Genetic Analysis of Bacterial Food Perception and its Influence on Foraging Behavior in *C. elegans*

by

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Abstract

The ability to adapt to changes in food conditions is critical for organismal homeostasis and survival. In this thesis, I explore the genetic and neuroendocrine mechanisms by which *C. elegans* evaluates bacterial food conditions and accordingly alters development and behavior. In Chapter One, I discuss the relationship between *C. elegans* and its bacterial diet. The influence of food on behavior suggests the presence of a gut-"brain" axis that senses and communicates information about nutritional state to the nervous system to elicit a behavioral response.

In Chapter Two, I characterize a gain-of-function allele of *scd-2,* the *C. elegans* Anaplastic Lymphoma Kinase (ALK) gene ortholog, *scd-2(syb2455),*which I designed based on an oncogenic mutation in *ALK.* While animals with loss-of-function mutations in *scd-2* are dauer-formation defective, *scd-2(syb2455)* animals enter dauer regardless of food conditions. In Chapter Three, I report that SCD-2 also regulates the food-dependent feeding and foraging behaviors known as dwelling and roaming; *scd-2(syb2455)* animals roam more and *scd-2* loss-of-function animals roam less than wild type. Additionally, in contrast to wild-type animals, which express the gene encoding the TGF-β signaling ligand DAF-7 exclusively in the ASI chemosensory neurons, *scd-2(syb2455)* animals constitutively express *daf-7* in both the ASI and ASJ neurons. The expression of *daf-7* in the ASJ neurons drives roaming in these animals. I demonstrate that *daf-7* expression in the ASJ neurons is also affected by food; ingested food in the pharynx inhibits *daf-7* expression in the ASJ neurons through SCD-2 signaling. From these data we propose a positive-feedback loop that regulates roaming behavior: in the absence of ingested food, active SCD-2 induces *daf-7* expression in the ASJ neurons to promote roaming, which further reduces food consumption. To further investigate how *daf-7* neuroendocrine signaling responds to nutritional state, in Chapter Four I describe a screen for satiety signals using the nutritional state-dependent *daf-7* expression in the ASJ neurons in males as a readout for communication along the gut-"brain" axis. This screen yielded a loss-of-function allele of *che-3* and a gain-of-function allele of *pdfr-1*. In Chapter Five, I discuss future directions for investigating how *C. elegans* interacts with its food environment.

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Chapter One

Introduction

Overview

The ability to sense and respond to changes in the environment is essential to an organism's survival. Attack from predator or pathogen, changes in temperature or osmolarity, or absence of food can represent a matter of life or death. Across kingdoms, eukaryotic organisms have developed both unique and conserved ways of adapting to changing food conditions. In this Introduction, I review conserved starvation response mechanisms as well as the effects of nutrient deprivation in the fruit fly *Drosophila melanogaster.* I additionally discuss satiety signaling in humans and its role in obesity. I then transition to the nematode *Caenorhabditis elegans* and the effects of changing food environments on its physiology, as well as known mechanisms of food sensing. Given the prominence of the *daf-7* TGF-β signaling pathway in Chapters Three and Four of this thesis, I end by reviewing the role of *daf-7* in food sensing and food-dependent phenotypes.

Conserved cellular starvation responses in eukaryotes

Diverse organisms are susceptible to starvation and respond to nutrient deprivation with widely conserved cellular programs. In response to low nutrient levels, as well as other stressful conditions, the Integrated Stress Response (ISR) identifies the loss of homeostasis and alters cellular programs to return to a balanced state or else activate apoptosis. Four key stresses initiate the ISR: amino acid deprivation, heme deprivation, double-stranded RNA, and endoplasmic reticulum stress. Each stress is sensed by a unique kinase that targets the eukaryotic translation initiation factor eIF2α, which stalls translation and initiates a transcriptional stress response when phosphorylated. GCN2, the conserved kinase that senses amino acid starvation, is activated by an increased cellular presence of deacetylated tRNA, among other factors (Masson, 2019).

Starvation-induced eIF2α phosphorylation is also essential for initiating autophagy, an intracellular degradation process that is conserved among eukaryotes (Kouroku et al., 2007; Noda & Inagaki, 2015). When autophagy is induced, a membrane sac called the isolation membrane engulfs a portion of the cytoplasm containing proteins and organelles, forming a double-membraned autophagosome. When the autophagosome fuses with a lysosome (or a vacuole in yeast or plants), the internal contents are degraded by lysosomal hydrolases. The degradation products are then released from lysosomes and recycled into metabolic and biosynthetic pathways (Noda & Inagaki, 2015). Autophagy-related genes, many of which were discovered in the yeast *Saccharomyces cerevisiae,* are regulated by numerous mechanisms that sense nutrients, energy, and stress. In addition to providing amino acids, sugars, fatty acids, and nucleosides when nutrient levels are low, autophagy also plays a role in removing aggregated proteins and damaged organelles that accumulate under diverse stressful conditions. Defects in autophagy contribute to numerous diseases in humans, including neurodegenerative diseases, cancer, infection, and metabolic diseases (White et al., 2015).

Regulation of autophagy by glucose levels is mediated in part by the target of rapamycin (TOR) kinase, which is found in one or both protein complexes TORC1/TORC2. When glucose is available, TORC1 is active and phosphorylates targets that are involved in anabolic metabolism and inhibit autophagy. When glucose is scarce, TORC1 is inhibited, anabolic metabolism is blocked, and autophagy is induced. The energy sensor AMP-activated protein kinase (AMPK) senses cellular AMP:ATP and ADP:ATP ratios and functions as an upstream regulator of TORC1 activity, among other factors such as tRNA synthetases (Leprivier & Rotblat, 2020). When glucose levels are low, energy is depleted, and AMPK is activated. Active AMPK phosphorylates and activates the TOR negative regulator tuberous sclerosis complex 2

(TSC2) and phosphorylates and inhibits the TORC1 component regulatory-associated protein of TOR (Raptor), resulting in the inhibition of the TORC1 and the induction of autophagy (Leprivier & Rotblat, 2020).

Effects of food deprivation on *Drosophila melanogaster*

To understand evolutionary adaptations to nutritional stress, researchers have conducted numerous selection experiments for starvation resistance in *Drosophila melanogaster* (Rion & Kawecki, 2007). These studies have proposed three physiological routes by which starvation resistance can evolve: enhanced resource accumulation, energy conservation, or starvation tolerance. In addition to evolutionary adaptations seen in starvation-resistant populations of *Drosophila*, individual animals retain plasticity to respond to mild nutritional stress (Rion & Kawecki, 2007). Both evolutionary and physiological adaptations to starvation involve increasing accumulation of lipids, alterations in carbohydrate and lipid metabolism, reduced reproduction, greater resistance to desiccation and oxidative stress, and prolonged development and lifespan (Djawdan et al., 1998; Harshman et al., 1999; Hoffmann & Harshman, 1999; Rion & Kawecki, 2007). *Drosophila* are able to heritably associate starvation with other cues; in one example, animals selected for starvation survival in the presence of decomposing lemon formed selection lines that increased their starvation resistance when exposed to lemon prior to starvation (Harshman et al., 1999). Additionally, starvation resistant lines show an increased body weight. This is likely due to either prolonged development allowing for prolonged period of larval feeding prior to pupation or generally increased feeding rate during development (Edgar, 2006).

Starvation can affect various locomotion and foraging behaviors in *Drosophila.* *Drosophila* larvae move by alternately extending their anterior end and retracting their posterior end, allowing them to move along foraging substrates, a behavior that leaves behind a trail called path length. *Drosophila* third instar larvae show genetically determined variability in their foraging strategies: "rover" animals have significantly longer path lengths than "sitters" on food, but their path length is indistinguishable on agar without food (Sokolowski, 1980, 2001). This difference is attributed to variation in a single gene called *foraging (for)* which encodes a cGMP-dependent protein kinase (PKG) (de Belle et al., 1989; Osborne et al., 1997). Both rover and sitter animals significantly decrease their path length when starved for four hours prior to testing on food, although rovers continue to have longer path lengths than sitters (Graf & Sokolowski, 1989). In adult *Drosophila,* food-deprived animals show increased velocity and total walked distance in a given period of time (Connolly, 1966). This behavior is hypothesized to increase the chances of a food encounter. In contrast, *Drosophila* selected for starvation resistance decrease locomotion to conserve energy, presumably having adapted to a lack of food for which to forage (Williams et al., 2004). Calorie-deprived animals also show suppressed sleep after twelve hours of starvation and subsequently increase their sleep upon refeeding (Keene et al., 2010).

Starvation influences food sensing and feeding behaviors in *Drosophila* by altering their gustatory and olfactory perception of food-related cues. Increased transcription of *sNPFR1* in odorant receptor neurons sensitizes these neurons to attractive food cues, while tachykinin suppresses the activity of aversion neurons (Ko et al., 2015; LeDue et al., 2016; Root et al., 2011). Octopaminergic/tyraminergic OA-VL neurons, which are in close proximity to the bitter sensory neurons, are inhibited by starvation and reduce the bitter sensory neuron responses,

resulting in starved *Drosophila* eating bitter foods they otherwise would have avoided (LeDue et al., 2016). Additionally, in response to nutritional stress, *Drosophila* larvae will eat both conspecific eggs and larval carcasses, which provide sufficient nutrition for the animals to develop into adults not significantly different in morphology from cornmeal-fed counterparts (Ahmad et al., 2015).

Obesity is a leading public health issue for humans

For many humans across the world, especially in industrialized countries, the consequences of eating too much food can be severe. Obesity is associated with reduced quality of life, reduced life expectancy, and increased risk of many diseases, including Type 2 diabetes, stroke, coronary artery disease, gallbladder disease, hyperlipidemia, hypertension, and several types of cancers (Withrow & Alter, 2011). Among individuals with the most severe obesity, a BMI of 40-44.9, 45-49.9, 50-54.9, or 55-59.9 kg/m^2 was associated with 6.5, 8.9, 9.8, and 13.7, years of life lost, respectively, compared to individuals of normal weight (Kitahara et al., 2014). Obesity is expensive for the healthcare system; obese patients typically experience medical costs 30% greater than those of their normal-weight peers (Withrow & Alter, 2011).

Although lifestyle interventions of diet and exercise can initially succeed at promoting weight loss and improving comorbidities, sustained effects are challenging as intervention adherence wanes over time (Piche et al., 2020). Pharmacologic treatments for obesity have expanded in recent years, including the recent approval of semaglutide for weight loss, but current options typically yield a modest weight loss of 3-15% of total body weight and can have significant side effects and costs (Fornes et al., 2022; Velazquez & Apovian, 2018). Although surgical interventions can lead to a loss of 20-40% of pre-surgery weight, they are invasive and

limited to individuals with the highest body mass indexes (Velazquez & Apovian, 2018). Understanding the genetic mechanisms driving food consumption and obesity could reveal unexplored avenues for its treatment and prevention.

Satiety signaling controls appetite in humans

The gut-brain axis (GBA) plays an essential role in altering feeding behaviors in response to nutritional state. In humans, presence of food in and distension of the gastrointestinal tract initiates secretion of hormones that inhibit further food intake. In contrast, by detecting the lack of recently consumed food, the GBA can increase appetite during a fasted state. There is evidence that dysregulation of GBA contributes to obesity in humans (Moris et al., 2022).

Food consumption triggers enteroendocrine cells located in the small intestine to secrete cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY), hormones which function to suppress appetite by binding to receptors on the vagus nerve. The macronutrient composition of a meal influences the secretion of these hormones, which are secreted to a lesser extent after a carbohydrate-rich meal than a protein-rich meal. Non-digestible carbohydrates can ferment into short-chain fatty acids, which elicit the secretion of GLP-1 and PYY. Chronic high fat intake can reduce the satiety effects of CCK and GLP-1. Frequent exercise has additionally been shown to increase the availability of CCK, GLP-1, and PYY (Moris et al., 2022). These hormones bind to receptors on the vagus nerve, which signals to the nucleus tractus solitarius (NTS) to reduce appetite. In addition to these vagally transmitted hormonal signals from the gut, signals from circulating nutrients and sensory information from the mouth carried by several cranial nerves also converge in the NTS to influence appetite (Harrold et al., 2012).

In contrast to these fast-acting satiety signals, adiposity signals detect longer-term changes in fat mass. Leptin is a hormone produced primarily in white adipose tissue and circulates in the blood in levels proportional to body mass and nutritional status. The production of leptin is primarily regulated by insulin (Obradovic et al., 2021). Leptin binds to receptors in the hypothalamus, where it inhibits appetite-stimulating neurons and stimulates appetite-inhibiting neurons. Leptin has also been shown to enhance the satiating power of CCK (Harrold et al., 2012). Hyperleptinemia and some degree of leptin insensitivity are typical in most cases of obesity, and some cases of severe obesity result from mutations affecting leptin signaling (Obradovic et al., 2021). Ongoing work is investigating the potential of leptin-based therapeutics to treat obesity.

Satiety and adiposity signals converge on hypothalamic nuclei in the brain to control appetite and food intake. These nuclei, along with other brain regions that control energy homeostasis, contain various neurotransmitters and neuropeptides that affect feeding behavior and body weight. Satiety signals include melanocortins, which are derived from the common precursor proopiomelanocortin (POMC); cocaine and amphetamine-regulated transcript (CART); and serotonin. Hunger signals include neuropeptide Y (NPY), melanin-concentrating hormone (MCH), and orexins such as OX-A and OX-B (Harrold et al., 2012).

The relatively recent rise in obesity suggests that much of what drives this disease is due to environmental and lifestyle changes, rather than inherited genetic variation. In fact, only about 20% of BMI variation can be attributed to genetics (Berthoud et al., 2017). The transition from low-calorie, high-fiber diets toward more energy-dense diets is considered a major contributor to the rise in obesity, as well as the increased exposure to enticing food advertisements, stress-induced overeating, and mindless food consumption (Berthoud et al., 2017). In these cases,

hedonic drive overrides the homeostatic mechanisms described above. The endogenous opioid system drives sensory pleasure from food, especially from foods with high sugar and fat content, and opioid antagonists have been shown to decrease food intake in rodents and in humans. Additionally, endocannabinoids increase consumption of the most palatable foods (Harrold et al., 2012). These hedonic mechanisms may provide additional potential therapeutic targets for obesity treatment.

C. elegans and its bacterial food environment

Caenorhabditis elegans is a free-living non-parasitic nematode, with adult hermaphrodites reaching one millimeter in length. Their genetic tractability, simple anatomy consisting of 959 cells in adult hermaphrodites, and high degree of genetic conservation with humans make *C. elegans* an easily tractable system in which to study diverse questions pertaining to animal physiology (Brenner, 1974). Throughout the past half-century, *C. elegans* has been host to countless genetic screens and numerous scientific breakthroughs, including the first complete metazoan cell lineage; identification and characterization of apoptosis, microRNAs, and RNA interference; the role of Ras signaling in metazoan development; and the first use of Green Fluorescent Protein (GFP) as a marker for gene expression (Beitel et al., 1990; Chalfie et al., 1994; Ellis & Horvitz, 1986; Fire et al., 1998; Hedgecock et al., 1983; Lee et al., 1993; Sulston & Horvitz, 1977).

The natural habitat of *C. elegans* is in microbe-rich decaying vegetative matter. In this environment, animals forage for bacterial food that can vary in nutritional quality, pathogenicity, and abundance. In contrast to wild *C. elegans,* which face constantly changing food conditions, laboratory-grown *C. elegans* are often fed monoxenic cultures of nonpathogenic *Escherichia coli*

OP50. Environmental food conditions can be experimentally manipulated by introducing different bacterial food species, treating bacterial food with antibiotics, or simply removing food entirely.

Different species of bacteria vary in their ability to support the growth and reproduction of *C. elegans.* Bacterial size, for instance, has been inversely correlated with food quality, as determined by the growth rate of animals fed a given food source. The large size of *Bacillus megaterium* makes it a very low-quality food source, while *Comamonas sp.* is small and supports the most rapid *C. elegans* growth (Avery & Shtonda, 2003). In addition to varying nutritional quality, some bacterial food is pathogenic to *C. elegans.* Some pathogens, such as *Pseudomonas aeruginosa* and *Serratia marcescens,* establish intestinal infections in animals and elicit locomotory problems, intestinal distension, and eventually premature death. Others, such as *Microbacterium nematophilum,* infect animals in a non-lethal manner by adhering to the cuticle of the animal causing swelling (Ewbank, 2002; Kim & Flavell, 2020). An additional class of pathogenic bacteria, including *Burkholderia pseudomallei,* produce fast-acting toxins that result in paralysis and rapidly kill *C. elegans* (Ewbank, 2002; O'Quinn et al., 2001).

External food cues can be uncoupled from the ingestion of bacteria by treating bacterial food with the antibiotic aztreonam. Aztreonam binds to penicillin-binding-protein-3, inhibiting cell wall synthesis in gram-negative bacteria and causing bacteria to form into filaments too long for *C. elegans* to ingest (Figure 1) (Gruninger et al., 2008; Rittenbury, 1990). Although the aztreonam-treated bacteria retains the chemosensory and mechanosensory cues present in untreated bacteria, it is unable to sustain *C. elegans* growth (Gruninger et al., 2008).

C. elegans have evolved numerous mechanisms enhance survival in diverse food conditions, which will be discussed in the following section.

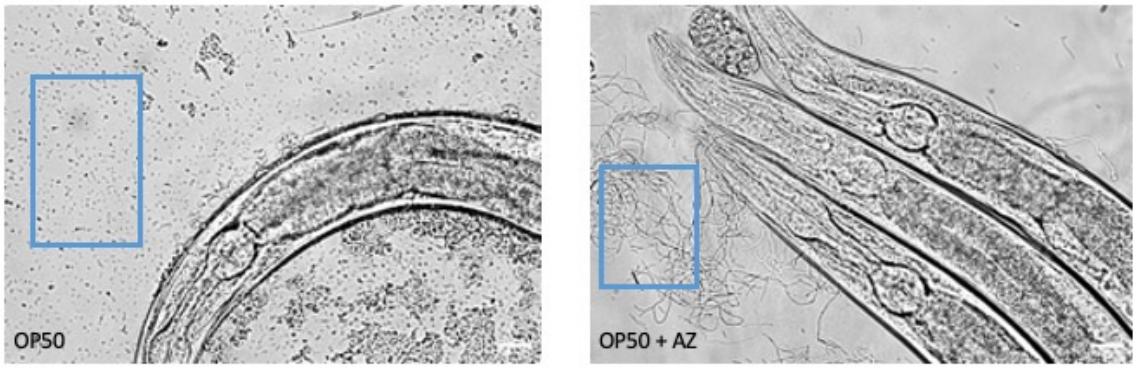

Figure 1. Aztreonam-treated OP50. Adult *C. elegans* and untreated OP50 (left), or aztreonam-treated OP50 (right). The food is highlighted in the blue boxes.

Effects of food conditions on *C. elegans*

A. Non-behavioral adaptations

If *C. elegans* eggs hatch in the complete absence of food, the developing larvae will arrest in the first larval (L1) stage. This phenomenon is termed L1 arrest. Animals in L1 arrest can survive for weeks without feeding and immediately resume development upon the return to edible food.

In contrast to L1 arrest, which is induced by the complete absence of food, dauer diapause is a state that *C. elegans* enter if larvae develop in crowded conditions with limited food (Cassada & Russell, 1975; Golden & Riddle, 1982, 1984a, 1984b, 1984c). Dauer larvae are morphologically distinct from their counterparts grown under more favorable conditions: they are radially constricted, contain an internal plug to close oral orifices, and possess a distinct cuticle with alae (Cassada & Russell, 1975; Riddle et al., 1981). Combined with a complete cessation in pumping, these features contribute to an enhanced resistance to environmental stressors (Cassada & Russell, 1975; Hu, 2007).

Crowded conditions for *C. elegans* are conveyed by a high concentration of a constitutively synthesized pheromone, consisting of a mix of sugars known as ascarosides (Butcher et al., 2007; Jeong et al., 2005). The relative amounts of pheromone and food signals determine whether an animal will enter dauer. In addition to high levels of pheromone and low levels of food, increased temperature promotes dauer arrest (Golden & Riddle, 1984b).

Over the past decades, four distinct genetic pathways governing dauer arrest have been characterized through the isolation and ordering of dauer-formation defective (Daf-d) and dauer-formation constitutive (Daf-c) mutants. These pathways are (1) the guanylyl cyclase pathway, (2) the insulin-like pathway, (3) the TGF-β-like pathway, and (4) the steroid hormone pathway

(Fielenbach & Antebi, 2008; Hu, 2007). Since the initial identification and characterization of these canonical dauer pathways, suppressor screens have been performed to identify additional regulators of dauer formation. A screen for suppressors of TGF-β-pathway Daf-c mutants revealed several new genes, including *scd-2* (suppressor of constitutive dauer arrest), which was found to encode a receptor tyrosine kinase orthologous to the human Anaplastic Lymphoma Kinase (ALK) (Inoue & Thomas, 2000; Reiner et al., 2008). Recent work from our lab has also identified a new role for KGB-1 JNK MAPK signaling in regulating dauer formation in parallel to the insulin-like and TGF-β-like pathways (Dogra et al., 2022).

Once animals have passed the decision points to enter dauer or L1 arrest, food deprivation can alter adult physiology. Hermaphrodites that are removed from food early in the fourth larval stage (L4) delay reproduction, develop into smaller adults with smaller oogenic germlines, and produce fewer viable progeny (Seidel & Kimble, 2011). Animals that are removed from food later in L4 or in adulthood demonstrate a phenomenon known as "bagging," a matricidal switch from oviparity to viviparity where embryos are retained and hatched inside the mother. This facultative viviparity allows embryos that would have hatched in the absence of food avoid L1 arrest by consuming parent body contents, so larvae reach the more durable dauer diapause. When reintroduced to food, these bagged larvae have similar lifespans and fertility to larvae from well-fed parents (Chen & Caswell-Chen, 2004). Food deprivation in reproductively mature animals also increases programmed cell death in the germline. This response is also seen under other stressful conditions, including oxidative stress and pathogen exposure (Salinas et al., 2006).

As has been documented in various organisms, dietary restriction during adulthood increases tolerance to oxidative and thermal stress and extends lifespan in *C. elegans* (Kaeberlein

et al., 2006; Lee et al., 2006). Following development on abundant UV-killed *E. coli,* animals fed a diet consisting of a 90% reduction in bacterial food beginning on day two of adulthood showed a 20% increase in lifespan, while those completely deprived of food (DR-FD) on day two of adulthood showed a lifespan extension of 50%. Transfer to DR-FD conditions on day eight of adulthood resulted in a similar lifespan extension, but transfer during L4 resulted in only a modest lifespan extension relative to control-fed animals (Kaeberlein et al., 2006). The extension in lifespan seen under dietary-restriction conditions is independent of several pathways that influence lifespan, such as insulin signaling or sirtuins (Kaeberlein et al., 2006; Lee et al., 2006).

In addition to adaptations to low levels of bacterial food, *C. elegans* has also developed an innate immune response to respond to exposure to pathogenic bacteria. Infection by *P. aeruginosa* induces *C. elegans* innate immunity, which incorporates cellular stress-response pathways including the Unfolded Protein Response and mitochondrial stress pathways (Kim & Flavell, 2020). Much of this immune response is thought to be induced by host damage resulting from infection rather than recognition of pathogen (Fletcher et al., 2019; Kim & Flavell, 2020; Melo & Ruvkun, 2012).

B. Behavioral Adaptations

C. elegans have additionally evolved many behavioral responses to food. The influence of food on behavior suggests the presence of a gut-"brain" axis in *C. elegans*, where the sensation of ingested food (or lack thereof) signals to the nervous system (the "brain") to influence behavior. Understanding the relatively simple nematode gut-"brain" axis could reveal insight into mechanisms of communication across organ systems and ways by which organisms adapt to changes in internal state.

C. elegans alter the feeding behavior known as pharyngeal pumping in response to changes in food environment and nutritional state. To conserve energy, animals deprived of food for 30 minutes will reduce pumping rates by about 50% compared to well-fed controls (Greer et al., 2008). Under conditions of no food, well-fed *C. elegans* mostly cease pumping, while animals that were starved for six hours prior to the assay will continue to pump (Avery & Horvitz, 1990). When animals that have been deprived of food for more than one hour are reintroduced to food, they exhibit serotonin-mediated hyperactive feeding for about an hour before returning to the pumping rates of *ad libitum* fed animals (Avery & Horvitz, 1990; Lemieux et al., 2015).

When well-fed animals encounter a lawn of food, they slow their locomotion, a behavior termed the basal slowing response, which is mediated by dopamine signaling. When food-deprived animals encounter a bacteria lawn, they exhibit an enhanced slowing response, slowing their locomotion more than well-fed animals in a process mediated by serotonin signaling (Sawin et al., 2000). Food deprivation also alters thermotaxis, as well-fed animals placed on a thermal gradient migrate toward their growth temperature, but starved animals disperse from their growth temperature (Hedgecock & Russell, 1975). This associative learning is also seen in animals that avoid NaCl after being grown in high-salt environments without food and is dependent on *scd-2* and *hen-1* (Ishihara et al., 2002).

Food conditions additionally influence male mate-searching behavior. In the absence of hermaphrodites, well-fed adult males voluntarily leave sources of food to find mating partners. Following starvation, males temporarily prioritize re-feeding over mate searching and remain on food for several hours before leaving food. This behavior is governed by sex- and food condition-specific chemosensory receptor expression as well as serotonin, insulin, Pigment-

Dispersing Factor (PDF) and TGF-β signaling (Barrios et al., 2012; Hilbert & Kim, 2017; Lipton et al., 2004; Ryan et al., 2014).

