response to starvation, the concentration of extracellular INS-1 may be too low to detect with GFP. Overexpression of INS-1 in a wild-type background induced a partially abnormal thermotaxis phenotype (Fig. 2B), which is consistent with the model that the secretion of INS-1 could modulate thermotactic behavior.

INS-1 antagonizes DAF-2 insulin-like signaling in food-associated thermotactic plasticity

A previous report suggested that INS-1 antagonizes DAF-2 insulin-like signaling for dauer formation in *C. elegans* (Pierce et al. 2001). We explored whether DAF-2 insulin-like signaling also functions in food-associated thermotactic behavioral plasticity by examining mutants of *daf-2*, a homolog of the insulin/IGF-1 receptor (Kimura et al. 1997), *age-1*, a homolog of PI-3-kinase (Morris et al. 1996), and *daf-16*, a forkhead-type transcriptional factor (Lin et al. 1997). The *daf-16(m26)* mutant, which is a suppressor of both the *daf-2* and the *age-1* mutant in dauer formation (Gottlieb and Ruvkun 1994; Larsen et al. 1995), showed a weak Aho phenotype (Fig. 3A). The *daf-2(e1368)* and *age-1(hx546)* mutants, however, normally avoided their cultivation temperature after a 3-h starvation (Fig. 3A). If INS-1 antagonizes DAF-2 insulin-like signaling for this integrative behavior, the behavioral responses of *daf-2(e1368)* and *age-1(hx546)* mutants to starvation might be opposite to the response of *ins-1(nr2091)* mutants. To test this hypothesis, we conducted a time course assay for starvation-induced temperature avoidance. As the cultivation time under starvation conditions increased, the fraction of wild-type animals that migrated to the cultivation temperature gradually decreased (Fig. 3C). By contrast, the fraction of *ins-1(nr2091)* mutants that migrated to the cultivation temperature did not decrease much even after a 3-h starvation (Fig. 3C). Consistent with our hypothesis, *age-1(hx546)* mutants started to avoid the cultivation temperature much earlier than wild-type animals (Fig. 3C). These results indicate that *age-1(hx546)* could associate cultivation temperature with starvation quicker than wild-type animals. *daf-2(e1368)* mutants showed a response similar to wild-type animals (Fig. 3C), which might be due to the fact that *daf-2(e1368)* is one of the weakest alleles in dauer formation (Gems et al. 1998). Because of developmental or behavioral defects, we were unable to examine starvation-induced temperature avoidance of *daf-2(mg43)* and *daf-2(e1370)* mutants, both of which are stronger alleles than *daf-2(e1368)* in dauer formation (Kimura et al. 1997; Gems et al. 1998; data not shown).

Double mutants were constructed and their food-associated thermotactic responses were tested to clarify the genetic interaction between the insulin-like signaling genes. With a 3-h starvation, both *daf-2* and *age-1* mutations partially suppressed the defective starvation-induced temperature avoidance of *ins-1* mutants, although *daf-2* did not suppress *daf-16* (Fig. 3A). These results are consistent with a model that INS-1 acts antagonistically

Figure 3. Insulin-like signaling, acting in cooperation with HEN-1, functions in thermotaxis interneurons. (A,B) Results of the thermotaxis assay for well-fed and starved wild-type, single-mutant, and double-mutant animals cultivated at 17°C. Single and double asterisks indicate $p < 0.05$ and $p < 0.002$, respectively. Fed animals, $n \geq 40$; starved animals, $n \geq 80$. Error bars indicate SEM. (C) Results of the thermotaxis assay from 0 (fed) to 3-h-starved animals cultivated at 17°C. Error bars indicate SEM ($n = 50$ animals for each time point). (D) Rescue experiments of the quicker avoidance defect of the *age-1(hx546)* mutant. Animals were cultivated under starvation conditions for 30 min at 17°C prior to the thermotaxis assay. Single and double asterisks indicate $p < 0.05$ and $p < 0.01$, respectively, for a comparison with starved *age-1(hx546)* mutants ($n \geq 40$ animals). Error bars indicate SEM.

on DAF-2 insulin-like signaling for food-associated thermotaxis behavior. *daf-16; ins-1* double mutants showed a stronger defect than *daf-16* single mutants (Fig. 3B), suggesting that *ins-1* is genetically downstream from *daf-16*, which is consistent with a feedback loop from *daf-16* to *ins-1*.

