The sensory system acts through a neuromedin U signaling pathway to mediate food type-dependent effects on lifespan

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Summary

In order to survive, the animal uses its sensory system to interpret the complexity of its environment. Interestingly, a subset of sensory neurons, which function in taste or olfaction, has been found to influence the lifespan of *C. elegans* and *Drosophila*. Although the mechanisms by which these neurons affect lifespan are unknown, the nature of these neurons suggest that the sensory influence on lifespan is mediated by food-derived cues.

This thesis shows that sensory neurons recognize different food types to affect *C. elegans* lifespan through a mechanism distinct from that of calorie restriction, which is commonly studied by restricting the levels of food intake. Moreover, this work identifies a neuropeptide signaling pathway, the neuromedin U pathway, which acts with the sensory system to affect *C. elegans* lifespan in response to specific food-derived cues, such as the lipopolysaccharide (LPS) structure of its *E. coli* bacterial food source.

The neuromedin U neuropeptide receptor *nmur-1*, which is expressed in sensory neurons, interneurons and the somatic gonad, shortens lifespan on *E. coli* food sources that have short LPS. In contrast, a neuromedin U ligand precursor, *nlp-44*, which is expressed in only two pairs of sensory neurons, lengthens lifespan in a food source-dependent manner. Genetic epistasis analysis suggests that *nlp-44* might encode ligands that act downstream of or in parallel to *nmur-1*. Since *C. elegans* has three other members of the neuromedin U receptor (NMUR) family, future studies are needed to determine whether *nlp-44* encodes different peptide ligands that could then act on several NMUR proteins, including NMUR-1.

The identification of a neuromedin U pathway that mediates the sensory recognition of food quality provides a genetic framework to elucidate further the mechanisms that underlie this sensory influence on lifespan. In addition, the observation that a neuropeptide signaling pathway promotes the sensory influence on lifespan raises the intriguing possibility that other neuropeptides might also affect lifespan in response to other types of sensory cues. These neuropeptide pathways might thus integrate different sensory information with signaling pathways already known to regulate lifespan.

Chapter I. General introduction

A. Gene–environment interactions

One of the fundamental questions in biology is how gene activities are affected by the environment. Biological systems, regardless of the level of organization, show remarkable plasticity in their adaptive mechanisms to a constantly changing environment. To appreciate fully the beauty of the interaction between genes and environment, I would like to give several examples of organismal plasticity in response to different environmental factors.

The *C. elegans* developmental programs are fascinating examples of such remarkable plasticity. Under optimal environments, *C. elegans* develops through four larval stages (L1-L4) before becoming reproductive adults. Hovever, harsh environmental conditions, such as overcrowding and starvation, which are reflected by a high ratio of worm-secreted dauer-inducing pheromones to food levels, or high temperatures, induce *C. elegans* first-stage larvae (L1) to develop into a specific non-feeding dormant stage, known as dauer, which has reduced metabolism (Riddle and Albert, 1997). This enables the worms to survive until environmental conditions become favorable, when the worms exit the dauer stage into reproductive adulthood (Riddle and Albert, 1997). This phenomenon of environmental-induced programmed quiescence is found not only in worms but also in many vertebrates, such as bats, some species of ground squirrels, bears and even one species of primates, the fat-tailed dwarf lemur (Dausmann et al., 2004). Under harsh environments and through a process known as hibernation, these vertebrates are able to undergo special dormant stages that have reduced metabolism (Dausmann et al., 2004).

An animal's adaptation to its environment can be acquired through genetic or epigenetic means. For example, *C. elegans* is found in many places in the world and every local *C. elegans* strain has evolved a unique adaptation to its environment. Indeed, in contrast to many *C. elegans* wild strains, like the Bristol N2, the CB4507 strain, which was isolated from a desert oasis, only responds to dauer-inducing pheromones at much higher temperatures, and thus reflects its unique adaptation to its hot desert environment (Reiner et al., 2008). This unique adaptation is caused by a mutation in a gene that modulates the response to dauer pheromones (Reiner et al., 2008).

At the same time, epigenetic modifications also allow animals to acquire a memory of their past environmental histories, which could enhance their survival in future exposures to the same environments. For example, *C. elegans* that underwent dauer formation in response to stress have global alterations in their chromatin state, *e.g.*, histone tail modifications, which correlate with changes in gene expression and physiology, including increased lifespan (Hall et al., 2010). In addition, there is a growing evidence for transgenerational effects, some of which are mediated by large-scale changes in DNA methylation, as shown in mammals, (Anway et al., 2005) and lead to inheritance not only of these epigenetic states but also to physiological changes across several generations (Kaati et al., 2007). Moreover, the *C. elegans* RNA-mediated interference (RNAi) response (Lu et al., 2005) is another possible example of an epigenetic transgenerational response elicited by the environment (Lu et al., 2005). *C. elegans* can be artificially infected with mammalian viruses and this triggers an antiviral response that requires the RNAi machinery (Lu et al., 2005). So, this suggests that

the transgenerational nature of RNAi in *C. elegans* (Fire et al., 1998) may help protect its offspring from future viral infections.

Food is one of the most important environmental factors that affect gene activities. For example, the quality of the food fed to the female larva of the honey bee (*Apis mellifera*) is a major determinant in the type of developmental caste program it will undergo, either that of the short-lived worker or that of the long-lived queen (Evans and Wheeler, 1999). In contrast to larvae that are destined to become workers, the larva that is designated to become a queen is fed throughout its development with a richer mixture of food, known as royal jelly, which contains salivary gland secretions from the provisioning worker bees (Evans and Wheeler, 1999). This nutritional control of different developmental programs, which lead not only to remarkably diverse behaviors but also lifespan, seems to involve epigenetic mechanisms, like DNA methylation (Kucharski et al., 2008). Indeed, the downregulation of DNA methyltransferase Dnmt3, a key player in DNA methylation, in recently hatched bee larvae have an effect that resembles that of royal jelly, *i.e.*, the induced development of queen honey bees (Kucharski et al., 2008).

Because of the food influence on gene activities, it is not surprising that animals also regulate their food consumption, as part of a feedback mechanism to maintain their internal homeostasis. Food consumption is dynamically regulated by the neuroendocrine system in response to the animal's current metabolic status, as well as its emotional and reproductive states (Saper et al., 2002; Morton et al., 2006; Murphy and Bloom, 2006). At the same time, food type could also have an effect on both the metabolic and emotional status of the animal,

e.g., the antidepressant effect of the spice saffron (Wang et al., 2010). Nonetheless, although these complex interactions between the animal's environment and gene activities are known, the mechanisms through which animals integrate such interactions remain unclear. The elucidation of these mechanisms is the focus of this thesis research.

B. The environmental effects on physiology are mediated by the sensory system

To regulate physiology, the neuroendocrine signaling pathways need meaningful information from the environment, which can be provided by the sensory system. The sensory system is part of the nervous system that is responsible for recognizing and processing sensory information. There are several types of sensory modalities, which include vision, hearing, somatosensation (touch and pain), thermosensation, olfaction (smell) and taste. The different types of sensory information are converted into changes in neuronal activities that in turn regulate the secretion of neurotransmitters, which further relay the information through neural circuitries that could ultimately control hormonal secretions (Alcedo et al., 2010). Some of the neural circuitries that process sensory information, *e.g.*, vision, olfaction and taste, lead to the hypothalamus, which serves as a master neuroendocrine regulator of many physiological processes: from food intake and metabolism to reproduction (Squire et al., 2003). Thus, through such circuitries, environmental cues can exert their effects on various physiological processes. Below I will give some examples of how sensory cues, *e.g.*, visual or chemosensory, can trigger systemic changes in mammalian physiology.

Many animals inherently display individual daily rhythms of biological processes, known as the circadian rhythm, which helps the organism to maintain functional periodicity according to the day/night cycle of the earth. Accordingly, light influences the mammalian circadian rhythm through the suprachiasmatic nucleus (SCN) of the hypothalamus, which is regarded as the master circadian pacemaker that regulates the clocks of all peripheral cells (Challet et al., 2003). The pacemaker role of the SCN was demonstrated by the reciprocal transplantation of the SCN tissue between wild type and a mutant hamster strain that has an arrhythmic circadian clock (Ralph et al., 1990). Neural grafts of wild-type SCN restored the circadian rhythmicity of arrhythmic animals whose own SCNs were removed (Ralph et al., 1990). Furthermore, the recipients' circadian rhythms exhibited the period of the donor genotype, regardless of the direction of the transplantation (wild type to mutant or vice versa) or the genotype of the host (Ralph et al., 1990).

Since the day/night cycle of the earth changes through the year, the circadian rhythm has to be reset from time to time, so that the animal functions synchronously with its environment. Light plays a major role in this synchronization, or so-called entrainment, of the circadian rhythm (Baehr et al., 1999; Hirayama et al., 2007). This entrainment of the clock occurs through the retinohypothalamic tract (Ding et al., 1994; Hattar et al., 2002; Provencio et al., 2002). Light is detected by the photopigment melanopsin in the photosensitive retinal ganglion cells (ipRGC), which transmits the photic information through their axons in the retinohypothalamic tract to the SCN (Squire et al., 2003). The SCN then interprets this information and coordinate the activities of the peripheral 'clocks', which involve the

secretion of different types of hormones (Wurtman et al., 1963; Wurtman et al., 1964; Kramer et al., 2005; Prosser et al., 2007).

Olfactory systems are also able to affect organismal physiology. Most mammals have a main olfactory system and an auxiliary olfactory system, known as the vomeronasal sense organ (Squire et al., 2003). The main olfactory system is used to find food, detect predators and prey and sample the environment by sensing volatile odorants. This system has three parts: the main olfactory epithelium, the main olfactory bulb and the olfactory cortex. The olfactory epithelium consists of approximately 2000 olfactory sensory neurons (OSNs), with each neuron expressing one allele of one of the 1000 odorant G protein-coupled receptor (GPCR) genes (Reed, 2004). OSNs that express the same olfactory receptors are randomly distributed throughout the epithelium, but their axons project into the same glomerulus in the main olfactory bulb (Reed, 2004). In the glomerulus, the OSNs make synapses with the dendrites of second-order neurons, *e.g.*, the mitral cells or the tufted cells, which in turn project their axons to the olfactory cortex (Reed, 2004). Besides the olfactory cortex, the information processed in the main olfactory bulb is also relayed through a parallel circuitry to the hypothalamus (Squire et al., 2003), which could explain how olfactory perception influences physiological processes that range from reproductive development (Yoon et al., 2005) to food intake (Zafra et al., 2006). With this in mind, the following is an example of an olfactory influence on physiology. Food odors have been reported to promote insulin secretion in the absence of food intake (Zafra et al., 2006). This process has been described as the cephalic/neural phase of food intake, which is required for optimal digestion (Zafra et al., 2006).

In contrast to the main olfactory system, the function of the vomeronasal organ (VNO) appears to be more specific to the regulation of instinctive behaviors in response to volatile and non-volatile pheromones (Bargmann, 1997). The VNO is separated from the main olfactory epithelium by a cartilaginous capsule (Squire et al., 2003). VNO neurons express two types of GPCRs that differ from each other and from the large family of olfactory receptors found in the main olfactory epithelium (Bargmann, 1997). Unlike the receptors in the olfactory epithelium, which are coupled to $G\alpha_{s\text{-}like}$ proteins (G_{olf}) to activate adenyl cyclase, VNO receptors are coupled to $G\alpha_{i/o}$ proteins that activate a different type of signaling through inositol 1,4,5-trisphosphate (Bargmann, 1997; Keverne, 1999). In addition, the pheromonal information perceived by the VNO is relayed to the accessory olfactory bulb and, again unlike information from the main olfactory system, is transmitted mainly, via the amygdala, to the hypothalamus to regulate reproductive, defensive and other behaviors (Keverne, 1999).

The gustatory system provides the animals with valuable information about the nature and quality of their food. There are five basic taste modalities: sweet, salty, sour, bitter and umami (Squire et al., 2003). These tastants are sensed by specialized structures known as taste buds that are found on the tongue of the animal (Squire et al., 2003). Each taste bud, of which humans have thousands, consists of approximately 50-150 receptor cells (Squire et al., 2003). Two cranial nerves innervate the tongue and carry taste information to the brain: the facial nerve (cranial nerve VII), which innervates the anterior two-thirds of the tongue, and the glossopharyngeal nerve (cranial nerve IX), which innervates the posterior one-third of

the tongue (Squire et al., 2003). A third cranial nerve (the vagus nerve, X) also receives taste information, which originates from the back part of the mouth (Squire et al., 2003). All three nerves carry taste information to a specific structure in the brainstem, known as the nucleus of the solitary tract, which transmits the information further to the thalamus and the cerebral cortex, as well as to the hypothalamus via a parallel circuit (Squire et al., 2003).

Taste stimuli have been reported to trigger various physiological responses. For example, taste perception can lead to salivary (Yamamoto, 1989) and pancreatic secretions (Ohara et al., 1988). In addition, like food odors and as part of the cephalic response to food intake, it has been shown that oral saccharine stimulation causes a glycemia-independent increase in peripheral insulin secretion (Berthoud et al., 1980). Moreover, there is a growing number of evidence that suggests that gustatory stimulation also affects organs that are not part of the digestive system. Gustatory stimulation with sucrose, NaCl, citric acid, quinine-HCl and monosodium glutamate has been reported to increase the heart rate of healthy university students, and this increase appears to differ with the concentration and type of taste solution (Horio, 2000). Furthermore, taste has been reported to induce perspiration (Lee, 1954).

C. The *C. elegans* sensory system

Despite the microscopic size and simpler morphology of *C. elegans*, it also has different types of sensory modalities: mechanosensation (touch), thermosensation, taste and olfactory perception. The adult *C. elegans* hermaphrodite has 302 neurons, the majority of which, including sensory neurons, develop during embryogenesis (White et al., 1986). The neuronal connectivities within the worm are well described, which include the identification

of roughly 5000 chemical synapses, 2000 neuromuscular junctions and 500 gap junctions (White et al., 1986).

C. elegans has 60 sensory neurons that detect various soluble and volatile compounds, tactile stimuli and temperature (Bargmann and Horvitz, 1991b; Bargmann and Horvitz, 1991a; Bargmann et al., 1993; Mori and Ohshima, 1995; Bargmann and Mori, 1997). Twenty-four of these sensory neurons are located in a pair of head sensory organs, called amphids (White et al., 1986). These sensory neurons extend dendrites to the tip of the head, where the ciliated endings of the dendrites are either directly or indirectly exposed to the environment (Perkins et al., 1986; White et al., 1986; Bargmann et al., 1993). Moreover, the axons of these sensory neurons join a large axon bundle, called the nerve ring, where they make synaptic connections with each other or with interneurons, which allow them to communicate with the rest of the *C. elegans*'s nervous system (White et al., 1986). Below I will briefly elaborate on the functions and circuitries of two types of sensory system within this animal.

C.1. The *C. elegans* olfactory system

C. elegans has three pairs of olfactory neurons, AWA, AWB and AWC, which are found in the amphid organs and detect volatile odors that can come from its food source, like bacteria (Bargmann et al., 1993). Two of these neuron pairs, AWA and AWC, are required for chemotaxis (Bargmann et al., 1993). AWAs detect a different set of odorants, *e.g.*, diacetyl, pyrazine, and 2,4,5-trimethylthiazole, while AWCs detect another set, *e.g.*, benzaldehyde, 2-butanone, isoamyl alcohol, 2,3-pentanedione, and 2,4,5-trimethylthiazole (Bargmann et al.,

1993). On the other hand, the third pair of olfactory neurons, AWB, is required for volatile avoidance, *e.g.*, from 2-nonanone (Bargmann and Mori, 1997; Troemel et al., 1997).

While the AWA pair and the AWB pair have been reported to exhibit functional symmetry, the AWC pair displays functional asymmetry (Troemel et al., 1999). One AWC neuron expresses the chemosensory GPCR *str-2* and is known as AWC^{ON}, while another does not and is known as AWC^{OFF} (Troemel et al., 1999). The decision to become AWC^{ON} or AWC^{OFF} is stochastic and mutually exclusive, so that the worm has one of each (Troemel et al., 1999). This asymmetric diversity has been proposed to be important for odor discrimination by segregating the perception of different odors into different neurons or combinations of neurons (Wes and Bargmann, 2001). For example, both AWCs detect benzaldehyde, while AWC^{ON} detects 2-butanone and AWC^{OFF}, 2, 3-pentanedione (Sagasti et al., 2001; Wes and Bargmann, 2001).

The three pairs of olfactory neurons synapse extensively to overlapping sets of interneurons (White et al., 1986): AWA to AIY and AIZ; AWB to AIZ; and AWC to AIY and AIB. However, the functional mechanisms behind these connectivities are just starting to be understood. Recently, a report has shown that the information from AWC neurons are processed through the activation of AIB interneurons and the inhibition of AIY interneurons through glutamate-mediated signaling (Chalasani et al., 2007). This type of information processing is similar to the relay of information from vertebrate photoreceptor cells to two classes of interneurons (Yang, 2004). Interestingly, similar to the photic inhibition of photoreceptor neuronal activities, the AWC neuronal activities are also inhibited by the

presence of odorants (Yang, 2004; Chalasani et al., 2007). Thus, this suggests that at least some odorants ultimately inhibit AIB interneuron function and activate AIY function.

C.2. The *C. elegans* gustatory system

Unlike the olfactory system, the *C. elegans* gustatory system consists of more sensory neuron pairs, some of which are found in the amphid organs (Bargmann and Horvitz, 1991a; Bargmann and Mori, 1997). Laser ablation experiments unraveled ten pairs of gustatory neurons: those in the head amphid organs – ADF, ADL, ASE, ASG, ASH, ASI, ASJ and ASK; and those found in the tail phasmid organs – PHA and PHB (Perkins et al., 1986; Bargmann and Horvitz, 1991a; Bargmann and Horvitz, 1991b; Hilliard et al., 2002). In addition to these neurons, at least six other neurons are proposed to have gustatory function, the IL2 neurons, which are also found in the worm's head region (Perkins et al., 1986). The gustatory neurons sense soluble, nonvolatile compounds, such as amino acids, salts, metabolites and the pheromone mixture of glycosides (Bargmann and Horvitz, 1991a). Some of these neurons function in chemoattraction to the above compounds, while others function in chemorepulsion, *e.g.*, ADL and ASH avoidance of metal salts and other noxious substances (de Bono et al., 2002; Hilliard et al., 2002). Like olfactory neurons, some of the gustatory neuron pairs are shown to have functional symmetry, whereas at least one of these neuron pairs also exhibit functional asymmetry, the ASEL (left ASE neuron), which detects Na+ ions, and the ASER (right ASE neuron), which detects the Cl^- ions (Bargmann and Horvitz, 1991a).

