

Plasticity of chemoreceptor gene expression: Sensory and circuit inputs modulate state-dependent chemoreceptors

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Animals dramatically modify their chemosensory behaviors when starved, which could allow them to alter and optimize their food-search strategies. Dynamic changes in the gene expression of chemoreceptors may be a general mechanism underlying food and state-dependent changes in chemosensory behaviors. In our recent study,¹ we identified chemoreceptors in the ADL sensory neuron type of *C. elegans* that are modulated by feeding state and food availability. Here, we highlight our recent findings by which sensory inputs into ADL, neuronal outputs from ADL, and circuit inputs from the RMG interneuron, which is electrically connected to ADL, are required to regulate an ADL-expressed chemoreceptor. This sensory and circuit-mediated regulation of chemoreceptor gene expression is dependent on cell-autonomous pathways acting in ADL, e.g. KIN-29, DAF-2, OCR-2 and calcium signaling, and circuit inputs from RMG mediated by NPR-1. Based on these findings, we propose an intriguing but speculative feedback modulatory circuit mechanism by which sensory perception of food and internal state signals may be coupled to regulate ADL-expressed chemoreceptors, which may allow animals to precisely regulate and fine-tune their chemosensory neuron responses as a function of feeding state.

Keywords: ADL, chemoreceptor, feeding state, food, gene regulation, neural circuit, plasticity, sensory input, sensory neuron

Abbreviations: OR, olfactory receptor; Obp, olfactory binding protein; SIK, salt-inducible kinase; ILP, insulin-like peptide; bHLH, basic helix-loop-helix; TRP, transient-receptor potential.

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Submitted: 02/13/2015

Accepted: 02/20/2015

<http://dx.doi.org/10.1080/21624054.2015.1023497>

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Commentary on: Gruner M, Nelson D, Winbush A, Hintz R, Ryu L, Chung SH, Kim K, Gabel CV, van der Linden AM. Feeding state, insulin and NPR-1 modulate chemoreceptor gene expression via integration of sensory and circuit inputs. *PLoS Genet* 2014; 10:e1004707; PMID:25357003; <http://dx.doi.org/10.1371/journal.pgen.1004707>

Modulating Chemoreceptor Genes: a General Strategy for Behavioral Plasticity

Growing evidence suggests that certain animals can alter their chemosensory neuron perception directly by

dynamically changing expression levels of chemoreceptor genes, thereby gating sensory stimuli to change chemosensory behavior. For example, previous studies in the mosquito *Anopheles gambiae* has shown that gene expression of the olfactory receptor, AgOr1, known to recognize an odor found in human sweat, is highly expressed before a blood feeding, but rapidly downregulated after a blood feeding.^{2,3} A recent global study showed that small changes in transcript levels of a number of olfactory receptor genes, were highly correlated with odor sensitivity changes within the antennae of *A. gambiae* subsequent to a blood feeding (Fig.1A).⁴ These findings supported the idea that mosquitoes may use expression changes of specific olfactory receptor genes to alter their behavioral sensitivity to host-specific odors, thereby diminishing host-seeking behavior after a blood meal. In addition to feeding state, changes in olfactory receptor and accessory (Obp) genes are also dependent on the sex of an animal. After mating, female *Drosophila* flies rapidly modify their olfactory behaviors to lower attraction to males such that they can focus on reproduction, and these behavioral changes are accompanied by modulation of different olfactory receptor genes (Fig.1B).⁵ In mice and zebrafish, a small subset of olfactory receptor genes is induced in temporal waves, which may reflect a mechanism for developmental acquisition of odorant discrimination.^{6,7}

Over 1500 predicted chemoreceptor genes have been identified in the genome of the nematode *Caenorhabditis elegans*,⁸ and unlike insects and mammals, each *C. elegans* chemosensory neuron type expresses multiple partially overlapping sets of

chemoreceptor genes (possibly >20), all of which are presumably linked to a common chemical response that is determined by the identity of the neuron and its synaptic targets.⁹ As each chemosensory neuron type expresses multiple chemoreceptor genes, it has been hypothesized by us and others that changing the expression of individual or distinct populations of chemoreceptor genes may allow *C. elegans* to selectively alter its response to specific or a subset of chemicals, without inadvertently altering the response to all chemicals sensed by that neuron. As expected, the expression of a subset chemoreceptor genes in *C. elegans* are modulated by neuronal activity, pheromone levels and developmental conditions.¹⁰⁻¹² As in other animals, dynamic changes in expression levels of chemoreceptor genes appear to directly alter chemosensory behaviors. For example, a recent study showed that altered expression level changes of the *odr-10* chemoreceptor gene, known to sense the food-related chemical diacetyl,¹³ modifies *C. elegans* male exploratory behavior¹⁴ (Fig. 1C). Thus, dynamic changes in the expression level of

chemoreceptor genes is observed across phyla, and may be a general strategy underlying behavioral plasticity. In this commentary, we highlight new potential mechanisms regarding the modulation of chemoreceptor gene expression by food and feeding state of *C. elegans*.¹