In the presence of different species of bacterial food, *C. elegans* show behavioral adaptations to optimize nutrition while avoiding potential pathogens. When presented with several non-pathogenic bacterial food sources, *C. elegans* exhibit preference for species with higher nutritional values. Furthermore, animals that have previously been exposed to high-quality food are more likely to leave a mediocre source of food than naïve animals (Shtonda & Avery, 2006). *C. elegans* have additionally evolved to avoid pathogenic bacteria. When faced with a choice between nonpathogenic *E. coli* OP50 and pathogenic *P. aeruginosa* PA14, animals will initially migrate toward PA14, only to reverse their choice after several hours of presumed PA14 intestinal infection (Zhang et al., 2005). When grown on a monoxenic PA14 lawn, *C. elegans* will leave the lawn after several hours of exposure, a behavior that enhances their survival on this pathogen (Meisel et al., 2014; Pujol et al., 2001; Reddy et al., 2009). A similar lawn avoidance behavior is seen on the pathogen *Serratia marcescens* (Pradel et al., 2007).

B.1 Feeding and Foraging Behavior

Food conditions additionally influence two-state feeding and foraging behavior; across the animal kingdom, foraging behavior increases in response to poor food conditions. For instance, starved *Portunus trituberculatus* and *Charybdis japonica* crabs increase the proportion of time spent searching for the *Ruditapes philippinarum* clams that they eat compared to when they are well fed (Sun et al., 2015). As described above, starved *Drosophila* increase their speed and distance traveled relative to fed animals (Connolly, 1966). Additionally, food deprived striped hamsters (*Cricetulus barabensis*) increase activity during their waking hours compared to

control animals fed *ad libitum* (Wen et al., 2018). This increased foraging seen in food-deprived animals is thought to have evolved to increase the chance of a food encounter.

On a standard *E. coli* OP50 lawn, *C. elegans* engages in a two-state feeding/foraging behavior known as roaming and dwelling (Ben Arous et al., 2009; Fujiwara et al., 2002). Well-fed wild-type animals spend about 80% of their time in a feeding state known as dwelling and 20% of their time in a foraging state known as roaming. Dwelling is characterized by a slow rate of locomotion as well as a high body bending angle, and roaming is characterized by faster locomotion and a lower body bending angle (Figure 2). As the bacterial lawn becomes less dense, and thus a less desirable food source, animals spend an increasing fraction of their time roaming. In the presence of inedible aztreonam-treated bacteria, animals transition to roaming about 80% of the time. Furthermore, dwelling has not been observed in the absence of food, as animals roam continuously. If animals are fasted for one hour prior to being reintroduced to bacterial food, they increase the proportion of time they spend dwelling to 90% (Ben Arous et al., 2009).

Genetic pathways that influence roaming and dwelling behavior include insulin, TGF-β, serotonin and PDF signaling, as well as chemosensation (Ben Arous et al., 2009; Flavell et al., 2013; Fujiwara et al., 2002). Animals with loss-of-function mutations in the serotonin biosynthetic enzyme TPH-1 or the serotonin receptor MOD-1 spend an increased fraction of time roaming, exhibiting both longer roaming states and shorter dwelling states (Flavell et al., 2013). Animals with loss-of-function mutations in both PDF ligands, PDF-1 and PDF-2, as well as mutations in the PDF receptor, PDFR-1, spend a greater fraction of time dwelling, with longer dwelling states and shorter roaming states than wild type animals (Flavell et al., 2013). Animals with loss-of-function mutations in the insulin receptor gene, *daf-2*, or the TGF-β ligand *daf-7*

increase their fraction of time dwelling under fed conditions (Ben Arous et al., 2009). *che-2* animals, which are defective in chemosensation, show an increased fraction of time dwelling compared to wild type animals. The effect of CHE-2 on dwelling was shown to function through the cGMP-dependent protein kinase EGL-4, and animals containing loss-of-function mutations in *egl-4* spending more time roaming than wild type animals (Fujiwara et al., 2002).

On especially high-quality food, like *E. coli* HB101 or *Comamonas* sp*.,* animals enter a third behavioral state known as quiescence, characterized by the complete cessation of movement and feeding and reduced sensory perception. Quiescence on high-quality food is dependent on the ability of animals to properly pump and absorb nutrients in the intestine. Longer quiescence states can be observed on high-quality food when animals have been fasted prior to refeeding. These refed animals initially dwell and feed actively but increase quiescence and decrease dwelling within three to six hours of refeeding. Quiescence behavior is regulated by insulin, cGMP, and TGF-β signaling through EGL-4 (You et al., 2008).

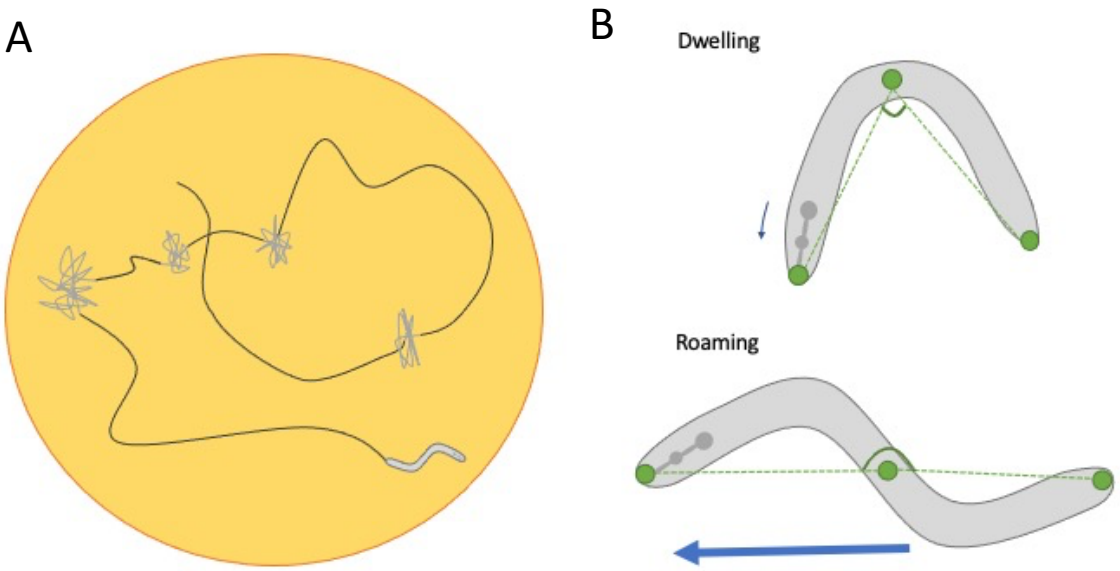

Figure 2. Roaming and Dwelling in *C. elegans.*

A. A schematic of *C. elegans* tracks on a uniform OP50 lawn. Dark gray regions indicate where the animal was roaming, and light gray regions indicate where the animal was dwelling.

B. Schematic of dwelling (top) and roaming (bottom) *C. elegans.* Dwelling animals are characterized by low speed (blue arrow) and a high bending angle (supplement of the angle made by the two ends and the midpoint of the *C. elegans* body, depicted in green). Roaming animals move at a higher speed and have a lower bending angle.

Food-sensing mechanisms in *C. elegans*

The ability of food to influence *C. elegans* physiology requires robust mechanisms of food sensation. The *C. elegans* nervous system consists of 302 neurons in adult hermaphrodites, and the genome of *C. elegans* encodes over 1000 chemoreceptor genes, many of which are sensitive to compounds in food (Bargmann, 1998). *C. elegans* are attracted to water-soluble factors associated with food, including cations, anions, alkaline pH, and cyclic nucleotides (Ward, 1973). Chemotaxis towards several attractants was found to be primarily dependent on the ASE chemosensory neurons; the ADF, ASG, and ASI neurons additionally play roles in a residual response to attractants after the ASE neurons are ablated (Bargmann & Horvitz, 1991). Additionally, volatile odorants associated with food have been shown to attract *C. elegans*; these volatile compounds include 2-butatone, diacetyl, isoamyl alcohol, and pyrazine and are detected in the ciliated AWC and AWA neurons (Bargmann et al., 1993). In addition to chemical cues, *C. elegans* can also detect bacteria through mechanosensation. The basal slowing response described above, for instance, is mediated by mechanically sensitive ion channels in ciliated dopaminergic neurons that react to the mechanical forces associated with moving across bacteria (Kim & Flavell, 2020; Sawin et al., 2000).

C. elegans are also sensitive to ambient oxygen levels, which are altered by bacterial metabolism. A thick *E. coli* lawn has an oxygen concentration of 17.1% in the center and 12.8% around the border, lower than the 21% ambient oxygen level (Gray et al., 2004). In the absence of food, *C. elegans* prefer a low oxygen level of about 8%, as this oxygen level is associated with bacterial metabolism. Oxygen sensing is mediated by the soluble guanylyl cyclase encoded by *gcy-35* expressed in the AQR, PQR, URX, and BAG neurons (Cheung et al., 2005; Gray et al., 2004). In the presence of food, N2 animals show a reduced avoidance of hyperoxia and are not

driven into the bacterial lawn as would be expected. This behavior has been attributed to the laboratory-acquired 215V allele of the gene *npr-1*. In contrast, wild isolates carry a 215F allele, which reduces NPR-1 activity relative to the 215V allele. These wild isolates maintain their hyperoxia avoidance in the presence of food and tend to migrate toward the border of a lawn where oxygen concentrations are lowest (de Bono & Bargmann, 1998; Kim & Flavell, 2020).

In addition to external factors signaling the presence of food in the environment, ingested food can also be detected by *C. elegans.* Serotonin signaling and the serotonergic NSM neurons are required for the enhanced slowing response and for stabilizing the dwelling state (Flavell et al., 2013; Sawin et al., 2000). The NSM neurons have a minor neurite that extends into the pharyngeal lumen, and these neurons are activated in response to the presence of food in the pharynx. The detection of food by the NSM neurons is dependent on the acid-sensing ion channels DEL-3 and DEL-7 which localize to the pharyngeal neurite and detect ingested bacteria (Rhoades et al., 2019). Detection of food in the intestine can also influence gene expression and behavior. Rictor/TORC2 acts in the intestine to sense food and regulate the expression of *daf-7* in the ASI neurons and *daf-28* in the ASI and ASJ neurons to influence the decision to enter dauer. In adult animals, Rictor/TORC2 senses food in the intestines and promotes dwelling in a PDF-2/PDFR-1-dependent manner (O'Donnell et al., 2018). Changes in cellular metabolite levels induced by food deprivation can additionally signal levels of ingested food. For instance, fasting reduces levels of kynurenine and kynurenic acid, which are byproducts of tryptophan metabolism, by about 70% (Lemieux et al., 2015). Reduced kynurenic acid levels in neurons signals through the NMDA receptor to influence serotonin signaling and feeding behavior (Lemieux et al., 2015).

daf-7 **and its role in adapting to changing food conditions**

TGF-β signaling has been implicated in numerous food-dependent phenotypes in *C. elegans*. As discussed above, the TGF-β signaling ligand DAF-7 was initially characterized for its role in signaling favorable growth conditions in developing larvae, with animals containing loss-of-function or null alleles of *daf-7* constitutively entering dauer. *daf-7* transcription in the ASI chemosensory neurons is responsive to the presence of dauer pheromone and food; animals containing a *pdaf-7::gfp* transcriptional reporter fail to express GFP as dauer larvae but induce GFP expression in the ASI neurons when reintroduced to food (Ren et al., 1996; Schackwitz et al., 1996).

In well-fed adult *C. elegans*, *daf-7* mutants show a 20-25% reduced pumping rate relative to wild-type animals. When these animals are food deprived, however, pumping rates are similar between *daf-7* and wild-type animals. This observation suggests that the difference seen between *daf-7* and wild-type animals in the presence of abundant food could be due to defects in sensing food in the *daf-7* mutant (Greer et al., 2008). Consistent with a perception of limited food conditions, *daf-7* animals accumulate 2.5-times as much fat as wild-type animals when well fed, similar to levels of dauer animals (Greer et al., 2008). Well-fed *daf-7* mutants have longer lifespans than wild-type animals (Shaw et al., 2007). Furthermore, dietary restriction does not further enhance the lifespan of *daf-7* mutants as it does in wild-type animals, suggesting that inhibition of *daf-7* is contributing to this dietary restriction-mediated lifespan extension (Fletcher & Kim, 2017).

DAF-7 expressed in the ASI neurons promotes quiescence; *daf-7* mutants on *E. coli* HB101 spend less time in a quiescent state and more time in a dwelling state after fasting and refeeding compared to wild type (Gallagher et al., 2013; You et al., 2008). On lower-quality *E. coli* OP50 food, where quiescence is not observed, *daf-7* animals spend more time dwelling and

less time roaming than wild type animals. *daf-7* animals increase the fraction of their time roaming when on inedible aztreonam-treated OP50 compared to untreated OP50, but still roam significantly less than wild-type animals (Ben Arous et al., 2009).

Although *daf-7* expression had previously been thought to be restricted to the ASI neurons, work from our lab has shown that under certain conditions, such as on pathogenic *P. aeruginosa* PA14 or in males, expression can be additionally induced in the ASJ chemosensory neurons. Induction of *daf-7* expression in the ASJ neurons promotes behavioral avoidance of PA14 (Meisel et al., 2014). Differential expression of *daf-7* in the ASJ neurons also regulates male mate-searching. When wild-type adult males are well fed, they express *daf-7* in both the ASI and ASJ neurons and readily leave a lawn of food to search for a mate. Well-fed male *daf-7* mutants, however, do not leave food to mate-search (Hilbert & Kim, 2017). This phenotype is similar to what is seen in starved males, which do not express *daf-7* in the ASJ neurons and will remain on food to feed for several hours. As starved males refeed, *daf-7* expression is reinduced in the ASJ neurons over several hours, prompting animals to leave food in search of a mate. The slower kinetics of *daf-7* induction in the ASJ neurons upon refeeding suggests that *daf-7* expression in males is influenced by the animal's internal nutritional state rather than the presence of external food cues (Hilbert & Kim, 2017).

Concluding remarks

The appropriate response to fluctuations in food conditions is essential for an organism to maintain homeostasis and survive in a rapidly changing environment. Across kingdoms, eukaryotes show both conserved and species-specific responses to nutrient deprivation. Animals have the advantage of modulating behavior in response to changing food conditions.

Nevertheless, the connection between the detection of changing food conditions and the ensuing behavioral adaptations is incompletely understood. Further elucidating how changes in food are perceived and communicated to the nervous system to execute a behavioral response in *C. elegans* could provide additional insight into not only how these animals sense and adapt to changing environments, but also the broader field of enteroception and gut-brain communication across organisms. In addition to its essential roles in responding to conditions in which food is limited and maintaining energy homeostasis, the human gut-brain axis has been observed to be dysregulated in obesity, a disease with an ever-increasing global prevalence. As some genes studied in this thesis are conserved between *C. elegans* and humans, insight into how *C. elegans* orthologs function to sense and respond to changes in food conditions could reveal similar roles for their human counterparts. This may lead to new therapeutic targets and means of prevention for obesity.

In Chapter Two of this thesis, I discuss the generation and characterization of a gain-of-function allele of *scd-2, scd-2(syb2455),* which we designed based on an ALK oncogenic mutation at a residue conserved in SCD-2. I describe the pheromone-dependent Daf-c phenotype of *scd-2(syb2455)* animals and propose a role for SCD-2 in food sensing. In Chapter Three, I demonstrate that SCD-2 regulates roaming and dwelling behavior by modulating the expression of *daf-7* in the ASJ neurons in response to changes in food conditions. Specifically, I show that ingested food detected in the pharynx inhibits the expression of *daf-7* in the ASJ neurons by signaling through SCD-2. The increase in *daf-7* expression in ASJ seen when animals are removed from edible food contributes to roaming behavior, and we propose a model in which *daf-7* expression in the ASJ neurons functions in a positive feedback loop with reduced food ingestion and SCD-2 signaling to stabilize the roaming state. In Chapter Four, I discuss a screen

performed to identify genes that signal satiety from the intestine to the ASJ neurons in male *C. elegans.* This screen yielded two mutants: one has a loss-of-function allele of *che-3,* and the other had a gain-of-function allele of *pdfr-1*. Taken together, the work presented in this thesis reveals how *C. elegans* sense food to influence neuroendocrine signaling and behavior, enhancing our understanding of food detection and signaling along the gut-brain axis.

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Chapter Two

Genetic analysis of SCD-2/ALK in the dauer developmental decision in *C. elegans*

Abstract

In response to stressful growth conditions, the developing larvae of *C. elegans* enter a developmental diapause known as dauer. Genetic analysis of dauer formation has identified multiple conserved signaling pathways in *C. elegans*, including insulin-like signaling and TGF-β-like signaling. Here, we characterize a hypermorphic allele of the *C. elegans ALK* ortholog, *scd-2,* which we generated by engineering an oncogenic mutation at a residue conserved between ALK and SCD-2. In contrast to the Daf-d phenotype seen in *scd-2* loss-of-function animals, we found that our gain-of-function *scd-2(syb2455)* allele confers a pheromone-dependent Daf-c phenotype. By performing epistasis experiments with our *scd-2(syb2455)* allele and Daf-d genes in known dauer-regulating pathways, we found that SCD-2 functions in parallel to insulin-like, TGF-β-like, and JNK MAPK signaling to regulate dauer formation. We also report that *scd-2(syb2455)* animals show enhanced susceptibility to starvation when arrested as L1s. The analysis of this new *scd-2* allele presented here and in Chapter Three suggests a role for SCD-2 in sensing and responding to unfavorable food conditions to influence development and behavior.

Introduction

In response to stressful growth conditions of high population density, limited food, and elevated temperature, *Caenorhabditis elegans* can arrest their growth and enter a developmental diapause state known as dauer (Golden & Riddle, 1982, 1984a, 1984b, 1984c). Dauer larvae are morphologically distinct from their counterparts grown under more favorable conditions: they are radially constricted, contain an internal plug to close oral orifices, and possess a distinct cuticle with alae (Cassada & Russell, 1975; Hu, 2007; Riddle et al., 1981). These features

increase resistance to environmental stressors, such as desiccation, and permit the animals to survive for long periods of time in this arrested state.

Four distinct genetic pathways governing dauer arrest have been characterized through the isolation and ordering of dauer-formation defective (Daf-d) and dauer-formation constitutive (Daf-c) mutants. These pathways are the guanylyl cyclase pathway, the insulin-like pathway, the TGF-β-like pathway, and the steroid hormone pathway (reviewed in (Fielenbach & Antebi, 2008; Hu, 2007)). Together these pathways integrate information about the environment and, depending on the conditions, signal to promote either reproductive growth or dauer arrest.

In *C. elegans,* the TGF-β-like signaling pathway is defined by the Daf-c genes *daf-1,-4,-7,-8,* and *-14* and the Daf-d genes *daf-3* and *daf-5.* DAF-7 is a TGF-β signaling ligand which binds to a heterodimeric receptor formed by DAF-1 and DAF-4. *daf-8 and -14* encode R-SMADs which inhibit the co-SMAD DAF-3, and *daf-5* encodes a Sno/Ski homolog that binds to DAF-3 to promote dauer development (Gumienny & Savage-Dunn, 2013; Patterson & Padgett, 2000). Since the initial identification and characterization of the canonical dauer pathways, suppressor screens have been performed to identify additional regulators of dauer formation. A screen for suppressors of TGF-β-pathway Daf-c mutants revealed several new genes, including *scd-2*, which was found to encode a receptor tyrosine kinase (Inoue & Thomas, 2000; Reiner et al., 2008). Animals with loss-of-function mutations in *scd-2* or its ligand *hen-1* are Daf-d (Inoue & Thomas, 2000; Reiner et al., 2008).

In addition to their roles in dauer formation, *scd-2* and *hen-1* have also been shown to influence sensory integration, associative learning, and memory. Animals with *hen-1* or *scd-2* mutations are defective in integrating two conflicting signals and show defects in crossing an aversive Cu^{2+} barrier to reach an attractive patch at diacetyl, despite wild-type responses to each

stimulus on its own (Ishihara et al., 2002; Shinkai et al., 2011; Wolfe et al., 2019). *scd-2* animals also fail to learn the pathogenicity of *P. aeruginosa* PA14 and do not avoid PA14 after a previous encounter with the pathogen (Wolfe et al., 2019). Additionally, *scd-2* and *hen-1* animals show defects in associating certain stimuli with lack of food. Wild type animals will migrate towards their growth temperature when well fed, but starved animals disperse from their growth temperature, having associated the temperature with a lack of food (Hedgecock & Russell, 1975). Similar responses are seen when animals are grown in the presence of NaCl with no food. However, *scd-2* and *hen-1* animals are defective in associating their growth conditions with lack of food and fail to disperse from growth conditions when starved (Ishihara et al., 2002).

The human ortholog of *scd-2* is the Anaplastic Lymphoma Kinase (ALK) gene. *ALK* was discovered after a rearrangement resulting in the fusion of the nucleophosmin (NPM1) gene with a previously uncharacterized receptor tyrosine kinase gene was identified in an anaplastic large-cell lymphoma cell line (Morris et al., 1994). *ALK* is expressed in the brain, small intestine, and testis, where it influences cell proliferation, differentiation, and survival in response to external stimuli (Holla et al., 2017). Genetic rearrangements resulting in gene fusions are common in *ALK*-dependent human cancers such as lung cancer, neuroblastoma, rhabdomyosarcoma, and renal cell carcinoma (Holla et al., 2017). Most current therapies for *ALK*-dependent cancers involve kinase inhibitors, including crizotinib, ceritinib, and alectinib, which target these *ALK* gene-fusion products. Unfortunately, secondary gain-of-function point mutations often develop in ALK gene fusion products treated with kinase inhibitors and can lead to disease progression after several months of treatment (Holla et al., 2017).

In addition to their role in driving treatment-resistant tumors in gene fusion-positive tumors, gain-of-function point mutations are also commonly seen as primary mutations in

neuroblastoma. Most oncogenic point mutations are seen in the kinase domain, with 85% of all *ALK* mutations seen at either F1174 or R1275 (Franco et al., 2013). F1174L, the most common point mutation, results in ALK autophosphorylation and cytokine-independent growth (Chen et al., 2008; Holla et al., 2017). Understanding the nature of these gain-of-function point mutations could be essential in developing new treatments and combatting drug resistance. Since these gain-of-function point mutations are primarily seen in tumors originating from neuronal cells, studying *scd-2*, which is expressed in the neurons, could additionally provide context-specific insight.

Recent work has additionally revealed a role for ALK in influencing metabolic phenotypes. A genome-wide association study (GWAS) looking for genetic variants associated with thinness (defined as the 6th percentile or below body mass index (BMI)) identified a variant in the first intron of *ALK* (Orthofer et al., 2020). RNAi-mediated *Alk* knockdown experiments in *Drosophila* identified that these animals had reduced triglyceride accumulation (Orthofer et al., 2020). Studies in *Drosophila* have also demonstrated a role for Alk in sparing the central nervous system during nutrient restriction, and expression of a dominant-negative Alk improves starvation survival (Cheng et al., 2011; Woodling et al., 2020). *Alk*$^{-/-}$ mice also displayed phenotypes associated with thinness, including reduced adiposity, increased energy expenditure, and resistance to obesity when fed a high-fat diet (Orthofer et al., 2020).

Here, we report the characterization of a gain-of-function allele of *scd-2*, containing a F1029L substitution that is conserved with the oncogenic F1174L ALK mutation. This *scd-2* allele confers a pheromone-dependent Daf-c phenotype and appears to function in parallel to TGF-β signaling, insulin signaling, and JNK MAPK signaling to influence dauer formation. We additionally found that this *scd-2(syb2455)* allele reduces survival of animals arrested as L1s

with no food. In conjunction with data presented in Chapter Three of this thesis, we propose a role for SCD-2 in sensing and/or relaying unfavorable food conditions.