INS-1 and HEN-1 act coordinately in food-associated thermotactic plasticity

Insulin-like signaling, in cooperation with the TGF- β and cyclic GMP pathways, is one of the important pathways for dauer formation. We tested whether dauer formation pathways other than the insulin-like signaling pathway are also involved in integrative behavior for temperature and feeding state. Dauer-defective (*Daf-d*) mutants for *daf-5*, *daf-6*, and *daf-12*, which encodes the TGF- β pathway member Sno/Ski (da Graca et al. 2004), a Patched-related protein that functions upstream of both the TGF- β pathway and the cyclic GMP pathway (Schackwitz et al. 1996; Perens and Shaham 2005), and a nuclear receptor (Antebi et al. 2000), respectively, did

not show the defects (Fig. 3B). Recently, Murakami et al. (2005) showed that the secreted protein TGF- β /DAF-7 is involved in memory acquisition of the cultivation temperature. However, *daf-7(e1372)* and *daf-1(m40)* mutants that had a deficit in one subunit of the TGF- β receptor did not show the defects, and neither the *daf-1* nor *daf-7* mutant suppressed the defect of *ins-1* mutants (Fig. 3B).

HEN-1, a secretory protein with an LDL receptor motif, is reported to be involved in food-associated thermotactic plasticity (Ishihara et al. 2002). To test for a genetic interaction between insulin-like signaling and HEN-1, we constructed double mutants. *daf-2* mutation did not suppress the defect of *hen-1* mutants. The *ins-1; hen-1* double mutant, however, showed a stronger mutant phenotype than *ins-1* or *hen-1* single mutants (Fig. 3B). These results suggest that INS-1 and HEN-1 act in parallel and that insulin-like signaling and HEN-1 signaling are major components in the regulation of food-associated thermotactic plasticity.

Insulin-like signaling functions in thermotaxis interneurons

Where is the target cell that receives and processes INS-1 to antagonize the DAF-2 insulin-like signaling pathway for temperature-starvation integrative behavior? To address this question, we conducted a cell-specific rescue experiment on *age-1(hx546)* mutants with a 30-min starvation. Expressing *age-1* cDNA in all neurons using the *unc-119* promoter rescued the quicker starvation-induced temperature avoidance defect of *age-1(hx546)* mutants (Fig. 3D). Expressing *age-1* cDNA in several neurons, including the AIZ, AIY, or RIA interneurons, all of which are essential interneurons for thermotaxis (Mori and Ohshima 1995), almost fully rescued the quicker avoidance defect of *age-1(hx546)* mutants (cf. Figs. 3D and 2E; Brockie et al. 2001). By contrast, expressing *age-1* cDNA in AFD thermosensory neurons by the *gcy-8* promoter, in sensory neurons by the *osm-6* promoter or in many neurons by the *unc-42* promoter did not rescue the defect (cf. Figs. 3D and 2E). These results suggest that AGE-1 (and probably the insulin-like signaling pathway) functions in thermotaxis interneurons for food-associated neural plasticity.

Calcium imaging of the thermotaxis interneurons

To analyze a physiological aspect of the insulin-like signaling in integrative behavior for food and temperature, we observed the changes in neuronal activity of the AIZ thermotaxis interneuron of live animals by measuring stimulus-evoked Ca^{2+} concentration changes using cameleon, a genetically encodable calcium indicator (Miyawaki et al. 1997; Kimura et al. 2004). The activity of the AIZ interneuron in wild-type animals cultivated at 17°C with food increased with warming and decreased with cooling, whereas the activity of the AIZ interneuron in the starved wild-type animals was much less responsive to temperature changes (Fig. 4A; Kuhara and Mori 2006). The AIZ interneuron of starved *ins-1(nr2091)* animals was as active as that of fed *ins-1(nr2091)* animals (Fig. 4B). These results suggest that INS-1 is required for the starvation-induced negative regulation of AIZ neuron activity. Calcium imaging on AFD thermosensory neurons revealed that feeding state did not influence the activity of AFD (Fig. 4C).

Figure 4. In vivo calcium imaging and model for food-associated thermotactic behavioral plasticity. (A,B) Calcium imaging of the AIZ interneuron in animals cultivated at 17°C under fed or starved conditions expressing the cameleon protein. (A) Wild-type animals. (B) *ins-1(nr2091)* mutants. $n = 10$ –13. Relative increases or decreases in the intracellular calcium concentration were measured as increases or decreases, respectively, in the YFP/CFP fluorescence ratio of the cameleon protein (Ratio Change). Temperature (Temp.) is shown as a black line at the bottom. (C) Calcium imaging of the AFD thermosensory neuron in wild-type animals grown at 20°C expressing the cameleon protein cultivated under fed or starved conditions ($n = 18$ –20). Temperature (Temp.) is shown as a black line at the bottom. (D) Model of the suggestive genetic pathway for modulation of integrative behavior between cultivation temperature and feeding state by insulin-like signaling based on the results presented in this study. DAF-7 is thought to be involved in memory acquisition of cultivation temperature (Murakami et al. 2005).