The postsynaptic partners of gustatory neurons overlap considerably with those of olfactory neurons, but gustatory neurons synapse more extensively to AIA, AIB and AIY interneurons (White et al., 1986). To promote chemotaxis or chemorepulsion, these first-order interneurons, downstream of the gustatory and olfactory neurons, communicate in turn with second-order interneurons (Gray et al., 2005). Subsequently, the second-order interneurons relay the information to motor neurons, which are required to regulate the worm's locomotion in response to different chemical cues (Gray et al., 2005).

D. Sensory influence on *C. elegans* physiology

C. elegans, like many other organisms, has its own life cycle (Figure 1), in addition to a wide range of behavioral and physiological traits. The life cycle of *C. elegans* is influenced by the sensory system and, as mentioned earlier, displays plasticity that ultimately enhances the animal's chance of survival. Favorable environmental conditions promote growth from L1 to L4 larvae, which molt into reproductive adults (Figure 1). On the other hand, L1 larvae exposed to overcrowding and low food availability are induced to enter an alternative developmental program, known as dauer arrest [Figure 1; (Golden and Riddle, 1982; Golden and Riddle, 1984)]. This switch in the developmental program is regulated by the ratio of dauer-inducing pheromones to food levels (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). Each worm constitutively secretes a dauer pheromone mixture (Golden and Riddle, 1982) of glycosides (Jeong et al., 2005; Butcher et al., 2007), and an increase in population density leads to an increase in dauer pheromones in the environment, as well as to low food levels. The L1 larva responds to this high pheromone/ food ratio by molting into a predauer stage, called the L2d, which feeds and stores nutrients in intestinal and hypodermal granules

(Riddle and Albert, 1997). If the dauer-inducing conditions persist, the L2d larvae molt into the dauer stage, which exhibits morphologically distinct features, like a plugged buccal cavity, a remodeled pharynx and cuticle and an arrested gonad (Riddle and Albert, 1997). The nonfeeding dauer larva also has decreased metabolism and increased resistance to stress (Riddle and Albert, 1997). Thus, once conditions improve, such as a lowering in the pheromone/food ratio, worms exit from the dauer stage and resume reproductive development.

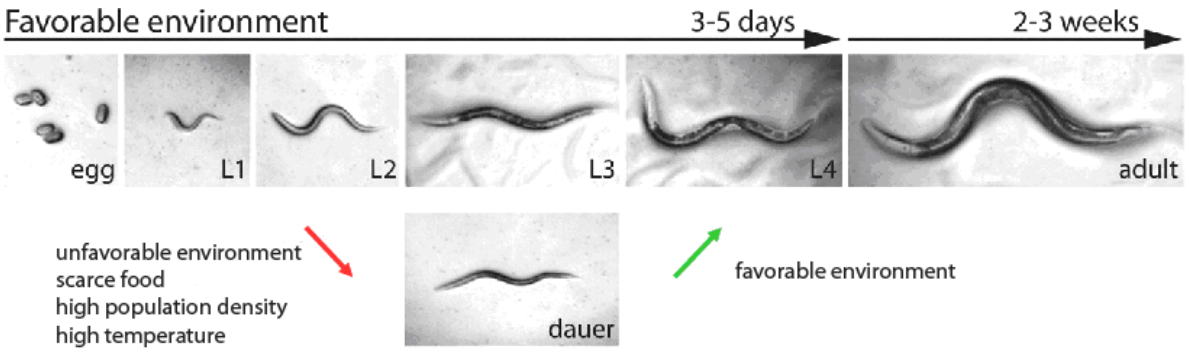

Figure 1. *C. elegans* life cycle [modified from (Fielenbach and Antebi, 2008)]. The worm undergoes four larval stages (L1-L4) before becoming an adult under optimal conditions. Harsh conditions induce L1 larvae to undergo dauer arrest to enhance survival until conditions become favorable again, upon which dauers exit into the fourth larval stage (L4).

Dauer formation is subject to active neuronal regulation (Bargmann and Horvitz 1991). The sensory neurons that regulate this process reside in a pair of major sensory organs, called amphids, which are located in the head region (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). The amphid neurons ASI, ADF, and ASG inhibit dauer formation, since their removal in L1 by laser ablation triggers transient dauer entry even in the presence of food (Bargmann and Horvitz, 1991b). In contrast, the amphid neurons ASJ and ASK promote dauer formation (Schackwitz et al., 1996). Interestingly, ASJ has a second function in that it

also promotes dauer exit under improved environmental conditions, since removal of ASJ in worms missing ASI, ADF and ASG underwent constitutive dauer arrest (Bargmann and Horvitz, 1991b).

These neurons regulate dauer formation by sensing food levels and/or dauer pheromone. ASI perceives food availability, since an abundance of food causes ASI to secrete DAF-7/ TGF-β, which is required for reproductive growth, whereas lack of food causes loss of *daf-7* expression from these neurons (Ren et al., 1996). In addition, the neurons ASJ and ASK have been shown to sense the dauer pheromone mixture: unlike unablated control worms, ablation of ASJ and/or ASK prevents dauer formation, even in the presence of high levels of dauer pheromone (Schackwitz et al., 1996). Moreover, the two chemoreceptors, SRBC-64 and SRBC-66, for dauer pheromones that have recently been identified are found to be expressed in ASK (Kim et al., 2009). The information these neurons receive, in terms of food levels versus dauer pheromone, are presumably integrated in downstream interneurons, like AIA, which receive synaptic inputs from ASI and ASK (White et al., 1986).

E. Sensory influence on lifespan

Aging is a multi-factorial process that involves a complex interaction between the environment of an animal and the signaling pathways that control its physiology. This interaction can also be mediated by the animal's sensory system, since the lifespan of *C. elegans*, as well as that of *Drosophila*, is influenced by mutations and treatments that inhibit the function of its sensory neurons (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Libert et al., 2007).

Under a specific condition, this sensory influence on lifespan involves a specific subset of sensory neurons (Alcedo and Kenyon, 2004). Laser ablation experiments revealed that the gustatory ASI and ASG neurons shorten adult lifespan (Figure 2), since ablation of either ASI or ASG alone extends lifespan (Alcedo and Kenyon, 2004). On the other hand, ablation of either the gustatory ASJ or ASK neurons completely suppressed the extended lifespan phenotype produced by ablation of ASI neurons, which suggest that ASJ and ASK function differently by lengthening lifespan [Figure 2; (Alcedo and Kenyon, 2004)]. Besides gustatory neurons, olfactory neurons also influence longevity (Figure 2), as ablation of AWA and AWC neurons extends adult lifespan (Alcedo and Kenyon, 2004).

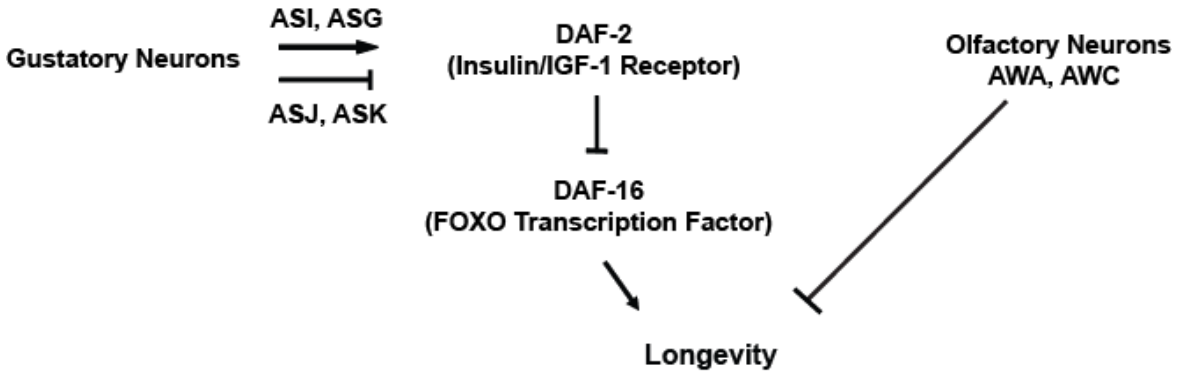

Figure 2. Certain gustatory neurons (ASI, ASG) and olfactory neurons (AWA, AWC) shorten lifespan, whereas other neurons (ASJ, ASK) lengthen lifespan. To affect lifespan, both classes of gustatory neurons appear to modulate insulin/IGF-1 signaling, while olfactory neurons appear to regulate a different signaling pathway.

These neurons influence lifespan, at least in part, by modulating the activity of the insulin/IGF-1 pathway (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004), which has been shown previously to affect worm lifespan (Kenyon et al., 1993). Null mutations in the

FOXO transcription factor *daf-16*, which acts downstream of the worm insulin/IGF-1 receptor DAF-2, can largely or completely suppress the extended lifespan of sensory mutants [(Figure 3); (Apfeld and Kenyon, 1999)] or ASI-ablated worms, respectively (Alcedo and Kenyon, 2004). Consistent with this model, sensory neurons have been shown to express several of the worm's forty insulin-like genes (Pierce et al., 2001; Li et al., 2003), which raises the possibility that environmental cues regulate the release of insulin-like ligands from sensory neurons to modulate the activity of the DAF-2 insulin/IGF signaling pathway (Figure 4).

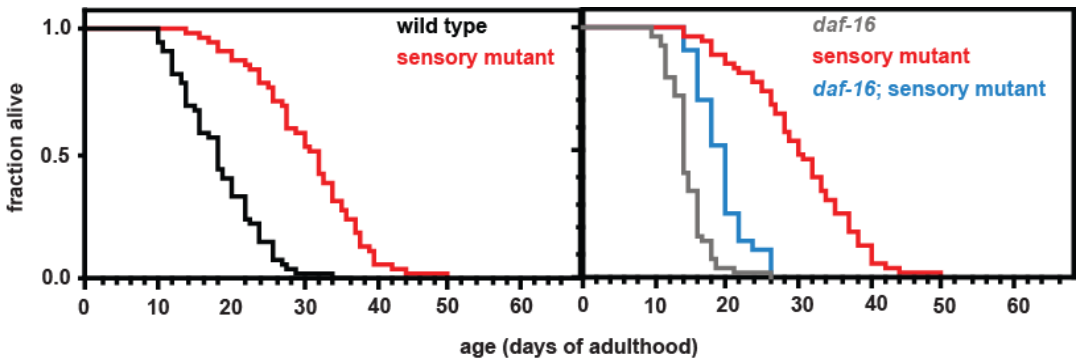

Figure 3. A sensory mutation extends lifespan in a *daf-16*-dependent and –independent manner. The lifespan of sensory mutants at 20°C are compared to wild type in the presence or absence of *daf-16* activity. This figure is modified from (Apfeld and Kenyon, 1999).

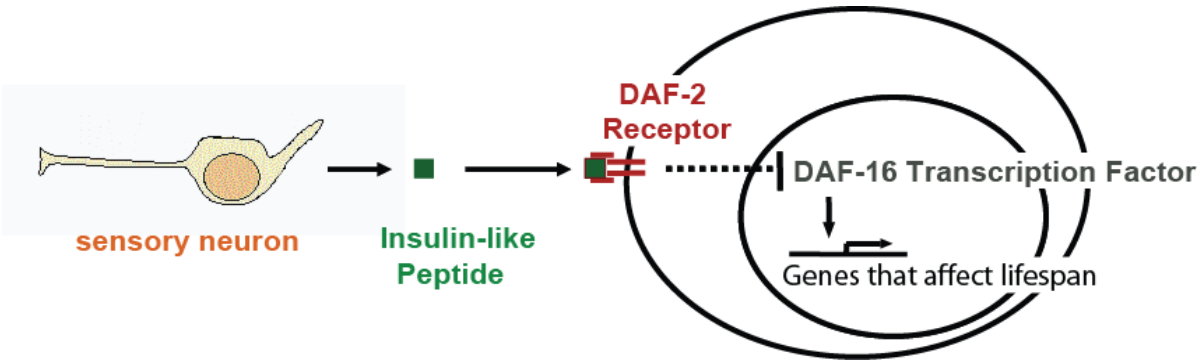

Figure 4. A model of how sensory cues modulate signaling pathways to affect lifespan.

The sensory influence on lifespan differs from the sensory influence on dauer formation. For example, the activity of the receptor is required during the first larval stage to regulate the dauer program (Riddle and Albert, 1997), while DAF-2 activity during early adulthood is sufficient to affect lifespan (Dillin et al., 2002). Moreover, the same high concentration of dauer pheromones that induce dauer formation is insufficient to promote changes in the animal's adult lifespan (Alcedo and Kenyon, 2004). It is possible that insulin-like ligands that modulate DAF-2 activity during development are distinct from the ligands that affect lifespan. These insulin-like peptides, which might be secreted from different cells, could bind DAF-2 with different affinities or act on DAF-2 in different cells to promote different signaling outputs.

At the same time, it is important to note that the sensory influence on lifespan is also partly independent of *daf-16*, since null *daf-16* mutations do not completely suppress the long lifespan of some sensory mutants [(Figure 3); (Apfeld and Kenyon, 1999)] or olfactory-neuron ablated worms (Alcedo and Kenyon, 2004). Thus, this suggests that different subsets

of sensory neurons, *e.g.*, gustatory versus olfactory neurons, affect lifespan through more than one pathway (Figure 2).

E.1. The DAF-2 insulin/IGF receptor pathway

One of the major and best understood signaling pathways that regulate lifespan is the insulin/IGF-1 pathway, which has been shown in worms, flies and mammals (Kenyon, 2005). Involvement of this pathway in lifespan regulation has been first demonstrated in *C. elegans*, where mutations in the *daf-2* gene, encoding an insulin/IGF-1 receptor ortholog (Kimura et al., 1997), were found to double worm lifespan (Kenyon et al., 1993). The lifespan extension caused by *daf-2* mutations requires the activity of *daf-16* (Kenyon et al., 1993), which encodes a transcription factor of the FOXO family (Lin et al., 1997; Ogg et al., 1997).

The DAF-2 receptor activates a conserved phosphoinositide (PI) 3-kinase pathway (Friedman and Johnson, 1988; Morris et al., 1996; Ogg and Ruvkun, 1998; Paradis and Ruvkun, 1998; Paradis et al., 1999; Hertweck et al., 2004) to affect lifespan, at least in part by regulating the nuclear localization of DAF-16 (Henderson and Johnson, 2001; Lee et al., 2001; Lin et al., 2001). In addition to DAF-16, DAF-2 also affects lifespan through the heat shock transcription factor HSF-1, which may act downstream of or in parallel to DAF-16 (Hsu et al., 2003; Morley and Morimoto, 2004; Cohen et al., 2006).

Through transcriptome analyses (Murphy et al., 2003) and bioinformatic investigation of *cis* regulatory regions (Lee et al., 2003), the DAF-2/DAF-16 pathway was found to regulate the

expression of genes that control *C. elegans* metabolism, stress and innate immune responses, which also affect lifespan. These genes include superoxide dismutase, metallothionine, catalase and glutathionine S-transferase, as well as genes involved in apolipoprotein synthesis, the glyoxylate cycle, microbicide function and amino acid turnover (Lee et al., 2003; Murphy et al., 2003). Other genes encode proteins with chaperone activity, *e.g.*, heat shock proteins (Lee et al., 2003; Murphy et al., 2003). The role of these genes in influencing lifespan has been confirmed with RNA-mediated interference, overexpression and mutant analyses (Sun et al., 2002; Lee et al., 2003; Melendez et al., 2003; Murphy et al., 2003; Walker and Lithgow, 2003). Thus, this pathway, which functions non-autonomously in many cells (Apfeld and Kenyon, 1998), appears to coordinate the expression of many genes in response to the animal's environment to optimize its survival.

The role of insulin/IGF-1 pathway in lifespan regulation is also conserved evolutionarily. For example, mutations in the *Drosophila* insulin receptor (IR) and its downstream substrate, the IRS *chico*, also extend lifespan (Clancy et al., 2001; Tatar et al., 2001). Furthermore, mice that lack one copy of its IGF-1 receptor (Holzenberger et al., 2003) or lack both copies of its insulin receptor in the adipose tissue (Bluher et al., 2003) or both copies of its IRS in the brain (Taguchi et al., 2007) live longer than wild-type.

E.2. Neuropeptide signaling pathways

Sensory neurons are known to express other non-insulin-like neuropeptides that could also mediate the sensory influence on lifespan. Neuropeptides are short sequences of amino acids that have diverse roles in the function and development of the nervous system, as well as in

other physiological processes. Neuropeptides make up a large class of signaling molecules not only in *C. elegans* but also in many species (Strand, 1999; Husson et al., 2007). They are processed post-translationally from proprotein precursors that can encode a single peptide or multiple peptides with different activities that exert their effects through G protein-coupled seven-pass membrane receptors (Strand, 1999; Husson et al., 2007). Neuropeptides can act as short-range signals, *e.g.*, neurotransmitters, or long-range signals, *e.g.*, hormones; they can also act as neuromodulators, *i.e.*, they elicit no effect on their own but activate or inhibit other signals depending on the metabolic state of the target cell (Strand, 1999). In fact, the same neuropeptide can sometimes act as a neurotransmitter on one target cell, a hormone on a different cell or a neuromodulator on other cells (Strand, 1999).

A single neuropeptide precursor can be differentially processed to give rise to distinct sets of neuropeptides in different cell types: either a single neuropeptide or multiple neuropeptides of the same or dissimilar compositions (Strand, 1999). A neuropeptide precursor (propeptide) is first cleaved by a signal peptidase in the endoplasmic reticulum to remove its signal peptide (Steiner, 1998). In the trans-Golgi network, the propeptide is then cleaved at its mono-, di-, or tribasic residues by proprotein convertases, which exposes the basic amino acid residues for further removal by carboxypeptidase E (Steiner, 1998; Strand, 1999). The removal of the basic residues next exposes a glycine, which donates an amino group in a subsequent amidation reaction (Strand, 1999). This amidation reaction is catalyzed by two enzymes, a peptidylglycine-α-hydroxylating monooxygenase (PHM) and a peptidyl-α-hydroxyglycine α-amidating lyase (PAL) (Eipper et al., 1993; Strand, 1999). This

modification is generally regarded as a mechanism that protects neuropeptides from degradation (Eipper et al., 1993; Strand, 1999). In addition, this modification provides biological activity to the neuropeptide, which is released from dense core vesicles that derive from the trans-Golgi network (Strand, 1999).