State-Dependent Plasticity in Chemoreceptors Expressed in ADL Neurons

Our recent work¹ focused on chemoreceptor genes expressed in the ADL sensory neuron type, which is known to express a multitude of chemoreceptors (>10),¹⁵ and respond to a wide variety of repulsive cues, e.g., odors,¹⁶ pheromone,¹⁷ and heavy metals.¹⁸ Using *gfp*-based expression analysis, we tested a subset of ADL-expressed chemoreceptors, and discovered that 2 chemoreceptor genes, *srh-234* and *srh-34*, are rapidly and reversibly modulated by fed and starved conditions of *C. elegans*. In fed animals, expression levels of *srh-234* is high but when animals are

starved, *srh-234* expression is rapidly downregulated until food is again available. Interestingly, changes in *srh-34* expression levels show an opposite phenotype compared to *srh-234* in fed and starved conditions. Using a *gfp*-based expression analysis of *srh-234*, we found several interesting new aspects of this state-dependent modulation of chemoreceptor genes: First, starvation modulation of *srh-234* expression depends both on sensory inputs from food and internal state signals. Second, multiple signaling pathways and transcriptional modules act cell-autonomously in ADL sensory neurons to couple food and internal state signals with chemoreceptor gene modulation. Third, circuit inputs from neurons other than ADL as well as ADL output modulate *srh-234* expression mainly through neuropeptide and insulin signaling.

Sensory inputs from food and internal state signals modulate chemoreceptor expression