Results

An F1029L variant of SCD-2 is hypermorphic

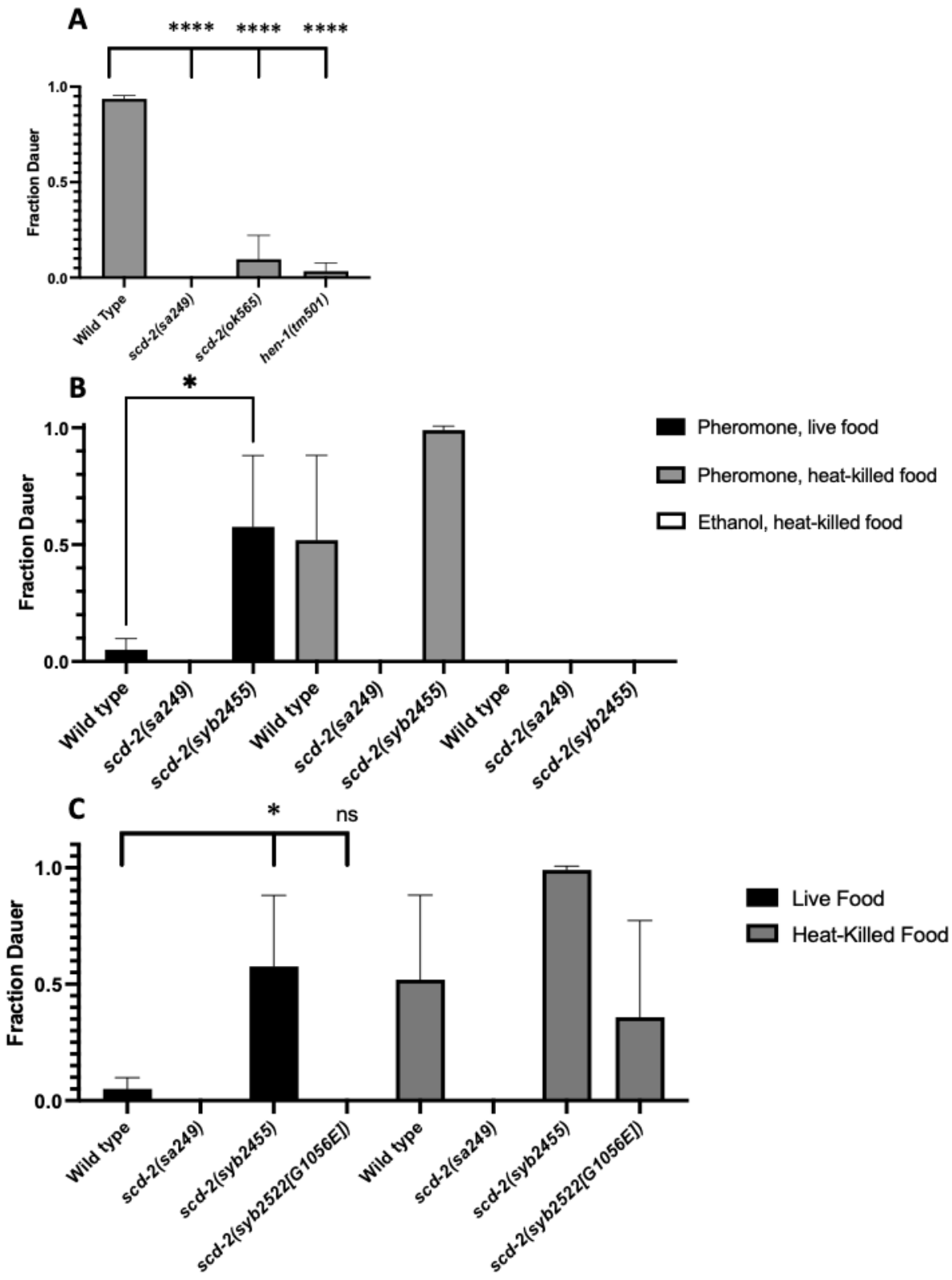
scd-2 was initially identified in a screen for suppressors of *daf-7* pathway constitutive dauer mutants (Inoue & Thomas, 2000). The two alleles of *scd-2* recovered from this screen, *scd-2(sa249)* and *scd-2(sa303),* were found to have a Daf-d phenotype in the absence of background *daf-7* pathway Daf-c mutations. Animals with mutations in *hen-1,* the gene encoding the ligand of SCD-2*,* are also Daf-d (Reiner et al., 2008) (Figure 1A).

SCD-2 and human ALK share overall amino acid sequence identity of 23% and similarity of 35%. Within the kinase domain, sequence identity is 47% and the similarity is 61% (Figure 2) (Reiner et al., 2008). The most common ALK gain-of-function point mutation, F1174L, occurs at a residue that is conserved in SCD-2 as F1029, and we hypothesized that an F1029L allele of *scd-2, scd-2(syb2455),* could be hypermorphic.

To assess the dauer-formation phenotype of our *scd-2(syb2455)*-carrying animals, we performed a dauer-formation assay broadly as has previously been described (Dogra et al., 2022; Neal et al., 2013). This assay incorporates the three conditions required for dauer formation, high pheromone concentration, low food abundance, and high temperature, to induce dauer larvae; high concentrations of purified pheromone are added to plates that are seeded with heat-killed food (which mimics low food levels) and incubated at 25˚C. Wild-type animals will enter dauer at high rates only when all three criteria are satisfied (Dogra et al., 2022). In contrast, Daf-d mutants, such as *scd-2* loss-of-function animals, fail to enter dauer under typical dauer-inducing

conditions. Many previously described Daf-c mutations, such as those in the insulin-signaling receptor gene, *daf-,2* or the gene encoding the TGF-β-signaling ligand *daf-7* lose the requirements for both low levels of food and high concentrations of pheromone and enter dauer constitutively at higher temperatures in the absence of pheromone and low levels of food.

We observed that *scd-2(syb2455)* gain-of-function animals formed dauers at a high rate even in the absence of poor-quality heat-killed food, which is necessary for wild-type animals to enter dauer (Figure 1B). Nevertheless, these animals remained sensitive to the presence of pheromone, and, like wild-type animals, required high concentrations of dauer pheromone to enter dauer (Figure 1B). A F1174I mutation in ALK is also oncogenic, and an independent F1029I allele of *scd-2* also conferred a Daf-c phenotype (Figure 1E) (Holla et al., 2017). Other ALK-conserved oncogenic mutations in *scd-2* did not confer Daf-c phenotypes: G1056E and Y1102C substitutions conferred wild-type dauer-entry phenotypes, and F1029S and K1132Q appeared to confer a Daf-d phenotype like loss-of-function mutations in *scd-2* (Figure 1C and Figure 1D). The *scd-2(syb2455)* allele was dominant to both wild type and *scd-2(sa249),* with both *scd-2(syb2455)/* wild type and *scd-2(syb2455)/scd-2(sa249)* trans heterozygotes showing the *scd-2(syb2455)* Daf-c phenotype, further supporting our classification of *scd-2(syb2455)* as gain-of-function (Figure 1E).


A
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****
****
Fraction Dauer
1.0
0.5
0.0
Wild Type
scd-2(sa249)
scd-2(ok565)
hen-1(tm501)
B
*
Fraction Dauer
1.0
0.5
0.0
Wild type
scd-2(sa249)
scd-2(syb2455)
Wild type
scd-2(sa249)
scd-2(syb2455)
Wild type
scd-2(sa249)
scd-2(syb2455)
Pheromone, live food
Pheromone, heat-killed food
Ethanol, heat-killed food
C
*
ns
Fraction Dauer
1.0
0.5
0.0
Wild type
scd-2(sa249)
scd-2(syb2455)
scd-2(syb2522[G1056E])
Wild type
scd-2(sa249)
scd-2(syb2455)
scd-2(syb2522[G1056E])
Live Food
Heat-Killed Food

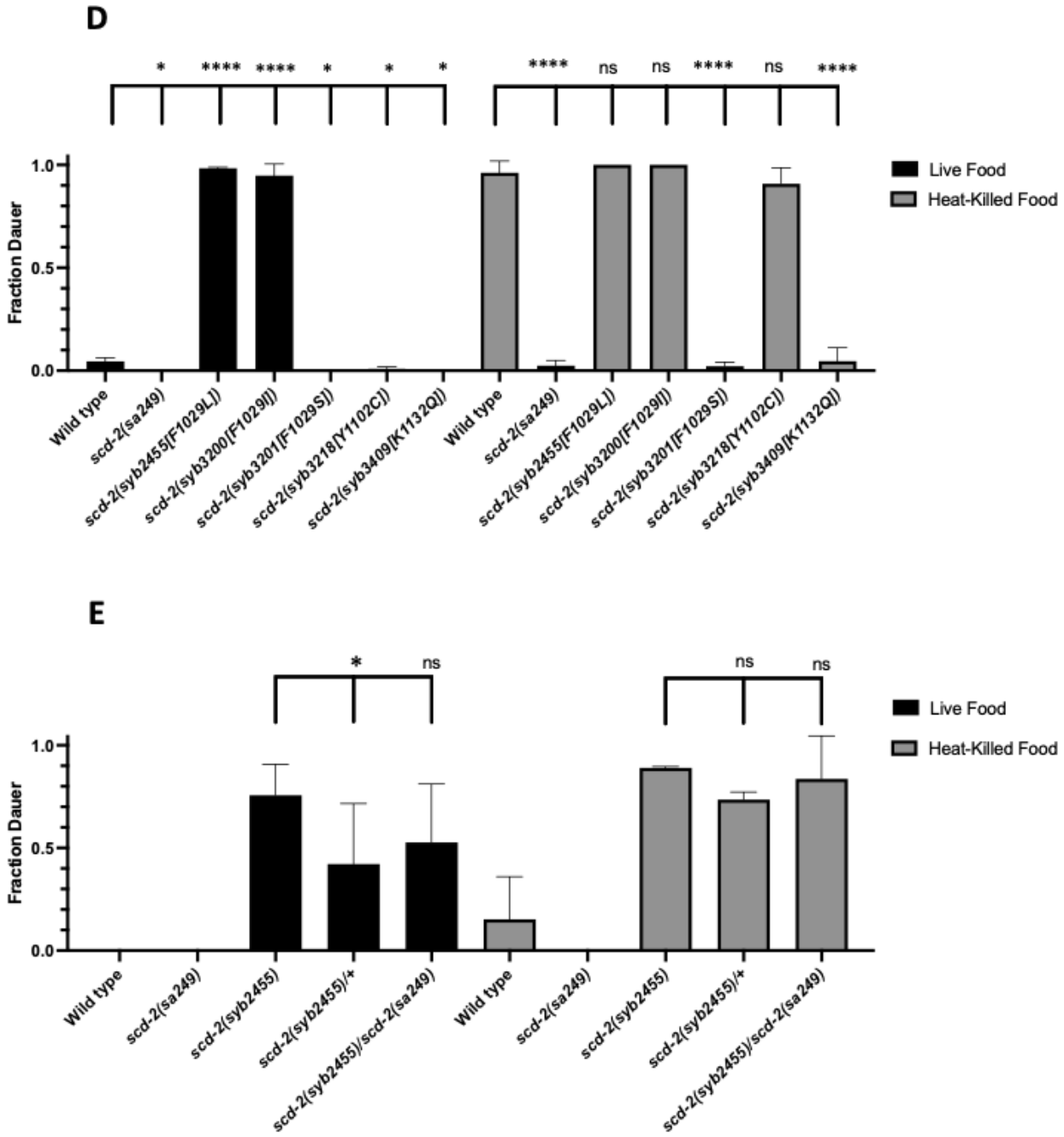

D
* **** **** * * *
**** ns ns **** ns ****
Fraction Dauer
1.0
0.5
0.0
Live Food
Heat-Killed Food
Wild type
scd-2(sa249)
scd-2(syb2455[F1029L])
scd-2(syb3200[F1029I])
scd-2(syb3201[F1029S])
scd-2(syb3218[Y1102C])
scd-2(syb3409[K1132Q])
Wild type
scd-2(sa249)
scd-2(syb2455[F1029L])
scd-2(syb3200[F1029I])
scd-2(syb3201[F1029S])
scd-2(syb3218[Y1102C])
scd-2(syb3409[K1132Q])
E
* ns
ns ns
Fraction Dauer
1.0
0.5
0.0
Live Food
Heat-Killed Food
Wild type
scd-2(sa249)
scd-2(syb2455)
scd-2(syb2455)/+
scd-2(syb2455)/scd-2(sa249)
Wild type
scd-2(sa249)
scd-2(syb2455)
scd-2(syb2455)/+
scd-2(syb2455)/scd-2(sa249)

Figure 1. A F1029L substitution in SCD-2 confers a hypermorphic phenotype.

A. Fraction of wild type, *scd-2(sa249), scd-2(ok565),* and *hen-1(tm501)* animals entering dauer on plates containing pheromone and heat-killed food. ****p<0.0001 by two-way student's t-test.

B. Fraction of wild type, *scd-2(sa249),* and *scd-2(syb2455[F1029L])* animals entering dauer on plates containing pheromone and live food (black bars), pheromone and heat-killed food (gray bars), or ethanol and heat-killed food. *p<0.05 by two-way student's t-test.

C. Fraction of wild type, *scd-2(sa249), scd-2(syb2455[F1029L]),* and *scd-2(syb2522[G1056E])* animals entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). ns not significant by two-way student's t-test.

D. Fraction of wild type, *scd-2(sa249), scd-2(syb2455[F1029L]), scd-2(syb3200[F1029I]), scd-2(syb3201[F1029S]), scd-2(syb3218[Y1102C]),* and *scd-2(syb3409[K1132Q])* animals entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). **** p<0.0001, * p<0.05, ns not significant by two-way student's t-test.

E. Fraction of wild type, *scd-2(sa249), scd-2(syb2455[F1029L]), scd-2(syb2455[F1029L])/*wild type heterozygotes, and *scd-2(syb2455[F1029L])/scd-2(s249)* heterozygotes entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). * p<0.05, ns not significant by two-way student's t-test.

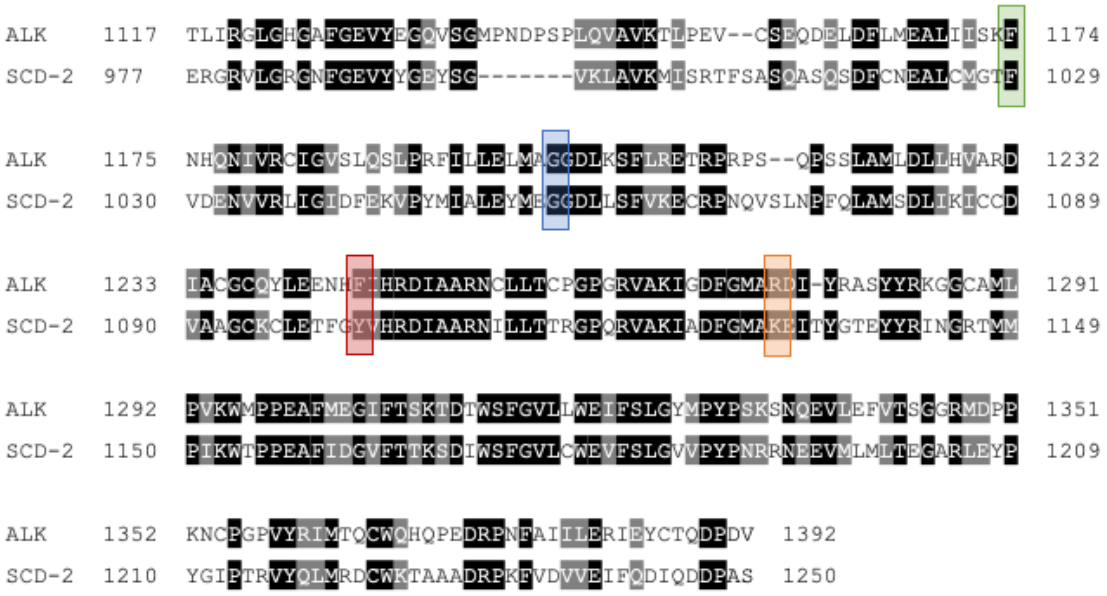


Figure 2. ALK and SCD-2 protein alignment

Alignment of human ALK (top) and *C. elegans* SCD-2 (bottom) kinase domain sequences. Identical residues are highlighted in black, conserved residues in gray. Conserved residues mutated in this study are highlighted: Green, F1174/F1029 (human/*C. elegans*); Blue, G1201/G1056; Red, F1245/Y1102; Orange R1275/K1132

scd-2 influences dauer formation in parallel to known dauer pathways

The isolation of a gain-of-function allele of *scd-2* allowed us to perform epistasis analysis with other known Daf-d alleles to see if we could place *scd-2* in a known dauer pathway. Given that *scd-2* was initially found as a suppressor of *daf-7* pathway constitutive dauer-forming mutants, it was initially hypothesized that SCD-2 could be acting downstream of this pathway. Loss-of-function alleles of *scd-2* incompletely suppress the Daf-c phenotypes of *daf-7, daf-4,* and *daf-8* (Reiner et al., 2008). Loss-of-function alleles of *scd-2* and *hen-1* were also found to influence DAF-3-mediated transcription in a *daf-7(e1372)* background, suggesting that DAF-3 may function downstream of SCD-2 (Reiner et al., 2008).

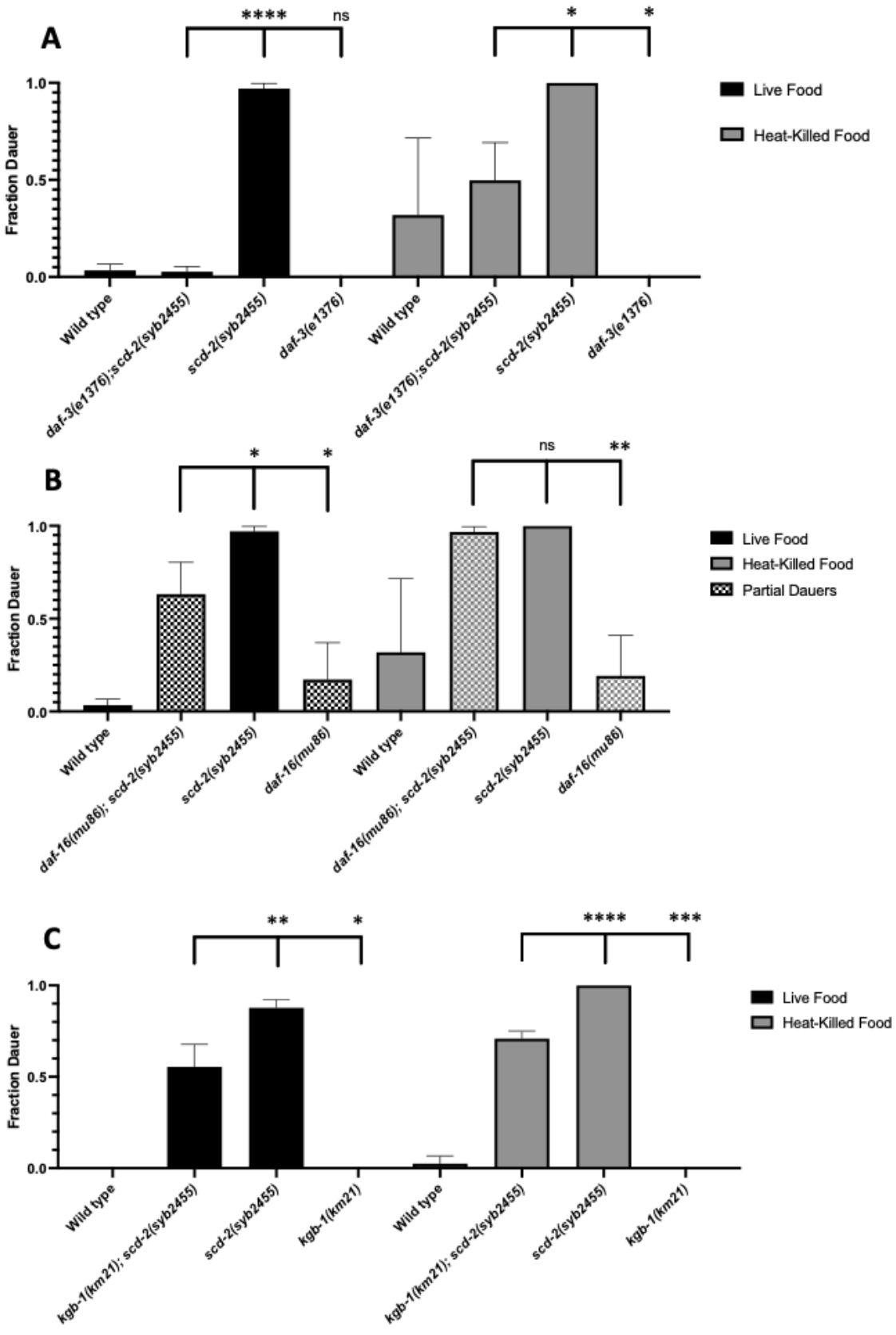
We sought to test the possibility that SCD-2 functions upstream of DAF-3 by examining if the Daf-c phenotype of *scd-2(syb2455)* animals could be suppressed by a loss-of-function allele of *daf-3.* We observed that *scd-2(syb2455); daf-3(e1376)* animals formed dauers at rates intermediate of those of each single mutant, indicating that *daf-3* incompletely suppresses the *scd-2(syb2455)* Daf-c phenotype. Therefore, if DAF-3 does function downstream of SCD-2, there are likely additional parallel pathways by which SCD-2 influences dauer formation (Figure 3A).

daf-2(e1370), a reduction-of-function mutation in the gene encoding the *C. elegans* insulin receptor, which confers a Daf-c phenotype, was found to completely suppress the Daf-d phenotypes of *scd-2(sa249)* and *scd-2(y386)* (Reiner et al., 2008). This could mean that DAF-2 functions downstream of or in parallel to SCD-2. To further investigate the relationship between *scd-2* and insulin signaling, I examined the effects of *scd-2(syb2455)* in a *daf-16* background (DAF-16 functions downstream of DAF-2 and confers a Daf-d phenotype (Gottlieb & Ruvkun, 1994)). The *scd-2(syb2455); daf-16(mu86)* double mutants entered dauer at high frequencies on

both live and heat-killed food; however, these dauers were morphologically distinct from both typical dauer and nondauer larvae, and likely represented the "partial dauers" seen in several double mutant strains with *daf-16* and a Daf-c mutation (Vowels & Thomas, 1992) (Figure 3B). Taken together, these findings likely indicate that SCD-2 functions in parallel to the *daf-2* insulin signaling dauer pathway to regulate dauer formation.

Our lab recently reported the characterization of the role of KGB-1 JNK MAPK signaling in dauer formation, functioning in parallel to the insulin-signaling and TGF-β-signaling pathways (Dogra et al., 2022). Mutations in the genes encoding the MAPKKK MLK-1, MAPKK MEK-1, and MAPK KGB-1 are all Daf-d. Like *scd-2(syb2455)* animals, animals with loss-of-function mutations in *vhp-1*, the gene encoding the MAPK phosphatase, also show a pheromone-dependent Daf-c phenotype, entering dauer at high rates regardless of food conditions but only if pheromone is present (Dogra et al., 2022). Additionally, *mlk-1* was recently found to be the same gene as *scd-4,* the *sa321* allele of which was recovered from the same screen for suppressors of *daf*-7 pathway Daf-c mutants as *scd-2* (Rasmussen et al., 2021).

Given these commonalities between the KGB-1 pathway and SCD-2, we wondered if SCD-2 might function in this *kgb-1* pathway. *kgb-1(km21)* animals are Daf-d, and *kgb-1(km21); scd-2(syb2455)* animals showed an intermediate phenotype between that of each single mutant (Figure 3C). We therefore concluded that SCD-2 functions at least partially in parallel to KGB-1 signaling to regulate dauer formation.


A
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Fraction Dauer
1.0
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Live Food
Heat-Killed Food
Wild type
daf-3(e1376);scd-2(syb2455)
scd-2(syb2455)
daf-3(e1376)
Wild type
daf-3(e1376);scd-2(syb2455)
scd-2(syb2455)
daf-3(e1376)
B
*
*
ns
**
Fraction Dauer
Live Food
Heat-Killed Food
Partial Dauers
Wild type
daf-16(mu86); scd-2(syb2455)
scd-2(syb2455)
daf-16(mu86)
Wild type
daf-16(mu86); scd-2(syb2455)
scd-2(syb2455)
daf-16(mu86)
C
**
*
****
***
Fraction Dauer
Live Food
Heat-Killed Food
Wild type
kgb-1(km21); scd-2(syb2455)
scd-2(syb2455)
kgb-1(km21)
Wild type
kgb-1(km21); scd-2(syb2455)
scd-2(syb2455)
kgb-1(km21)

Figure 3. *scd-2* influences dauer formation in parallel to known dauer signaling pathways

A. Fraction of wild type, *daf-3(e1376); scd-2(syb2455), scd-2(syb2455),* and *daf-3(e1376)* animals entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). **** $p<0.0001$, * $p<0.05$, ns not significant by two-way student's t-test.

B. Fraction of wild type, *daf-16(mu86); scd-2(syb2455), scd-2(syb2455),* and *daf-16(mu86)* animals entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). Checkered bars represent partial dauers. ** $p<0.01$, * $p<0.05$, ns not significant by two-way student's t-test.

C. Fraction of wild type, *kgb-1(km21); scd-2(syb2455), kgb-1(km21),* and *scd-2(syb2455)* animals entering dauer on plates containing pheromone and live food (black bars) or pheromone and heat-killed food (gray bars). **** $p<0.0001$, *** $p<0.001$, **$p<0.01$, * $p<0.05$ by two-way student's t-test.

SCD-2 influences L1 arrest survival

When *C. elegans* eggs hatch in the complete absence of food, the developing larvae will arrest in the first larval stage (L1) (Baugh, 2013). L1 arrest contrasts with dauer diapause, which is triggered by crowded conditions and limited food, in that animals are less stress-resistant and cannot survive for as long without food (Baugh, 2013). There is some genetic overlap between regulators of L1 arrest and dauer, including *daf-2* insulin signaling (Baugh & Sternberg, 2006), and given the role of *scd-2* in dauer, we wondered if it also had any effects on L1 arrest. We assayed the ability of animals to survive L1 arrest for long periods of time and recover to the L4 larval stage or adulthood when reintroduced to food. We found that both wild type and *scd-2(sa249)* animals were able to survive as L1s for 7-10 days without feeding and retain the capacity to grow to L4 when reintroduced to food. In contrast, we observed that *scd-2(syb2455)* animals lost the ability to recover to L4 or adulthood after 2-4 days of starvation, suggesting potential abnormalities in detecting starvation and entering L1 arrest and/or the metabolic reprogramming necessary for survival (Figure 4).