We recently found that the interneuron-deficient calcineurin mutant *tax-6(sensory+, inter-)* also showed a defect in association between temperature and feeding state at 17°C (Fig. 3A; Kuhara and Mori 2006). TAX-6 calcineurin is required for temperature-starvation integrative behavior in both of two directly connected interneurons, AIZ and RIA, and like INS-1, TAX-6 is required for starvation-induced regulation of AIZ activity (Kuhara and Mori 2006). To investigate the genetic interaction between calcineurin-mediated signaling and insulin-like signaling in thermotaxis interneurons, we constructed *daf-2; tax-6(sensory+, inter-)* mutants. The food-associated thermotactic behavior defect of the *tax-6(sensory+, inter-)* mutation was not suppressed by the *daf-2* mutation (Fig. 3A), which is consistent with the possibility that TAX-6 calcineurin acts downstream from DAF-2 in thermotaxis interneurons. We also investigated whether DAF-16 is involved in transcription of *tax-6* by comparing the expression of a *tax-6::GFP* translational fusion gene in a wild-type background with expression of the fusion gene in a *daf-16(mu86)* deletion mutant background. We could not find any differences in expression level (data not shown). These results are consistent with the possibility that *daf-16* is not required for the transcription of *tax-6*.

A neuroendocrine system modulates the neural circuit important for integrative behavior

Our results propose a model for food-associated thermotactic plasticity (Fig. 4D). During association between cultivation temperature and starvation, INS-1 is secreted from several neurons and antagonizes insulin-like signaling by inhibiting the activity of DAF-2 receptor. DAF-16 may be activated, probably through AGE-1, and a feedback loop from DAF-16 to INS-1 might exist. HEN-1 might also be secreted from AIY or ASE neurons (Ishihara et al. 2002). We thus suggest that a neuroendocrine system is important for modulating the neural circuit that underlies the integrative behavior.

Murakami et al. (2005) argued that AGE-1 acts in AIY neurons to enhance isothermal tracking, which is one aspect of thermotaxis. Insulin-like signaling is required for salt chemotaxis learning, where animals pre-exposed to the chemoattractant NaCl under starvation condition exhibit reduced chemotactic response to NaCl (Tomioka et al. 2006). These reports also implicate the importance of insulin-like signaling in behavioral plasticity.

What are the targets of DAF-16 in integrative behavior for temperature and food? Insulin-like signaling is a part of the dauer formation pathway, which has many feedback loops (Schackwitz et al. 1996). The results of the present study are consistent with a feedback loop in insulin-like signaling for this integrative behavior. Assuming the existence of a feedback loop, one clue for the targets of DAF-16 in the thermotactic plasticity might be found in a report by Murphy et al. (2003), which suggested that the insulin homologs INS-2, INS-7, INS-18, and INS-21 are likely to be direct or indirect targets of DAF-16. Likewise, it is plausible that any of these insulin-like molecules act agonistically on DAF-2 to activate the insulin-like pathway in food-associated thermotactic plasticity. We believe that these issues are critical for further study.

Materials and methods

Strains and genetics

The standard techniques were used for culturing and handling *C. elegans*. For details and strains, see Supplemental Material.

Behavioral assays

A radial temperature-gradient assay was performed as described previously (Mori and Ohshima 1995; Mohri et al. 2005). The locomotory rate assay was performed according to a previous report (Sawin et al. 2000). The interaction assay was also performed as previously described (Ishihara et al. 2002). For details, see Supplemental Material.

Molecular biology and germline transformation

Standard methods for molecular biology and germline transformation were used. For details and vectors, see Supplemental Material.

In vivo calcium imaging and data analysis

In vivo calcium imaging was performed essentially according to Kimura et al. (2004) and Kuhara and Mori (2006). For details, see Supplemental Material.

Acknowledgments

We thank G. Ruvkun, C. Wolkow, P. Hu, and J. Xu for kindly providing *ins-1(nr2091)*, *daf-2(mg43)*, and *age-1(mg305)* mutants; T. Ishihara for *hen-1(tm501)* mutants, A. Fire for pPD plasmids; O. Hobert and Y. Okochi for the AIY and *lin-11* promoters; B. Wedel, D. Garbers, and H. Inada for the *gcy-8* promoter; Y. Tanizawa for the *glr-3*

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short promoter; P. Swoboda and H. Sasakura for the *osm-6* promoter; J. Sze for the *tph-1* promoter; J. McGhee for the *ges-1* promoter; H. Kagoshima for the *ceh-14* promoter; all the members of Mori Laboratory for technical advice and stimulating discussions; A. Coulson and R. Shownkeen for cosmid; and the *C. elegans* Sequence Consortium for updating the *C. elegans* genome information. The *Caenorhabditis* Genetic Center provided some of the strains used in this study. This work was supported by Grant-in-Aid for Scientific Research on Priority Areas-Molecular Brain Science from the MEXT (00210010) and by HFSPO (to I.M.). I.M. is a Scholar of the Institute for Advanced Research of Nagoya University.

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Genes Dev. 2006, **20**:

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