When we discovered that an ADL-expressed chemoreceptor is regulated by

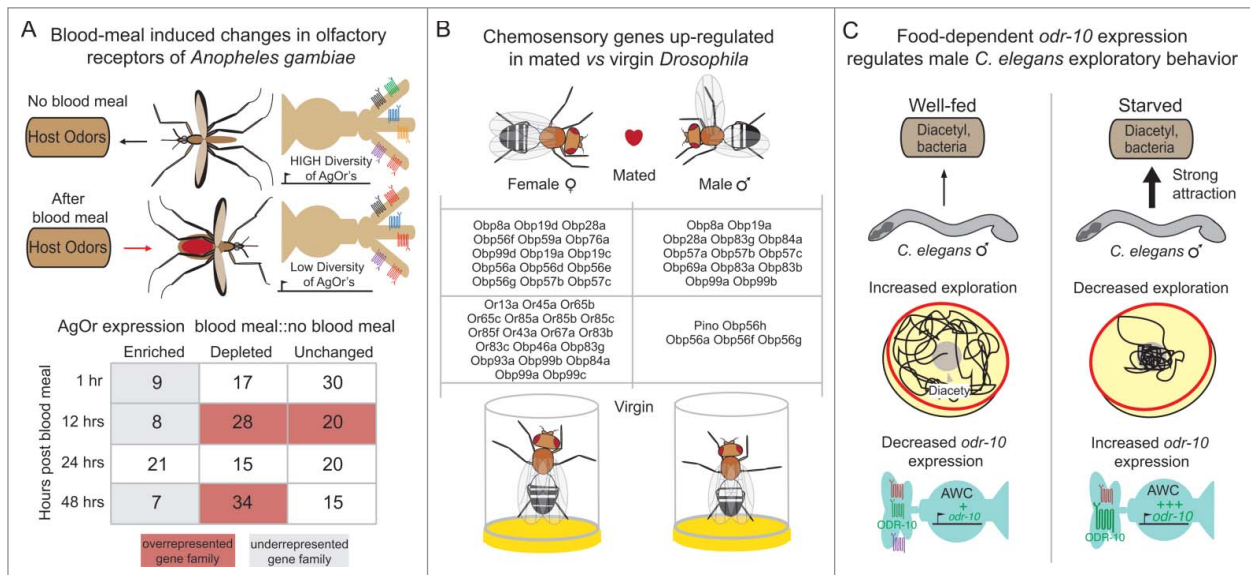


Figure 1. Dynamic expression changes in chemoreceptor genes are correlated with behavioral transitions in invertebrates. Modifying chemoreceptor gene expression can gate sensory responses preventing environmentally inappropriate behaviors. (A) In mosquitoes (*Anopheles gambiae*) prior to a blood meal females are highly attracted to host odorants. Following a blood meal, attraction is rapidly lost provoking dispersal. Changes in the expression levels of olfactory receptor (Or) genes are correlated with the transition from host-seeking to host leaving. Data shown is from.⁴ (B) After mating, *Drosophila* males and females change expression of many olfactory genes including olfactory receptors (Ors) and olfactory-binding proteins (Obps). Data shown is from.⁵ (C) The chemoreceptor, *odr-10*, detects the food-related odorant, diacetyl,¹³ and in males is expressed exclusively in the AWC sensory neuron type.¹⁴ In males, the expression of *odr-10* is modulated by food with low expression in well-fed conditions and high expression after starvation. These dynamic changes in expression levels of *odr-10* could explain changes in exploratory foraging exhibited by *C. elegans* males when food is abundant. Data shown is from.¹⁴

the feeding state of *C. elegans* (fed vs starved), one of the first questions we asked is whether the reduced *srh-234* expression phenotype observed in starved animals is due to either internal nutritional signals associated with a decrease in food ingestion, or due to sensory inputs that are associated with decreased food presence. Using methods such as Functional Rescue In Single-Sensory Cilia (FRISSC),¹⁹ and sub-cellular laser-surgery²⁰ experiments of the ADL dendrite, we found that sensory inputs from food directly mediated by ADL sensory neurons are required to regulate *srh-234* expression. Furthermore, when we examined *srh-234* expression in animals exposed to bacterial food that can be normally sensed by *C. elegans* but not eaten,²¹ we found that internal nutritional signals are required to regulate *srh-234* expression. These results suggested that modulation of *srh-234* expression is dependent on both sensory perception and ingestion of food. Prior to our study, it was known that the presence and absence of food can elicit rapid changes in ADL-mediated repulsive responses, and this short-term plasticity occurs through serotonergic and dopaminergic signaling pathways.²² We found that key neuromodulators, e.g. serotonin, dopamine, tyramine, and octopamine, all of which whom signal hunger and satiety, do not effect *srh-234* expression. In contrast to short-term plasticity, state-dependent mechanisms of long-term plasticity in ADL-mediated responses are more likely to involve changes in chemoreceptor gene expression. It is therefore tempting to speculate that increased *srh-234* expression levels in ADL may allow fed animals to be less tolerant of repulsive cues detected by *srh-234* (i.e. perhaps enhancing discrimination toward certain food-related odors) whereas decreased *srh-234* expression in ADL following long periods of starvation may allow starved animals to be more tolerant to these repulsive cues (i.e., perhaps being less discriminatory with food-related odors). This regulation of chemoreceptor gene expression could allow starved *C. elegans* to modulate changing priorities in food-searching behavior

under stress-full conditions such as starvation.

Multiple cell-autonomous pathways in ADL regulate chemoreceptor gene expression

Our work shows that multiple, in parallel acting pathways function cell-autonomously in ADL to convey food and internal state signals to regulate *srh-234* expression (Fig. 2):¹

1. SIK pathway. Previously, we showed that KIN-29 salt-inducible kinase (SIK) regulates a subset of chemoreceptor genes including *srh-234* by inhibiting the gene repressive functions of the MEF-2 MADS-domain containing transcription factor.^{12,23} We found that *mef-2* is essential for the reduced *srh-234* expression in starved animals but dispensable when fed, suggesting that during starvation MEF-2 may repress but not drive *srh-234* expression. MEF2 is known to bind a consensus MEF2 binding site upstream of a consensus E-box sequence (CANNTG) where basic helix-loop-helix (bHLH) factors are negatively regulated.²⁴ We suggest that a similar transcriptional module may operate at the *srh-234* promoter. In this mechanism, bHLH factors may drive *srh-234* expression via the candidate E-box site (CACCTG) during feeding conditions (Fig. 2A) but in starved conditions, MEF-2 likely binds directly to the candidate MEF2 site in the *srh-234* promoter and repress bHLH factor(s) leading to the reduction of *srh-234* expression (Fig. 2B). Consistent with this hypothesis, we recently identified candidate bHLH factors that may act in a complex manner at the *srh-234* promoter to drive its expression via the E-box site (Gruner and van der Linden, pers. comm). Many of the 34 predicted bHLH factors in the *C. elegans* genome that either can form homodimers (9 bHLH factors) or heterodimers (21 bHLH factors)²⁵ have a well-characterized role in cell-specification and nervous system development, which may overshadow their role in orchestrating

state-dependent regulation of chemoreceptor genes.