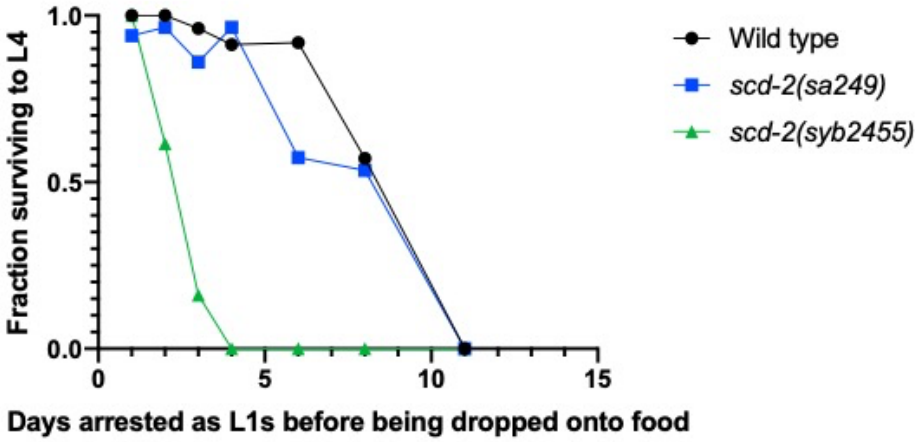

Figure 4. *scd-2(syb2455)* animals have reduced survival as arrested L1s. Fraction of wild type (black), *scd-2(sa249)* (blue) or *scd-2(syb2455)* (green) surviving to L4 vs. the number of days the animals were arrested as L1s before being dropped onto *E. coli* OP50 food.

Discussion

SCD-2 and HEN-1 function in parallel to insulin, TGF-beta, and JNK MAPK signaling to regulate dauer formation.

Here, we describe a gain-of-function allele of *scd-2* that confers a pheromone-dependent Daf-c phenotype. This allele has facilitated epistasis experiments with Daf-d genes in pathways known to influence dauer formation to complement experiments previously performed with *scd-2* loss-of-function alleles and Daf-c genes. Analysis of *scd-2(syb2455);daf-3(e1376), scd-2(syb2455);daf-16(mu86),* and *scd-2(syb2455);kgb-1(km21)* double mutants revealed that all double mutant strains investigated had phenotypes intermediate of the two single mutants analyzed.

Experiments in which the expression of a DAF-3-dependent transcriptional reporter was found to be partially dependent on *scd-2* and *hen-1* had resulted in the conclusion that DAF-3 functioned downstream of SCD-2 (Reiner et al., 2008). However, in our work, we saw that *daf-3(e1376)* was only partially able to suppress the Daf-c phenotype of *scd-2(syb2455).* Although this could be consistent with DAF-3 sometimes functioning downstream of SCD-2, the incomplete suppression suggests that *scd-2* signaling functions at least partially in parallel to *daf-3* to promote dauer formation.

Although *daf-2(e1370)* was capable of fully suppressing the Daf-d phenotype of loss-of-function mutations in *scd-2*, I found *daf-16(mu86)* was not able to completely suppress the Daf-c phenotype of *scd-2(syb2455)* animals. This suggests that insulin-like signaling does not function to regulate dauer entry downstream of SCD-2, as is one possible explanation for the *daf-2* result. Instead, I found that *scd-2(syb2455);daf-16(mu86)* enter dauer at levels intermediate to the two single mutants on live food and levels closer to *scd-2(syb2455)* animals on heat-killed food. If

SCD-2 was truly functioning upstream of insulin signaling, we would expect *daf-16(mu86)* to suppress the Daf-c phenotype of *scd-2(syb2455).* As has been previously described, the animals containing a *daf-16(mu86)* mutation formed dauers morphologically distinct from both typical dauer or nondauer larvae. These animals are termed "partial dauers" and are hypothesized to be capable of making the decision to enter dauer but cannot execute proper entry into dauer (Vowels & Thomas, 1992).

Finally, I found that SCD-2 appears to function in parallel to the KGB-1 JNK MAPK pathway to regulate dauer formation. Because the dauer entry phenotype of *kgb-1(km21);scd-2(syb2455)* animals was intermediate of both single mutant phenotypes on both live and heat-killed food, we conclude that these genes regulate dauer formation independently of each other.

SCD-2 influences L1 arrest

Here we report that *scd-2(syb2455)* animals show reduced survival as arrested L1s than either wild-type or *scd-2(sa249)* animals. This result is somewhat surprising, as we might expect that if SCD-2 plays a role in shortening survival as arrested L1s, then *scd-2(sa249)* animals would have prolonged survival. It is possible that this result could be explained by the relative strength of the alleles; if *scd-2(syb2455)* causes a greater increase in SCD-2 activity than *scd-2(sa249)* causes a decrease, it is possible that *scd-2(sa249)* would be able to stull function within a physiological range. Nevertheless, *scd-2(sa249)* is predicted to be a kinase-null allele, and preliminary investigation of *scd-2(ok565)* deletion animals suggests that these animals also show wild-type survival when arrested as L1s (data courtesy of Brian Vassallo) (Reiner et al., 2008).

Previously, autophagy has been shown to be essential for survival as arrested L1s; animals with mutations causing either insufficient or excessive autophagy do not survive for as

long as arrested L1s as wild type animals do (Kang et al., 2007). Given our hypothesized role of SCD-2 in communicating unfavorable food conditions (see below), it is possible that autophagy is hyperactivated in the *scd-2(syb2455)* animals, resulting in their reduced survival in starvation conditions. A suppressor screen for this enhanced susceptibility to starvation has been performed by Brian Vassallo, and mutant alleles are being characterized. These alleles could reveal insight into how *scd-2* mediates L1 arrest survival and may also play roles in other SCD-2-dependent phenotypes.

SCD-2 is inhibited by food

In contrast to other Daf-c mutants such *daf-7* or *daf-2,* we saw that *scd-2(syb2455)* animals still required dauer pheromone for dauer entry; the dauer-formation phenotype of *scd-2(syb2455)* results specifically from an insensitivity to food conditions when making the dauer decision. We thus hypothesize that *scd-2(syb2455)* conveys a "bad food" or "no food" signal. In contrast to the *scd-2(syb2455)* animals, *C. elegans* with loss-of-function alleles in *scd-2* and *hen-1* do not form dauers under conditions where food is limited, suggesting that these animals may constitutively sense the presence of ingested food even in its absence. Given that loss-of-function alleles of *scd-2* mimic abundant food conditions and gain-of-function alleles of SCD-2 mimic limited food conditions, we propose that food inhibits SCD-2.

Our proposed role for *scd-2* in food sensing is consistent with an emerging role for ALK in regulating body weight. The implications of this correlation will be further discussed in Chapter Three.

Materials and Methods

***C. elegans* strains:** *C. elegans* was maintained on *E. coli* OP50 as previously described (Brenner, 1974). Constitutive dauer strains were grown at 16˚C. See Table 1 for a complete list of strains used in this study.

Design of potential *scd-2* gain-of-function alleles: We performed a protein alignment of *C. elegans* SCD-2 and human ALK in NCBI BLAST. Using a list of known oncogenic ALK mutations (Holla et al., 2017), we screened these residues for conservation or similarity between the *C. elegans* and human protein sequences. Genome editing was done by SunyBiotech using CRISPR technology.

Dauer assay: One-hundred microliters of pheromone mix (containing ascarosides ascr#2, ascr#3, ascr#5, and ascr#8 each at a concentration of 20 μM in 10% ethanol) was added to 3.5 cm plates (volume ~4 ml) made with Noble agar and without peptone, resulting in an effective plate concentration of 0.5 μM for each ascaroside. The plates were seeded with 40 μl of either a mix containing 99% heat-killed/1% live or 100% live *E. coli* OP50. The 1% live food was necessary in the heat-killed condition to prevent the worms from arresting development before reaching dauer (Qi et al., 2017). Gravid animals were bleached and ~100-150 eggs were dropped onto prepared plates. Plates were incubated for 72 hours at 25˚C, then dauer and nondauer animals were counted.

L1 arrest survival: Assays were performed as previously described (Kang et al., 2007). Gravid animals were bleached on day 0, and each day, ~50 arrested L1s were dropped onto 3 NGM plates seeded with *E. coli* OP50 for each genotype, and the number of L1s dropped was counted. Plates were scored 48-96 hours for survival to L4 or adulthood, and the fraction of the arrested L1s that were dropped onto the plate that survived to L4 was calculated. The average of the 3

replicates for each genotype and day was plotted. The assay was continued until no animals of any genotype survived to L4.

Strain Name	**Genotype**	**Source**
N2	Wild type	Caenorhabditis Genetics Center (CGC)
JT249	*scd-2(sa249)*	CGC
RB783	*scd-2(ok565)*	CGC
JC2154	*hen-1(tm501)*	CGC
PHX2455	*scd-2(syb2455[F1029L])*	This study/SunyBiotech/Chapter Three
PHX2522	*scd-2(syb2522[G1056E])*	This study/SunyBiotech
PHX3200	*scd-2(syb3200[F1029I])*	This study/SunyBiotech
PHX3201	*scd-2(syb3201[F1029S])*	This study/SunyBiotech
PHX3218	*scd-2(syb3218[Y1102C])*	This study/SunyBiotech
PHX3409	*scd-2(syb3409[K1132Q])*	This study/SunyBiotech
CB1376	*daf-3(e1376)*	Horvitz Lab
ZD2608	*daf-3(e1376);scd-2(syb2455)*	This study
CF1038	*daf-16(mu86)*	CGC
ZD2607	*daf-16(mu86);scd-2(syb2455)*	This study
KU21	*kgb-1(km21)*	CGC
ZD2609	*kgb-1(km21);scd-2(syb2455)*	This study

Table 1. *Caenorhabditis elegans* strains used in this study.

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Chapter Three

SCD-2/ALK regulates foraging behavior in response to nutrient conditions by activating a DAF-7 neuroendocrine positive-feedback loop in *C. elegans*

Abstract

The ability to detect and rapidly respond to changes in environmental conditions, such as limited food, is essential for organismal homeostasis and survival. Here, we show that the *C. elegans* ALK ortholog SCD-2 responds to fluctuations in levels of ingested food to influence feeding and foraging behaviors known as dwelling and roaming by modulating *daf-7* expression in the ASJ neurons. We observe that the ingestion of bacterial food inhibits *daf-7* expression in the ASJ neurons in a SCD-2-dependent manner, and an *scd*-2 gain-of-function mutant shows constitutive *daf-7* expression in the ASJ neurons independent of bacterial food conditions. This food- and *scd-2*-dependent expression of *daf-7* in the ASJ neurons drives roaming behavior. We propose that SCD-2-mediated *daf-7* expression functions in a positive-feedback loop to stabilize the roaming state in the continued absence of ingested food. This neuroendocrine-mediated positive-feedback loop facilitates rapid behavioral adaptations to constantly fluctuating food environments.

Introduction

Organisms must be able to both sense changes in their environment and respond accordingly to survive. Fluctuations in temperature or osmolarity or the appearance of a pathogen or predator can quickly present a life-or-death situation. The absence of nutritious food represents a stressor for most organisms, and numerous mechanisms exist to both detect (e.g., chemoreceptors) and respond to (e.g., autophagy and reduced protein translation) nutritional stress. Animals can additionally alter behavior in response to starvation to enhance survival. Food deprivation causes hyperactivity in *Drosophila melanogaster*, a seemingly paradoxical adaptation that increases the chance of a food encounter (Connolly, 1966; Yang et al., 2015).

Striped hamsters (*Cricetulus barabensis*) similarly increase foraging behavior at night when starved, coupled with enhanced rest during the day compared to hamsters fed *ad libitum* (Wen et al., 2018).

The roundworm *Caenorhabditis elegans* provides a simple model in which to study the sensing of and response to changing food environments. *C. elegans* are bacterivores that eat microbes growing on decomposing organic matter. In this context, animals encounter nutritious and pathogenic bacteria, as well as areas where bacterial food is absent, and have evolved numerous mechanisms to adapt to these variable conditions. Food deprivation is known to influence many aspects of *C. elegans* physiology, including behavior such as locomotion, mate-searching, and thermotaxis (Hedgecock & Russell, 1975; Lipton et al., 2004; Sawin et al., 2000).

On food, *C. elegans* engages in two-state foraging and feeding behavior; these states are known, respectively, as roaming and dwelling (Ben Arous et al., 2009; Fujiwara et al., 2002). Roaming animals travel quickly across a bacteria lawn and turn infrequently, whereas dwelling animals remain in a smaller area, with slower movement and more frequent turns. The relative time spent in each of these states is proportional to food abundance and quality; animals in more optimal food environments favor dwelling (Ben Arous et al., 2009). Genetic analysis has revealed several pathways that contribute to roaming and dwelling behaviors. Serotonin signaling promotes dwelling behavior in opposition to Pigment-Dispersing Factor (PDF) signaling, which promotes roaming (Flavell et al., 2013). The acid-sensing ion channels (ASICs) encoded by the genes *del-3* and *del-7* have also been implicated in feeding and foraging behaviors (Rhoades et al., 2019). These channels function in the NSM neurons to detect the presence of ingested food in the pharynx, and animals lacking these channels are more active than wild type. The *C. elegans* TGF-β signaling ligand encoded by *daf-7* has also been shown to

contribute to foraging behavior, with *daf-7* animals spending more time in the dwelling state than wild-type animals (Ben Arous et al., 2009).

daf-7 expression was previously thought to be restricted to the ASI neurons, with these neurons showing dynamic expression of *daf-7* in response to changing environmental conditions, such as crowding and food levels (Ren et al., 1996; Schackwitz et al., 1996). Work from our lab using both transcriptional GFP reporters and fluorescence *in situ* hybridization (FISH) has shown that in response to certain external or internal cues, such as on pathogenic *P. aeruginosa* PA14 or in males, *daf-7* expression is dynamically induced in the ASJ neurons (Hilbert & Kim, 2017; Meisel et al., 2014). Under conditions where we see *daf-7* expressed in the ASJ neurons, animals engage in exploratory behavior, such as mate-searching.

In Chapter Two, I characterized a gain-of-function allele of *scd-2, scd-2(syb2455). scd-2(syb2455)* animals enter dauer constitutively in the absence of heat-killed bacterial food, a factor that is necessary to induce dauer formation in wild-type animals (Dogra et al., 2022; Golden & Riddle, 1982). Despite their insensitivity to bacterial food conditions, these *scd-2(syb2455)* animals remain dependent on the presence of pheromone to enter dauer, suggesting that they are specifically defective in food sensing. In contrast, animals with loss-of-function alleles of *scd-2* are dauer-formation defective, and do not enter dauer in the presence of pheromone and heat-killed bacterial food. These phenotypes have motivated us to hypothesize that SCD-2 signaling might respond to the presence of bacterial food; specifically the ingestion of food inhibits SCD-2. Thus, animals with loss-of-function mutations in *scd-2* constitutively sense food through low SCD-2 activity even in the absence of food, while animals with a gain-of-function mutation in *scd-2* are insensitive to the inhibition of SCD-2 by food and constitutively sense a lack of food.

Given the role of *scd-2* in sensing food during development to regulate dauer entry described in Chapter Two, as well as the reported roles of ALK in thinness (Orthofer et al., 2020), we wanted to further examine the role of SCD-2 in regulating food-sensitive phenotypes. Here we examine the role of SCD-2 in the regulation of roaming behavior. We demonstrate that SCD-2 promotes roaming through the upregulation of *daf-7* expression in the ASJ neurons in a food-dependent manner. This SCD-2 dependent *daf-7* neuroendocrine response acts in a positive-feedback loop to allow for rapid behavioral state transitions in response to fluctuations in food conditions.

Results

SCD-2 promotes roaming behavior

After investigating the role of SCD-2 in sensing food conditions in the dauer developmental decision, we asked if *scd-2* could influence the food-dependent two-state feeding/foraging behavior known as roaming and dwelling. On nutritious food, wild-type animals spend most of their time dwelling, a behavior marked by a low movement speed and high body bending angle. As food conditions worsen, animals will decrease the fraction of time dwelling and increase the fraction of time roaming, a state marked by high speed and low curvature (Ben Arous et al., 2009). We observed that animals with loss-of-function mutations in *scd-2* dwelled more than wild-type animals, with *scd-2(sa249)* animals roaming 1% of the time and *scd-2(ok565)* animals roaming for 7% of the time, compared to 21% in wild type (Figure 1A). Animals with a loss-of-function allele of *hen-1*, the gene that encodes the ligand of SCD-2, also dwelled more than wild type, spending just 4% of time roaming (Figure 1A). When we probed the roaming and dwelling behavior of *scd-2(syb2455)* gain-of-function animals, we found

an increase in roaming behavior, with *scd-2(syb2455)* animals roaming for 47% of the time, compared to the 14% observed in wild-type animals (Figure 1B).

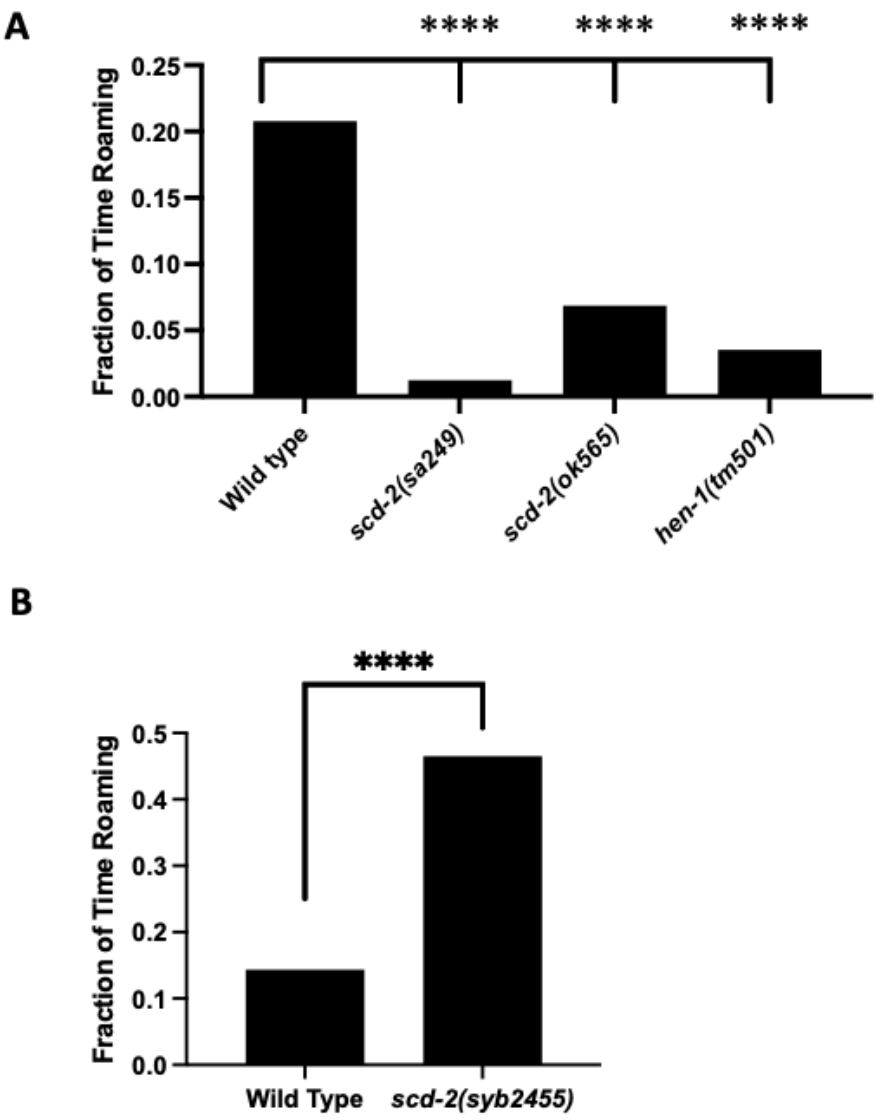


Figure 1. SCD-2 promotes roaming

A. Fraction of time spent in roaming state for wild type, *scd-2(sa249), scd-2(ok565)* and *hen-1(tm501)* animals. Wild type vs. *scd-2(sa249)* $\chi^2 = 1709.60$, $p<0.0001$, wild type vs. *scd-2(ok565)* $\chi^2 = 579.18$, $p<0.0001$, wild type vs. *hen-1(tm501)* $\chi^2 = 1176.25$, $p<0.0001$

B. Fraction of time spent in roaming state for wild type and *scd-2(syb2455)* animals.*)* $\chi^2 = 1284.49$, $p<0.0001$

SCD-2 influences *daf-7* expression in the ASJ neurons to alter roaming behavior

Our lab has previously reported that *daf-7* expression is induced in the ASJ chemosensory neurons in response to the pathogen *Pseudomonas aeruginosa* PA14 (Meisel et al., 2014). A screen for animals defective in upregulating *daf-7* in the ASJ neurons in response to PA14 recovered an allele of *hen-1.* Subsequent analysis has revealed that animals with loss-of-function mutations in *scd-2* are also defective in upregulating *daf-7* on PA14 (Figure 2A). When we assayed the *daf-7* expression phenotype of the *scd-2(syb2455)* gain-of-function animals, we saw that these animals constitutively express *daf-7* in the ASJ neurons on nonpathogenic *E. coli* OP50, a condition in which wild-type animals do not express *daf-7* in the ASJ neurons (Figure 2B).

Previous analysis of roaming and dwelling behavior has identified DAF-7 as a contributor to roaming behavior (Ben Arous et al., 2009). Since we see both increased roaming and increased *daf-7* expression in the ASJ neurons in the *scd-2(syb2455)* animals, we wondered if *daf-7* expression specifically in the ASJ neurons could contribute to roaming in these *scd-2(syb2455)* animals. Previous work from our lab has also linked *daf-7* expression in the ASJ neurons with general exploratory behavior such as male mate-searching and pathogen avoidance, further supporting our hypothesis that *daf-7* in the ASJ neurons could drive roaming (Hilbert & Kim, 2017; Meisel et al., 2014)

Consistent with previously reported findings, we found that *daf-7(e1372)* and *daf-7(ok3125)* animals dwelled more than wild type animals, spending just 9% and 5% of their time roaming, respectively, compared to 19% in wild type (Figure 3A) (Ben Arous et al., 2009). We next asked whether roaming behavior could be specifically influenced by *daf-7* expressed in the ASJ neurons. We found that in a *daf-7(ok3125)* background, rescue of *daf-7* cDNA under the

ASJ-specific *trx-1* promoter increased the fraction of time animals spent roaming from <1% seen in the non-transgenic *daf-7(ok3125)* controls to 5% or 7%, suggesting that *daf-7* expression in the ASJ neurons does indeed promote roaming (Figure 3B).

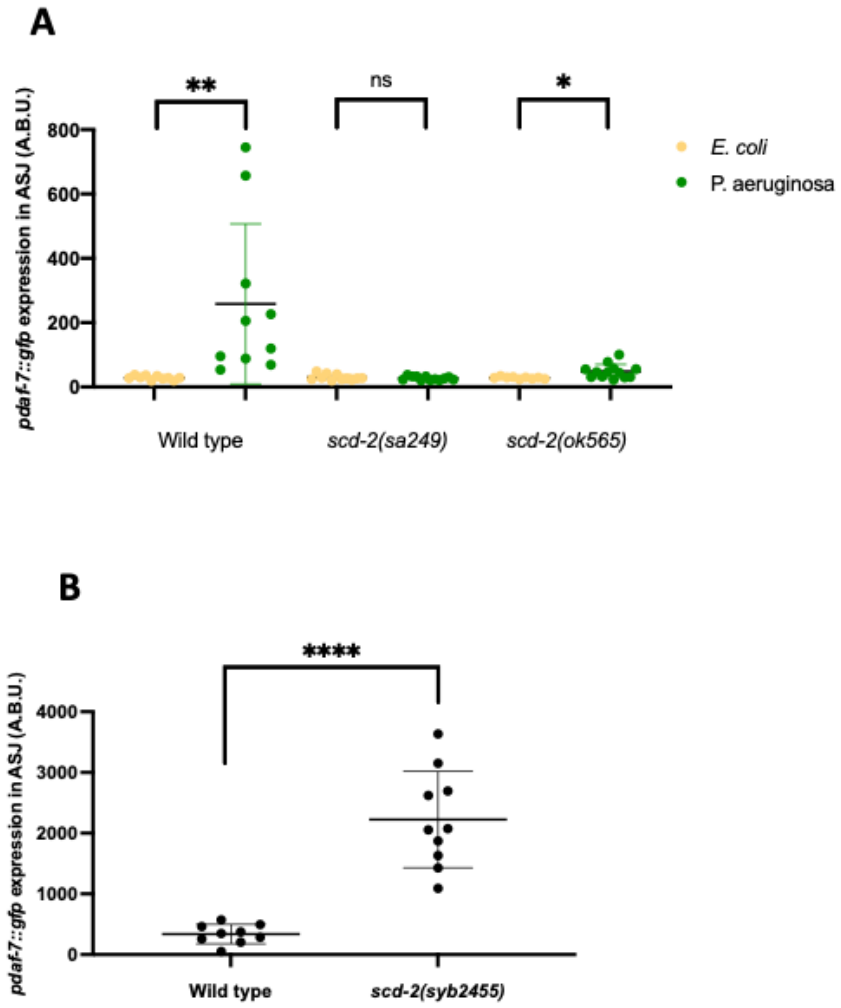


Figure 2. SCD-2 influences the expression of *daf-7* in the ASJ neurons

A. Expression of *pdaf-7::gfp* in ASJ in wild type, *scd-2(sa249),* or *scd-2(ok565)* animals on *E. coli* OP50 or *P. aeruginosa* PA14. **p<0.01, *p<0.05, ns not significant as determined by two-way t test. Error bars represent SD.

B. Expression of *pdaf-7::gfp* in ASJ in wild type or *scd-2(syb2455)* animals on *E. coli* OP50 **** p<0.0001 as determined by two-way t test. Error bars represent SD.