Since the molecular mechanisms underlying neuropeptide-mediated functions remain poorly understood, *C. elegans*, with its simple nervous system, known neuronal connectivities and completely sequenced genome, provides a unique opportunity for a systematic analysis of neuropeptidic function. To date, *C. elegans* is predicted to have 113 neuropeptide genes that encode over 250 distinct neuropeptides (Bargmann, 1998; Husson et al., 2007). From these genes, forty encode insulin-like peptides, thirty-one encode FMRFamide-related peptides, and forty-two encode non-insulin, non-FMRFamide-related neuropeptides (Bargmann, 1998; Husson et al., 2007).

The *C. elegans* genome also contains four proprotein convertase genes: *kpc-1, egl-3*/*kpc-2*, *aex-5/kpc-3* and *bli-4/kpc-4* (Thacker and Rose, 2000). At least two of these convertases have been shown to be expressed in neurons, *egl-3/kpc-2* and *bli-4/kpc-4* (Thacker et al., 1995; Thacker and Rose, 2000; Kass et al., 2001). The loss-of-function mutations in two of these convertases, *kpc-1* and *egl-3/kpc-2*, result in slow growth and defects in egg-laying, mechanosensation and locomotion (Thacker and Rose, 2000; Kass et al., 2001; Jacob and Kaplan, 2003). On the other hand, loss-of-function mutations in *bli-4/kpc-4* result in lethality (Thacker et al., 1995). Together these suggest that proprotein convertases cleave precursors whose neuropeptides have diverse functions.

C. elegans also has a neural-specific carboxypeptidase E, *egl-21*, which is expressed in a large fraction of neurons (Jacob and Kaplan, 2003), and at least one gene that encodes a protein needed for the amidation of its neuropeptides (Sieburth et al., 2005). Moreover, the worm genome is predicted to have more than 1000 GPCRs, some of which function as neuropeptide receptors (Bargmann, 1998). Mutations in some of these neuropeptide receptors have implicated these proteins in regulating processes, like feeding behavior (de Bono and Bargmann, 1998; Keating et al., 2003; Bendena et al., 2008).

F. Aims of the thesis

The fact that gustatory and olfactory neurons influence longevity suggests that the sensory influence on lifespan could be mediated by food-derived sensory cues. However, the observation that not every gustatory neuron affects lifespan (Alcedo and Kenyon, 2004) also suggests that the sensory influence on lifespan cannot be explained simply through the regulation of general food intake. Consistent with this hypothesis, sensory-impaired worms do not look like calorically-restricted worms (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004), which are also long-lived but have decreased food intake (Klass, 1977). Thus, it is possible that the worm, through its sensory system, adjusts its rate of aging in response to more specific sensory cues, *e.g.*, food quality.

Gustatory and olfactory neurons are known to express neuropeptides other than insulin-like peptides (Li et al., 1999; Nathoo et al., 2001); however, the identities of non-insulin-like neuropeptides and their downstream components that influence longevity remain unknown.

Since these sensory neurons may affect physiology, and consequently lifespan, by controlling the release of neuropeptides in response to food quality, I aim to identify new neuropeptide signaling pathways that mediate the sensory influence on lifespan.

Thus, I plan to answer the following questions:

(1) What non-insulin-like neuropeptide signaling pathway(s) are regulated by the sensory system to influence lifespan?

(2) From which cells do these pathways function to influence lifespan?

(3) How do these pathways modulate the activities of already known longevity-influencing pathways?

Chapter II. Results

A neuromedin U receptor acts with the sensory system to mediate food type-dependent effects on *C. elegans* lifespan

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*These authors contributed equally to this work.

Abstract

The type of food source has previously been shown to be as important as the level of food intake in influencing lifespan. Here we report that different subsets of sensory neurons mediate the effects of different food sources on *C. elegans* longevity. We also show that the sensory system acts with a homolog of mammalian neuromedin U receptors, *nmur-1*, to affect lifespan in a food source-dependent manner. Wild-type *nmur-1*, which is expressed in the somatic gonad, sensory neurons and interneurons, shortens lifespan only on specific *E. coli* food sources—an effect that is dependent on the type of *E. coli* lipopolysaccharide (LPS) structure. Moreover, the food type-dependent effect of *nmur-1* on lifespan is different from that of food-level restriction. Together our data suggest that *nmur-1* processes information from specific food cues to influence lifespan and other aspects of physiology.

Introduction

The sensory systems of *C. elegans* and *D. melanogaster* have been shown to modulate the lifespan of these animals [1-4]. This sensory influence involves subsets of gustatory and olfactory neurons [2,3] that either shorten or lengthen lifespan, which suggests that (i) some of the cues that affect lifespan are food-derived and that (ii) these cues can exert different effects on lifespan. Since a reduction in food levels can increase lifespan [5], it is possible that the sensory system influences lifespan by simply regulating the animal's general food intake, and, indeed, the sensory system has been implicated in the lifespan effects of food-level restriction in *Drosophila* [3]. On the other hand, the sensory influence on lifespan, at least in *C. elegans*, can be uncoupled from sensory effects on feeding rate, development and reproduction [1,2]. Since the lifespan effect of food-level restriction has been linked to changes in feeding rates and decreased development and reproduction [5], this suggests that the sensory system also affects lifespan through other mechanism(s).

The *C. elegans* hermaphrodite has 60 sensory neurons with dendrites that terminate in ciliated endings [6]. These specialized structures contain dedicated sensory receptors [7,8] and are thus the sites of recognition for different types of environmental cues, including gustatory, olfactory, thermal and mechanical stimuli [9]. Within its natural environment, *C. elegans* encounters various types of bacteria that can serve as food sources. Similar to the sensory influence on lifespan, some of these food sources have been shown to alter lifespan independently of development and reproduction [10]. At the same time, not all but only a subset of food-sensing neurons influence the lifespan of *C. elegans* grown on the standard laboratory food source [2], *E. coli* OP50 [11]. Together these data raise the possibility that

sensory neurons promote the lifespan effects of different food sources through a mechanism distinct from that of food-level restriction.

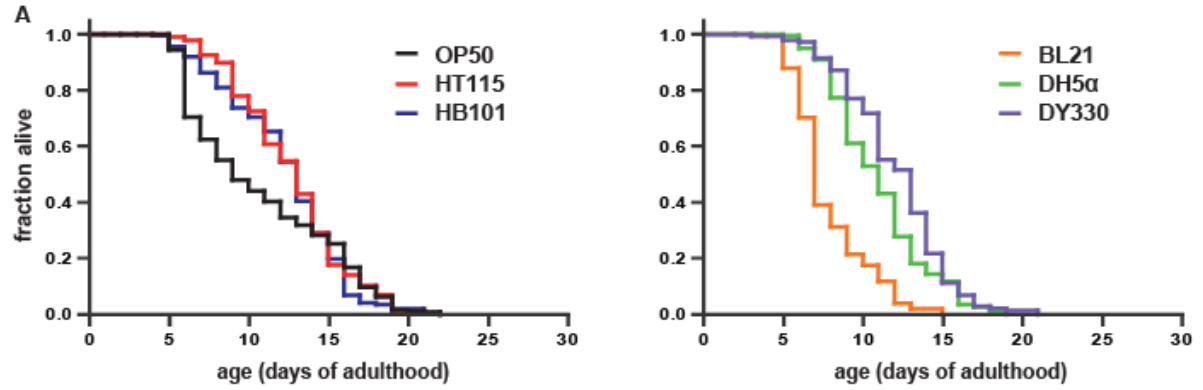
In this study, we have investigated the role of the sensory system in the food-source influence on *C. elegans* lifespan and the signaling pathway(s) that might be involved in this process. We show that the *C. elegans* sensory system recognizes food types to affect longevity. We also identify (i) the neuromedin U receptor *nmur-1* as a neuropeptide signaling pathway involved in this process and (ii) a food-derived cue, the *E. coli* lipopolysaccharide (LPS) structure, which elicits the *nmur-1* response.

Results

The Sensory System Mediates the Effects of Food Types on Lifespan

Wild-type *C. elegans* have altered lifespan on different *E. coli* strains (Figures 1A and 1B). Indeed, we found that at 25°C the mean lifespan of wild-type worms is shorter on OP50 than on HT115 (Figures 1A and 1B), another food source that is widely used [12,13]. To test the hypothesis that sensory perception contributes to these food source-dependent effects, we measured the lifespan of sensory mutants on OP50 and HT115 at this temperature.

The gene *daf-10* encodes an ortholog of an intraflagellar transport complex protein that is required for cilia formation in a subset of sensory neurons [14,15] (Table S1). We observed that the lifespan of *daf-10* mutants is extended compared to that of wild type to the same extent (44% vs. 46%; Table 1) when grown on either OP50 or HT115 (Figure 1C), which suggests that some sensory neurons shorten lifespan independently of these two food sources.



B

Wild-type Lifespan at 25°C

Food Source	Mean Lifespan ± SEM (Days)	% OP50	*P* Value Against OP50 (Logrank)	*P* Value Against OP50 (Wilcoxon)	% HT115	*P* Value Against HT115 (Logrank)	*P* Value Against HT115 (Wilcoxon)
OP50	10.7 ± 0.3				**- 16**	0.02	< 0.0001
BL21	7.9 ± 0.3	**- 26**	< 0.0001	0.0002	**- 38**	< 0.0001	< 0.0001
HB101	12.3 ± 0.3	**+ 15**	0.23	0.0001	- 3	0.42	0.46
HT115	12.7 ± 0.3	**+ 19**	0.02	< 0.0001			
DY330	12.1 ± 0.3	**+ 13**	0.52	0.0002	- 5	0.07	0.19
DH5α	11.0 ± 0.2	+ 3	0.23	0.02	**- 13**	< 0.0001	< 0.0001

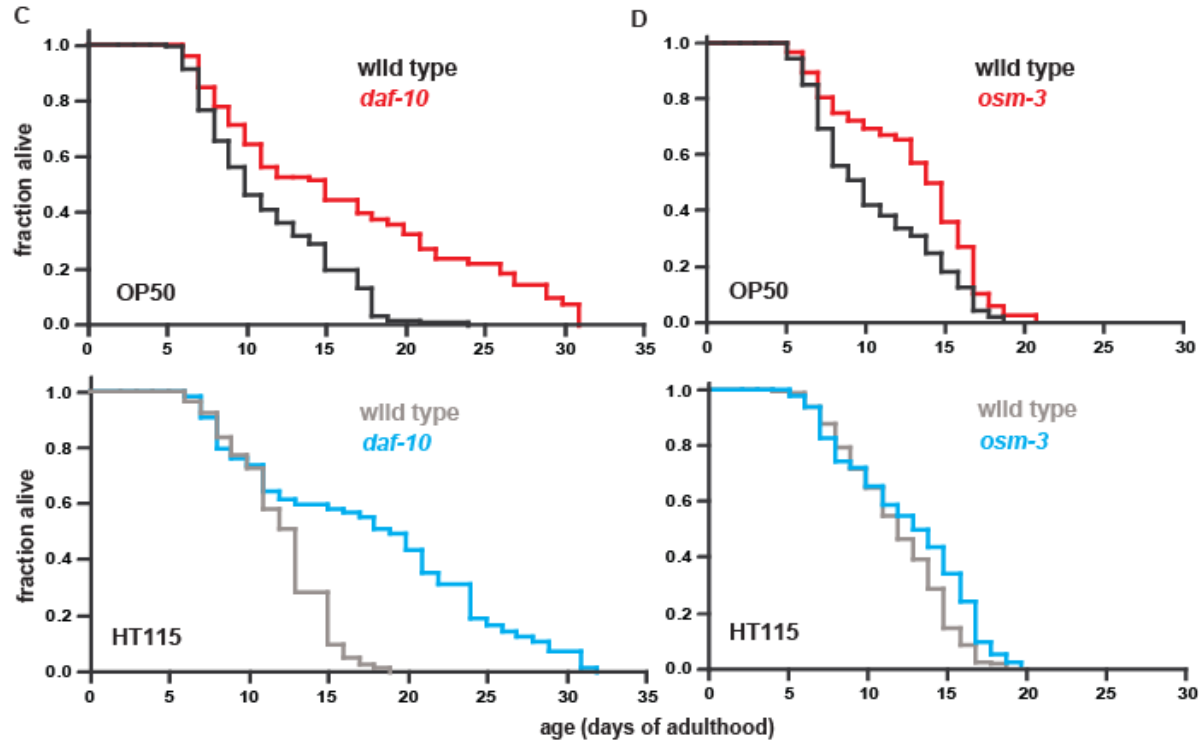


Figure 1. Sensory neurons mediate the different effects of various types of food sources on lifespan. (**A**) Wild-type survival plots on different *E. coli* food sources and (**B**) statistics for cumulative data

from 2 to 3 independent trials at 25°C (see Table S3 for statistics on individual trials). The % OP50 and % HT115 in **B** refer to mean lifespan changes relative to the two standard food sources. Bold underlined values indicate a significant difference in survival (Wilcoxon $P \leq 0.01$) on a given food source compared to either OP50 or HT115. Logrank test results are given for comparison (see Materials and Methods). (**C**) The lifespan of *daf-10(m79)* sensory mutants compared to wild type on two different *E. coli* food sources. The curves in this and subsequent panels represent cumulative data. Detailed data on these and subsequent survival analyses can be found in Tables 1 or S3. (**D**) The lifespan of wild type and *osm-3(n1540)* sensory mutants on different *E. coli* strains.

In contrast, worms that carry mutations in *osm-3*, which encodes a kinesin motor protein required for cilia formation in a different subset of sensory neurons [16,17] (Table S1), live long relative to wild type only when grown on OP50, but not when grown on HT115 (Figure 1D; Table 1). This implies that at least some of the *osm-3*-expressing neurons sense the lifespan-influencing difference(s) between these food sources.

nmur-1 Affects Lifespan in a Food Source-Dependent Manner

Since *osm-3* functions in cilia structure formation rather than in directly sensing or translating food-derived cues, we searched for non-structural genes that would act with the sensory system to regulate the food source-dependent effects on lifespan. Candidate genes would include those encoding sensory receptors and downstream signaling molecules, like neuropeptides and their receptors, which help transmit or modulate sensory information. Unlike individual sensory receptors specific for single cues, a single downstream factor may affect the integration of several cues, which would make the effects of this class of genes more readily detectable.

The *C. elegans* genome has more than 75 neuropeptide-like genes and more than 1000 G-protein-coupled receptors, some of which function as neuropeptide receptors [9,18-21]. We focused on a subset of these genes based on the availability of mutations and on the evidence that their homologs in other animals regulate feeding and metabolism [19,21-25]. We compared the lifespan of the different mutants on OP50 and HT115.

While most neuropeptide signaling pathways had no effect on lifespan on the two food sources tested (Table S2), we found that animals carrying the deletion mutation

Table 1. Cumulative adult lifespans at 25°C

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Sensory mutants									
OP50: Wild type	11.3 ± 0.4	15	130/150 (2)						
OP50: *daf-10(m79)*	16.3 ± 0.9	22	78/148 (2)	**+ 44**	< 0.0001	< 0.0001			
HT115: Wild type	12.1 ± 0.3	15	124/140 (2)				+ 7	0.94[a]	0.02[a]
HT115: *daf-10(m79)*	17.7 ± 0.8	24	101/140 (2)	**+ 46**	< 0.0001	< 0.0001	+ 9	0.29[a]	0.13[a]
OP50: Wild type	10.5 ± 0.3	14	187/220 (3)						
OP50: *osm-3(n1540)*	12.9 ± 0.3	17	192/250 (3)	**+ 23**	< 0.0001	< 0.0001			
HT115: Wild type	11.8 ± 0.2	15	198/220 (3)				**+ 12**	0.10[a]	0.0002[a]
HT115: *osm-3(n1540)*	12.6 ± 0.3	16	219/250 (3)	+ *7*	< 0.0001	0.02	- 2	0.37[a]	0.49[a]
nmur-1 food-dependence									
OP50: Wild type	10.7 ± 0.3	16	240/290 (4)						
OP50: *nmur-1*	15.0 ± 0.3	18	230/292 (4)	**+ 40**	< 0.0001	< 0.0001			
BL21: Wild type	7.9 ± 0.3	9	52/150 (2)				**- 26**	< 0.0001[a]	0.0002[a]
BL21: *nmur-1*	10.1 ± 0.5	14	85/150 (2)	**+ 28**	0.0001	0.002	**- 33**	< 0.0001[a]	< 0.0001[a]
HB101: Wild type	12.3 ± 0.3	15	193/220 (3)				**+ 15**	0.23[a]	< 0.0001[a]
HB101: *nmur-1*	15.1 ± 0.2	17	173/220 (3)	**+ 23**	< 0.0001	< 0.0001	+ 1	0.25[a]	0.38[a]
HT115: Wild type	12.7 ± 0.3	15	186/220 (3)				**+ 19**	0.02[a]	< 0.0001[a]
HT115: *nmur-1*	13.3 ± 0.3	16	166/210 (3)	+ 5	0.06	0.08	**- 11**	< 0.0001[a]	< 0.0001[a]
DY330: Wild type	12.1 ± 0.3	14	137/150 (2)				**+ 13**	0.52[a]	0.0002[a]
DY330: *nmur-1*	12.8 ± 0.3	15	126/150 (2)	+ 6	0.05	0.11	**- 15**	< 0.0001[a]	< 0.0001[a]
DH5α: Wild type	11.0 ± 0.2	13	196/220 (3)				+ 3	0.23[a]	0.02[a]
DH5α: *nmur-1*	13.2 ± 0.2	16	197/220 (3)	**+ 20**	< 0.0001	< 0.0001	**- 12**	< 0.0001[a]	< 0.0001[a]
Rescue experiments									
Line 1									
OP50: *jxEx4*	10.1 ± 0.4	14	69/83 (1)						
OP50: *nmur-1; jxEx12*	11.9 ± 0.5	15	54/66 (1)	**+ 18**	0.01[b]	0.009[b]			
OP50: *nmur-1; jxEx4*	14.4 ± 0.4	17	73/88 (1)	**+ 42**	< 0.0001[b]	< 0.0001[b]	**+ 21**	0.0003[c]	0.0001[c]

Table 1 (Continued)