2. Insulin signaling: As internal nutritional signals in *C. elegans* are conveyed through an insulin pathway, we examined *srh-234* expression in *daf-2* insulin-like receptor mutants. We found that the function of DAF-2 and its downstream target DAF-16 FOXO transcription factor are specifically required in ADL neurons to regulate *srh-234* expression. In this mechanism, DAF-2 regulates *srh-234* by inhibiting the gene expressive functions of DAF-16 during feeding (Fig. 2A), but in starved conditions, DAF-16 likely functions as a repressor leading to reduced *srh-234* expression (Fig. 2B). The mechanism by which DAF-2/DAF-16 signaling regulates *srh-234* expression in ADL cell-autonomously remains unclear, as there are no clear DAF-16 binding sites present in the *srh-234* promoter used in our study.¹ These results suggest that DAF-2 in ADL via DAF-16 is conveying signals about the nutritional status of *C. elegans* to promote *srh-234* expression.
3. TRPV signaling: The TRPV channels, OCR-2 and OSM-9, can form heterodimers, and are involved in the regulation of chemoreceptor genes, such as the *odr-10* chemoreceptor.^{26,27} We found that expression of the *ocr-2*, but less *osm-9*, is necessary and sufficient in ADL to regulate *srh-234* expression. The OCR-2-mediated regulation of *srh-234* is independent of MEF-2 or DAF-16 function, suggesting that yet unknown transcription factors act downstream of OCR-2 to regulate *srh-234*. However, based on the recent identification of a functional nuclear localization signal in OCR-2,²⁸ it remains possible that OCR-2 itself may directly regulate *srh-234* expression.
4. Intracellular calcium levels: Calcium signaling mediated by voltage-dependent calcium channels are highly linked to gene regulatory mechanisms. When we examined mutants of voltage-gated calcium channels, *egl-19* and *unc-36*, we found that they reduce *srh-234* expression during feeding. Conversely, when we examined a *gf*

mutation in *egl-19*, predicted to increase calcium influx into cells,²⁹ we found that *egl-19(gf)* restores the reduced *srh-234* expression phenotype

during starvation. Calcium-dependent signaling mediated by OCR-2 may alter neuronal gene expression in *C. elegans*.³⁰ We found that

overexpression of *egl-19(gf)* specifically in ADL neurons could bypass the requirement of OCR-2 as well as KIN-29, DAF-2 and NPR-1 (see