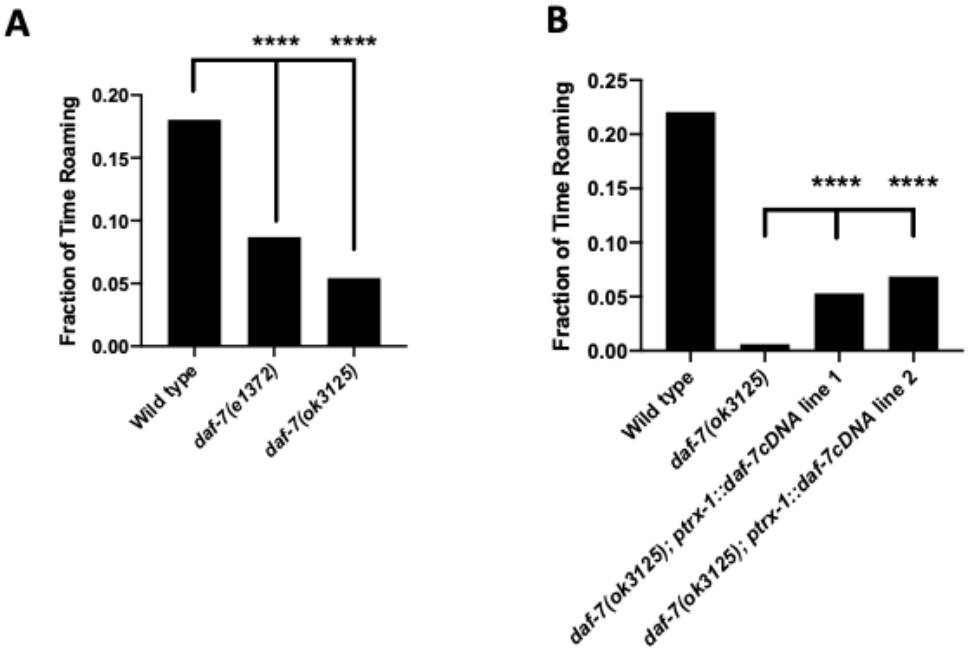


Figure 3. *daf-7* expression in the AJS neurons causes roaming.

A. Fraction of time spent in roaming state for wild type, *daf-7(e1372),* and *daf-7(ok3125)* animals. Wild type vs. *daf-7(e1372)* $\chi^2 = 29.89$, $p<0.0001$. Wild type vs. *daf-7(ok3125)* $\chi^2 = 191.31$, $p<0.0001$

B. Fraction of time spent in roaming state for wild type, *daf-7(ok3125),* and two independent lines with *daf-7* rescued in the ASJ neurons in a *daf-7(ok3125)* background. *daf-7(ok3125)* vs. rescue line 1 $\chi^2 = 119.99$, $p<0.0001$. *daf-7(ok3125)* vs. rescue line 2 $\chi^2 = 168.67$, $p<0.0001$

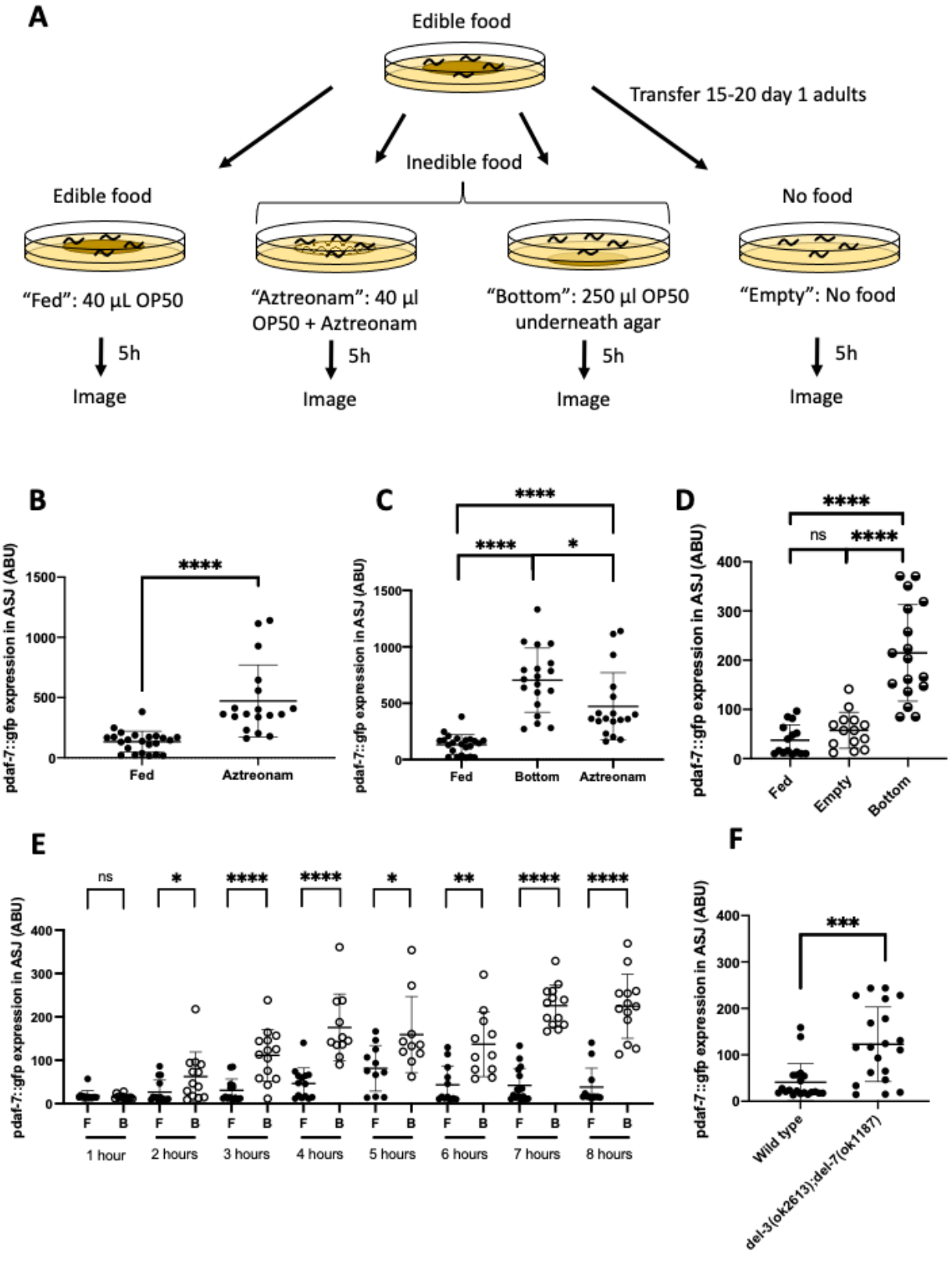
Ingested food inhibits *daf-7* expression in the ASJ neurons through SCD-2

Considering our observation that *daf-7* expression in the ASJ neurons promotes roaming, and that animals increase their time in the roaming state as food conditions worsen, we wondered if *daf-7* expression in the ASJ neurons is regulated by food. In contrast to the normal *E. coli* OP50 food source, which does not induce *daf-7* expression in the ASJ neurons, we observed that when animals were fed OP50 treated with aztreonam, an antibiotic that causes the bacteria to form inedible long strands (Gruninger et al., 2008), *C. elegans* express *daf-7* in both the ASI and ASJ neurons (Figures 4A and 4B). Given that *daf-7* is not expressed in the ASJ neurons when hermaphrodites are grown on edible food, we hypothesized that ingestion of food inhibits *daf-7* expression in the ASJ neurons. Consistent with this hypothesis, we also see *daf-7* expressed in the ASJ neurons when OP50 is seeded under the agar of the plates and thus cannot be eaten by *C. elegans* (Figures 4A and 4C). The *daf-7* induction that we see in the ASJ neurons on inedible food is consistent with the stark increase in roaming seen when animals are on aztreonam-treated food (Ben Arous et al., 2009). Interestingly, we do not see *daf-7* expression in the ASJ neurons in the complete absence of food, suggesting that an external food signal is required for the induction of *daf-7* in ASJ in the absence of ingested food (see Chapter Five) (Figures 4A and 4D).

We next asked whether the inhibition of *daf-7* expression in the ASJ neurons due to ingested food was caused by food sensed early after it was consumed or if there was a longer-term gut-to-ASJ signal at play. The kinetics of *daf-7* induction in the ASJ neurons when animals are moved from edible to inedible food showed that *daf-7* in the ASJ neurons is induced relatively quickly (~ 3 hours) after animals are removed from edible food (Figure 4E). Considering the time constant for GFP fluorophore formation of ~ 4 hours, this rapid

accumulation of signal suggests that the absence of ingested food is sensed almost immediately to influence *daf-7* transcription (Heim et al., 1994). We thus hypothesized that the ingestion cue is sensed early in the digestive tract, such as in the pharynx. As a complementary genetic approach, we examined the role of the ASICs encoded by *del*-3 and *del-7,* which are expressed in the minor neurite of the NSM neurons where they detect food in the pharynx, in inhibiting *daf-7* expression in the ASJ neurons on edible food (Rhoades et al., 2019). We saw that *del-3;del-7* animals express *daf-7* in the ASJ neurons on edible food, suggesting that these channels could be necessary for sensing the ingested food that typically inhibits *daf-7* expression (Figure 4F).

Given our proposed model in which ingested food signals through SCD-2 to regulate roaming via *daf-7* expression in the ASJ neurons, we hypothesized that *daf-7* expression in the ASJ neurons would not respond to changes in food conditions in *scd-2* mutant backgrounds. When we investigated the effects of *scd-2* signaling on food sensing, we found that animals with loss-of-function alleles of either *scd-2* or *hen-1* were defective in upregulating *daf-7* in the ASJ neurons in response to inedible food (Figure 4G and 4H). In contrast, as described above, we found that *scd-2(syb2455)* animals constitutively expressed *daf-7* in ASJ even in the presence of ingested food (Figure 4H). We saw a minimal change in *daf-7* expression in the ASJ neurons when animals are moved from edible food to inedible food (wild type inedible food:edible food = 8.14, *scd-2(syb2455)* inedible food:edible food = 1.72) (Figure 4H). The insensitivity of *scd-2(syb2455)* to the ingested food signal is consistent with a role for SCD-2 in mediating the upregulation of *daf-7* expression in the ASJ neurons in the absence of ingested bacterial food.


A
Edible food
Transfer 15-20 day 1 adults
Inedible food
Edible food
No food
"Fed": 40 µL OP50
"Aztreonam": 40 µl OP50 + Aztreonam
"Bottom": 250 µl OP50 underneath agar
"Empty": No food
5h
Image
B
pdaf-7::gfp expression in ASJ (ABU)
****
Fed
Aztreonam
C
****
*
Fed
Bottom
Aztreonam
D
ns
Fed
Empty
Bottom
E
ns
*
1 hour
2 hours
3 hours
4 hours
5 hours
6 hours
7 hours
8 hours
F
***
Wild type
del-3(ok2613);del-7(ok1187)

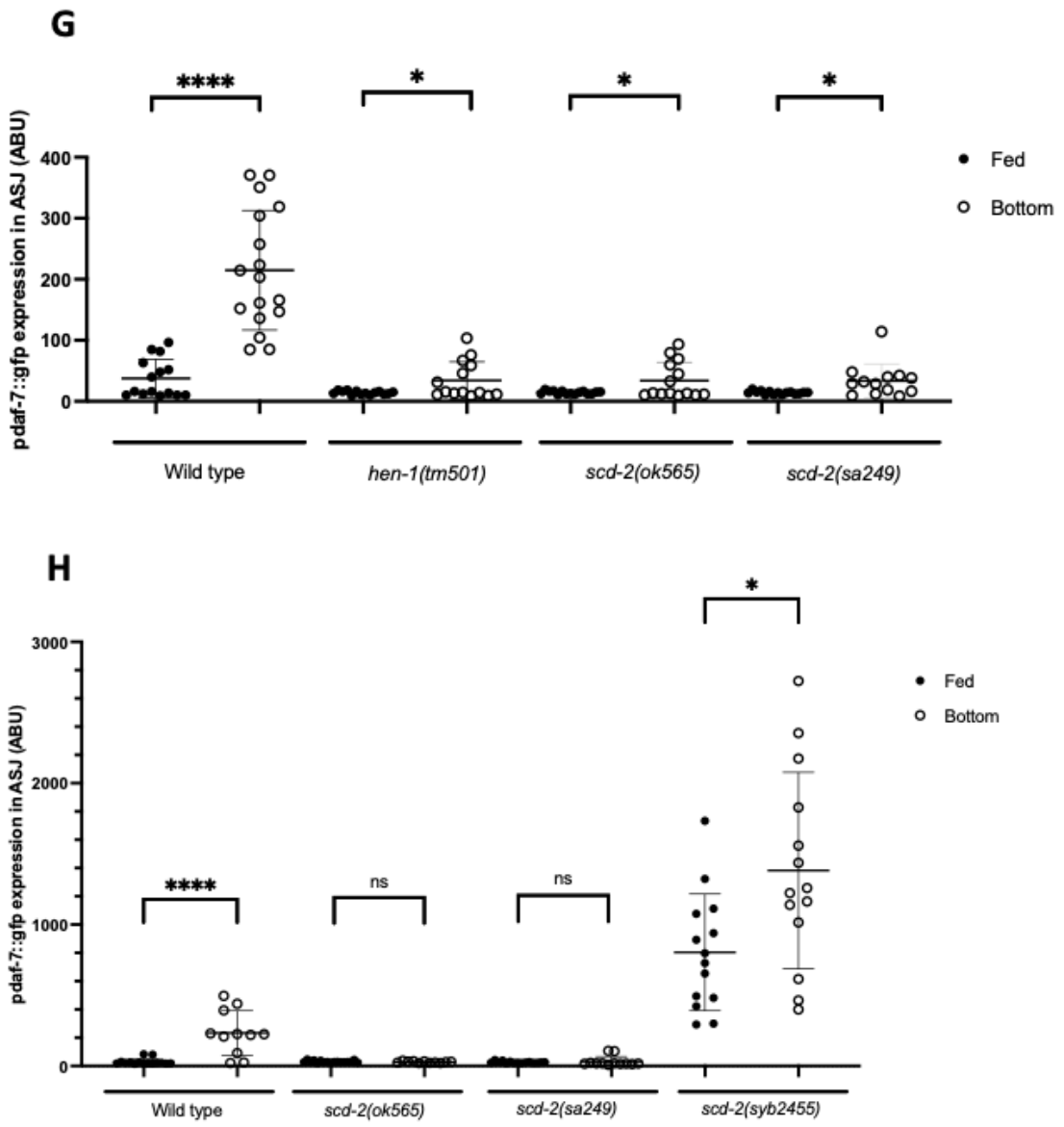


Figure 4. Ingestion of food inhibits *daf-7* expression in ASJ through SCD-2.

A. A diagram of food conditions used in these assays

B. Expression of *pdaf-7::gfp* in ASJ in animals fed untreated OP50 ("Fed") and aztreonam-treated OP50 ("Aztreonam). ****p<0.0001 as determined by two-way t-test. Error bars represent SD

C. Expression of *pdaf-7::gfp* in ASJ in animals fed untreated OP50 ("Fed"), on plates where untreated OP50 was seeded under the agar ("Bottom"), and animals fed aztreonam-

treated OP50 (“Aztreonam). ****p<0.0001, *p<0.05 as determined by two-way t-test. Error bars represent SD

D. Expression of *pdaf-7::gfp* in ASJ in animals fed OP50 (“Fed”), on plates with no food (“Empty”), or on plates where OP50 was seeded under the agar (“Bottom”). ****p<0.0001, ns not significant as determined by two-way t-test. Error bars represent SD.

E. Expression of *pdaf-7::gfp* in ASJ in animals 1, 2, 3, 4, 5, 6, 7, or 8 hours after being moved from growth plates containing edible OP50. Animals were moved at times so that all were 72 hours post egg-drop at time of imaging. “F” refers to fed animals on plate conditions with untreated OP50, and “B” refers to animals transferred to plates with OP50 seeded on the bottom. ****p<0.0001, ** p<0.01 *p<0.05, ns not significant as determined by two-way t-test. Error bars represent SD

F. Expression of *pdaf-7::gfp* in ASJ in wild type animals or *del-3(ok2613);del-7(ok1187)* animals on edible food. ***p<0.001 as determined by two-way t-test. Error bars represent SD

G. Expression of *pdaf-7::gfp* in ASJ in wild type, *scd-2(sa249), scd-2(ok565)*, or *hen-1(tm501)* on OP50 (“Fed”) or plates with OP50 seeded underneath the agar (“Bottom”). Error bars represent SD. ****p<0.0001, *p<0.05 as determined by two-way t test. Error bars represent SD.

H. Expression of *pdaf-7::gfp* in ASJ in wild type, *scd-2(ok565), scd-2(sa249)*, or *scd-2(syb2455)* on edible OP50 (“Fed”) or plates with OP50 seeded underneath the agar (“Bottom”). ****p<0.0001, *p<0.05, ns not significant as determined by two-way t-test. Error bars represent SD

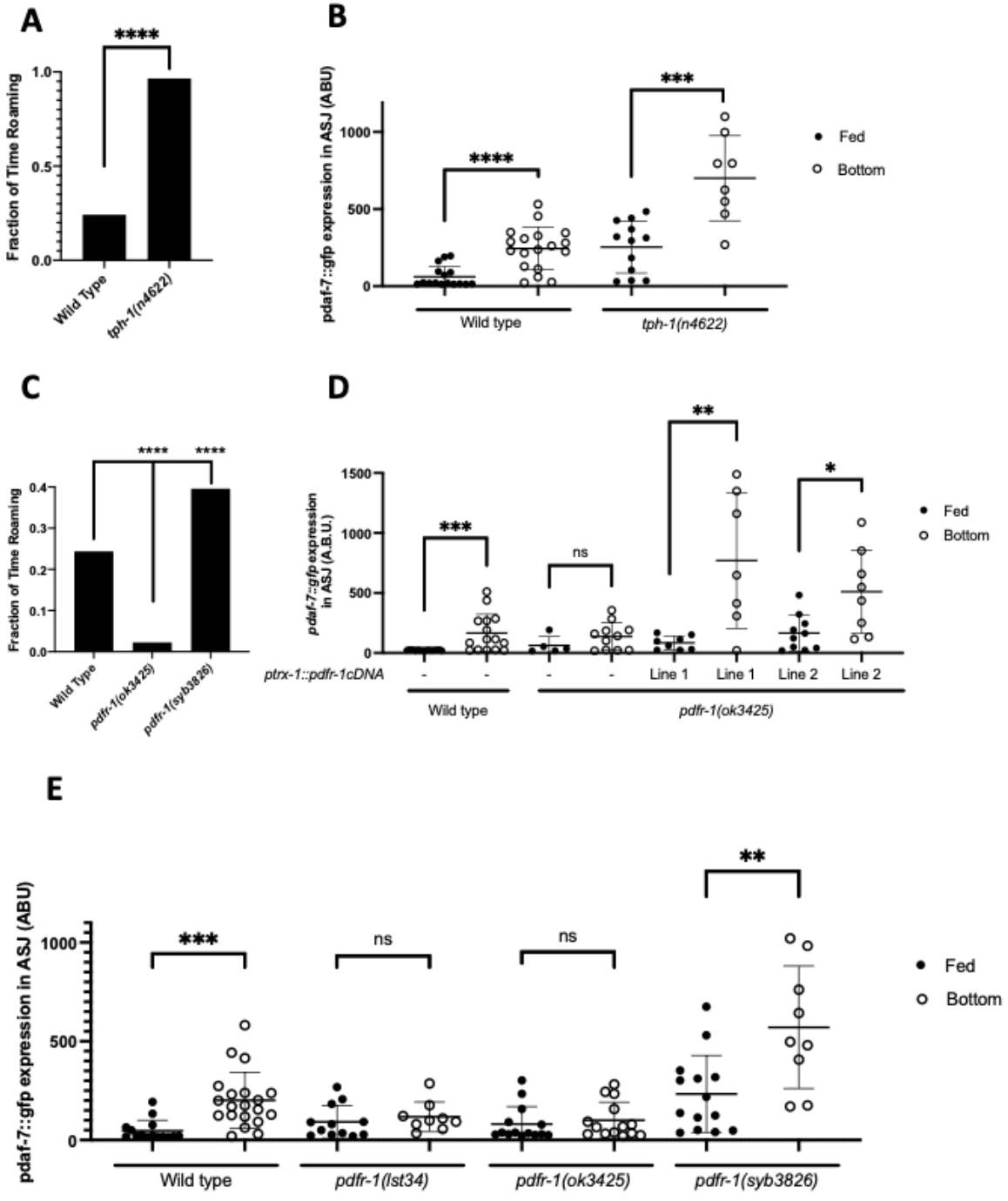
Roaming behavior and *daf-7* expression in the ASJ neurons are regulated by common factors.

The identification of the role of *daf-7* expression in the ASJ neurons in promoting roaming behavior led us to question if other known conditions that affect roaming and dwelling do so by modulating *daf-7* expression in the ASJ neurons. Conversely, we wondered if under conditions where *daf-7* expression is induced in the ASJ neurons, animals roam more. *tph-1,* the gene encoding the enzyme that catalyzes the rate-limiting step of serotonin biosynthesis, has previously been reported to contribute to dwelling behavior, with *tph-1* mutants spending 97% of their time roaming, compared to 24% seen in wild type (Figure 5A) (Flavell et al., 2013). Consistent with the correlation between *daf-7* expression in the ASJ neurons and roaming, we see that *tph-1(n4622)* animals constitutively express *daf-7* in the ASJ neurons regardless of their food environment (Figure 5B). Nevertheless, this increased *daf-7* expression is still able to be modulated in response to internal food cues, suggesting that serotonin signaling functions in parallel to the ingested food signal to influence *daf-7* expression.

The Pigment-Dispersing Factor (PDF) receptor PDFR-1 and its ligands PDF-1 and PDF-2 have also been previously shown to modulate roaming and dwelling behavior, with animals with loss-of-function mutations in *pdfr-1* or *pdf-1* and *pdf-2* showing a greater fraction of their time dwelling than wild-type animals (Figure 5C) (Flavell et al., 2013). We observed that *pdfr-1(ok3425)* animals spend just 3% of their time roaming, compared to 24% in wild type. From a screen done in parallel to this work and described in Chapter Four of this thesis, we recovered an allele of *pdfr-1* with a Ser325Phe substitution, which likely is gain-of-function. I observed that *pdfr-1(syb3826)* animals increase the fraction of their time roaming compared to wild-type animals, spending 40% of their time in the foraging state (Figure 5C).

Animals with loss-of-function mutations in *pdfr-1* do not show a *daf-7* transcriptional response in the ASJ neurons to changing food conditions (Figures 5D and 5E). Expression the *pdfr-1* cDNA exclusively in the ASJ neurons in a *pdfr-1(ok3425)* background rescued the sensitivity of *daf-7* expression in the ASJ neurons to changing food cues, suggesting the site-of-action of PDFR-1 is in the ASJ neurons (Figure 5D). In contrast, *pdfr-1(syb3826)* gain-of-function animals constitutively show expression of *daf-7* in the ASJ neurons when fed edible OP50, and this expression is further upregulated on inedible food, suggesting that *pdfr-1* also regulates *daf-7* expression in ASJ and roaming in parallel to the ingested food cue (Figure 5E).

Additionally, we saw that under other conditions where *daf-7* is expressed in the ASJ neurons, such as in males, roaming was also increased. Males, which as well-fed adults constitutively express *daf-7* in the ASJ neurons, spent 86 % of their time roaming, compared to 21% in hermaphrodites (Figure 5F).


A
****
Fraction of Time Roaming
1.0
0.5
0.0
Wild Type
tph-1(n4622)
B
***
****
pdaf-7::gfp expression in ASJ (ABU)
1000
500
0
Fed
Bottom
Wild type
tph-1(n4622)
C
****
****
Fraction of Time Roaming
0.4
0.3
0.2
0.1
0.0
Wild Type
pdfr-1(ok3425)
pdfr-1(syb3826)
D
**
*
***
ns
pdaf-7::gfp expression in ASJ (A.B.U.)
1500
1000
500
0
Fed
Bottom
ptrx-1::pdfr-1cDNA
-
-
-
-
Line 1
Line 1
Line 2
Line 2
Wild type
pdfr-1(ok3425)
E
**
***
ns
ns
pdaf-7::gfp expression in ASJ (ABU)
1000
500
0
Fed
Bottom
Wild type
pdfr-1(lst34)
pdfr-1(ok3425)
pdfr-1(syb3826)

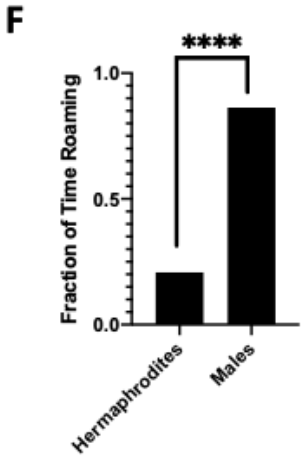


Figure 5. *daf-7* expression in the ASJ neurons and roaming behavior share common regulators.