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Line 2									
OP50: *jxEx4*	12.8 ± 0.4	17	109/127 (2)						
OP50: *nmur-1; jxEx40*	14.6 ± 0.5	19	89/152 (2)	**+ 14**	0.002[b]	0.01[b]			
OP50: *nmur-1; jxEx4*	17.4 ± 0.4	21	120/144 (2)	**+ 36**	< 0.0001[b]	< 0.0001[b]	**+ 19**	< 0.0001[c]	< 0.0001[c]
HT115: *jxEx4*	11.2 ± 0.3	13	128/152 (2)						
HT115: *nmur-1; jxEx40*	13.3 ± 0.3	16	146/169 (2)	**+ 19**	< 0.0001[b]	< 0.0001[b]			
HT115: *nmur-1; jxEx4*	12.8 ± 0.2	15	146/162 (2)	**+ 14**	< 0.0001[b]	< 0.0001[b]	*- 4*	0.004[c]	0.18[c]
Line 2 and second set of controls									
OP50: *jxEx14*	12.5 ± 0.5	15	69/80 (1)						
OP50: *nmur-1; jxEx40*	12.8 ± 0.5	15	45/80 (1)	+ 2	0.75[b]	0.71[b]			
OP50: *nmur-1; jxEx14*	15.1 ± 0.5	18	46/70 (1)	**+ 21**	0.002[b]	0.0003[b]	**+ 18**	0.0006[c]	0.0008[c]
E. coli LPS-dependence									
OP50: Wild type	11.9 ± 0.8	17	34/40 (1)						
OP50: *nmur-1*	17.5 ± 0.5	19	31/40 (1)	**+ 47**	< 0.0001	< 0.0001			
CS180: Wild type	14.3 ± 0.2	17	205/240 (3)						
CS180: *nmur-1*	15.1 ± 0.2	17	202/240 (3)	+ 5	0.05	0.03			
CS2198: Wild type	13.2 ± 0.3	15	141/160 (2)				**- 8**	0.006[d]	0.001[d]
CS2198: *nmur-1*	15.8 ± 0.3	19	134/161 (2)	**+ 20**	< 0.0001	< 0.0001	+ 3	0.05[d]	0.19[d]
CS2429: Wild type	13.3 ± 0.2	16	213/240 (3)				**- 7**	0.01[d]	0.0003[d]
CS2429: *nmur-1*	16.2 ± 0.3	19	188/240 (3)	**+ 22**	< 0.0001	< 0.0001	**+ 7**	< 0.0001[d]	0.002[d]
CS1861: Wild type	13.4 ± 0.5	17	72/80 (1)				- 3	0.99[d]	0.61[d]
CS1861: *nmur-1*	14.5 ± 0.5	17	63/80 (1)	+ 8	0.17	0.12	- 5	0.56[d]	0.51[d]
daf-10 epistasis									
OP50: Wild type	11.3 ± 0.4	15	130/150 (2)						
OP50: *nmur-1*	15.4 ± 0.4	18	126/150 (2)	**+ 36**	< 0.0001	< 0.0001			
OP50: *daf-10(m79)*	16.3 ± 0.9	22	78/148 (2)	**+ 44**	< 0.0001	< 0.0001			
OP50: *daf-10; nmur-1*	21.1 ± 0.8	26	75/150 (2)	**+ 87**	< 0.0001	< 0.0001	**+ 29**	0.002[e]	< 0.0001[e]

Table 1 (Continued)

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
daf-10 epistasis									
HT115: Wild type	12.1 ± 0.3	15	124/140 (2)						
HT115: *nmur-1*	12.2 ± 0.4	15	55/70 (1)	+ 1	0.76	0.82			
HT115: *daf-10(m79)*	17.7 ± 0.8	24	101/140 (2)	**+ 46**	< 0.0001	< 0.0001			
HT115: *daf-10; nmur-1*	21.8 ± 1.0	25	32/70 (1)	**+ 80**	< 0.0001	< 0.0001	**+ 23**	0.02[e]	0.002[e]
osm-3 epistasis									
OP50: Wild type	10.5 ± 0.3	14	187/220 (3)						
OP50: *nmur-1*	14.0 ± 0.3	17	188/220 (3)	**+ 33**	< 0.0001	< 0.0001			
OP50: *osm-3(n1540)*	12.9 ± 0.3	17	192/250 (3)	**+ 23**	< 0.0001	< 0.0001			
OP50: *osm-3; nmur-1*	15.2 ± 0.2	18	220/250 (3)	**+ 45**	< 0.0001	< 0.0001	**+ 18**	< 0.0001[f]	< 0.0001[f]
							+ 9	0.005[g]	0.009[g]
HT115: Wild type	11.8 ± 0.2	15	198/220 (3)						
HT115: *nmur-1*	12.7 ± 0.2	15	182/220 (3)	**+ 8**	0.008	0.009			
HT115: *osm-3(n1540)*	12.6 ± 0.3	16	219/250 (3)	*+ 7*	< 0.0001	0.02			
HT115: *osm-3; nmur-1*	13.8 ± 0.2	17	217/250 (3)	**+ 17**	< 0.0001	< 0.0001	**+ 10**	0.01[f]	0.002[f]
							+ 9	< 0.0001[g]	0.001[g]
CS180: Wild type	13.8 ± 0.2	16	143/160 (2)						
CS180: *nmur-1*	14.4 ± 0.2	17	143/160 (2)	*+ 4*	0.002	0.03			
CS180: *osm-3(n1540)*	15.5 ± 0.2	17	134/160 (2)	**+ 12**	< 0.0001	< 0.0001			
CS180: *osm-3; nmur-1*	15.7 ± 0.2	17	136/160 (2)	**+ 14**	< 0.0001	< 0.0001	+ 1	0.24[f]	0.24[f]
							+ 9	< 0.0001[g]	< 0.0001[g]
CS2429: Wild type	12.8 ± 0.3	16	152/160 (2)						
CS2429: *nmur-1*	14.2 ± 0.3	17	147/160 (2)	**+ 11**	< 0.0001	0.0007			
CS2429: *osm-3(n1540)*	15.3 ± 0.3	18	140/160 (2)	**+ 20**	< 0.0001	< 0.0001			
CS2429: *osm-3; nmur-1*	14.8 ± 0.3	18	148/160 (2)	**+ 16**	< 0.0001	< 0.0001	- 3	0.32[f]	0.32[f]
							+ 4	0.02[g]	0.05[g]
daf-2 epistasis on OP50									
Wild type	11.6 ± 0.5	15	62/70 (1)						
nmur-1	15.3 ± 0.4	17	61/70 (1)	**+ 32**	< 0.0001	< 0.0001			
daf-2(e1370)	33.3 ± 1.2	39	54/70 (1)	**+ 188**	< 0.0001	< 0.0001			

Table 1 (Continued)

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
daf-2 epistasis on OP50									
daf-2(e1370); nmur-1	36.7 ± 1.1	43	59/71 (1)	**+ 216**	< 0.0001	< 0.0001	+10	0.02[h]	0.05[h]
Wild type	11.8 ± 0.5	15	76/80 (1)						
nmur-1	14.8 ± 0.4	17	72/80 (1)	**+ 25**	< 0.0001	< 0.0001			
daf-2(e1368)	21.0 ± 1.1	29	62/80 (1)	**+ 78**	< 0.0001	< 0.0001			
daf-2(e1368); nmur-1	24.4 ± 0.8	29	67/80 (1)	**+ 107**	< 0.0001	< 0.0001	+16	0.24[i]	0.06[i]
daf-16 independence on OP50									
Wild type	11.7 ±0.3	15	128/141 (2)						
nmur-1	16.6 ± 0.5	19	57/70 (1)	**+ 42**	< 0.0001	< 0.0001			
daf-16(mu86)	9.5 ± 0.3	13	125/140 (2)	**- 19**	< 0.0001	< 0.0001			
daf-16; nmur-1	11.8 ±0.3	13	127/139 (2)	0	0.30	0.99	**+ 24**	< 0.0001[j]	< 0.0001[j]
aak-2 independence on OP50									
Wild type	11.2 ± 0.5	14	69/80 (1)						
nmur-1	14.8 ± 0.3	17	110/130 (1)	**+ 32**	< 0.0001	< 0.0001			
aak-2(ok524)	10.8 ± 0.5	13	66/80 (1)	- 4	0.49	0.74			
nmur-1 aak-2	13.8 ± 0.4	16	64/80 (1)	**+ 23**	0.007	0.0001	**+ 28**	0.0001[k]	< 0.0001[k]
hsf-1 independence on OP50									
Wild type	11.0 ± 0.3	14	102/140 (2)						
nmur-1	14.7 ± 0.4	18	99/140 (2)	**+ 34**	< 0.0001	< 0.0001			
hsf-1(sy441)	7.3 ± 0.1	8	248/624 (2)	**- 34**	< 0.0001	< 0.0001			
hsf-1; *nmur-1*	8.2 ± 0.2	10	102/744 (2)	**- 25**	< 0.0001	< 0.0001	**+ 12**	< 0.0001[m]	0.0004[m]
pmk-1 independence on OP50									
Wild type	12.4 ± 0.3	16	197/240 (3)						
nmur-1	15.1 ± 0.3	18	189/240 (3)	**+ 22**	< 0.0001	< 0.0001			
pmk-1(km25)	12.4 ± 0.3	15	178/291 (3)	0	0.62	1.0			
pmk-1; *nmur-1*	14.2 ± 0.3	17	166/300 (3)	**+ 15**	0.02	< 0.0001	**+ 15**	0.002[o]	< 0.0001[o]

Table 1. Adult lifespan on different *E. coli* food sources at 25°C. We assayed wild type and mutant worms in parallel in independent trials (details shown in Table S3) and we show statistics from the cumulative experiments on different *E. coli* strains. The 75th percentile is the age when the fraction of worms alive in each group falls below 0.25. The first number in the fourth column is the number of worms observed as having died, while the second number gives the total number of worms in each experiment, including worms that were censored during the course of the assay. The numbers in parentheses in the fourth column indicate the number of trials performed. Worms that crawled off the plate, exploded or bagged were censored at the time of the event, allowing these worms to be incorporated into the data set until the censor date and to avoid loss of information. Differences that are significant ($P \leq 0.01$) according to the Wilcoxon test, which in most cases are also significant according to the logrank test, are underlined and in boldface type. Differences that are significant only according to the logrank test are italicized. The % difference between wild type and mutants under different conditions is indicated in the fifth column. The % difference between certain groups of worms that are specified by the superscripted symbols is shown in the eighth column. The superscripted symbols indicate the following: [a], compared to the cumulative data for the same genotype assayed on OP50; [b], compared to *jxEx4[myo-3p::rfp]* or *jxEx14[myo-3p::rfp]* on the same food source; [c], compared to the rescue line on the same food source; [d], compared to the cumulative data for the same genotype assayed on CS180; [e], compared with *daf-10(m79)* tested on the same food source; [f], compared with *osm-3(n1540)* tested in parallel on the same food source; [g], compared with *nmur-1(ok1387)* assayed in parallel on the same food source; [h], compared with *daf-2(e1370)*; [i], compared with *daf-2(e1368)*; [j], compared to the cumulative data for *daf-16(mu86)*; [k], compared with *aak-2(ok524)*; [m], compared to the cumulative data for *hsf-1(sy441)*; and [o], compared to the cumulative data for *pmk-1(km25)*.

(*ok1387*) within the gene *C48C5.1* live long on OP50 but not on HT115 (Figures 2A and 2B; Tables 1, S2 and S3). *C48C5.1* is predicted to encode a seven-transmembrane neuropeptide receptor (Figure S1) with homology to mammalian neuromedin U receptors (NMURs), whose peptide ligand, neuromedin U, has been shown to regulate food intake [24]. We renamed *C48C5.1* as *nmur-1*, since our study makes it the first phenotypically characterized member of the worm NMUR family, of which there are at least three other members—*nmur-2* (*K10B4.4*), *nmur-3* (*F02E8.2*) and *nmur-4* (*C30F12.6*). As a confirmation that the wild-type function of *nmur-1* is to shorten lifespan in a food source-dependent manner, we were able to rescue the long-life phenotype of the *nmur-1* mutation on OP50 with the wild-type *nmur-1* genomic locus, without shortening lifespan on HT115 (Figure 2G; Tables 1 and S3).

nmur-1 Acts with the Sensory System to Mediate the Food-Source Effects on Lifespan

Next, we asked whether sensory neurons regulate the food source-dependent effects on lifespan through *nmur-1*. We found that loss of *nmur-1* still considerably increases the lifespan of *daf-10* sensory mutants on OP50 (Figure 3A; Table 1), which indicates that *nmur-1* acts in parallel at least to some *daf-10*-expressing neurons. Surprisingly, loss of *nmur-1* extends the lifespan of *daf-10* mutants also on HT115 (Figure 3B; Table 1), which may suggest that the lifespan of *nmur-1* mutants becomes food source-independent in the absence of *daf-10* activity. Thus, *nmur-1* appears to be subject not only to activation by certain environmental cues but also to inhibition by others.

In contrast, animals that carry both *nmur-1* and *osm-3* mutations have a lifespan phenotype similar to that of *nmur-1* single mutants on OP50 and HT115 (Figures 3C and 3D;

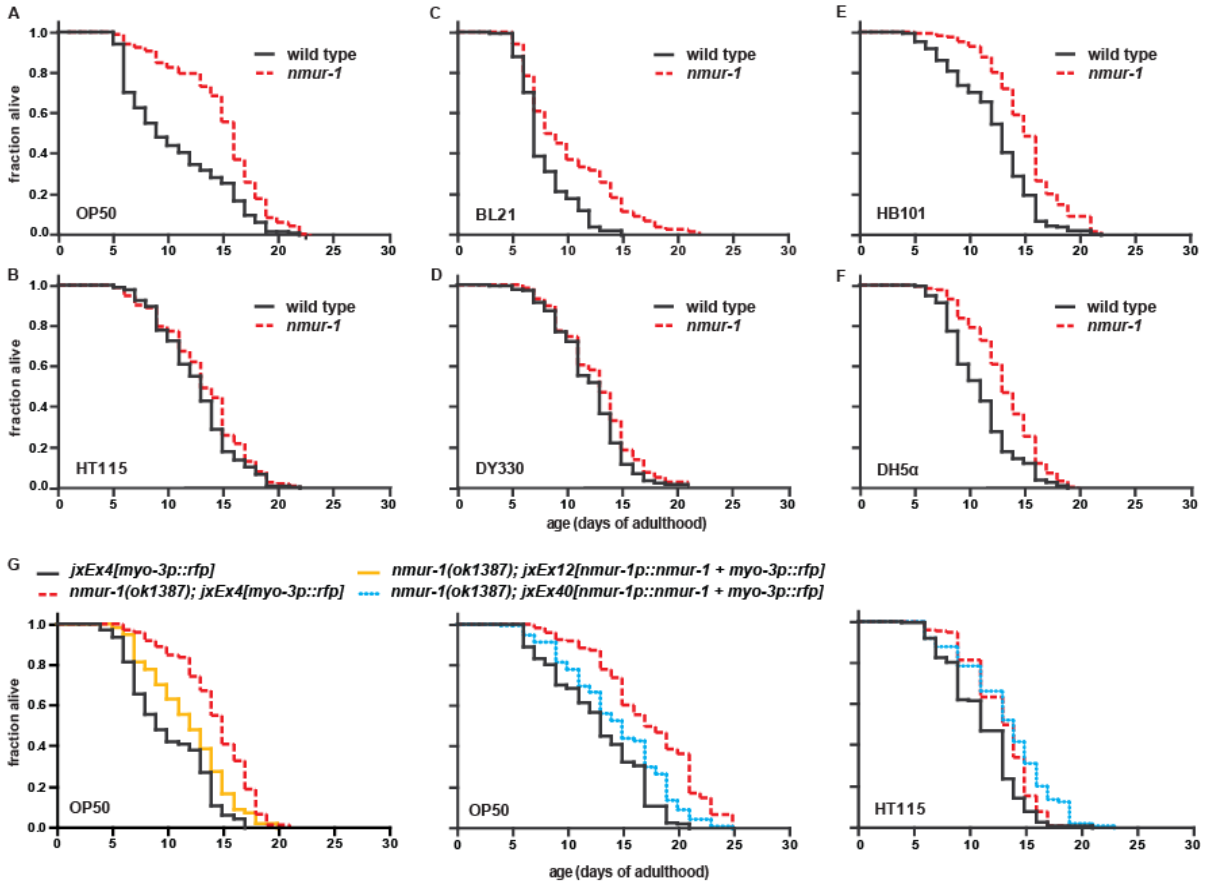


Figure 2. *nmur-1* modulates lifespan in a food source-dependent manner. (**A-F**) Lifespan of wild-type and *nmur-1(ok1387)* worms on different *E. coli* strains, which are indicated in the lower left corner of each panel. (**G**) The wild-type *nmur-1* genomic locus can rescue the food source-dependent long-life phenotype of *nmur-1(ok1387)* on OP50, without shortening lifespan on HT115. The lifespan of the two rescue lines, *nmur-1(ok1387); jxEx12* and *nmur-1(ok1387); jxEx40*, are compared to wild-type and *nmur-1* mutant worms that carry the *myo-3p::rfp* coinjection marker, *jxEx4*, alone.