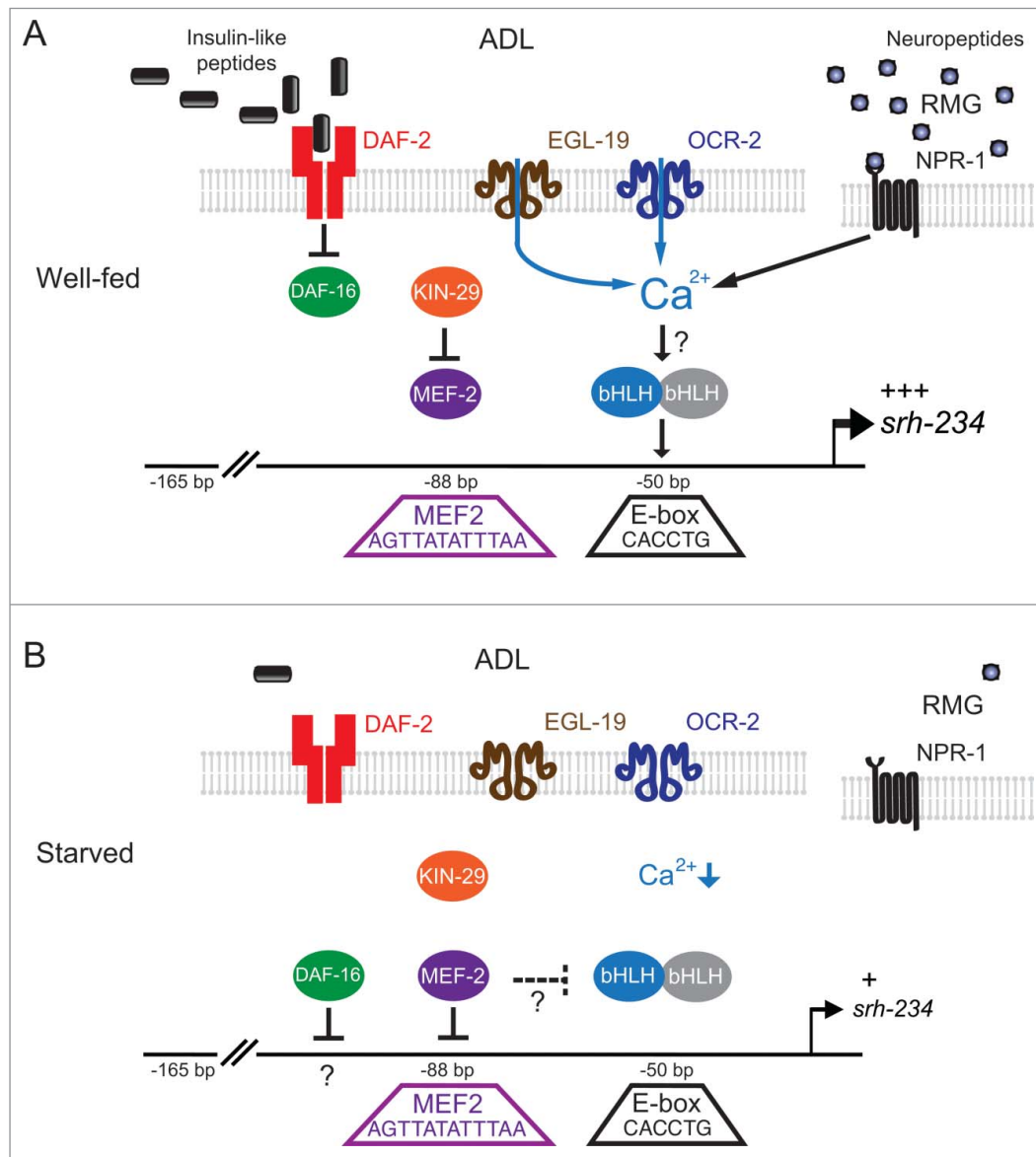


Figure 2. A model by which sensory inputs and cell-autonomous pathways in the ADL sensory neuron regulate chemoreceptor gene expression. **(A)** When well-fed (in the presence of food), expression of chemoreceptor *srh-234* is increased in the polymodal nociceptive neuron ADL. The transient-receptor potential (TRPV) channels, OCR-2 and OSM-9 (not shown), are essential for the perception of food and the cell-autonomous activation of *srh-234* expression. L-type voltage-activated Ca^{2+} channel, EGL-19, when constitutively activated is sufficient to increase *srh-234* expression, suggesting Ca^{2+} couples food perception with transcriptional changes. The insulin-like receptor, DAF-2, acting in a DAF-16-dependent manner is also essential for *srh-234* expression. The neuropeptide receptor, NPR-1, expressed in the RMG interneuron, and regulates *srh-234* expression in ADL likely via gap junctions (**Fig. 3**). The MEF-2 transcription factor is inhibited by the salt-inducible kinase, KIN-29, and prevented from binding a candidate MEF2 site in the *cis*-regulatory region of *srh-234*. A candidate E-box motif was found about 30 bp downstream of a MEF2 site in the *srh-234* promoter.¹ **(B)** When starved (in the absence of food), OCR-2 activity and intracellular Ca^{2+} levels in ADL are likely decreased. Low agonistic insulin-like peptides (ILPs) or possibly increased antagonistic ILPs lower DAF-2 activity and allow DAF-16 nuclear translocation. Although DAF-16 is necessary for repressing *srh-234* expression during starvation, there are no predicted binding sites within the 165 bp *cis*-regulatory region of *srh-234*. MEF-2 is no longer inhibited by KIN-29 allowing DNA binding, and possible inhibition of bHLH-mediated activation of *srh-234* at the E-box site. Taken together, during starvation MEF-2 and DAF-16 may repress *srh-234* expression, while bHLH factors may drive *srh-234* expression during feeding (Gruner and van der Linden, pers. comm.) The epistatic relationship between the different signaling pathways in ADL neurons remains to be fully defined. Data shown is from.¹