A. Fraction of time spent in roaming state for wild type and *tph-1(n4622)* animals. χ^2 = 4575.48, p<0.0001

B. Expression of *pdaf-7::gfp* in the ASJ neurons of wild type and *tph(n4622)* animals on edible OP50 ("Fed") or plates with OP50 seeded underneath the agar ("Bottom"). ****p<0.0001, ***p<0.001, as determined by two-way t-test. Error bars represent SD

C. Fraction of time spent in roaming state for wild type, *pdfr-1(ok3125)*, and *pdfr-1(syb3826)* animals. Wild type vs. *pdfr-1(ok3425)* χ^2 = 2278.24, p<0.0001, Wild type vs. *pdfr-1(syb3826)* χ^2 = 484.47, p<0.0001

D. Expression of *pdaf-7::gfp* in the ASJ neurons of wild type, *pdfr-1(lst34), pdfr-1(ok3425),* or *pdfr-1(syb3826)* animals on edible OP50 ("Fed") or plates with OP50 seeded underneath the agar ("Bottom"). ****p<0.0001, **p<0.01, *p<0.05, ns not significant as determined by two-way t-test. Error bars represent SD

E. Expression of *pdaf-7::gfp* in the ASJ neurons in wild type, *pdfr-1(ok3425),* and two independent ASJ-specific *pdfr-1* cDNA rescue lines on edible OP50 ("Fed") or plates

with OP50 seeded underneath the agar ("Bottom"). ***$p<0.001$, **$p<0.01$, *$p<0.05$, ns not significant as determined by two-way t-test. Error bars represent SD.

F. Fraction of time spent in roaming state for wild type hermaphrodites and males. $\chi^2 = 4926.86$, $p<0.0001$

Discussion

SCD-2 is inhibited by ingested food

Here, we report a role for the *C. elegans* ALK ortholog, SCD-2, in promoting roaming behavior in response to changing food conditions. The results in this chapter, in conjunction with the data presented in Chapter Two, show that animals with loss-of-function mutations in *scd-2* mimic the constitutive presence of ingested food, while animals with a gain-of-function mutation in *scd-2* sense the constitutive absence of ingested food. These observations led us to model in which SCD-2 is inhibited by ingested food. Animals with loss-of-function mutations in *scd-2* interpret the low SCD-2 activity as an ingested food signal, which explains the Daf-d phenotype, increased dwelling, and lack of *daf-7* expression in the ASJ neurons on inedible food. In contrast, in *scd-2(syb2455)* animals, SCD-2 is constitutively active, as it would be in the absence of ingested food. This is consistent with the Daf-c phenotype, increased roaming, and constitutive expression of *daf-7* in the ASJ neurons on edible food seen in *scd-2(syb2455)* animals.

Bacterial food ingestion regulates roaming and dwelling behavior through SCD-2 signaling and *daf-7* expression in the ASJ neurons

The role for *daf-7* expression in ASJ in promoting roaming behavior was suggested by a correlation between conditions in which *daf-7* expression is induced in the ASJ neurons and an increase in exploratory behavior, as well as a previously reported observation that *daf-7* mutants dwell more than wild-type animals (Ben Arous et al., 2009; Hilbert & Kim, 2017). Here, we additionally demonstrate that the presence of a transgene where expression of the *daf-7* cDNA is driven by the ASJ-specific *trx-1* promoter increases roaming in the *daf-7(ok3125)* genetic background, suggesting a causal relationship between *daf-7* expression in the ASJ neurons and

roaming. However, the fact that roaming is not fully restored to wild-type levels when *daf-7* expression is restricted to the ASJ neurons suggests that *daf-7* produced in other cells likely also contributes to roaming.

Taken together, the data in this chapter support a model in which bacterial food ingestion regulates roaming and dwelling behavior via SCD-2 and *daf-7* expression in the ASJ neurons. When animals are on abundant edible food, the ingestion of this food inhibits SCD-2, which results in no *daf-7* expression in the ASJ neurons, and no *daf-7*-driven roaming, so the animals dwell. In contrast, when animals are removed from edible food, SCD-2 is no longer inhibited, resulting in the upregulation of *daf-7* expression in the ASJ neurons, which drives roaming (Figure 6).

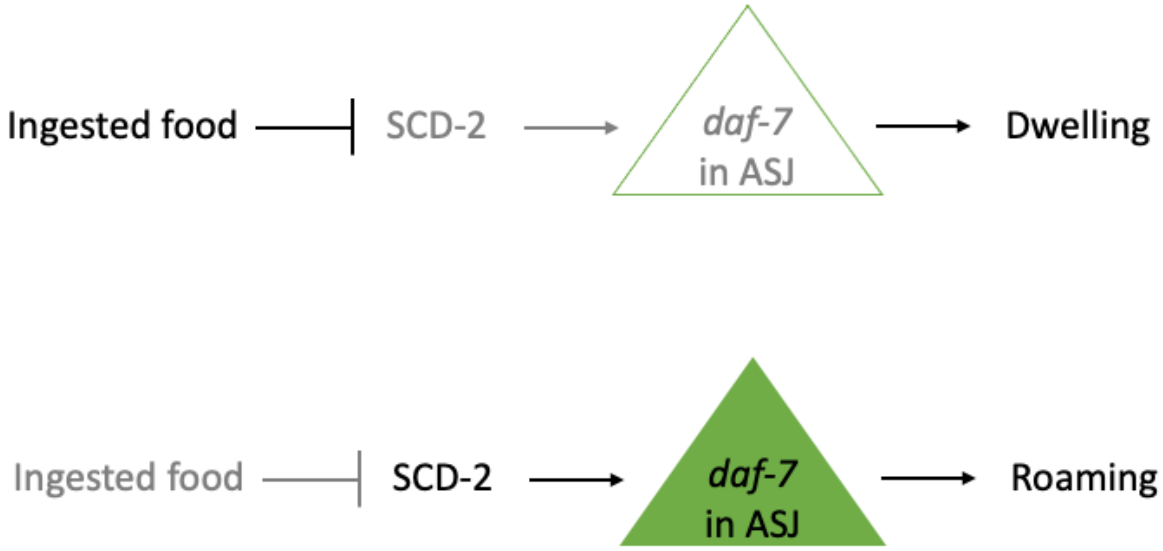


Figure 6. SCD-2 responds to ingested food to influence *daf-7* expression in the ASJ neurons and roaming and dwelling. A model for how ingested food signals through SCD-2 and DAF-7 to regulate roaming and dwelling. In the presence of ingested food, SCD-2 is inhibited, so *daf-7* is not expressed in the ASJ neurons and animals dwell. In the absence of ingested food, active SCD-2 upregulates *daf-7* expression in the ASJ neurons, which promotes roaming.

Dynamic SCD-2 mediated *daf-7* expression in the ASJ neurons creates a positive-feedback loop that facilitates rapid transitions between roaming and dwelling states.

The change in *daf-7* expression levels in the ASJ neurons upon exposure to different food cues and the relationship between *daf-7* expression in the ASJ neurons and roaming create a regulatory paradigm where an animal can sense and quickly adapt its behavior to changing food environments. *C. elegans* that are not currently eating but can sense food in the vicinity, and thus express *daf-7* in the ASJ neurons, would logically induce roaming behavior to facilitate finding this food; however, an animal actively ingesting food, resulting in the inhibition of *daf-7* expression in the ASJ neurons, would want to remain dwelling in its current food environment.

In the absence of ingested food, an external food cue (further characterized in Chapter Five) is still necessary to induce the expression of *daf-7* in the ASJ neurons, possibly because it is less risky to forage for food when there are cues that there is food to be found in the vicinity. Well-fed animals are exposed to both this external food cue that induces *daf-7* expression in the ASJ neurons as well as the ingestion cue that inhibits *daf-7* expression in the ASJ neurons, yet the *daf-7* expression pattern of these animals is restricted to the ASI neurons. We propose that there is a hierarchy within these opposing stimuli: the inhibitory ingested signal is stronger than the soluble food cue, so when animals are exposed to both cues, *daf-7* is not expressed in the ASJ neurons. In this case, the ingestion of food suppresses any inclination toward roaming, as it is more advantageous for an animal on edible food to stay and eat.

The observed effects of food environment on *daf-7* expression in the ASJ neurons via SCD-2 and the influence of DAF-7 on roaming behavior have led us to propose a positive-feedback loop where DAF-7 stabilizes the roaming state as animals navigate their surroundings (Figure 7). As animals in a patchy food environment encounter a region with less edible food,

SCD-2 is no longer inhibited, and *daf-7* expression is induced in the ASJ neurons due to loss of the inhibitory signal from SCD-2. This increase in *daf-7* briefly promotes roaming behavior. As animals roam, they ingest less bacteria, further reducing inhibition of SCD-2 and promoting *daf-7* expression in the ASJ neurons, further stabilizing roaming. This positive feedback loop allows for a more rapid response to local environments with less or no food. Once a roaming animal encounters edible food and begins to eat, SCD-2 is inhibited by food ingestion, resulting in a downregulation of *daf-7* expression in the ASJ neurons, promoting a dwelling state in which the animal can continue to eat. Further food ingestion further inhibits SCD-2 and reduces *daf-7* expression in the ASJ neurons, stabilizing this dwelling state. Our proposed feedback loop is consistent with the previously reported observation that *daf-7* animals are defective in leaving a lawn of depleting food (Milward et al., 2011), since there would be no induction of *daf-7* in the ASJ neurons as less food is consumed to promote roaming and lawn leaving.

Although all experimental evidence we have at this point supports this model, a few key additional experiments could increase our confidence. First, I am in the process of investigating the effect of an ASJ neuron-specific *daf-7* knockout strain, generated by a floxed *daf-7* and ASJ-specific *ptrx-1::cre* transgene. The increased roaming seen in the ASJ-specific *daf-7* cDNA rescue in the *daf-7(ok3125)* background supports the idea that *daf-7* expression from the ASJ neurons contributes to roaming. However, the *ptrx-1::daf-7* cDNA transgene is likely not expressing *daf-7* at its endogenous levels. If this ASJ-specific *daf-7* knockout shows an increased dwelling phenotype relative to wild type, then this would support our model.

Second, our model predicts that *daf-7* mutants would have both longer dwelling states and shorter roaming states than wild-type animals. In the absence of *daf-7,* dwelling animals that suddenly encounter a region with less food would not increase their *daf-7* transcription levels in

response, and the resulting cascade of more roaming, less eating, and more *daf-7* would not follow. There would thus likely take a stronger stimulus to promote a transition to roaming, resulting in prolonged dwelling states. In contrast, when *daf-7* animals are roaming, their decreased food consumption would not trigger an increase in *daf-7* levels to stabilize the roaming state. Thus, these animals could more easily transition back to dwelling. Analyzing the state durations in these animals could provide further evidence in support of the positive-feedback regulation.

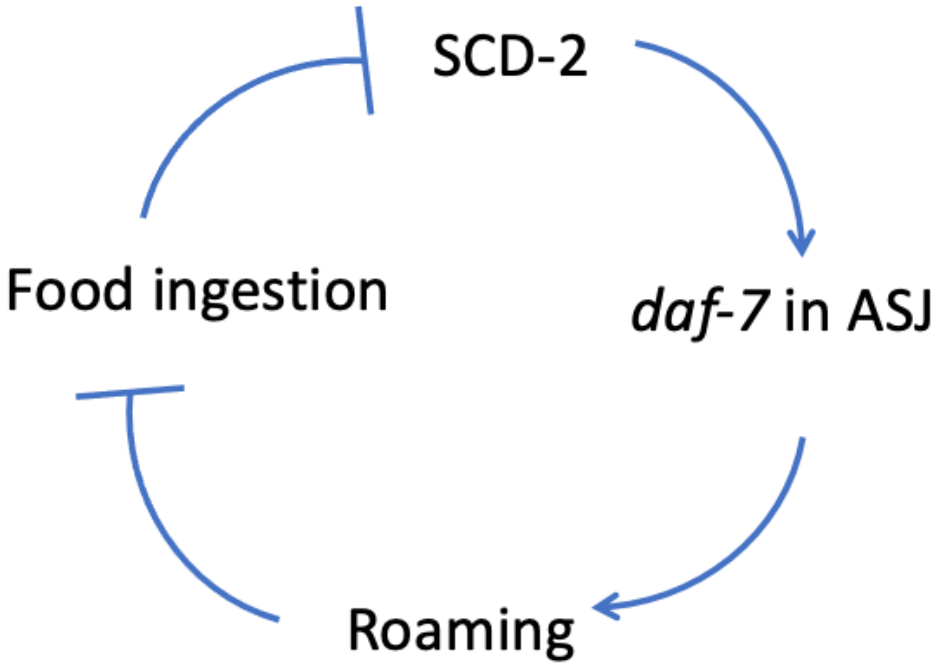


Figure 7. SCD-2-mediated *daf-7* expression in the ASJ neurons stabilizes the roaming state via a positive-feedback loop. A model by which *daf-7* expression in the ASJ neurons increases roaming, increased roaming results in less food ingested, less food ingested results in less inhibition of SCD-2, and SCD-2 promotes more *daf-7* expression in the ASJ neurons, representing a positive-feedback loop.

Roaming behavior and *daf-7* expression in the ASJ neurons are influenced by common factors

tph-1 and *pdfr-1* have previously been reported to influence roaming and dwelling behavior (Flavell et al., 2013). We have observed that in genetic backgrounds where roaming is favored, such as *tph-1* loss-of-function or *pdfr-1* gain-of-function animals, *daf-7* expression is induced in the ASJ neurons. The *daf-7* upregulation in these animals, especially *tph-1(n4622)* mutants, is relatively modest compared to the increase in roaming behavior, suggesting that TPH-1 and PDFR-1 do not regulate roaming and dwelling exclusively through modulation of *daf-7* expression in the ASJ neurons. Conversely, induced *daf-7* expression in the ASJ neurons of these animals could result from the fact that they roam more than wild-type animals and thus are ingesting less food. Furthermore, the increased *daf-7* expression in the ASJ neurons in *tph-1(n4622)* and *pdfr-1(syb3826)* animals is still sensitive to inhibition from ingested food, suggesting that these genes function at least partially in parallel to the sensing of ingested food to modulate *daf-7* expression in the ASJ neurons.

The observation of increased roaming under conditions where *daf-7* is expressed in the ASJ neurons, such as in males, further solidifies the correlation between *daf-7* expression in the ASJ neurons and roaming. However, whether the increased *daf-7* expression in the ASJ neurons is entirely responsible for the increase in roaming seen under these conditions is not known. It is likely that in addition to its causal role in increasing roaming, the expression of *daf-7* in the ASJ neurons also serves as a marker for a roaming-prone state in which other genetic factors additionally drive roaming behavior. Sexually dimorphic expression of *daf-7* in the ASJ neurons will be further discussed in Chapter Four.

Defining the SCD-2 site of action

At present, our analysis of the role of *scd-2* in food perception and foraging behavior lacks insight into its site of action. Cloning of the *scd-2* cDNA proved challenging and hindered efforts for cell- and tissue-specific rescue experiments. As an alternative approach, I am currently generating several cell- and tissue-specific *scd-2* knockout lines using a floxed allele of *scd-2* and crossing in different transgenes with Cre driven by various promoters. I am initially looking into neurons where SCD-2 was initially reported to function in its roles in sensory integration and associative learning, such as the NSM and AIA neurons, in addition to pan-neural and pan-sensory-neural promoters (Shinkai et al., 2011; Wolfe et al., 2019). We hope that by elucidating where SCD-2 is functioning, we will gain mechanistic insight into how food is sensed.

Further examination of the role of HEN-1, the ligand of SCD-2, in food sensing and promoting roaming behavior could also provide mechanistic insight into the regulation of roaming and dwelling in response to changing food environments. HEN-1 expression has been demonstrated to be restricted to the ASE and AIY neurons, pointing to a potential role for these neurons in sensing ingested food to regulate HEN-1 upstream of SCD-2 (Ishihara et al., 2002).

A role for ALK/SCD-2 in thinness

The role of SCD-2 in regulating the physiological response to ingested food in *C. elegans* is consistent with the growing body of work tying ALK and its orthologs to thinness and metabolic phenotypes in humans, mice and *Drosophila* (Cheng et al., 2011; Orthofer et al., 2020; Woodling et al., 2020). Our model in which SCD-2 is inhibited by ingested food is consistent with various phenotypes seen in *ALK*$^{-/-}$ mice and *Alk* RNAi *Drosophila*, including increased

energy expenditure and reduced triglyceride levels (Orthofer et al., 2020). If reduced ALK activity in mice and *Drosophila* is typically associated with ingested food, as it appears to be in *C. elegans*, these *ALK*$^{-/-}$ mice and *Alk* RNAi *Drosophila* might behave like animals that are constantly eating and engage in compensatory mechanisms of increased energy expenditure and triglyceride metabolism to maintain body-weight homeostasis. Further characterization of the mechanism by with SCD-2 and ALK regulate food-sensing and metabolism could reveal new factors governing body weight and have implications in the treatment and prevention of obesity.

Materials and Methods

***C. elegans* strains:** *C. elegans* was maintained on E. coli OP50 as previously described (Brenner, 1974). Daf-c strains were grown at 16˚C. See Table 1 for a complete list of strains used in this study.

Preparation of food condition plates: Unless otherwise indicated, all assays were performed on NGM plates with no peptone. Aztreonam-treated bacteria was prepared as previously reported (Gruninger et al., 2008), and 40 µL aztreonam-treated food was added to plates containing 10 µg/mL aztreonam. “Fed” plates for *daf-7* quantification were seeded with 40 µL OP50 grown overnight in a shaking LB culture at 37˚C, and “Bottom” plates were seeded with 250 µL OP50, and the agar was inverted with a spatula immediately prior to adding animals. PA14 was grown as previously described and seeded onto SKA plates, as were *E. coli* OP50 controls for these experiments (Meisel et al., 2014).

***pdaf-7::gfp* quantification assays:** Plates of gravid animals were bleached and eggs were dropped onto NGM plates seeded with OP50 and grown at 20˚C for 67 hours, unless otherwise

noted. On the day of imaging, 15-20 day 1 adult animals were transferred by picking to assay plates, where they were incubated for 5 hours at 20˚C.

Animals to be imaged were mounted on glass slides with agarose pads and 50 mM sodium azide or 5 mM Levamisole. All imaging for pictures were conducted on the Zeiss Axioimager Z1. Quantification of GFP brightness was derived from maximum fluorescence values within the ASJ neurons in FIJI.

Statistical analysis was performed in Prism.

Roaming/dwelling assay: Animals were egg-laid and grown at 20˚C for 72 hours. 10 cm NGM plates without peptone were seeded with 2mL stationary phase *E. coli* OP50 grown overnight in LB. Assays were performed with ~20 animals inside a 6 cm copper ring placed in the center of the seeded plate and recorded at 3.75 frames per second for 90 minutes. Videos were analyzed using MBF Biosciences WormLab software. Measurements of speed and bending angle were averaged over 10 second intervals, and values for each 10-second interval were plotted on a scatter plot of speed vs. bending angle. Quantification of fraction of time spent roaming or dwelling was done by segregating the points of the scatter plots by a horizontal line whose placement was based on the distribution of points in the control condition for each experiment. Values for this speed cutoff ranged from y=7 to y=12. Points falling above the line were classified as roaming, and those below the line were classified as dwelling. Statistical significance was determined by χ^2 analysis of the distribution of points classified as roaming or dwelling between genotypes or conditions.

Strain Name	Genotype	Source
N2	Wild type	Caenorhabditis Genetics Center (CGC)
JT249	*scd-2(sa249)*	CGC
RB783	*scd-2(ok565)*	CGC
JC2154	*hen-1(tm501)*	CGC
PHX2455	*scd-2(syb2455)*	This study/Chapter Two/SunyBiotech
FK181	*ksIs2[pdaf-7::gfp; rol-6(su1006)]*	CGC
ZD2540	*ksIs2; scd-2(sa249)*	This study
ZD930	*ksIs2; scd-2(ok565)*	This study
ZD918	*ksIs2; hen-1(tm501)*	This study
ZD2605	*ksIs2; scd-2(syb2455)*	This study
CB1372	*daf-7(e1372)*	CGC
ZD715	*daf-7(ok3125)*	(Meisel et al., 2014)
ZD695	*daf-7(ok3125);qdEx34[ptrx-1::daf-7;pges-1::GFP]*	(Meisel et al., 2014)
ZD696	*daf-7(ok3125);qdEx35[ptrx-1::daf-7;pges-1::GFP]*	(Meisel et al., 2014)
ZD2632	*ksIs2; del-3(ok2613);del-7(ok1187)*	This study
MT14984	*tph-1(n4622)*	Horvitz Lab
ZD667	*ksIs2; tph-1(n4622)*	This study
ZD2079	*pdfr-1(ok3425)*	(Hilbert & Kim, 2018)
PHX3826	*pdfr-1(syb3826)*	This study/Chapter Four/SunyBiotech
ZD1852	*ksIs2; pdfr-1(lst34); him-5(e1490)*	(Hilbert & Kim, 2018)
ZD1987	*ksIs2; pdfr-1(ok3425); him-5(e1490)*	(Hilbert & Kim, 2018)
ZD2633	*ksIs2; pdfr-1(syb3826); him-5(e1490)*	This study
ZD2083	*ksIs2; pdfr-1(ok3425); him-5(e1490); qdEx[ptrx-1::pdfr-1(B)::2A::mCherry +pofm-1::GFP] #1*	(Hilbert & Kim, 2018)
ZD2084	*ksIs2; pdfr-1(ok3425); him-5(e1490); qdEx[ptrx-1::pdfr-1(B)::2A::mCherry +pofm-1::GFP] #2*	(Hilbert & Kim, 2018)

Table 1. Complete list of *C. elegans* strains used in this study

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Chapter Four

Genetic analysis of determinants of sexually dimorphic *daf-7* expression in the ASJ neurons of *C. elegans* males

Abstract

Communication along the gut-brain axis is essential for controlling food intake in response to changing nutritional state. In the roundworm *Caenorhabditis elegans,* information about nutritional state is communicated from the intestine to the ASJ neurons in males to influence the expression of the TGF-β signaling ligand gene *daf-7.* Well-fed adult males express *daf-7* in both the ASI and ASJ chemosensory neurons; in contrast, starved adult males express *daf-7* only in the ASI neurons. Here, we describe a forward genetic screen for mutant males that continue to express *daf-7* in the ASJ neurons when starved, indicating a breakdown of intestine-neuron signaling in response to nutritional state. From this screen, we recovered two mutant strains: one contains a loss-of-function mutation in *che-3,* and the other contains a gain-of-function mutation in *pdfr-1.* Follow-up analysis revealed that neither of these genes was directly involved in satiety signaling, but instead the mutations resulted in non-starved-male-specific upregulation of *daf-7* expression in the ASJ neurons.

Introduction

Communication between the gut and brain is an important regulator of satiety in many animals. Key to the control of food intake is signaling from the digestive system to the brain to induce or inhibit feeding in response to nutritional state. In humans, numerous satiety signals sense the presence of food in the gastrointestinal tract and signal through the vagus nerve to the hindbrain to inhibit further food intake. These fast-acting satiety signals work in concert with adiposity signals, such as leptin and insulin, which detect changes in fat mass. These hormones bind to receptors in the hypothalamus, where they affect expression of neuropeptides that

regulate appetite. These signals fluctuate in response to periods of feeding and fasting to maintain body weight homeostasis (Harrold et al., 2012).

Throughout its life, the roundworm *Caenorhabditis elegans* encounters diverse environments where bacterial food can vary in its pathogenicity, nutritional quality, and abundance. If food is removed during development, *C. elegans* arrest their growth until the return to more favorable conditions (Baugh, 2013; Cassada & Russell, 1975; Hu, 2007). Adult *C. elegans* facing limited food conditions can also adapt their physiology and behavior, with food conditions influencing body and germline size, egg-laying behavior, lifespan, locomotion, foraging, and olfaction (Ben Arous et al., 2009; Chen & Caswell-Chen, 2004; Colbert & Bargmann, 1997; Hedgecock & Russell, 1975; Kaeberlein et al., 2006; Lee et al., 2006; Sawin et al., 2000; Seidel & Kimble, 2011).

Numerous studies have shown that the TGF-β signaling ligand DAF-7 is important in the regulation of various food-dependent areas of *C. elegans* physiology, including the dauer developmental decision, satiety quiescence, and fat storage (Gallagher et al., 2013; Greer et al., 2008; Ren et al., 1996; Schackwitz et al., 1996; You et al., 2008). While well-fed hermaphrodites express *daf-7* exclusively in the ASI chemosensory neurons, well-fed males express *daf-7* in both the ASI and ASJ neurons. Work presented in Chapter Three of this thesis supports a model in which *daf-7* expression in the ASJ neurons is sensitive to both ingested food, which is sensed through SCD-2 to inhibit *daf-7* expression in the ASJ neurons, and an external soluble food cue that induces *daf-7* expression in ASJ (further discussed in Chapter Five). The upregulation of *daf-7* expression in the ASJ neurons on inedible food is seen in both males and hermaphrodites (Figure 1), and the dynamic expression of *daf-7* in the ASJ neurons in response to changes in the food environment regulates feeding and foraging behavior.

In addition to its role in food seeking, male behavior is additionally influenced by the need to find a mate. To this end, in the absence of hermaphrodites, well-fed males will voluntarily leave sources of food for prolonged periods of time to search for a hermaphrodite mate (Lipton et al., 2004). The expression of *daf-7* in the ASJ neurons promotes this food-leaving behavior in males (Hilbert & Kim, 2017). Following starvation, males temporarily prioritize refeeding over mate searching and remain on food for several hours before leaving in search of a mate (Lipton et al., 2004). These starved animals do not express *daf-7* in their ASJ neurons (Hilbert & Kim, 2017).

Because of the prolonged period in which a mate-searching male may be away from food, it is essential that this behavior be regulated by the overall nutritional state of the animal, rather than the short-term cues of food ingestion or external soluble signals. Because *daf-7* expression in this context regulates mate-searching, *daf-7* expression in the ASJ neurons must also share this nutritional-state-dependent regulation. Consistent with this hypothesis, previous work from our lab has demonstrated that reintroduction of starved males to a nutritious food source allows *daf-7* transcription in the ASJ neurons to resume on a scale of hours, suggesting that this *daf-7* expression in the ASJ neurons is dependent on internal satiety cues rather than the presence of external food signals or short-term food ingested cues (Hilbert & Kim, 2017).