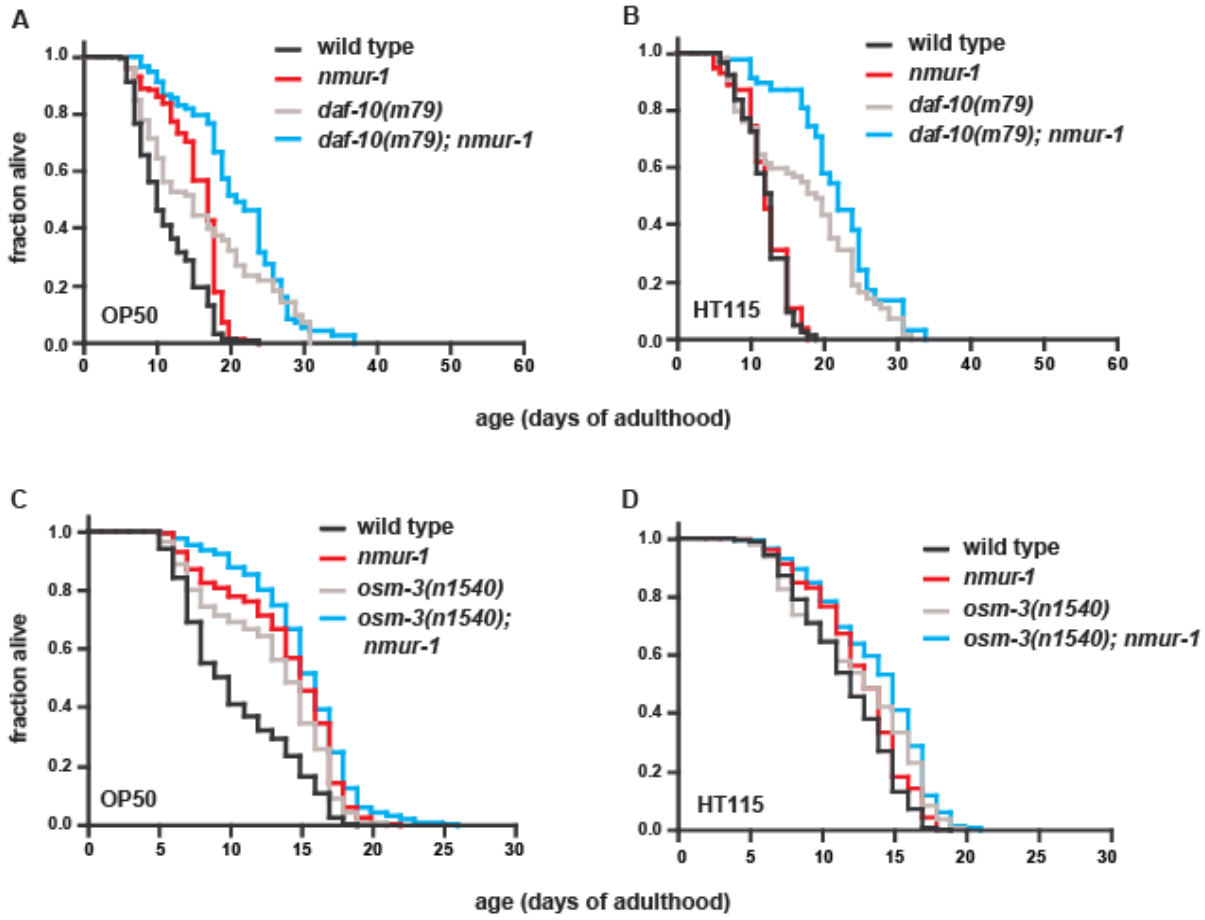


Figure 3. *nmur-1* acts with the sensory system to regulate lifespan. (**A-B**) The effects of *nmur-1* on the lifespan of *daf-10(m79)* mutants as measured on *E. coli* OP50 and HT115. (**C-D**) The effects of *nmur-1* on the lifespan of *osm-3(n1540)* mutants as compared on *E. coli* OP50 and HT115.

Table 1). This suggests that *nmur-1* acts with *osm-3* either in a subset of *osm-3*-expressing sensory neurons or in downstream cells. We observed expression of a *gfp* reporter for *nmur-1* in the spermathecae of the somatic gonad, in several different types of sensory neurons, some of which co-express *osm-3* [16] (Table S1), and in interneurons (Table 2), some of which receive inputs from, or modulate the activity of, *osm-3*-expressing sensory neurons [6]. This expression pattern, together with the genetic interaction between the mutations in *nmur-1* and *osm-3*, suggests that *nmur-1* plays a role in the processing of sensory information derived by the worm from various food sources.

The Effect of *nmur-1* on Lifespan Involves the *E. coli* LPS Structure

We then explored the possible differences between OP50 and HT115, which might be recognized by the worm. OP50 is derived from an *E. coli* B strain [11], whereas HT115 is from an *E. coli* K-12 strain [26,27]. To determine whether *nmur-1* affects lifespan only on B strains but not on K-12 strains, we measured the lifespan of *nmur-1* mutants on other bacteria derived from these two lineages. Interestingly, we found that *nmur-1* mutants live long consistently on the B strain BL21 [28], and on HB101 (Figures 2C and 2E; Tables 1 and S3), a K-12 strain that contains a large stretch of B strain genomic DNA [29]. In contrast, the *nmur-1* long-life phenotype is absent on another K-12 strain, DY330 [30], and only occasionally present on the K-12 strain DH5α(Figures 2D and 2F; Tables 1 and S3) [31]. Together these data suggest that *nmur-1* affects lifespan in a largely B strain-dependent manner.

Table 2. *nmur-1p::gfp* expression at 25°C

Cell/Tissue	**Type**	**Function**
ADFL/R	Amphid sensory neuron	Chemosensation[a], serotonergic neuron[b]
ADLL/R	Amphid sensory neuron	Chemosensation[a], nociception[c]
AFDL/R	Amphid sensory neuron	Thermosensation[d]
OLQDL/R, OLQVL/R	Outer labial sensory neuron	Mechanosensation[e]
AIAL/R (?)	Interneuron[f]	Integrates chemosensory information[f, g]
AIZL/R	Interneuron[f]	Integrates chemo-[f, g] and thermosensory[d] information
AVKL/R	Interneuron[f]	Unknown
DVA	Interneuron[f]	Stretch-receptor-mediated proprioception[h]
PVT	Interneuron[f]	Unknown
RICL/R	Interneuron[f]	Unknown
RIH	Interneuron[f]	Unknown
SDQL/R	Interneuron[f]	Unknown
PDA	Motor neuron[f]	Innervates posterior body wall muscles
ALA[f]	Neuron	Unknown
SIBDL/R[f]	Neuron	Unknown
SIBVL/R[f]	Neuron	Unknown
and 2 other head neurons		
Spermatheca	Somatic gonad	Reservoir for maturing spermatids and adult sperm

Table 2. *nmur-1p::gfp* expression. The superscripted symbols indicate the references that describe the types and known functions of the corresponding neurons: [a], [6]; [b], [74]; [c], [75]; [d], [76]; [e], [77]; [f], [78]; [g], [79]; and [h], [80]. The question mark indicates that the neuron expressing *nmur-1p::gfp* is likely to be AIA, since its position and morphology are consistent with those known for AIA, but this particular identification remains to be confirmed.

Although the B and K-12 strains clearly would have many differences, one of the few well-characterized molecular differences between these strains lies in the LPS structures (Figure 4A) on their outer membranes [32-34]. Since the LPS of the K-12 strain [33,34] has a longer outer core than the LPS of the B strain [32], we tested whether LPS structure influences lifespan. We compared wild-type and *nmur-1* mutant worms on *E. coli* K-12 mutants that have truncated LPS to worms grown on the corresponding K-12 parent strain. We found that wild-type worms live shorter on the LPS truncation mutants CS2198 and CS2429 [35,36] than on the isogenic parent strain CS180 (Tables 1 and S3), which expresses wild-type K-12 LPS [35]. On the other hand, *nmur-1* mutants live long compared to wild type only on the LPS truncation mutants (Figures 4C and 4D; Tables 1 and S3), but not on the K-12 parent strain (Figure 4B; Tables 1 and S3).

To exclude the possibility that all changes to the LPS will elicit the *nmur-1* response, we also measured the lifespan of worms grown on the K-12 strain CS1861 that expresses the *Shigella dysenteriae* 1 O Antigen fused to the end of the full-length K-12 LPS [36]. We observed no lifespan difference between wild type and *nmur-1* mutants on this strain (Figure 4E; Table 1). Together our data suggest that a short *E. coli* LPS structure can shorten worm lifespan in an *nmur-1*-dependent manner.

In contrast to *nmur-1*, we found that *osm-3* can affect lifespan independently of the *E. coli* LPS structure (Figures 4F and 4G; Table 1), which indicates that at least some of the *osm-3*-expressing neurons detect other food-derived cues. However, even on the CS180 and CS2429 bacterial food sources, *nmur-1* and *osm-3* appear to act together in influencing lifespan, since *osm-3*; *nmur-1* double mutants have the same lifespan phenotype as *osm-3* or *nmur-1* single mutants (Figures 4F and 4G; Table 1).

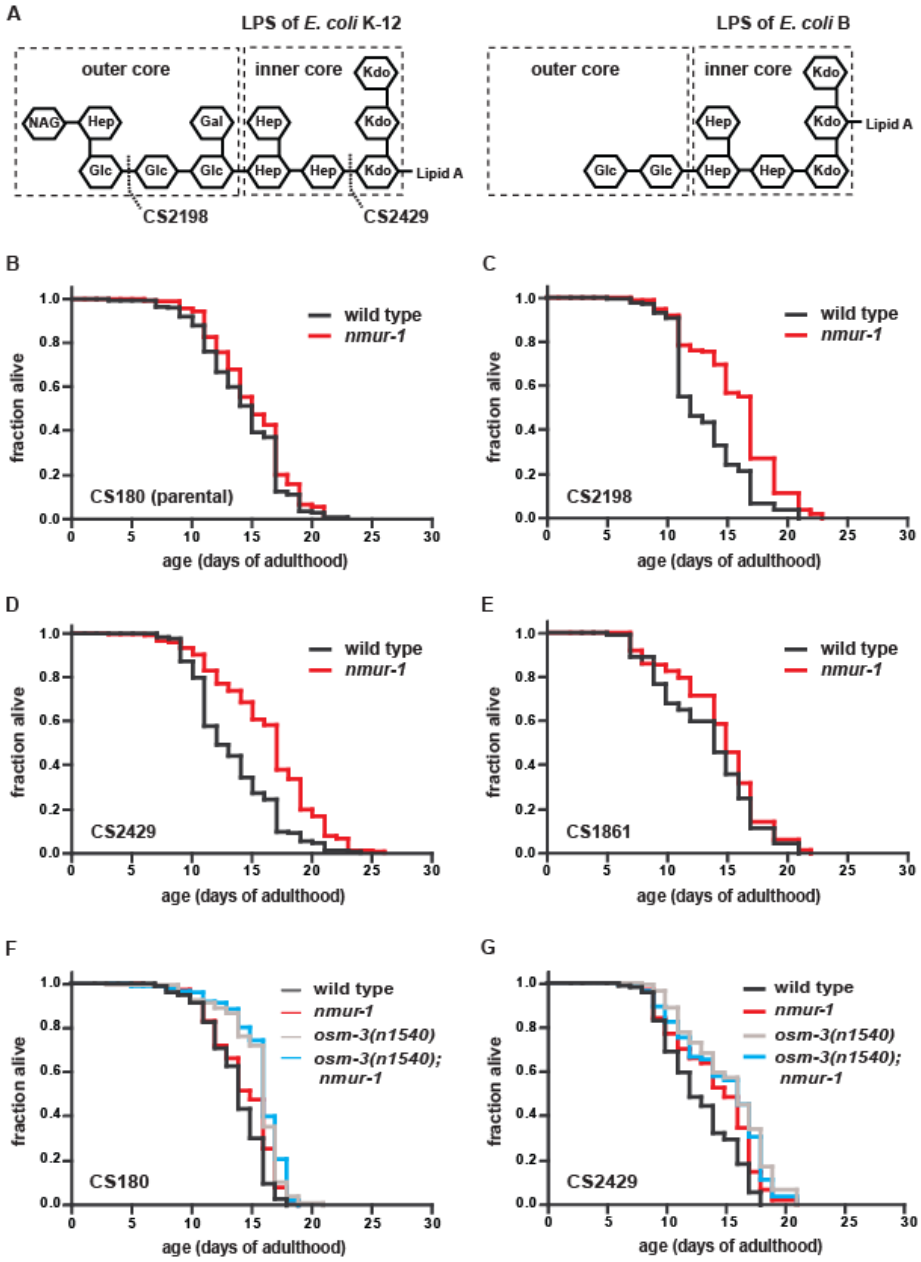


Figure 4. The *E. coli* LPS structure influences *C. elegans* lifespan in an *nmur-1*-dependent manner. (**A**) The LPS structures of *E. coli* K-12 and B strains have different sugar compositions [32,33]. Strain CS180 expresses wild-type K-12 LPS. Strains CS2198 and CS2429 are isogenic derivatives of CS180 and express the indicated truncated forms of K-12 LPS. Strain CS1861 is derived from CS180 and expresses the *Shigella dysenteriae* 1 O Antigen attached to the tip of the full-length K-12 LPS. (**B-E**) Survival curves of wild-type and *nmur-1* mutant worms on CS180, CS2198, CS2429 and CS1861. (**F-G**) The lifespan of worms carrying mutations in *nmur-1* and/or *osm-3* as compared on *E. coli* strains with different LPS structures.

The *nmur-1* Food-Source Effect on Lifespan Is Distinct from That of General Food-Level Restriction

Food type [37] and sensory neurons [3,38] have been shown to mediate the lifespan extension induced by dietary restriction (DR), which is commonly studied through restriction of food levels. Thus, the food-type dependent effects on lifespan we observe might reflect different levels of DR experienced by wild type and mutant worms on the various food sources. To address this possibility, we measured the feeding rates, speed of development, total progeny and the rates of reproduction of wild type and *nmur-1* mutants on five different *E. coli* strains (Figures S2 and S3), since DR is known to change these parameters [5]. For comparison, we used a genetic model of DR [39], a mutation in *eat-2* that impairs pharyngeal function [40], which leads to decreased feeding rates on both OP50 and HT115 (Figure S4A). Interestingly, unlike the *nmur-1* mutation, the *eat-2* mutation increases lifespan on both OP50 and HT115 (Figure S4C), which suggest that the food-type effects of *nmur-1* are not the same as general DR. Moreover, we observed no correlation between lifespan and feeding rates or lifespan and development of wild type or *nmur-1* mutants on the different food sources (Figures 5A, 5B, S2A-S2D, S3A and S3B), which are also unlike the reported effects of DR [5].

As expected for a genetic model of DR, we found that the lifespan extension conferred by the *eat-2* mutation is accompanied by a decrease in total progeny on OP50 and HT115 (Figures 5C and S4B). Surprisingly, we also found that wild-type worms grown on different food sources do exhibit an inverse correlation between lifespan and number of progeny, but that *nmur-1* mutants can still live long without a proportionate decrease in total progeny (Figures 5C, S2E and S3C). This suggests that the food source-dependent effects

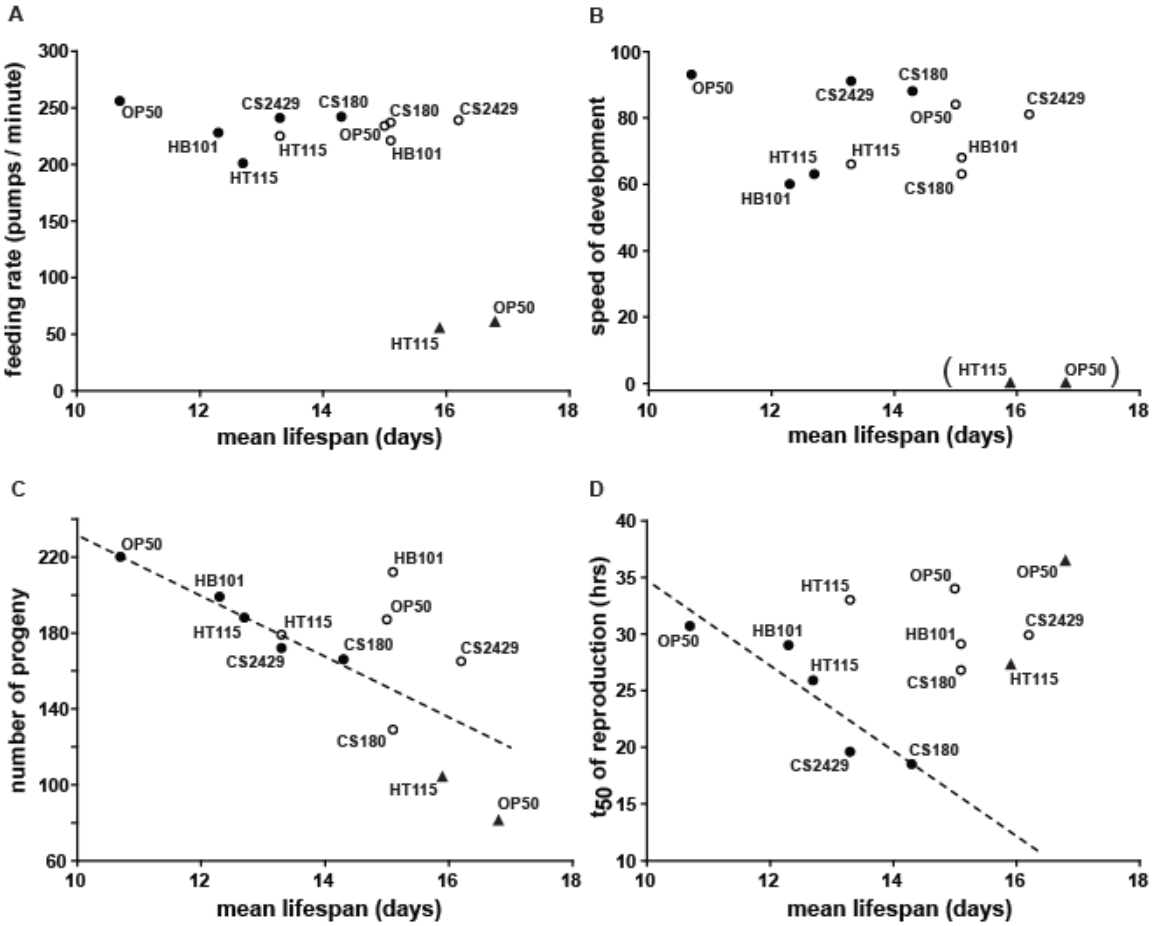


Figure 5. *nmur-1* exerts its effects on lifespan without inducing signs of food-level restriction. (**A-D**) The correlations of lifespan with feeding, development and reproduction are shown across five different food sources. The figures are compiled from the lifespan data in Tables 1 and S3 and from the data on feeding rates, developmental rates, progeny numbers and reproduction rates presented in Figures S2, S3 and S4. Pharyngeal pumping rates of young adults (**A**) and speed of development (**B**) do not correlate with mean lifespan for wild-type (closed circles) and *nmur-1* mutant worms (open circles), nor for the combined data, but are strongly reduced in food level-restricted *eat-2(ad1116)* mutants (closed triangles). The parentheses around the *eat-2* mutant data in **B** mean that the mutant speed of development falls outside the range of our index (see Material and Methods). Progeny number (**C**) and t_{50} of reproduction (**D**) are inversely correlated with mean lifespan of wild-type worms (closed circles; $P = 0.003$ for total progeny and $P = 0.029$ for reproduction time). The dotted lines are the regression lines for total progeny and reproduction time ($R^2 = 0.960$ and 0.837, respectively) calculated from the wild-type data alone. *nmur-1* (open circles) exerts an additional, reproduction-independent effect on lifespan, as suggested by the deviation from the regression lines of the corresponding data points for OP50, HB101 and CS2429.

on lifespan have reproduction-dependent and reproduction-independent components, the latter of which is uncovered by the *nmur-1* mutation. Interestingly, we also observed that food sources that increase wild-type lifespan induce the animals to reproduce faster (Figure 5D), which not only differs from *eat-2* mutants (Figure 5D) but is also the inverse of the effects shown for food-level restriction on rates of reproduction [5,41]. At the same time, we again saw no correlation between the *nmur-1* mutant lifespan and its rates of reproduction on the different *E. coli* strains (Figures 5D, S2F and S3D).