below) in regulating *srh-234* expression. These results suggest that increased calcium influx in ADL increases *srh-234* expression during feeding (Fig. 2A), whereas low calcium levels reduces the expression of *srh-234* during starvation (Fig. 2B). Thus, perhaps intracellular calcium levels in ADL neurons are modulated by an animals' feeding state; however, we were unable to detect changes in 'basal' calcium levels in fed or starved animals using current calcium imaging tools available in *C. elegans*. Nevertheless, our genetic experiments with *egl-19(gf)* provided support that calcium levels in ADL neurons regulate *srh-234* expression, possibly by directly influencing bHLH-mediated transcription (Fig. 2).³¹

Taken together, we identified multiple key components, e.g., KIN-29, OCR-2, DAF-2, and calcium channels, that function cell-autonomously in ADL to regulate *srh-234* expression. Signaling mediated by these pathways converge on a transcriptional module that includes MEF-2, DAF-16 and bHLH factors. We suggest that these signaling pathways and transcriptional regulators in ADL neurons couple the perception of food and internal nutritional signals to regulate a subset of ADL-expressed chemoreceptor genes.

Circuit inputs and ADL outputs regulate chemoreceptor gene expression

Chemoreceptor expression is primarily regulated by sensory inputs from the environment, but our recent work identified unexpected requirements for circuit inputs into ADL and outputs from ADL in the regulation of *srh-234* expression (Fig. 3):¹

1. Circuit inputs from RMG via NPR-1: Recent elegant work proposed the hub-and-spoke model explaining the circuit properties underlying aggregative behavior and pheromone responses.³² In this circuit, RMG interneurons (hub) is the major site for the NPR-1 neuropeptide receptor (a homolog of neuropeptide Y, NPY) to alter pheromone responses mediated by the gap-junction connected sensory neurons, ASK and ADL (spokes).^{17,32} We found that *npr-1* is

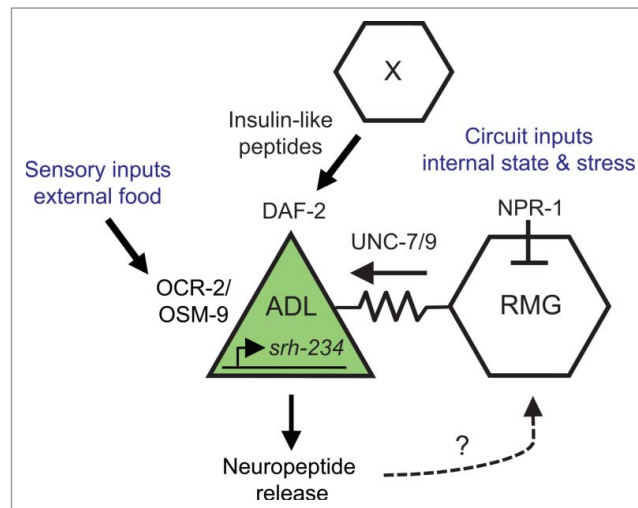


Figure 3. A speculative model for sensory and circuit inputs, and modulatory feedback from ADL to regulate chemoreceptor gene expression. Circuit inputs from RMG interneurons mediated by the neuropeptide receptor, NPR-1, possibly through the action of gap-junctions, UNC-7/9, regulate *srh-234* expression in ADL. Insulin-like peptides (ILPs) from yet unknown neurons (Neuron X), or ADL itself, target DAF-2 acting cell-autonomously in ADL to regulate *srh-234* expression. In addition to circuit inputs, ADL perceives sensory inputs from external food likely transduced via the TRPV channels, OCR-2 and OSM-9, acting cell-autonomously in ADL. This in turn may alter the output of ADL by releasing yet unknown neuropeptides from ADL to regulate NPR-1 activity in RMG. Taken together, sensory and circuit inputs are integrated through multiple signaling pathways to fine-tune expression levels of *srh-234* in ADL depending on the perception of food and an internal state of starvation. Data shown is from.¹