In this chapter, we used this differential *daf-7* expression in the male ASJ neurons as a specific readout for the integration of satiety cues from the intestine to the nervous system and performed a screen for adult males that continue to express *daf-7* in their ASJ neurons when starved. We aimed to identify mutations in genes that detect lack of food in the intestine, relay this sensation to the ASJ neurons, or receive this communication in the ASJ neurons. In addition to uncovering new insights into how *C. elegans* sense nutritional state and integrate these cues to

influence behavior, we hoped our screen could reveal conserved genes that regulate satiety with potential implications for the prevention or treatment of obesity in humans.

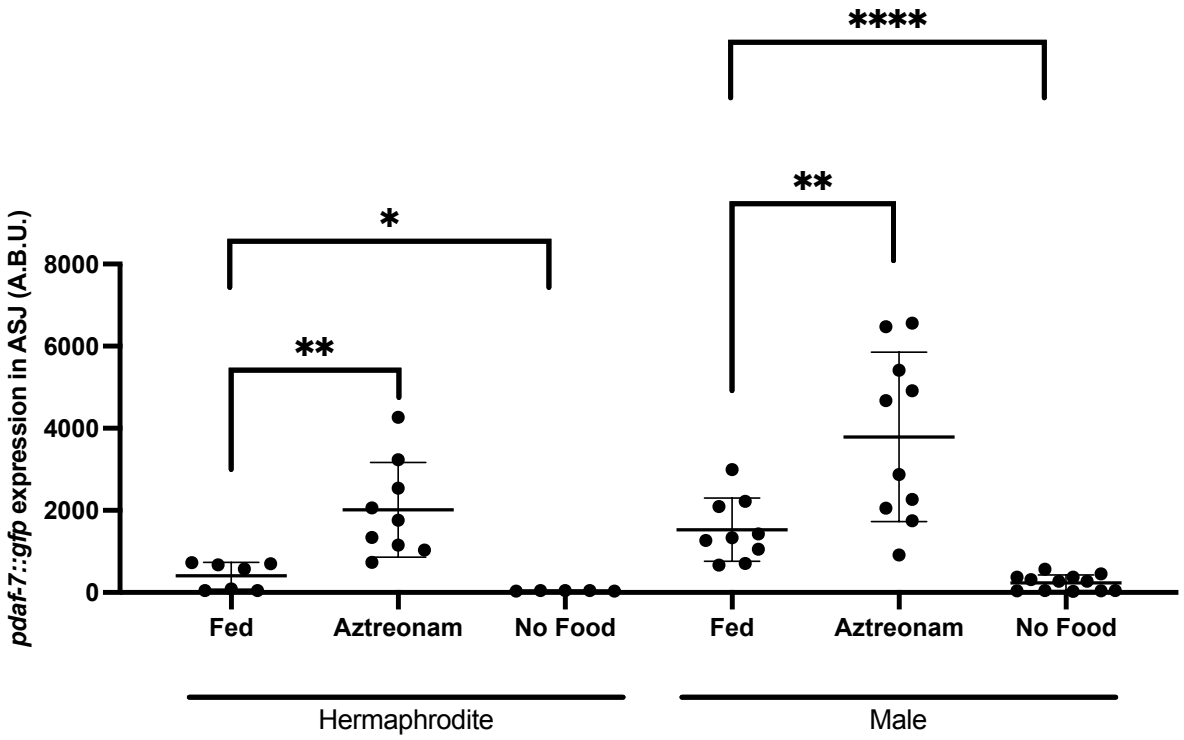

Figure 1. *daf-7* expression in the ASJ neurons is sensitive to food cues in both hermaphrodites and males. Compared to expression in well-fed animals, *daf-7* expression in the ASJ neurons is upregulated on inedible food (Aztreonam-treated bacteria) in both males and hermaphrodites. In the complete absence of food, *daf-7* is not expressed in the ASJ neurons in males or hermaphrodites.

Results

We performed a clonal screen for mutant male *C. elegans* that express *daf-7* in their ASJ neurons when starved. To improve our chances of finding genes that were specifically involved in the satiety response, rather than abnormal ASJ neuron differentiation or detection of short-term food cues that influence *daf-7* in ASJ in both males and hermaphrodites, we looked for mutant strains where well-fed males and hermaphrodites had wild-type *daf-7* expression patterns. Our starting strain contained an integrated *pdaf-7::gfp* reporter for visualizing *daf-7* expression and a *him-5(e1490)* allele which enhances the spontaneous formation of males (Meneely et al., 2012). After mutagenizing our P0s, we singled about 1625 F1s, allowing us to screen approximately 3250 haploid genomes. From this screen we obtained two mutant strains, *qd385 and qd388,* which expressed *daf-7* in the ASJ neurons in starved males but not in hermaphrodites (Figure 2).

Following backcrossing and whole genome sequencing, we identified *qd388* as an allele of *che-3*, a gene that encodes a dynein isoform that is important for the development of chemosensory cilia (Wicks et al., 2000). Our *g880a* allele potentially disrupted a splice site acceptor, and the retained intron is 41 base pairs long, resulting in a downstream frameshift. When a fosmid containing a wild-type copy of *che-3* was injected into animals with the *che-3(qd388)* allele, starved males displayed the wild-type phenotype and expressed *daf-7* only in the ASI neurons (Figure 3).

Previous analysis of *che-3(e1124)* animals from our lab has shown that hermaphrodites constitutively expressed *daf-7* in both the ASI and ASJ neurons (Park, 2019). It is possible that we did not see *daf-7* expression in hermaphrodite ASJ neurons with our *che-3(qd388)* allele because it is weaker than *e1124*, and males are more sensitized to express *daf-7* in the ASJ

neurons than hermaphrodites, or because of an additional mutation in the background that repressed *daf-7* expression in the hermaphrodite ASJ neurons. *che-3* animals have previously been described as dye-filling defective. Although I was able to see some dye-filling in my *qd388* animals, it was not seen in every animal and often was dimmer than wild-type animals (Figure 4).

Our other allele, *qd385,* was a mutation in the gene *pdfr-1*, causing an S325F substitution. *pdfr-1* encodes the *C. elegans* ortholog of the Pigment Dispersing Factor (PDF) receptor. PDFR-1 and its ligand PDF-1 are essential for male mate-searching behavior. Previous work from our lab has implicated *pdfr-1* in the regulation of *daf-7* expression in the ASJ neuron in males, with males containing loss-of-function alleles of *pdfr-1* failing to express *daf-7* in the ASJ neurons when well fed (Barrios et al., 2012; Hilbert & Kim, 2018). Given our observed phenotype of constitutive *daf-7* expression in the ASJ neurons of males, we wondered if our *qd385* allele could represent a *pdfr-1* gain-of-function allele. To ensure that the S325F substitution was responsible for the *daf-7* expression phenotype, rather than a background mutation, we generated a S235F allele using CRISPR, *pdfr-1(syb3826),* and observed that both males and hermaphrodites with this allele constitutively express *daf-7* in the ASJ neurons (Figure 5A). During analysis of our *qd385* mutant, we saw only occasionally expression of *daf-7* in ASJ in hermaphrodites; we hypothesize that additional background mutations in this strain could have occasionally repressed *daf-7* expression in hermaphrodite ASJ neurons.

Further analysis of this *pdfr-1(syb3826)* allele revealed that the S325F substitution indeed likely resulted in a hypermorphic PDFR-1, as it showed the opposite phenotype as *pdfr-1* loss-of-function animals in multiple assays in addition to *daf-7* expression. On most food sources, animals divide their time between a foraging stake known as roaming, marked by high speed and

low bending angle, and a feeding state known as dwelling, marked by low speed and high bending angle (Ben Arous et al., 2009). PDFR-1, as well as its ligands PDF-1 and PDF-2, has been previously reported to promote exploratory roaming behavior; loss-of-function mutations in these genes lead to animals spending a greater fraction of their time dwelling (Figure 5B) (Flavell et al., 2013). In contrast, animals with the gain-of-function *pdfr-1(syb3826)* allele spend less time dwelling and more time roaming, a phenotype that is discussed further in Chapter Three of this thesis (Figure 5B).

Additionally, I have found that the *pdfr-1(syb3826)* animals show the opposite dauer-formation phenotype as animals with loss-of-function alleles of *pdfr-l.* When *C. elegans* larvae develop in the presence of high concentrations of dauer pheromone and low-quality heat-killed wild-type animals will enter dauer at high frequency, while live food is sufficient to suppress dauer entry even in the presence of pheromone (Figure 5C, wild type bars). Animals with loss-of-function alleles of *pdfr-1* show a Daf-d (dauer-formation defective) phenotype: in the presence of dauer pheromone and low-quality heat-killed food, these animals enter dauer at low frequency (Figure 5C, *pdfr-1(lst34)* bars). In contrast, animals with the *pdfr-1(syb3826)* allele show a dauer-formation constitutive (Daf-c) phenotype; these animals enter dauer at a higher rate than wild type, even under favorable growth conditions of live food (Figure 5C, *pdfr-1(syb3826)* bars).

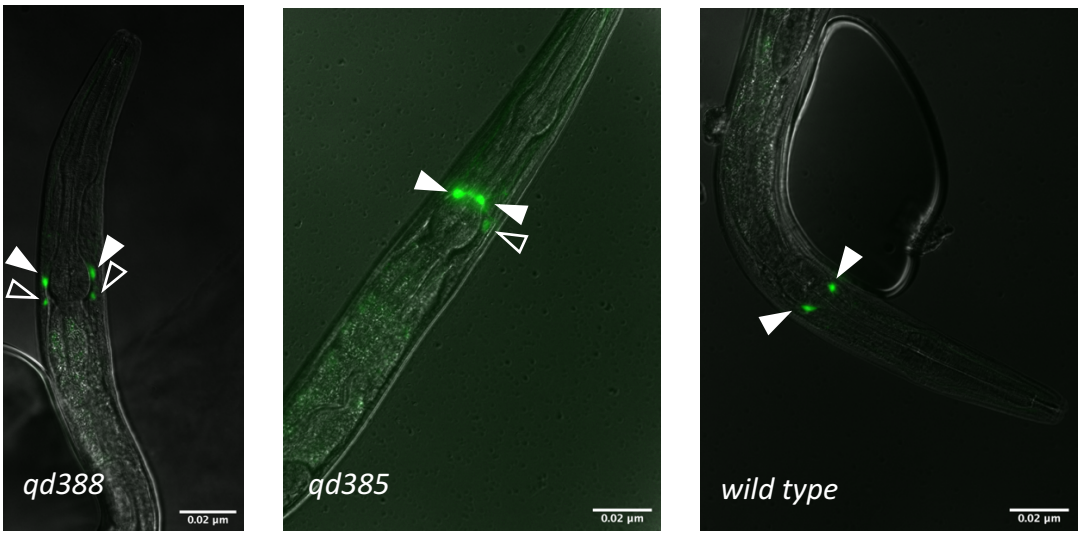

Figure 2. *qd388* and *qd385* males express *daf-7* in their ASJ neurons when starved.

Representative images of starved males of each of two mutants recovered from our screen and wild type starved males. Closed arrowheads point to ASI neurons, and open arrowheads point to ASJ neurons.

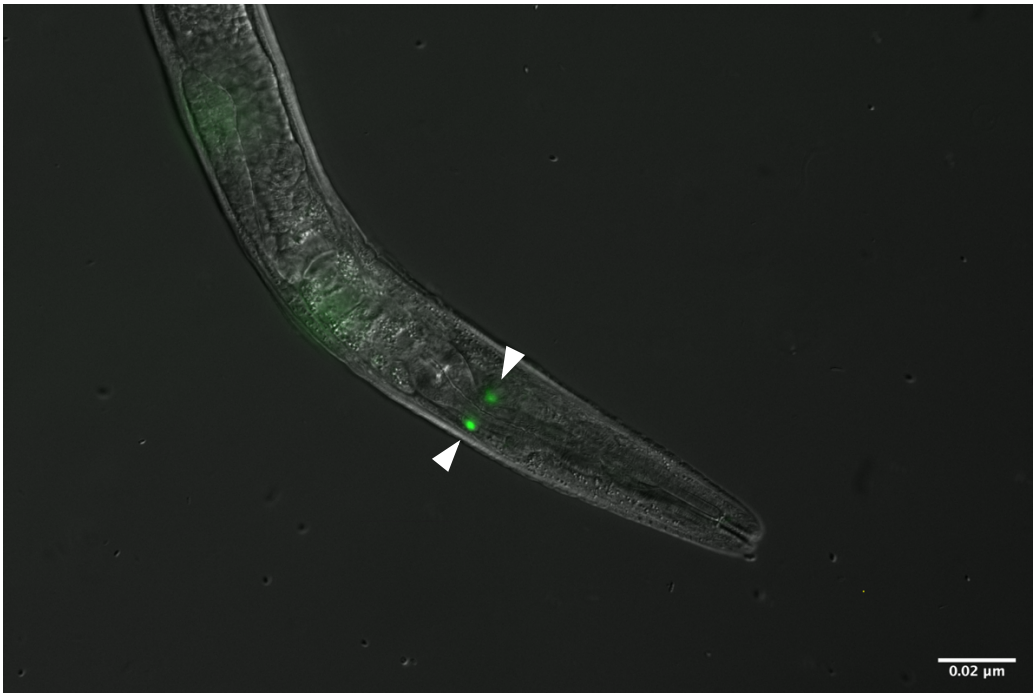

Figure 3. A fosmid containing a wild-type copy of *che-3* rescues the mutant phenotype of *qd388* males. Representative image of a starved adult male *che-3(qd388)* animal expressing *pdaf-7::gfp* with an injected Fosmid containing wild type *che-3* (WRM067cB09). Closed arrowheads point to ASI neurons.

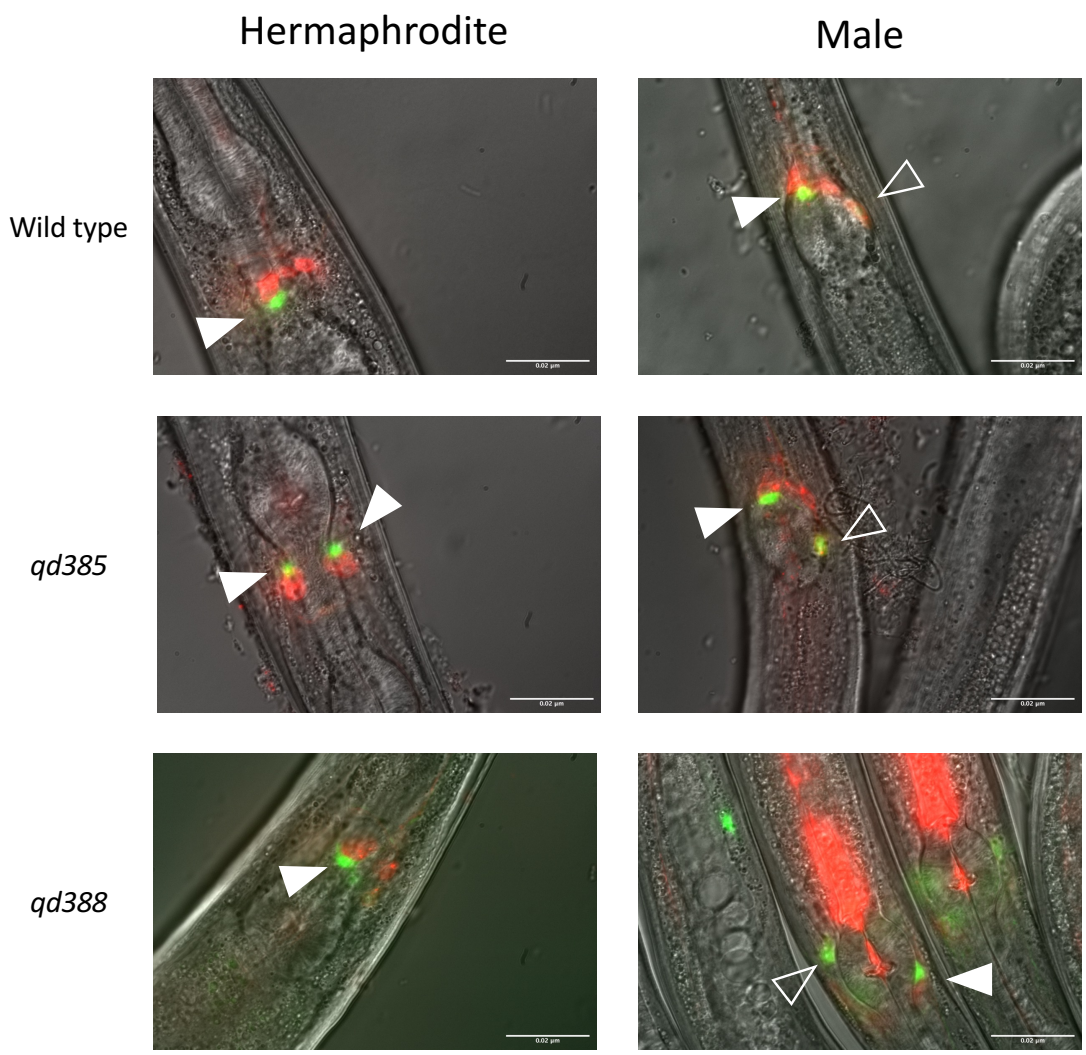

Figure 4. Dye-filling of *qd385* and *qd388* animals. Representative images of fed wild type, *qd385,* and *qd388* hermaphrodites and males expressing *pdaf-7::gfp* and incubated with DiI. Closed arrowheads point to ASI neurons, open arrowheads point to ASJ neurons.

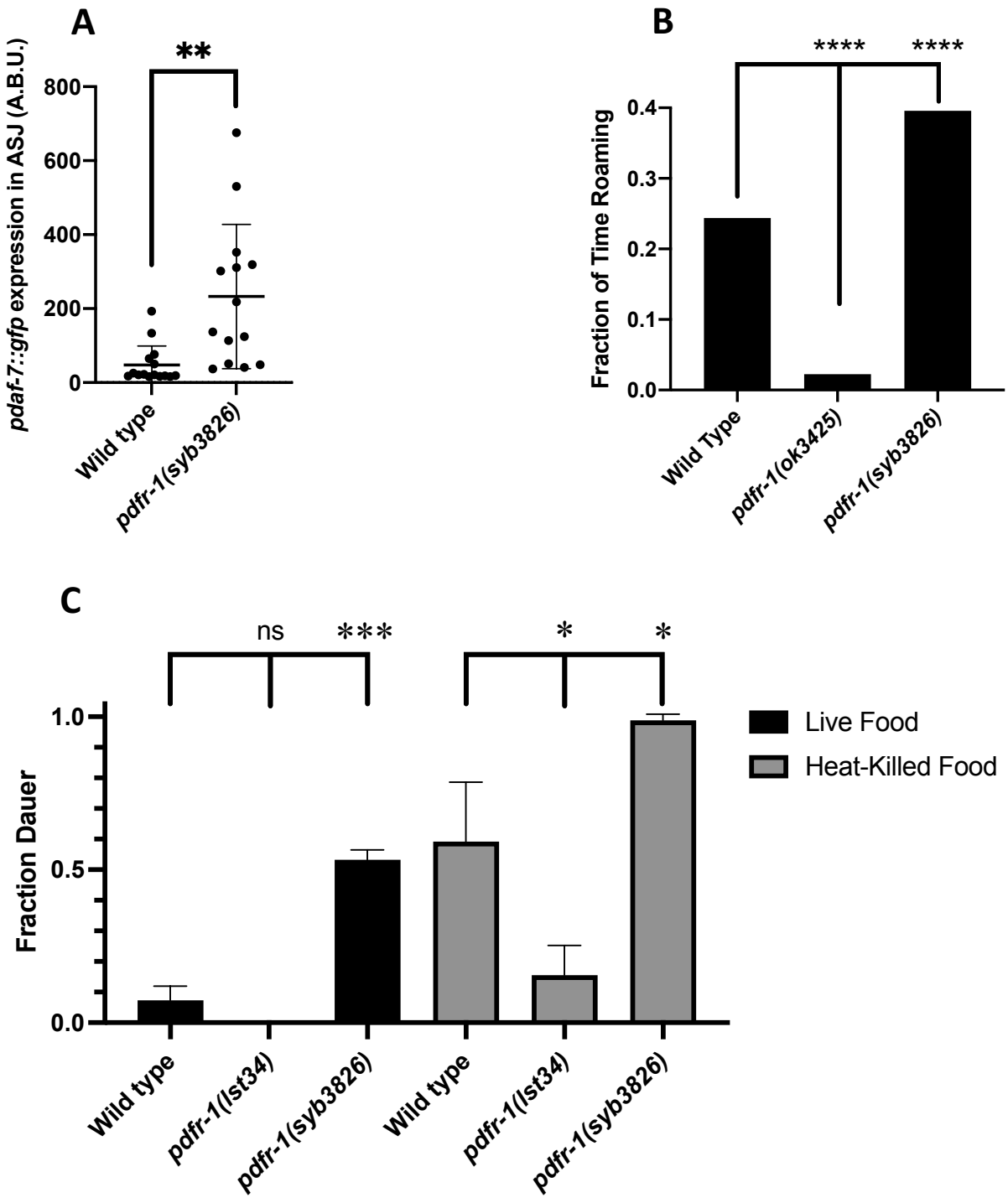
A
**
pdaf-7::gfp expression in ASJ (A.B.U.)
800
600
400
200
0
Wild type
pdfr-1(syb3826)
B
****
****
Fraction of Time Roaming
0.4
0.3
0.2
0.1
0.0
Wild Type
pdfr-1(ok3425)
pdfr-1(syb3826)
C
ns
***
*
*
Fraction Dauer
1.0
0.5
0.0
Live Food
Heat-Killed Food
Wild type
pdfr-1(lst34)
pdfr-1(syb3826)
Wild type
pdfr-1(lst34)
pdfr-1(syb3826)

Figure 5. *pdfr-1(syb3826)* is a gain-of-function allele.

A. Quantification of *pdaf-7::gfp* expression in the ASJ neurons in wild type and *pdfr-1(syb3826)* hermaphrodites. **p<0.01 as determined by two-way t test. Error bars represent SD.

B. Fraction of time spent in roaming state for wild type, *pdfr-1(syb3826),* and *pdfr-1(ok3125).* Wild type vs. *pdfr-1(ok3425)* $\chi^2 = 2278.24$, p<0.0001, Wild type vs. *pdfr-1(syb3826)* $\chi^2 = 484.47$, p<0.0001

C. Fraction of animals entering dauer on plates containing dauer pheromone. Plates were seeded with either 100% live (black) or 99% heat-killed/1% live (gray bars) food. Each bar represents the average fraction of animals that entered dauer over 4 plates of ~100-150 animals. Error bars represent standard deviation. Significance based off two-way student's t-test to matching wild-type food condition. ns not significant, * p<0.05, ***p<0.001

Discussion

Our screen for male *C. elegans* that continue to express *daf-7* in the ASJ neurons when starved yielded two mutants: *qd388,* an allele of *che-3,* and *qd385,* an allele of *pdfr-1. che-3* is required for the structure and function of sensory cilia in ciliated neurons, including the ASJ neurons. Loss-of-function alleles of *che-3* have been noted for their defects in both chemotaxis toward the attractive salt ammonium acetate and aversion to copper sulfate (Wicks et al., 2000). Although the dye-filling capacity of our *qd388* animals is not entirely consistent with the complete absence of dye-filling previously observed in *che-3* animals (Wicks et al., 2000), our new allele may represent a weaker defect in chemosensory cilia structure, allowing some dye-filling while still altering *daf-*7 expression. Given the known effects of *che-3* on chemosensory neurons, and the observation that *che-3(e1124)* hermaphrodites constitutively express *daf-7* in the ASJ neurons, we hypothesize that *qd388's* effect on *daf-7* expression in starved males is due to abnormal chemosensation rather than changes in satiety signaling.

The other mutant recovered from our screen, *qd385,* is a S325F substitution in *pdfr-1*. Given the known role of *pdfr-1* in regulating *daf-7* expression in the ASJ neurons of males, and the fact that the *pdfr-1(syb3826)* allele induces *daf-7* in the ASJ neurons in both starved males and hermaphrodites, it is unlikely that *pdfr-1* represents a novel satiety regulator. Instead, we believe that the gain-of-function allele causes constitutive *daf-7* expression in the ASJ neurons independently of satiety signaling from the intestine.

Previous work from our lab found that ASJ neuron-specific expression of a gain-of-function variant of the adenylate cyclase ACY-1, which functions downstream of PDFR-1, was sufficient to induce *daf-7* expression in hermaphrodite ASJ neurons but not in the ASJ neurons of starved males (Hilbert & Kim, 2018). This result suggested that the effects of starvation

functioned downstream of or in parallel to PDFR-1-ACY-1 signaling. Our finding that *daf-7* was still expressed in the ASJ neurons of starved *pdfr-1(qd385)* males suggests that the effects of starvation and PDFR-1-ACY-1 signaling occur in parallel to each other to modulate *daf-7* expression in ASJ, and that the *pdfr-1* gain-of-function allele perhaps increases PDFR-1-ACY-1 signaling to a greater degree than the *acy-1* gain-of-function allele. The S325F gain-of-function allele has facilitated our study of the role of *pdfr-1* in modulating roaming behavior, as discussed in Chapter Three.