Since our data show that the effects of *eat-2* on *C. elegans* physiology differ from those of *nmur-1* or the different food sources, this suggests that the effects of the food sources and *nmur-1* on lifespan can be distinct from food-level restriction. Consistent with this idea, we observed that, unlike long-lived, food level-restricted animals that have decreased lipid storage [5], *nmur-1* mutants do not exhibit gross changes in overall lipid levels compared to wild type (data not shown).

nmur-1 Acts At Least Partly Independently of *daf-16* and *hsf-1*

Next, we asked whether *nmur-1* acts through the insulin/IGF-1 *daf-2* pathway, which has been shown to regulate worm lifespan [42,43]. We found that removing *nmur-1* does not significantly increase the lifespan of insulin/IGF-1 receptor *daf-2* mutant worms (Figures 6A and 6B; Table 1). However, loss of *nmur-1* can still extend the lifespan of worms carrying a null mutation in *daf-16* (Figure 6C; Table 1), a FOXO transcription factor acting downstream of *daf-2* [42,44,45]. Thus, our data suggests that *nmur-1* acts either with *daf-2* but at least partly independently of *daf-16*, or in parallel to the *daf-2*/*daf-16* pathway.

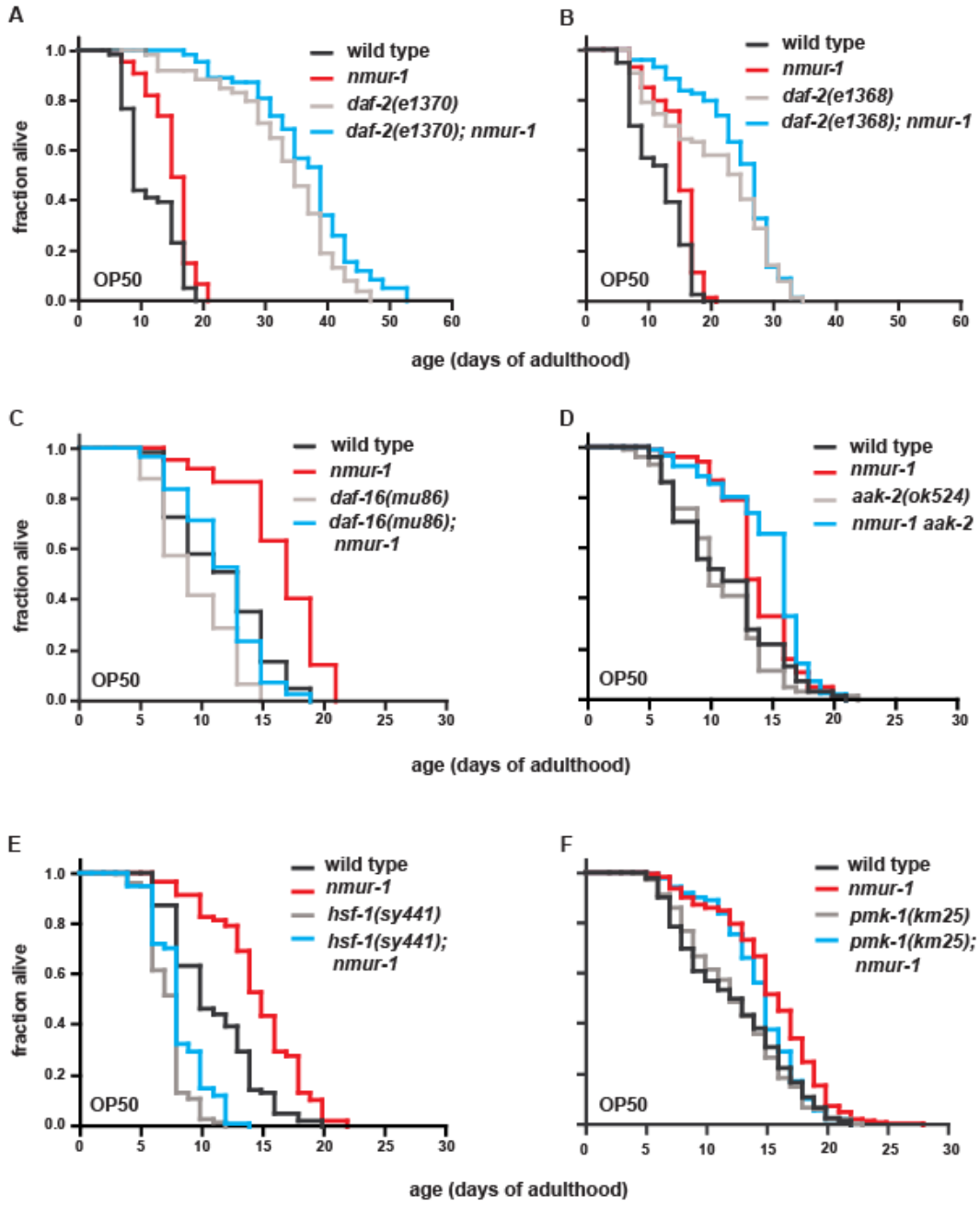


Figure 6. *nmur-1* acts at least partly independently of *daf-16* and *hsf-1*. (**A**-**B**) The effect of *nmur-1* on *daf-2* lifespan is shown for two different *daf-2* reduction-of-function mutant backgrounds. Since *daf-2* mutants undergo developmental arrest at 25°C, all strains in these experiments were grown at 20°C until the first day of adulthood, when the worms were shifted to 25°C to initiate lifespan measurements. (**C**) The effect of *nmur-1* on lifespan as assayed in a *daf-16(mu86)* null background. (**D**-**F**) The effect of *nmur-1* on the respective lifespan of *aak-2(ok524)*, *hsf-1(sy441)* or *pmk-1(km25)* mutants.

To identify other factors required for *nmur-1* to affect lifespan, we tested how removal of *nmur-1* would affect the short lifespan caused by mutations in genes proposed to act independently of *daf-16* [46-48]. We found that loss of *nmur-1* can still extend the lifespan of animals with a mutation in either (i) the AMP-dependent kinase *aak-2* (Figure 6D; Table 1), which regulates energy metabolism [46]; (ii) the heat shock transcription factor *hsf-1* (Figure 6E; Table 1), which regulates stress response [47,49,50]; or (iii) the p38 MAPK *pmk-1* (Figure 6F; Table 1), which regulates innate immunity [48,51]. Although none of these factors appears essential for *nmur-1* function, we did observe partial suppression of the *nmur-1* phenotype in the *hsf-1* mutant background. This could suggest that *nmur-1* affects lifespan by acting through several parallel pathways that include *hsf-1* and/or *daf-16*.

Discussion

Food is a complex environmental factor that affects many physiological processes, including lifespan. In the laboratory, *C. elegans* are grown on agar plates, on which the bacterial lawn that serves as the food source presumably provides a large part of the worm's chemosensory and mechanosensory inputs. Thus, the previous finding that some gustatory and olfactory neurons function either to shorten or lengthen *C. elegans* lifespan [2] makes it likely that food-derived cues affect longevity through the sensory system.

Some sensory neurons have been shown to be required for the prolonged lifespan conferred by DR [3,38], *i.e.*, under conditions of limited food availability. However, the fact that the sensory system also modulates lifespan when food is abundant suggests that the

sensory influence on lifespan involves more than one mechanism, as illustrated in this and previous studies [1,2].

Sensory Neurons Recognize Food Types to Affect Lifespan

If food-derived cues alter lifespan through the sensory system, then it is likely that impairment of a specific set of sensory neurons that detect a given set of cues would affect lifespan only on some food sources. In this study, we provide the most detailed investigation so far of the interdependence between food and sensory perception in regulating *C. elegans* longevity. We show not only that wild-type lifespan is modulated by different *E. coli* food sources (Figures 1A and 1B) but also that three sensory genes have food source-dependent effects on lifespan.

Mutations in two of these genes - *osm-3* and *nmur-1* - increase lifespan on OP50, but not on HT115 (Figures 1D and 2; Tables 1 and S3). Since the effects of these mutations are non-additive (Figures 3C and 3D; Table 1), this suggests that *osm-3 and nmur-1* influence lifespan through a common mechanism. On the other hand, a mutation in the third gene, *daf-10*, not only extends lifespan on both OP50 and HT115 (Figure 1C; Table 1) but also alters the food type-dependence of the *nmur-1* effect on lifespan (Figures 3A and 3B; Table 1).

Together with their requirement in the formation of the sensory cilia in subsets of neurons [15,16], the *osm-3* and *daf-10* data are consistent with a role for sensory perception in the food source-dependent effects on lifespan. In addition, the identification of a neuropeptide receptor gene, *nmur-1*, that interacts with *osm-3* and *daf-10* (Figures 3, 4F and

4G; Table 1), suggests a mechanism through which the sensory system mediates the effects of specific food cues on lifespan. The *nmur-1* expression in sensory neurons and interneurons (Table 2) suggests that *nmur-1* modulates the transduction of signals downstream of the sensory receptors. Based on the observed interactions among these three genes, we propose the following model: (i) *osm-3*-expressing sensory neurons detect the presence of certain food-derived cues and transmit this information through an *nmur-1*-dependent pathway; and (ii) a different set of *daf-10*-expressing neurons detects other food cues that inhibit *nmur-1* activity.

According to this model, the expression patterns (Table S1) of *osm-3* [16] and *daf-10* [15] should help define the candidate sensory neurons that might recognize the food cues that shorten or extend lifespan through *nmur-1*. *daf-10* is necessary for proper cilia morphology in the mechanosensory CEP neurons and some unidentified neurons in the head and tail sensory organs called the amphids and phasmids, respectively [15]. Several amphid neurons also express *osm-3* [16]: these include two pairs of gustatory neurons, ASI and ASG, that have been found to shorten lifespan on OP50 [2]; and two other gustatory neuron pairs that co-express *nmur-1* - ADF, which by itself has no lifespan effect on OP50 [2], and ADL. In addition, *osm-3* is expressed outside of the amphid organs in the IL2 inner labial head neurons and in the phasmid tail neurons [16], all of which have been proposed to have chemosensory function [15,52].

A Neuropeptide Receptor of the Neuromedin U Receptor Family Mediates the Sensory Influence on Lifespan

Our discovery of a food source-dependent function for the *C. elegans nmur-1* gene is consistent with the known food-associated activities of other members of the NMUR signaling pathway in mammals [24,53] and insects [54,55]. In mammals, NMUR2, the receptor isoform expressed in the central nervous system, and its ligand, the octapeptide NMU-8, have been implicated in the regulation of food intake and energy expenditure [24,53]. In *Drosophila*, the gene *hugin* encodes two of the peptide ligands, PK-2 and HUG-γ, recognized by two of four NMUR isoforms [54-56]. *hugin* regulates not only the food-seeking behavior and feeding rate of larvae but also affects the rate of food intake of adult flies in a food type-dependent manner [54]. Like *hugin*, we find that *nmur-1* exerts food-type specific effects on feeding rate (Figures S2A-S2C), although the *nmur-1* regulation of this process appears to be parallel to its regulation of lifespan (Figure 5A). Similar to the neuronal expression of *nmur-1*, *Drosophila hugin* is expressed in interneurons that appear to relay gustatory information [54]. At present, a potential role for the fly or mammalian NMUR signaling pathways in the regulation of lifespan has not been reported. However, the evolutionary conservation of several aspects of NMUR signaling leads us to speculate that the effects on lifespan of this system might also be conserved across species.

The *Drosophila* neuromedin U (NMU) signaling system also includes a second neuropeptide precursor gene, *capability*, that encodes three other peptide ligands, CAPA-1, CAPA-2 and CAPA-3 (also called PK-1), that can activate three of the fly NMUR isoforms [56]. The *C. elegans* homolog of *capability*, *nlp-44*, has recently been identified [57]. Like *capability,* it is predicted to give rise to three peptides, one of which activates the receptor

encoded by *nmur-2* [57]. A mutation of *nmur-2* gives no lifespan phenotype on the food sources we have tested (Table S2), but it will be interesting to determine whether peptides derived from *nlp-44* can also activate NMUR-1.

A role of *nmur-1* in the sensory influence on lifespan is supported by its expression in a number of sensory neurons and interneurons (Table 2). However, it remains possible that sensory cues regulate *nmur-1* activity at the level of the somatic gonad, which is the only non-neuronal tissue that expresses the *nmur-1* reporter gene (Table 2). At the same time, the expression of *nmur-1* in a relatively large number of cells also makes it likely that the parallel effects of *nmur-1* on lifespan, feeding rate, development and reproduction (Figures 5A-5D, S2 and S3) are mediated by its activity in different subsets of cells.

The food source-dependent activities of *nmur-1* raise the possibility that other neuropeptide signaling pathways - many of which are associated with the sensory system [18-20,25] - will also affect lifespan or other aspects of physiology only under specific conditions. Although most of the neuropeptide signaling pathways we have screened so far on two food sources show no effect on lifespan (Table S2), it remains possible that they will have effects on other food types. Thus, the large repertoire of neuropeptides and their receptors in *C. elegans* might serve to translate environmental complexity into appropriate physiological responses.

The Bacterial LPS Represents a Food-Derived Cue that Influences Lifespan

We find that wild-type worms live shorter on the *E. coli* B strains BL21 and OP50 than on K-12 strains, like HT115 and DY330 (Figures 1A and 1B). Conversely, the *nmur-1*

mutation causes reproducible lifespan extensions on the B strains but not on the K-12 strains (Figure 2; Tables 1 and S3). Since B and K-12 strains differ in their LPS structure, we have tested the lifespan effects of specific alterations in the K-12 LPS that mimic aspects of the B strain LPS (Figure 4A). Although the effect of LPS on wild-type lifespan is not large, wild-type worms do live longer on full-length than on truncated forms of the K-12 LPS (Tables 1 and S3). We also find that the *nmur-1* effect on lifespan is LPS-dependent and suppressed by full-length K-12 LPS, but not by its truncated versions (Figure 4; Tables 1 and S3).

Although the LPS experiments were carried out in isogenic bacterial backgrounds, the effects of the LPS alterations might be indirect since they could lead to secondary changes in bacterial metabolism or surface structure. Indeed, LPS truncations have been shown to interfere with the expression of outer membrane proteins, increase capsule polysaccharide levels and redistribute phospholipids from the inner to the outer leaflet of the outer membrane (ref. 58 and references therein). However, these secondary changes have only been observed with mutations that disrupt the inner core of the LPS, like the mutation present in the CS2429 strain (Figure 4A), and thereby compromise the integrity of the outer membrane [58]. No such effects have been reported for truncations that affect only the LPS outer core, like the mutation in CS2198 (Figure 4A), and, thus, the observation that *nmur-1* extends lifespan on both CS2429 and CS2198 argues for a direct effect of the bacterial LPS on worm lifespan. Direct recognition of LPS is biologically plausible: LPS is the predominant component of the outer membrane of gram-negative bacteria, and is consequently used by multicellular organisms from diverse phyla to recognize bacteria in the context of defense against pathogens [59,60].

Nevertheless, the LPS structure is clearly only one of potentially many food-derived cues that influence worm lifespan. This is most evident from the LPS-independent lifespan phenotype of *osm-3* mutants (Figures 4F and 4G; Table 1), and from the fact that the lifespan extension by the *nmur-1* mutation is greater on OP50 than on any other strain with a similar, short LPS (Figure 2; Tables 1 and S3). Thus, changes in lifespan are likely triggered by different sets of sensory neurons in response to a variety of food-derived cues, and loss of *nmur-1* interferes with the detection of several of these cues.

The LPS dependence of the *nmur-1* phenotype makes it conceivable that *nmur-1* may regulate stress-related and innate immune responses elicited by different food sources. We find that *nmur-1* can still affect lifespan in the absence of either of three genes, *daf-16*, *hsf-1* and *pmk-1*, all of which have major roles in stress responses and innate immunity [47-51,61-63]. However, the mutations in *daf-16* and *hsf-1* can partly suppress the *nmur-1* lifespan phenotype (Figure 6; Table 1), which makes it possible that the *nmur-1* influence on lifespan requires a combination of mechanisms that involve *daf-16*, *hsf-1* and/or other factors.

Food-Type Effects on Lifespan Can Be Distinct from Food-level Restriction

We find that the food-source influence on wild-type lifespan is strongly correlated with reproductive effects (Figures 5C and 5D), in that increases in lifespan are accompanied not only by a decreased number of total progeny but also a faster rate of reproduction. One possible interpretation of this data is that the different reproductive profiles cause the food source-dependent differences in wild-type lifespan. Indeed, with the exception of BL21, the bacterial diets we have tested seem to affect initial survival more than late-age survival. This

is supported by age-specific force of mortality plots (Figure S5A): the different food sources alter wild-type mortality primarily before day 10 of adulthood, but have little effect thereafter. It is conceivable that damage inflicted on somatic tissues [64] or neglect of somatic maintenance and repair during reproduction [65] are important determinants of early mortality. In agreement with this idea, we find that long-lived *glp-1* mutant worms [66], which are sterile because they generate few or no germ cells [67], have very similar lifespan, at least on OP50, HT115, CS180 and CS2429 (W. M., unpublished data). This suggests that the food type-dependent effects on wild-type lifespan are indeed germline-dependent. In contrast, we find that *nmur-1* exerts an additional effect on lifespan that is largely independent of reproduction (Figures 5C and 5D) and also appears to be independent of *glp-1* on OP50 and CS2429 (B. A. and W. M., unpublished data). Accordingly, the *nmur-1* mutation can affect mortality prior to day 10 of adulthood (OP50 and CS180; Figure S5B) on the food sources that significantly reduce the total progeny of *nmur-1* mutants (compare OP50 and CS180 in Figures 5C, S2E and S3C). At the same time, *nmur-1* mutants show reduced mortality after day 10, but not past day 16, of adulthood on the short LPS strains OP50 and CS2429 (Figure S5B), the latter of which has no effect on the *nmur-1* mutant number of progeny (Figure S3C). Thus, our findings imply that food sources affect lifespan through both reproduction-dependent and reproduction-independent mechanisms, with the second being uncovered by the *nmur-1* mutation.