necessary and sufficient when expressed in RMG to restore the reduced *srh-234* expression in ADL of *npr-1* mutants during feeding, which is dependent on the function of the innexin gap-junction components, *unc-7* and *unc-9*. Based on these results, we suggest a potential circuit mechanism for NPR-1-mediated regulation of *srh-234* expression similar to the proposed RMG hub-and-spoke circuit.³² In this circuit (Fig. 3), high activity of NPR-1 inhibits RMG in fed conditions, perhaps by silencing the gap-junction circuit as previously proposed,³² resulting in increased *srh-234* expression in ADL. Conversely, when NPR-1 activity is reduced or absent in starved conditions, gap junction-mediated communication is open in the RMG circuit, which in turn may decrease *srh-234* expression. NPY-like signaling in both *C. elegans* and *Drosophila* appears to be a central regulator of stress responses.^{33,34} It is therefore possible that NPR-1 signaling in RMG conveys stress information associated with starvation to regulate *srh-234* expression in ADL.

Several properties of this NPR-1 gap-junction circuit in the regulation of *srh-234* expression remains to be determined. For instance, although our results suggest that *unc-7/9* gap-junctions are required for NPR-1-mediated regulation of *srh-234*, expression of *unc-7* in either RMG or ADL did not restore the *srh-234* expression phenotype of *npr-1 unc-7* double mutants. A recent study revealed a more complex picture of gap-junction networks, such that an electrically-coupled circuit may function as a co-incidence detector, in which changes in the number of active neurons within the circuit may generate different responses.³⁵ It is therefore possible that *unc-7/9* may be required in other neurons, besides RMG and ADL, consistent with our results showing that pan-neuronal expression of *unc-7* partially restored the *srh-234* expression phenotype of *npr-1 unc-7* doubles.

2. Circuit inputs mediated by insulin-like peptides: We found that a specific *gf* mutation in the *daf-28* insulin-like peptide (ILP), predicted to block

agonistic ILPs from binding to the DAF-2 receptor,³⁶ reduces *srh-234* expression in feeding conditions similar as *daf-2* mutants. Based on these findings, we suggest that the DAF-2 receptor acting cell-autonomously in ADL neurons may be targeted by ILPs from neurons other than ADL (Fig. 3). Interestingly, in salt-chemotaxis learning, DAF-2 acts in ASE sensory neurons, and is targeted by the INS-1 ILP released from AIA interneurons.³⁷ We found that *ins-1* mutants do not alter *srh-234* expression. However, there are over 40 ILPs predicted in the *C. elegans* genome³⁸ that together act in a complex manner to regulate olfactory responses,^{39,40} and a comprehensive study of these ILPs may provide clues about how they regulate chemoreceptor gene expression as a function of feeding state.

3. Neuronal outputs of ADL: We found that enhancing neuropeptide secretion in ADL by overexpressing *pkc-1(gf)*⁴¹ strongly increases the expression of *srh-234* during feeding conditions, suggesting that altering ADL output regulates *srh-234* expression (Fig. 3). Prior to our study, it was shown that OCR-2 may couple the perception of food to UNC-31-dependent secretion of insulin-like peptides from ADL neurons to regulate L1 starvation survival.⁴² Consistent with these findings, we found that overexpressing *pkc-1(gf)* in ADL can partially restore the reduced *srh-234* expression phenotype of *ocr-2* mutants, and this regulation appears to be dependent on the function of NPR-1 (Fig. 3). Taken together, these results suggest that perhaps the release of neuropeptides such as ILPs from ADL may in turn modulate NPR-1 in RMG to regulate *srh-234* expression, revealing the ability of feedback modulation by which chemoreceptor gene expression in chemosensory neurons are regulated.

Conclusions

Our recent study¹ provides the first illustration of circuit level inputs and

potential feedback modulatory mechanisms through which chemoreceptor genes expressed in chemosensory neurons are dynamically altered in response to changes in food availability and an internal state of starvation. Based on our results, we suggest that circuit inputs from RMG interneurons mediated by NPR-1 signaling are integrated with signals from the perception of food and internal state associated with starvation (and other stress information) through multiple pathways acting cell-autonomously in ADL, which in turn may regulate NPR-1 in RMG through the release of yet unknown neuropeptides from ADL. Given that the free-living nematode *C. elegans* has high anatomical and functional similarity with parasitic nematodes that seek out their host using chemical cues,⁴³ it would be intriguing to speculate that certain parasitic nematodes use similar state-dependent mechanisms of chemoreceptor gene modulation to transition between host-seeking and leaving behavior.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was funded by grants from the National Science Foundation IOS1353014, and the National Institute of General Medical Sciences P20GM103650 and P20GM103440.

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