Despite our recovery of two mutants that expressed *daf-7* in the ASJ neurons of starved males, our screen ultimately did not identify genes that functioned as satiety signals in *C. elegans*. This is potentially due to several limitations of our screen. First, our phenotype of *daf-7* expression in the ASJ neurons of starved males was not specific for abnormal satiety signaling, as we had hoped it would be. As we observed, additional background mutations or weak defects in chemosensation could result in constitutive *daf-7* expression in the ASJ neurons being observable only in males, despite a gene influencing *daf-7* expression in the ASJ neurons of both sexes. Additionally, the clonal screen design made the screening of a large number of genomes difficult; it is likely that a larger screen would have uncovered more mutants, some in genes more directly related to satiety signaling. Alternative screen designs are discussed in Chapter Five.

Materials and Methods

Strains: *C. elegans* was maintained on E. coli OP50 as previously described (Brenner, 1974). All strains were grown at 20˚C except for starved plates from singled F1s prior to retesting,

which were stored at 16˚C until ready to be scored. A complete list of strains used in this study is presented in Table 1.

Preparation of food condition plates: Unless otherwise indicated, all assays were performed on NGM plates with no peptone. Aztreonam-treated bacteria was prepared as previously reported (Gruninger et al., 2008), and 40 µL aztreonam-treated food was added to plates containing 10 µg/mL aztreonam. *E. coli* OP50-seeded plates ("Fed") for *daf-7* quantification were seeded with 40 µL OP50 grown overnight in a shaking LB culture at 37˚C. "No food" plates were unseeded. Animals were transferred to assay plates as L4s 16 hours prior to imaging.

Screen Design: L4 ZD1005 animals were mutagenized with 47 mM EMS (methanesulfonic acid, ethyl ester, Sigma #M-0880). Animals were allowed to recover overnight and the following day P0s were egg-laid. When F1s were L4 stage, ~1625 hermaphrodites were singled onto OP50 plates which were allowed to starve out. To assay clonal populations of F2s-F4s, we chunked starved plates and perform the starvation assay described below, looking for populations where ~1/4 of adult males expressed *daf-7* in ASJ but hermaphrodites did not express *daf-7* in ASJ.

Plates where starved males expressed *daf-7* in ASJ were retested twice to confirm the phenotype, then backcrossed three times (*qd388*) or four times (*qd385*) to ZD1005 animals. Whole genome sequencing was performed on both *qd385* and *qd388* animals, as well as the starting ZD1005 strain. Polymorphisms that were homozygous in the mutant strains and occurred in coding sequences were prioritized as causal candidates.

Starvation assay: 10-15 gravid adult hermaphrodites were transferred to a fresh OP50-seeded plate to lay eggs that were grown at 20˚C for 50 hours. The late-L4 animals were washed off the plates in 1 mL M9 buffer and washed three times in 1 mL M9. The animals were dropped onto

an unseeded NGM plate without peptone and grown for an additional 48 hours. The animals were then assayed on a fluorescent dissecting microscope for *pdaf-7::gfp* expression pattern.

Imaging and quantification of *Pdaf-7p*::*gfp* levels: Animals to be imaged were mounted on glass slides with agarose pads and 50 mM sodium azide. Quantification of GFP brightness was derived from maximum fluorescence values within the ASJ neurons in FIJI. All imaging for pictures were conducted on the Zeiss Axioimager Z1.

Generation of *che-3* fosmid-rescue strains: Genomic rescue of *che-3* was done by injection of the WRM067cB09 fosmid from the Moerman fosmid library. The fosmid was injected at a concentration of 50 ng/µL into the *qd388* mutant strain, along with a plasmid containing *pofm-1::gfp* as a co-injection marker, also at 50 ng/µL. Two independent lines were analyzed for *pdaf-7::gfp* expression in the ASJ neurons of starved males.

Dye-filling assay: DiI was diluted from a stock solution of 2mg/mL in dimethyl formamide 1:200 in M9. 15-20 day 1 adult worms were transferred to 150 µLdiluted stain, incubated for 3 hours in the dark at room temperature, and imaged as described above.

Dauer assay: One hundred microliters of pheromone mix (containing ascarosides ascr#2, ascr#3, ascr#5, and ascr#8 each at a concentration of 20 µM in 10% ethanol) was added to 3.5 cm plates (volume ~4 mL) made with Noble agar and without peptone, resulting in an effective plate concentration of 0.5 µM for each ascaroside. The plates were seeded with 40 µL of either a mix containing 99% heat-killed/1% live or 100% live *E. coli* OP50. The 1% live food was necessary in the heat-killed condition to prevent the worms from arresting development before reaching dauer (Qi et al., 2017). Gravid animals were bleached and ~100-150 eggs were dropped onto prepared plates. Plates were incubated for 72 hours at 25˚C, then dauer and nondauer animals were counted.

Roaming/dwelling assay: Animals were egg-laid and grown at 20˚C for 72 hours. 10 cm NGM plates without peptone were seeded with 2mL stationary phase *E. coli* OP50 grown overnight in LB. Assays were performed with ~20 animals inside a 6cm copper ring placed in the center of the seeded plate and recorded at 3.75 frames per second for 90 minutes. Videos were analyzed using MBF Biosciences WormLab software. Measurements of speed and bending angle were averaged over 10 second intervals. Quantification of fraction of time spent roaming or dwelling was done by segregating the points of the scatter plots by a horizontal line placed based on the distribution of points in the wild-type condition. Points falling above the line were classified as roaming, and those below the line were classified as dwelling.

Strain Name	Genotype	Source
ZD1005	*ksIs2[pdaf-7::gfp+rol-6(su1006)];him-5(e1490)*	(Hilbert & Kim, 2017)
ZD2578	*che-3(qd388); ksIs2; him-5(e1490), 3x backcrossed*	This study
ZD2604	*pdfr-1(qd385); ksIs2; him-5(e1490), 4x backcrossed*	This study
LSC39	*pdfr-1(lst34)*	Horvitz lab
ZD2079	*pdfr-1(ok3425), 6x backcrossed*	(Hilbert & Kim, 2018)
PHX3826	*pdfr-1(syb3826)*	This study
ZD2633	*pdfr-1(syb3826); ksIs2; him-5(e1490)*	This study

Table 1. A complete list of *C. elegans* strains used in this study

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Chapter Five

Unanswered questions and future directions

Work presented in this thesis has demonstrated the genetic basis by which *C. elegans* perceive bacterial food to influence exploratory behavior and developmental arrest. I have shown that the *C. elegans* ALK ortholog, SCD-2*,* plays a key role in the behavioral and developmental adaptations in response to changing food environments. Questions remain from our work, including the nature of the external food cue necessary for *daf-7* expression in the ASJ neurons observed in Chapter Three, and how SCD-2 is functioning to perceive and signal the presence of ingested food in the pharynx. Additionally, I propose several options for further screens for satiety signals that sense starvation in males and relay this signal to the ASJ neurons to influence *daf-7* expression.

Identifying a soluble food cue that induces *daf-7* expression in the ASJ neurons and determining how it is perceived by *C. elegans*

In Chapter Three, I reported that, although we see induction of *daf-7* expression in the ASJ neurons when animals are on inedible food, we do not see *daf-7* expression in the complete absence of food, despite the absence of an ingested food cue (Figure 1A). This suggests the existence of a component of inedible food that induces *daf-7* expression in the ASJ neurons. We have seen that both aztreonam-treated food and food seeded under the agar were capable of upregulating *daf-7* in the ASJ neurons, but we found that when animals were on plates where OP50 had been spotted on the lid, such that the animals were in contact with volatile cues from the OP50 but not soluble cues, *daf-7* was not expressed in the ASJ neurons (Figure 1B). This suggested that a soluble cue from the bacteria lawn was responsible for upregulating *daf-7* in the ASJ neurons in the absence of ingested food.

We next investigated the nature of this soluble food cue. First, we asked if *daf-7* expression was induced in the ASJ neurons by an *E. coli*-specific compound. We tested three other bacteria species, JUb023 (*Xanthomonas sp./Stenotrophomonas sp*.), JUb267 (*Chryseobacterium sp*.), and JUb269 (*Pseudomonas fluorescens*), and saw that each strain was able to induce *daf-7* in the ASJ neurons when seeded underneath the agar, despite not inducing *daf-7* expression in the ASJ neurons when ingested (Figure 1C). We also prepared extracts of OP50 and found that these retained the ability to induce *daf-7* in the ASJ neurons, suggesting that metabolically active bacteria were not necessary for *daf-7* expression in the ASJ neurons (Figure 1D).

As I was performing these experiments, I noticed occasional expression of *daf-7* in the ASJ neurons of animals on NGM plates with no food. After several weeks of troubleshooting and evaluating various components of the NGM plates, I discovered that peptone was upregulating *daf-7* expression in the ASJ neurons, although this induction was suppressed by the addition of edible food (Figure 1E). I found that LB was also able to induce *daf-7* expression in the ASJ neurons (Figure 1F). Given the high protein content of both peptone and LB, I wondered if amino acids could be responsible for the upregulation of *daf-7* expression in the ASJ neurons. Analysis of individual single amino acids found that none was able to induce *daf-7* expression to the degree seen with LB or peptone (data not shown).

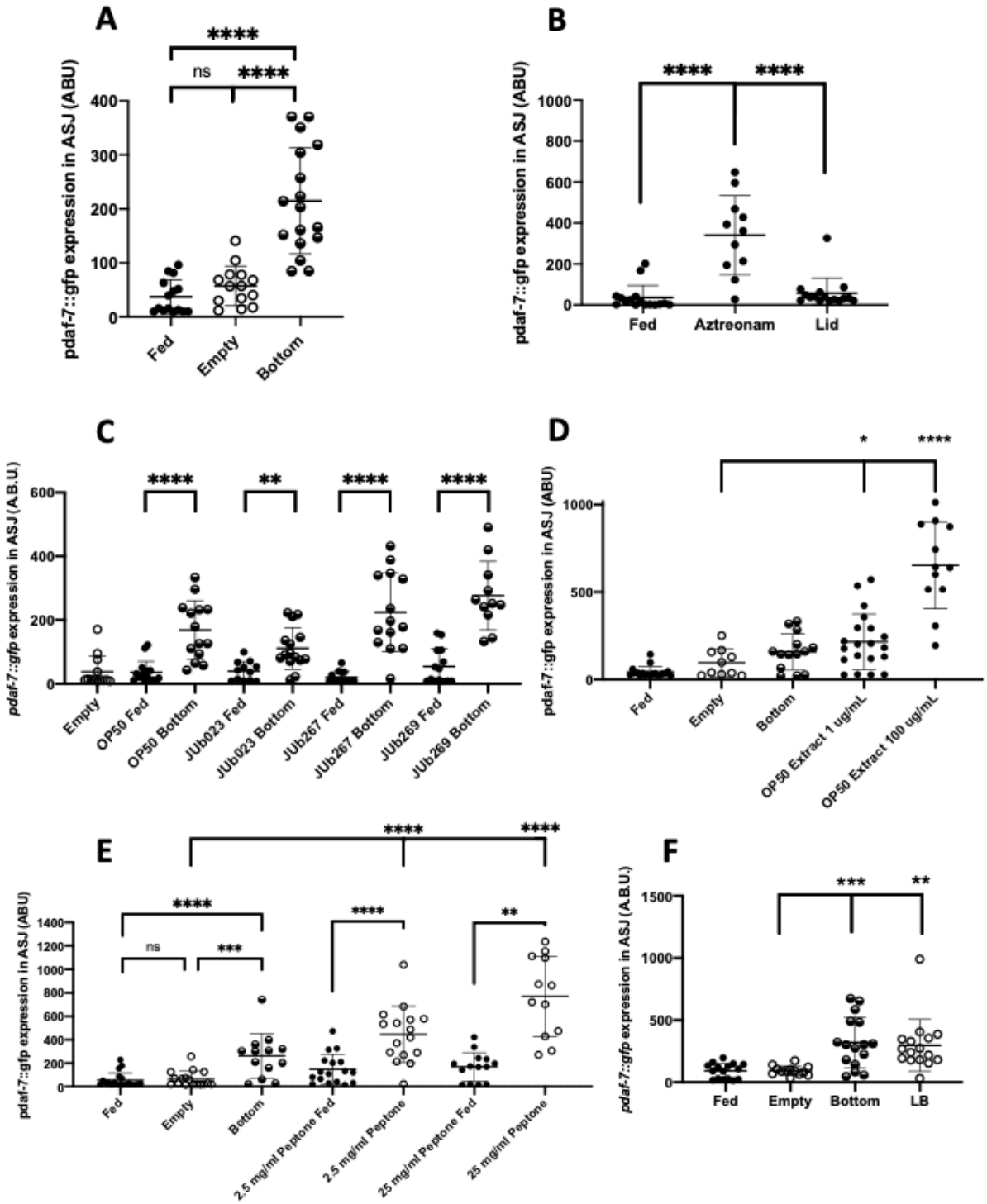
The precise nature and identity of the soluble cue that signals nearby food remains unknown. I believe that identifying the food-specific cues that modulate *daf-7* expression will broaden our understanding of the relationship between *C. elegans* and its microbial food environment and propose several experimental routes to identify this cue.

Fractionation of OP50 extract and peptone has identified some fractions capable of inducing *daf-7* expression in the ASJ neurons. In the case of fractionated OP50 extract, there was no clear fraction that contained all activity, and given this result, our collaborators who performed the fractionation discouraged us from sub-fractionation (Figure 2A). Our fractionated peptone also yielded somewhat discouraging results, although there is possibly some activity in fractions 7 and 12 that could be further investigated (Figure 2B). It is possible in each fractionation experiment that the mixing of several fractions is necessary for full activity, or that a compound was lost during the fractionation process.

One alternative approach to identify the external food signal is a candidate-based screen with compounds that signal bacterial food to *C. elegans* in other contexts, such as NAD+, which acts as a food signal to promote dauer exit (Mylenko et al., 2016). An additional approach to identify soluble signals is to screen bacteria mutants for an inability to induce *daf-7* expression in the ASJ neurons when seeded on the bottom of the plate. The Keio collection is a library of nearly 4000 *E. coli* K12 strains with single genes knocked out (Baba et al., 2006). Screening through this library for strains lacking the soluble cue required to upregulate *daf-7* in the ASJ neurons in the absence of ingested food could identify the external food cue that *C. elegans* sense. However, if multiple compounds act as food signals independently of each other, such that the loss of only one signal does not prevent *daf-7* upregulation, they could be missed by this screen.

As a complementary approach, a forward genetic screen for *C. elegans* defective in upregulating *daf-7* in the ASJ neurons when exposed to inedible food could be performed. Mutants from this screen could potentially include receptors for specific nutrients. Follow-up analysis to ensure that these mutants are still able to upregulate *daf-7* expression in the ASJ

neurons in other contexts, such as in males or on PA14, would be necessary to ensure that recovered mutants do not contain grosser ASJ-neuron defects (Hilbert & Kim, 2017; Meisel et al., 2014). This screen may additionally uncover mutants that constitutively signal the ingestion of food, hence constitutively inhibiting *daf-7* expression, which could provide their own insight into the nature and detection of the ingested food cue.


A
pdaf-7::gfp expression in ASJ (ABU)
****
ns
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Fed
Empty
Bottom
B
pdaf-7::gfp expression in ASJ (ABU)
****
****
Fed
Aztreonam
Lid
C
pdaf-7::gfp expression in ASJ (A.B.U.)
****
**
****
****
Empty
OP50 Fed
OP50 Bottom
JUb023 Fed
JUb023 Bottom
JUb267 Fed
JUb267 Bottom
JUb269 Fed
JUb269 Bottom
D
pdaf-7::gfp expression in ASJ (ABU)
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Fed
Empty
Bottom
OP50 Extract 1 ug/mL
OP50 Extract 100 ug/mL
E
pdaf-7::gfp expression in ASJ (ABU)
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****
ns
***
****
**
Fed
Empty
Bottom
2.5 mg/ml Peptone Fed
2.5 mg/ml Peptone
25 mg/ml Peptone Fed
25 mg/ml Peptone
F
pdaf-7::gfp expression in ASJ (A.B.U.)
***
**
Fed
Empty
Bottom
LB

Figure 1. A soluble food cue promotes expression of *daf-7* in the ASJ neurons.

A. Expression of *pdaf-7::gfp* in ASJ in animals fed OP50 ("Fed"), on plates with no food ("Empty"), or on plates where OP50 was seeded under the agar ("Bottom"). ****p<0.0001, ns not significant as determined by two-way t-test. Error bars represent SD.

B. Expression of *pdaf-7::gfp* in ASJ in animals fed untreated OP50 ("Fed"), fed aztreonam-treated OP50 ("Aztreonam"), or on plates where 40 µl OP50 was seeded on a small patch of agar poured inside the lid of the plate ("Lid"). ****p<0.0001 as determined by two-way t-test. Error bars represent SD.

C. Expression of *pdaf-7::gfp* in ASJ in animals either fed different bacteria species or on plates where different bacteria species were seeded underneath the agar. ****p<0.0001, ** p<0.01 as determined by two-way t-test. Error bars represent SD.

D. Expression of *pdaf-7::gfp* in ASJ in animals fed OP50 ("Fed"), on plates with no food ("Empty"), on plates where OP50 was seeded under the agar ("Bottom"), or on plates containing no food but with either 100 ug/ml or 1 ug/ml OP50 extract. ****p<0.0001, *p<0.05 as determined by two-way t-test. Error bars represent SD.

E. Expression of *pdaf-7::gfp* in ASJ in animals fed OP50 ("Fed"), on plates with no food ("Empty"), on plates where OP50 was seeded under the agar ("Bottom"), or with 2.5 mg/ml or 25 mg/ml peptone with or without OP50. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, ns not significant as determined by two-way t-test. Error bars represent SD.

F. Expression of *pdaf-7::gfp* in ASJ in animals fed OP50 (“Fed”), on plates with no food (“Empty”), on plates where OP50 was seeded under the agar (“Bottom”), or with 250 μL LB. ***$p<0.001$, **$p<0.01$ as determined by two-way t-test Error bars represent SD.

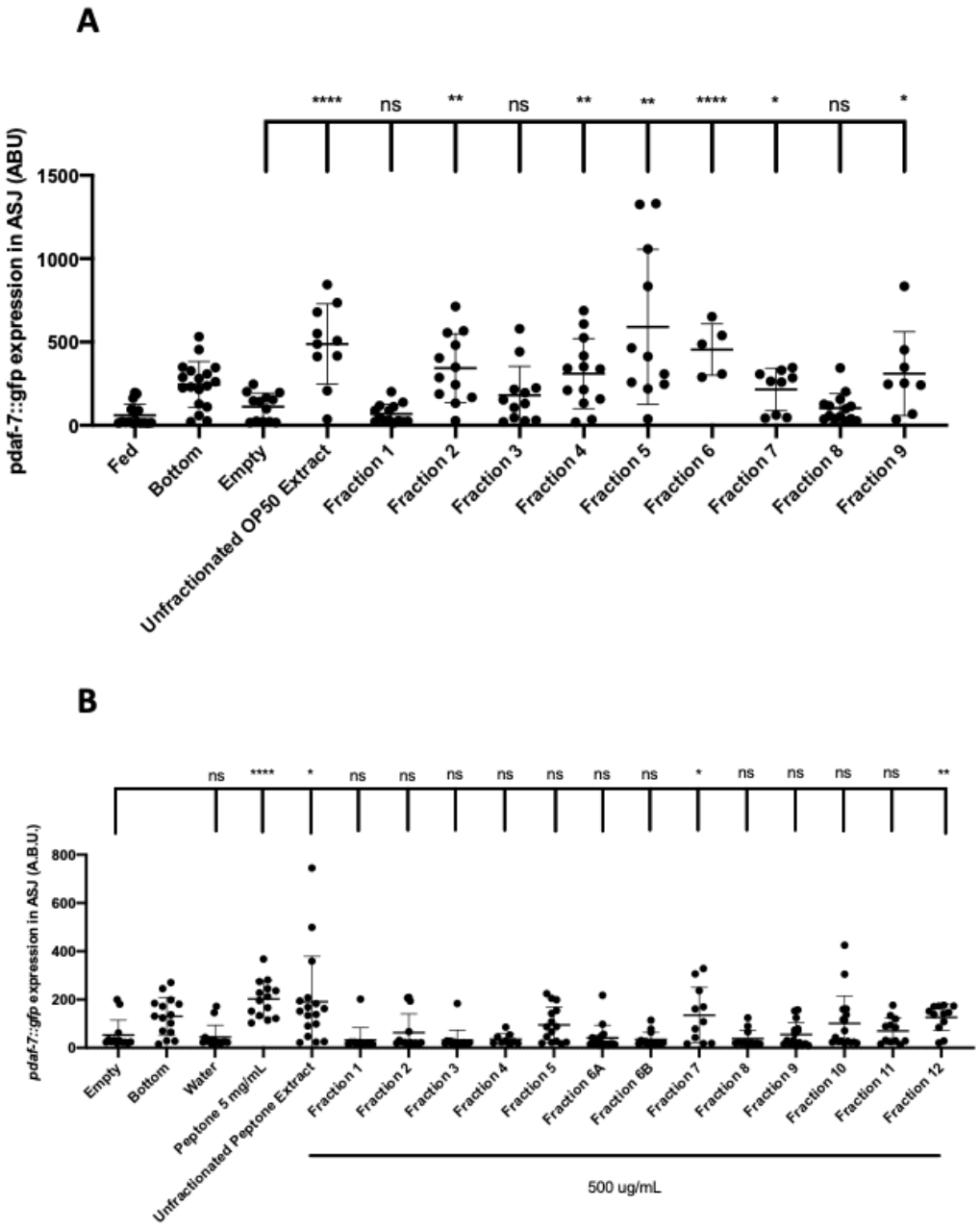

Figure 2. **Fractionation of OP50 extract and Peptone.**

Expression of *pdaf-7::gfp* in ASJ in wild type animals animals on OP50 ("Fed"), no food ("Empty"), or plates with OP50 seeded underneath the agar ("Bottom") or on fractionated OP50 extract (2A) or peptone (2B). ****$p<0.0001$, **$p<0.01$, *$p<0.05$, ns not significant as determined by two-way t-test. Error bars represent SD

Identification of additional genes in the *scd-2* pathway

In Chapters Two and Three, I introduced a gain-of-function allele of *scd-2* containing a F1029L substitution. This gain-of-function allele provides many pathways for follow-up analysis. Brian Vassallo, another Kim lab graduate student, performed two screens for suppressors of the *scd-2(syb2455)* Daf-c and reduced L1 arrest survival phenotypes. Several candidate genes have been identified, with further analysis and characterization ongoing. Genes functioning downstream of *scd-2* to promote dauer entry and L1 arrest survival could further elucidate the role of *scd-2* as a food sensor. Furthermore, if these genes are conserved in humans, they could additionally provide further insight into ALK signaling and potential mechanisms behind ALK's role in cancer and thinness.

Identification of new satiety signals

In Chapter Four of this thesis, I described a clonal screen for males that continue to express *daf-7* in the ASJ neurons when starved. The primary aim of this screen was to find genes that were involved in sensing the presence or absence of food in the intestine, transmitting this sensation to the ASJ neurons, and/or receiving this signal. Although the genes we found were ultimately not directly involved in satiety signaling, modified screens for this phenotype could uncover new satiety signals in *C. elegans*. Since the phenotype we were screening for was specific to males, we performed a clonal screen. This allowed us to propagate hermaphrodite siblings of any male that displayed the mutant phenotype. However, given the space constraints of storing many singled F1s and the time it took to screen so many plates, just two haploid genomes at a time, we suggest that this screen be performed differently if it were to be repeated.

One strategy for a new screen involves mutagenizing the same starting strain as our screen, ZD1005 (*ksIs2[pdaf-7::gfp; rol-6(su1006)]; him-5(e1490))*. Instead of singling F1s and performing a clonal screen, however, in this non-clonal screen, F2 animals would be starved, and males would be scored for *daf-7* expression in the ASJ neurons. Any mutant male would be crossed to a ZD1005 hermaphrodite and F2 hermaphrodites from this cross that yield a mutant F3 population would be further analyzed. A caveat to this approach is that mutants might be lost in the backcross due to unsuccessful mating, although using a starting strain without the dominant *rol-6(su1006)* allele on the transgene would likely enhance mating success.

An additional alternative approach to this screen is to work in hermaphrodites with a masculinized nervous system. Overexpression of the *fem-3* cDNA under the pan-neuronal *rab-3* promoter is sufficient for masculinization of the hermaphrodite nervous system, and hermaphrodites with a masculinized nervous system express *daf-7* in their ASJ neurons like males (Hilbert & Kim, 2017; Mehra et al., 1999). A screen using hermaphrodites with a masculinized nervous system could provide a way to look at male-specific neuronal gene expression in animals capable of self-fertilization. Prior to performing this screen, it would be essential to confirm that these hermaphrodites with a masculinized nervous system still show a *daf-7* expression response in the ASJ neurons to starvation.

One drawback of these nonclonal screen designs is that we would be screening multiple F2s at a time, raising the risk that an animal with a mutant phenotype may be overlooked. Additionally, these non-clonal screens would likely recover many mutants with abnormal *daf-7* expression in the ASJ neurons not specific to starved males, since we would not be able to eliminate mutants based off the phenotype of hermaphrodite siblings. Nevertheless, the ability to screen many more haploid genotypes is a significant advantage over our clonal screen design.

We hope that, with more mutant genomes analyzed and additional backcrossing to ensure that *daf-7* is not expressed in the hermaphrodite ASJ neurons, future iterations of this screen could successfully take advantage of this *daf-7* expression phenotype to identify new satiety signals.

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