In contrast to the longevity-promoting effect of food-level restriction [5,41], the food type-dependent effects on lifespan that we observe not only have a reproduction-independent component (Figures 5C and 5D) but are also independent of alterations in feeding rate and developmental rate (Figure 5A and 5B) and can occur in the absence of gross changes in

lipid levels (data not shown). In addition, our data show that different food types and *nmur-1* affect initial mortality without decreasing late-age mortality (Figure S5), unlike food-level restriction, which decreases the slope of the mortality trajectory and thus slows the rate of aging [68]. These data lead us to propose that these two forms of dietary influence on lifespan employ distinct, but possibly overlapping, mechanisms.

More recently, another study [69] has shown that different DR regimens for *C. elegans* require different signaling pathways to affect lifespan. However, some of these regimens altered not only food levels but also the nature of food sources. In fact, at least one of these protocols, which lowered protein levels, does not decrease but increase reproduction (ref. 69 and references therein), which suggests that the lifespan effect of protein restriction, unlike that of other DR protocols, is partly reproduction-independent. Our data might help explain some of these findings, if one assumes that the net consequence on lifespan of some DR protocols represents a mix of independent effects from food-level restriction and food-type dependence. In the future, it would be of interest to determine whether the food type-dependent effects on lifespan will also require the activities of genes, *e.g.*, the NFE2-related protein *skn-1* [38] and the FOXA transcription factor *pha-4* [70], that have been implicated in the longevity-promoting effects of DR.

Materials and Methods

Worm Strains and Bacterial Strains. All worm mutant strains used in this study were backcrossed 6 times to our lab wild-type (N2) strain, with the exception of *nmur-1(ok1387)*, which was backcrossed 8 times, and *eat-2(ad1116)*, which was outcrossed once, before

generation of different mutant combinations and any phenotypic analysis. The different worm mutant alleles used are indicated within the figures, supplementary tables and their legends. Worms were grown for at least two generations at 25^{o}C on the same food source used in a given phenotypic analysis, unless otherwise stated.

The *E. coli* strains used were: OP50 [11], HT115 [rnc14::ΔTn10 λ(DE3) of W3110] [13,26,27], BL21(DE3) [28], DY330(DE3) [Δ(argF-lacZ)U169 gal490*(IS2) pglΔ8 rnc<>cat λcI857 Δ(cro-bioA) of W3110] [30], HB101 [29], DH5α [31], CS180 [rfa+] [35], CS2198 [rfaJ19::Tnlac Δlac pyrD+ of CS180] [35], CS2429 [rfaC^{-} of CS180] [36] and CS1861 (CS180 transformed with a plasmid that confers chloramphenicol resistance and encodes the proteins required for the expression of *Shigella dysenteriae* 1 O Antigen fused to the parent strain K-12 LPS) [36].

Transgenic Worms. We generated two independent rescue lines using standard methods: *nmur-1(ok1387); jxEx12[nmur-1p::nmur-1 + myo-3p::rfp]* and *nmur-1(ok1387); jxEx40[nmur-1p::nmur-1 + myo-3p::rfp]*. The rescue fragment, which is a 7.96 kb-long PCR fragment of the wild-type *nmur-1* genomic locus (injected at 100 ng/ul), includes the 2.9-kb sequence upstream of the *nmur-1* start codon and the 1-kb sequence downstream of the correct stop codon (see Figure S1). The *myo-3p::rfp* (gift of Cori Bargmann) was used as a coinjection marker (injected at 100 ng/ul). As controls, we also generated wild-type and *nmur-1* mutant worms that carry the *myo-3p::rfp* coinjection marker alone.

We observed that the extrachromosomal array *jxEx12* has a large number of arrested embryos and larvae, whereas the extrachromosomal array *jxEx40* produces ~13% arrested

larvae (25 arrested worms/196 total worms). These additional phenotypes might be due to a hyperactive NMUR-1 pathway caused by overexpression of the gene from its extrachromosomal copies.

To determine the expression pattern of *nmur-1*, we generated a transcriptional *gfp* reporter construct (*nmur-1p::gfp*; based on the pPD117.01 vector; gift from A. Fire), in which the *gfp* is flanked by the 2.9-kb sequence upstream of the *nmur-1* start codon and by the 1-kb sequence downstream of the correct stop codon, including the newly identified 3' UTR (see Figure S1). In addition, sequences from the four largest introns, 1, 4, 8 and 10, which may contain regulatory sequences required for expression, were fused downstream of the 1-kb 3' *cis* sequences. This construct was injected into wild-type worms at a concentration of 100 ng/µl, and two independent transgenic lines, *jxEx36* and *jxEx37*, were recovered, which show identical patterns of *gfp* expression.

Bacterial Culture and Assay Plate Preparation. All bacterial strains were grown from single colonies in Luria-Bertani medium overnight at 37°C. However, the medium used to grow the chloramphenicol-resistant strain CS1861 was supplemented with 100 µg/ml chloramphenicol. Nematode-growth agar plates (6 cm in diameter; ref. 11) were seeded with 100 µl bacterial culture and were allowed to dry at room temperature (23°C). Seeded plates were stored at room temperature and used within 5 days.

Lifespan Assays. The survival analyses of all worm strains on the different bacteria were initiated on the first day of adulthood and carried out at 25°C. Throughout their reproductive

period, the worms were transferred daily to new plates to separate them from their progeny. We used the JMP 5.1 (SAS) software to determine Kaplan-Meier estimates of survival probabilities and mean lifespan, and for all statistical comparisons. *P* values were determined by the logrank and Wilcoxon tests. The logrank test, which places more weight on larger survival times, is appropriate when comparing differences between groups of animals, whose ratio of hazard functions (ratio of mortality rates) stays approximately constant over time [71]. However, when the hazard ratios do not stay constant with time, as when one survival curve shows more early deaths than another (*e.g.*, wild type on OP50 vs. wild type on HB101 or HT115 in Figures 1A and 1B), the Wilcoxon test is more appropriate for comparing differences between groups [71]. We found that the Wilcoxon test is more sensitive to the lifespan differences we see in most of our experiments, since the *nmur-1* mutation and most bacterial food sources clearly affect mean lifespan more than the maximum lifespan, which in fact violates the logrank test assumption of constant hazard ratios. Here we refer to a Wilcoxon *P* value of ≤ 0.01 as a significant difference between the various groups of animals. For comparison, we report both the Wilcoxon and logrank test results in all tables.

For mortality plots, the age-specific force of mortality was calculated as $F_x = -\ln(1-D_x)$, where D_x is the probability of death between day x-1 and x of adulthood [72]. At least five independent trials of a given lifespan experiment were used to calculate means and standard errors of F_x, which were plotted on a log scale against age.

Measurements of Feeding Rate, Progeny Number and Rate of Development. Feeding rates were determined on the first and fourth day of adulthood at 25°C by measuring the animals' pharyngeal pumping rates, which reflect the rates at which they eat bacteria [73]. The pumps of the pharyngeal bulbs of individual worms were counted 3 to 5 times over periods of 30 seconds. Each resulting mean value was then doubled to get "pumps per minute". A two-way ANOVA test was used to compare the different genotypes on different food sources and *P* values were calculated with the Tukey post-test.

Developmental rate differences were determined through a population-based assay at 25°C. First-stage (L1) larvae that had hatched within a 2-hour time window were collected and allowed to develop for 36.5 hours. At this point, the number of second-stage (L2), third-stage (L3) and fourth-stage (L4) larvae, as well as of young adult (YA) or gravid adult (GA) worms were counted. The chi square test was used to compare the resulting stage distributions across food sources or worm genotypes.

Total progeny and temporal profiles of egg-laying were determined at 25°C by culturing L4 larvae singly on plates of the appropriate food source. The worms were then transferred to new plates regularly until they stopped laying eggs. The eggs were allowed to hatch and the larval progeny were then counted. Two-way ANOVA and the Tukey post-test were used to compare the total number of progeny of genotypes across food sources. To ensure that the data followed a normal distribution, it was necessary to incorporate a statistical censoring procedure to exclude outliers (worms with very low number of progeny) from the data set before the ANOVA test. Briefly, this involved identification of outliers and calculation of standard deviation (SD) for the remaining set. Then, from the full data set, we excluded worms that had produced less progeny than the mean minus 2.5 times SD.

In general, this procedure led to exclusion of worms with a progeny number smaller than 90, which corresponded to ~4% of the total data set. The exception is *nmur-1* mutant worms feeding on HB101, for which two classes of worms seem to exist: one with a large number of progeny and another with a small number of progeny. In this particular case, censoring caused 25% of worms to be excluded from the analysis, and the remaining data set to be biased considerably towards a larger progeny number, as can be seen in Figure S2E.

The temporal profiles of egg-laying were determined from the same statistically censored populations of worms. The Hill function, $P(t) = P_{max} * t^n/(t^n + t_{50}^n)$ was used to fit the cumulative number of progeny over time, where t denotes time, P_{max} is the total number of progeny, n the Hill coefficient and t_{50} the time until half of the progeny is produced. In Figures S2F and S3D, the data was normalized to P_{max}.

For statistical assessments of correlations between mean lifespan and feeding, development or reproduction on different food sources, we used the Pearson Product Moment test. To determine the correlation between lifespan and development, the stage distributions of the original data were used to calculate "speed of development" values, which are the percentages of worms scored as either young or gravid adults in the corresponding assays. *eat-2* mutants have a value of zero on this scale because no mutant worms reached adulthood within 36.5 hrs after egg-laying. To correlate lifespan and rate of reproduction, the t_{50} of the fitted temporal reproduction profiles was used.

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SUPPORTING ONLINE MATERIAL

Supplementary Table 1. Sensory neurons affected by cilium-structure genes

Gene	Sensory Neurons Affected	Function
daf-10	Amphid sensory neurons	Chemosensation[a,b], mechanosensation[a,c], Thermosensation[a,d]
	Phasmid sensory neurons	Chemosensation[a,e]
	CEP	Dopaminergic mechanosensory neuron[a,f]
osm-3	ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK	Amphid sensory neurons[a]; chemosensation[b]
	IL2	Inner labial sensory neurons[a]
	PHA, PHB	Phasmid sensory neurons[a]; chemosensation[e]

Table S1. Sensory neurons affected by cilium-structure genes. The subsets of sensory neurons affected by two sensory genes, *daf-10* [1] and *osm-3* [2]. The two genes affect partly overlapping subsets of neurons. The superscripted symbols indicate the references that identify the neurons and their corresponding functions: [a], [3]; [b], [4-6]; [c], [7]; [d], [8]; [e], [9]; and [f], [10].

Supplementary Table 2. Adult lifespans of neuropeptide and neuropeptide receptor mutants tested at 25°C

ORF/ Treatment		Allele	Homolog	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)
E. coli OP50							
C30F12.6	*nmur-4*	*ok1381*	Thyrotropin-releasing hormone-like receptor/ Neuromedin U-like receptor	57/80	+ 4	0.55	0.82
C48C5.1	*nmur-1*	*ok1387*	Neuromedin U-like receptor	61/70	**+ 34**	0.002	0.0003
C50H2.1	*fshr-1*	*ok778*	Follicle stimulating hormone receptor	63/176	- 5	0.39	0.28
F35B12.7	*nlp-24*	*tm2105*	Opioid-like neuropeptide	50/68	+ 9	0.13	0.09
F35C11.1	*nlp-5*	*tm2125*	Allatostatin/ galanin-like neuropeptide	85/98	+ 8	0.05	0.10
F56B6.5	*npr-16*	*ok1541*	Somatostatin-like receptor	61/80	+ 7	0.28	0.32
H02I12.3	*tag-89*	*ok514*	Thyrotropin-releasing hormone-like receptor	64/80	+ 9	0.21	0.26
K10B4.4	*nmur-2*	*ttTi8340*	Neuromedin U-like receptor	66/80	+ 2	0.74	0.56
ZK455.3	*npr-9*	*tm1652*	Galanin-like receptor	67/80	+ 2	0.42	0.84

ORF/ Treatment		Allele	Homolog	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)
E. coli HT115							
C30F12.6	*nmur-4*	*ok1381*	Thyrotropin-releasing hormone-like receptor/ Neuromedin U-like receptor	58/80	- 7	0.56	0.27
C48C5.1	*nmur-1*	*ok1387*	Neuromedin U-like receptor	50/60	- 6	0.51	0.32
C50H2.1	*fshr-1*	*ok778*	Follicle stimulating hormone receptor	36/70	- 8	0.04	0.06
F35B12.7	*nlp-24*	*tm2105*	Opioid-like neuropeptide	58/70	+ 5	0.54	0.43
F35C11.1	*nlp-5*	*tm2125*	Allatostatin/ galanin-like neuropeptide	60/70	+ 3	0.31	0.61
F56B6.5	*npr-16*	*ok1541*	Somatostatin-like receptor	65/71	+ 1	0.84	0.94
H02I12.3	*tag-89*	*ok514*	Thyrotropin-releasing hormone-like receptor	64/70	+ 6	0.32	0.28
K10B4.4	*nmur-2*	*ttTi8340*	Neuromedin U-like receptor	51/79	- 8	0.10	0.30
ZK455.3	*npr-9*	*tm1652*	Galanin-like receptor	60/70	+ 8	0.06	0.13

Table S2. Adult lifespans of neuropeptide and neuropeptide receptor mutants tested at 25°C. We measured the lifespan of *C. elegans* grown on OP50 or HT115 and that carry mutations in genes that encode either neuropeptides or neuropeptide receptors. These neuropeptides or neuropeptide receptors show homologies to members of different neuropeptide signaling pathways in other animals, which are involved in regulating their feeding behavior and metabolism [11-16]. The statistical analyses performed on these experiments are as described in the legend of Table 1.

Supplementary Table 3. Individual trials of adult lifespans on different food sources at 25°C

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Sensory mutants									
Trial 1 - OP50: Wild type	11.7 ± 0.5	15	71/80						
Trial 1 - OP50: *daf-10(m79)*	15.8 ± 1.3	27	39/78	+ *35*	0.004	0.15			
Trial 1 - HT115: Wild type	12.2 ± 0.4	15	68/70				+ 4	0.79[a]	0.17[a]
Trial 1 - HT115: *daf-10(m79)*	17.3 ± 1.1	24	56/70	**+ 42**	< 0.0001	0.004	+ 9	0.29[a]	0.15[a]
Trial 2 - OP50: Wild type	10.9 ± 0.5	15	59/70						
Trial 2 - OP50: *daf-10(m79)*	17.6 ±1.2	24	39/70	**+ 61**	< 0.0001	< 0.0001			
Trial 2 - HT115: Wild type	11.9 ± 0.4	15	56/70				+ 9	0.58[a]	0.05[a]
Trial 2 - HT115: *daf-10(m79)*	18.2 ± 1.1	24	45/70	**+ 53**	< 0.0001	0.0002	+ 3	0.75[a]	0.62[a]
Trial 1 - OP50: Wild type	10.9 ± 0.5	15	59/70						
Trial 1 - OP50: *osm-3(n1540)*	13.7 ± 0.6	17	52/70	**+ 26**	0.0002	0.0005			
Trial 1 - HT115: Wild type	11.9 ± 0.4	15	56/70				+ 9	0.58[a]	0.05[a]
Trial 1 - HT115: *osm-3(n1540)*	11.4 ± 0.6	15	55/70	- 4	0.49	0.25	**- 17**	0.08[a]	0.01[a]
Trial 2 - OP50: Wild type	9.2 ± 0.5	12	61/70						
Trial 2 - OP50: *osm-3(n1540)*	12.5 ± 0.4	15	78/100	**+ 36**	< 0.0001	< 0.0001			
Trial 2 - HT115: Wild type	12.3 ± 0.4	15	66/70				**+ 34**	0.0003[a]	< 0.0001[a]
Trial 2 - HT115: *osm-3(n1540)*	13.2 ± 0.4	16	90/100	+ 7	0.03	0.09	+ 6	0.11[a]	0.16[a]
Trial 3 - OP50: Wild type	11.3 ± 0.5	15	67/80						
Trial 3 - OP50: *osm-3(n1540)*	12.8 ± 0.6	16	62/80	+ *13*	0.01	0.06			
Trial 3 - HT115: Wild type	11.3 ± 0.4	15	76/80				0	0.50[a]	0.91[a]
Trial 3 - HT115: *osm-3(n1540)*	12.7 ± 0.5	16	74/80	+ *12*	0.004	0.02	- 1	0.42[a]	0.95[a]
nmur-1 food-dependence									
Trial 1 - OP50: Wild type	10.5 ± 0.5	14	73/80						
Trial 1 - OP50: *nmur-1*	15.7 ± 0.5	18	65/80	**+ 50**	< 0.0001	< 0.0001			
Trial 1 - BL21: Wild type	7.4 ± 0.6	7	20/80				**- 30**	0.001[a]	0.003[a]
Trial 1 - BL21: *nmur-1*	9.9 ± 0.6	13	44/80	**+ 34**	0.007	0.004	**- 37**	< 0.0001 [a]	< 0.0001[a]
Trial 1 - HB101: Wild type	13.2 ± 0.5	16	74/80				**+ 26**	0.008[a]	< 0.0001[a]
Trial 1 - HB101: *nmur-1*	16.4 ± 0.5	19	70/80	**+ 24**	< 0.0001	< 0.0001	+ 4	0.17[a]	0.37[a]

Suppl. Table 3 (Continued)

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Trial 1 - HT115: Wild type	12.4 ± 0.4	15	70/80				**+ 18**	0.12[a]	0.0007[a]
Trial 1 - HT115: *nmur-1*	13.9 ± 0.5	17	66/80	+ 12	0.04	0.02	**- 11**	0.001[a]	0.002[a]
Trial 1 - DY330: Wild type	11.9 ± 0.4	14	73/80				**+ 13**	0.28[a]	0.001[a]
Trial 1 - DY330: *nmur-1*	13.0 ± 0.4	15	72/80	+ 9	0.05	0.08	**- 17**	< 0.0001[a]	< 0.0001[a]
Trial 1 - DH5α: Wild type	12.2 ± 0.4	15	69/80				**+ 16**	0.26[a]	0.001[a]
Trial 1 - DH5α: *nmur-1*	13.6 ± 0.4	16	74/80	+ 11	0.04	0.02	**- 13**	< 0.0001[a]	< 0.0001[a]
Trial 2 - OP50: Wild type	10.1 ±0.6	14	48/70						
Trial 2 - OP50: *nmur-1*	13.8 ±0.5	16	52/72	**+ 37**	< 0.0001	< 0.0001			
Trial 2 - BL21: Wild type	8.1 ± 0.4	10	32/70				- *20*	0.001[a]	0.05[a]
Trial 2 - BL21: *nmur-1*	10.4 ± 0.7	15	41/70	+ *28*	0.008	0.09	**- 25**	0.002[a]	0.0002[a]
Trial 2 - HB101: Wild type	11.5 ± 0.4	14	57/70				+ 14	0.48[a]	0.10[a]
Trial 2 - HB101: *nmur-1*	13.9 ±0.3	15	55/70	**+ 21**	< 0.0001	< 0.0001	+ 1	0.25[a]	0.62[a]
Trial 2 - HT115: Wild type	11.9 ±0.3	14	57/70				+ 18	0.51[a]	0.02[a]
Trial 2 - HT115: *nmur-1*	12.6 ±0.4	15	50/70	+ *6*	0.003	0.08	- 9	0.03[a]	0.05[a]
Trial 2 - DY330: Wild type	12.4 ± 0.4	14	64/70				**+ 23**	0.02[a]	0.005[a]
Trial 2 - DY330: *nmur-1*	12.5 ± 0.4	15	54/70	+ 1	0.72	0.64	- *9*	0.01[a]	0.03[a]
Trial 2 - DH5α: Wild type	10.3 ± 0.2	12	62/70				+ 2	0.07[a]	0.72[a]
Trial 2 - DH5α: *nmur-1*	13.1 ± 0.4	15	62/70	**+ 27**	< 0.0001	< 0.0001	- 5	0.13[a]	0.11[a]
Trial 3 - OP50: Wild type	10.9 ± 0.6	16	57/70						
Trial 3 - OP50: *nmur-1*	15.2 ± 0.5	18	52/70	**+ 39**	< 0.0001	< 0.0001			
Trial 3 - HB101: Wild type	11.9 ±0.5	16	62/70				+ 9	0.74[a]	0.19[a]
Trial 3 - HB101: *nmur-1*	14.7 ± 0.3	16	48/70	**+ 24**	< 0.0001	< 0.0001	- 3	0.06[a]	0.07[a]
Trial 3 - DH5α: Wild type	10.4 ±0.4	12	65/70				- 5	0.12[a]	1.0[a]
Trial 3 - DH5α: *nmur-1*	12.8 ±0.3	16	61/70	**+ 23**	< 0.0001	< 0.0001	**- 16**	< 0.0001[a]	< 0.0001[a]
Trial 4 - OP50: Wild type	11.3 ± 0.7	17	62/70						
Trial 4 - OP50: *nmur-1*	15.1 ± 0.6	19	61/70	**+ 34**	0.002	0.0003			
Trial 4 - HT115: Wild type	13.8 ± 0.5	17	59/70				**+ 22**	0.17[a]	0.004[a]
Trial 4 - HT115: *nmur-1*	13.0 ± 0.6	15	50/60	- 6	0.51	0.32	**- 14**	0.004[a]	0.01[a]

Suppl. Table 3 (Continued)

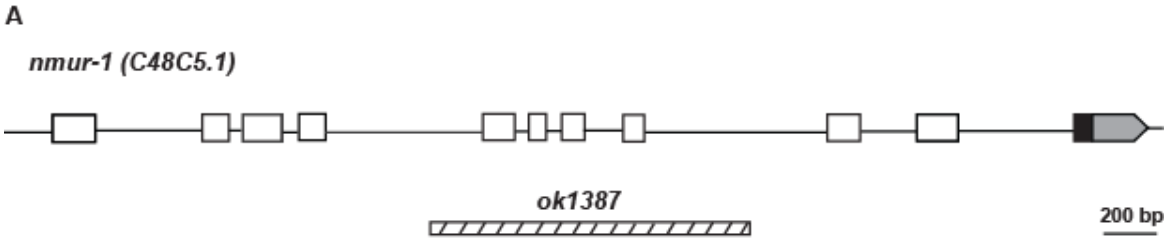
Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Rescue experiments									
Line 2									
Trial 1									
OP50: *jxEx4*	12.5 ± 0.6	16	45/52						
OP50: *nmur-1; jxEx40*	14.1 ± 0.7	18	44/72	+ *13*	0.01[b]	0.11[b]			
OP50: *nmur-1; jxEx4*	17.4 ± 0.6	21	55/70	**+ 39**	< 0.0001[b]	< 0.0001[b]	**+ 23**	0.0007[c]	0.001[c]
Trial 2									
OP50: *jxEx4*	13.1 ± 0.6	17	64/75						
OP50: *nmur-1; jxEx40*	14.9 ± 0.7	19	45/80	+ 14	0.03[b]	0.02[b]			
OP50: *nmur-1; jxEx4*	17.4 ± 0.6	21	65/74	**+ 33**	< 0.0001[b]	< 0.0001[b]	**+ 17**	0.006[c]	0.006[c]
HT115: *jxEx4*	11.3 ± 0.4	13	58/72						
HT115: *nmur-1; jxEx40*	13.2 ± 0.5	17	80/89	**+ 17**	0.001[b]	0.005[b]			
HT115: *nmur-1; jxEx4*	13.3 ± 0.3	15	77/82	**+ 18**	0.0003[b]	0.0001[b]	+ 1	0.31[c]	0.84[c]
Trial 3									
HT115: *jxEx4*	11.1 ± 0.4	14	70/80						
HT115: *nmur-1; jxEx40*	13.5 ± 0.5	16	66/80	**+ 22**	< 0.0001[b]	< 0.0001[b]			
HT115: *nmur-1; jxEx4*	12.3 ± 0.3	14	69/80	+ 11	0.05[b]	0.02[b]	**- 9**	0.001[c]	0.01[c]
E. coli LPS-dependence									
Trial 1 - CS180: Wild type	13.8 ± 0.4	16	71/80						
Trial 1 - CS180: *nmur-1*	15.2 ± 0.4	17	65/80	+ 10	0.03	0.03			
Trial 1 - CS2198: Wild type	13.6 ± 0.4	16	69/80				- 1	0.73[d]	0.54[d]
Trial 1 - CS2198: *nmur-1*	15.7 ± 0.4	19	66/80	**+ 15**	0.0003	0.0009	+ 3	0.14[d]	0.35[d]
Trial 1 - CS2429: Wild type	. ±0.4	16	72/80				0	0.97[d]	0.84[d]
Trial 1 - CS2429: *nmur-1*	16.9 ± 0.5	19	68/80	**+ 22**	< 0.0001	< 0.0001	**+ 11**	0.001[d]	0.007[d]
Trial 1 - CS1861: Wild type	13.4 ± 0.5	17	72/80				- 3	0.99[d]	0.61[d]
Trial 1 - CS1861: *nmur-1*	14.5 ± 0.5	17	63/80	+ 8	0.17	0.12	- 5	0.56[d]	0.51[d]

Suppl. Table 3 (Continued)

Strain/Treatment	Mean Lifespan ±SEM (Days)	75th Percentile (Days)	No. of Animals Observed/ Total Initial Animals	% Wild type	*P* Value Against Wild type (Logrank)	*P* Value Against Wild type (Wilcoxon)	% of Specified Groups	*P* Value Against Specified Groups (Logrank)	*P* Value Against Specified Groups (Wilcoxon)
Trial 2 - OP50: Wild type	11.9 ± 0.8	17	34/40						
Trial 2 - OP50: *nmur-1*	17.5 ± 0.5	19	31/40	**+ 47**	< 0.0001	< 0.0001			
Trial 2 - CS180: Wild type	14.9 ± 0.4	17	69/80						
Trial 2 - CS180: *nmur-1*	15.4 ± 0.4	17	64/80	+ 3	0.35	0.32			
Trial 2 - CS2198: Wild type	12.8 ± 0.3	15	72/80				**- 14**	0.0004[d]	< 0.0001[d]
Trial 2 - CS2198: *nmur-1*	15.9 ± 0.4	17	68/81	**+ 24**	< 0.0001	< 0.0001	+ 3	0.20[d]	0.41[d]
Trial 2 - CS2429: Wild type	13.2 ± 0.4	17	69/80				**- 11**	0.05[d]	0.001[d]
Trial 2 - CS2429: *nmur-1*	16.4 ± 0.7	21	52/80	**+ 24**	0.0002	0.0001	+ 6	0.02[d]	0.25[d]
Trial 3 - CS180: Wild type	14.3 ± 0.4	17	65/80						
Trial 3 - CS180: *nmur-1*	14.7 ± 0.4	17	73/80	+ 3	0.88	0.0.52			
Trial 3 - CS2429: Wild type	12.8 ± 0.4	14	72/80				**- 10**	0.03[d]	0.007[d]
Trial 3 - CS2429: *nmur-1*	15.4 ± 0.5	18	68/80	**+ 20**	0.0001	< 0.0001	+ 5	0.05[d]	0.17[d]

Table S3. Individual trials of adult lifespans on different food sources at 25°C. The analyses performed here are as described in the legend of Table 1. The superscripted symbols indicate the following: [a], compared to the same genotype assayed in parallel on OP50 in independent trials; [b], compared to *jxEx4[myo-3p::rfp]* on the same food source in independent trials; [c], compared to the rescue line on the same food source in independent trials; and [d], compared to the same genotype assayed in parallel on CS180 in independent trials.

A



B

```
1    atgttgcaagcttgcctaaacaccaccgaagaccaatgtgattgccttgcattcaattgt
      M  L  Q  A  C  L  N  T  T  E  D  Q  C  D  C  L  A  F  N  C   20
61   ccaattgtttatagtcattcggaaagcgaaaaagaagcgtgttacatggagcactgcttt
      P  I  V  Y  S  H  S  E  S  E  K  E  A  C  Y  M  E  H  C  F   40
121  atttcaaaacgagcactggatgacgtcacgttgtataaggtgactgctctttacattttc
      I  S  K  R  A  L  D  D  V  T  L  Y  K  V  T  A  L  Y  I  F   60
181  attttcttagttggtgtaattggaaacactacaacctgcttagtcatgaaaaaacatccc
      I  F  L  V  G  V  I  G  N  T  T  T  C  L  V  M  K  K  H  P   80
241  atgatgaaaacccatgcaagcatgtatctcatgaatctggcggtttcggacttggtcacg
      M  M  K  T  H  A  S  M  Y  L  M  N  L  A  V  S  D  L  V  T  100
301  ttatgcgtgggtttaccgtttgaagtaatgatgaactggaatcagtacccatggccattt
      L  C  V  G  L  P  F  E  V  M  M  N  W  N  Q  Y  P  W  P  F  120
361  ccggattacatatgcaacttgaaagcgctcattgcggaaacaacgagttccgtttctatt
      P  D  Y  I  C  N  L  K  A  L  I  A  E  T  T  S  S  V  S  I  140
421  ctgacaattctgatttttgcaattgaacgttatgtagcggtgtgtcatccacttttttcta
      L  T  I  L  I  F  A  I  E  R  Y  V  A  V  C  H  P  L  F  L  160
481  atgaaggttcaaccattcaagagaaatattggaactataattggctttacttggatttttc
      M  K  V  Q  P  F  K  R  N  I  G  T  I  I  G  F  T  W  I  F  180
541  tctatcctttgtgctatgccctttgcgatccatcaccgagccgattacattatgaaaagc
      S  I  L  C  A  M  P  F  A  I  H  H  R  A  D  Y  I  M  K  S  200
601  tggccagggacagacaacagaataccggttaaatcttcaaaaatgtgcatgatagcagtg
      W  P  G  T  D  N  R  I  P  V  K  S  S  K  M  C  M  I  A  V  220
661  atgtttgaaccaaagctagcgtcaacttttaagattctatttcacttctctgccatagca
      M  F  E  P  K  L  A  S  T  F  K  I  L  F  H  F  S  A  I  A  240
721  ttctttgcactcccactgtttacaattgtaattctctatgcaagaattgcatgtaaggta
      F  F  A  L  P  L  F  T  I  V  I  L  Y  A  R  I  A  C  K  V  260
781  tccagcaacagaacaattcaaccaggcgaacttgatatcactgaggaactgcaaatgaga
      S  S  N  R  T  I  Q  P  G  E  L  D  I  T  E  E  L  Q  M  R  280
841  atcaatgcaatttttatgtgcaatcgtttcggctttcttcatctgctaccttccgtttcaa
      I  N  A  I  L  C  A  I  V  S  A  F  F  I  C  Y  L  P  F  Q  300
901  ttgcaacgtctcttgtttttctattttgataatgaagttattttgacatgggtcaatcag
      L  Q  R  L  L  F  F  Y  F  D  N  E  V  I  L  T  W  V  N  Q  320
961  tatatgtattttatctcaggattccttttctatcttgccactatcatcaatcctattgcc
      Y  M  Y  F  I  S  G  F  L  F  Y  L  A  T  I  I  N  P  I  A  340
1021 tataaccttgcatccagccgttttcgaagagcattcaaagacattcttattgattactgt
      Y  N  L  A  S  S  R  F  R  R  A  F  K  D  I  L  I  D  Y  C  360
1081 tggagaggaggatctgagcgttatccaagaagctcattcagcaaatatagcttagctcat
      W  R  G  G  S  E  R  Y  P  R  S  S  F  S  K  Y  S  L  A  H  380
1141 actcctctcaggcaagccatgtccaaccgggttccaattctcgactccaaaacaaacgca
      T  P  L  R  Q  A  M  S  N  R  V  P  I  L  D  S  K  T  N  A  400
1201 taacttcaaaacattgtcccaaaataatattatttgtacatatattttattttgtctcca
      *
1261 cattatttttaatttttttttctccctcttcaatatttgtaaaccttgtctcatatttttc

1321 ctgctaattcgatatcatgtagtttgcattacgggggcatacttcattctatactttatt

1381 tcaatctgccaaataaaaagtgtaggttttctaaaaaaaaaaaaa
```

Figure S1. Gene architecture and coding sequence of *nmur-1*. (**A**) The gene structure of *nmur-1* (*C48C5.1*) predicted by WormBase (version WS207; www.wormbase.org) consists of only ten exons (shown in white). However, upon isolation and sequencing of the *nmur-1* cDNA, we found that the *nmur-1* gene locus includes a terminal eleventh exon (shown in black) that encodes an additional 22 amino acids and is followed by a 210 bp 3' UTR (gray). The extent of the *ok1387* deletion is indicated by the hatched bar. (**B**) The *nmur-1* cDNA sequence along with its translated protein sequence. The arrowheads indicate exon-intron boundaries within the DNA sequence, the 3' UTR is italicized and the poly-A sequence used for priming the reverse transcription of the mRNA is framed. Within the protein sequence, the predicted seven transmembrane domains are underlined. The revised protein sequence shows 45% similarity and 27% identity to human NMUR1 and 47% similarity and 29% identity to human NMUR2 [13].

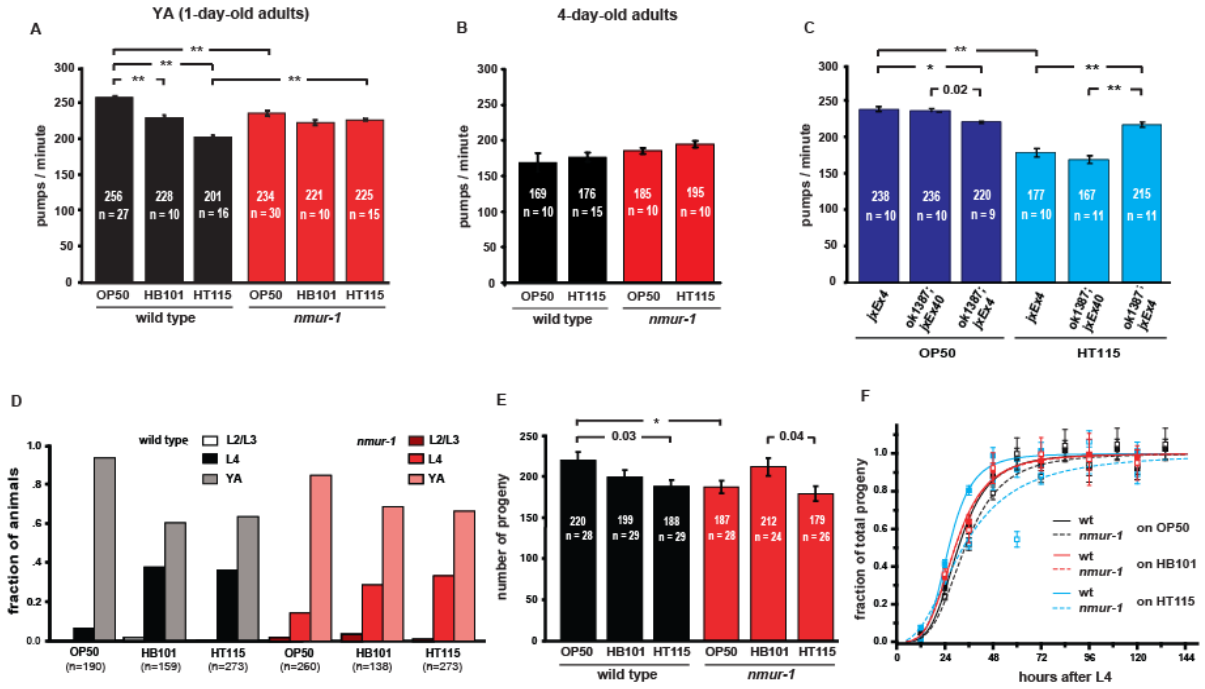


Figure S2. *nmur-1* modulates food source-dependent effects on feeding rate, development and reproduction. (**A-B**) Pharyngeal pumping rates of wild-type and mutant worms on different bacteria. Rates are expressed as mean pumps per minute and determined from the indicated number (n) of worms. ** indicates $P \leq 0.001$ in this and subsequent panels. Since wild type and *nmur-1* mutants pump at a similar rate on HB101, a food source that does increase mutant lifespan compared to wild type (Figure 2E), the *nmur-1* regulation of lifespan and feeding rate presumably involve two distinct pathways. (**C**) The wild-type *nmur-1* genomic locus can also rescue the feeding rate phenotypes of *nmur-1* mutants on OP50 ($P = 0.02$) and HT115 ($P \leq 0.001$). The rescued worms are compared to wild-type and *nmur-1* mutant worms that carry the *myo-3p::rfp* coinjection marker alone. * indicates $P \leq 0.01$ in this and later panels. (**D**) Distribution of developmental stages of wild-type and mutant worms at 36.5 hours after hatching on different bacteria. L2 indicates second-stage; L3, third-stage; L4, fourth-stage larvae; and YA, young adults. Although both wild type and mutants develop faster on OP50 than on HB101 or HT115 ($P < 0.001$ for either genotype), mutants develop slower than wild type on OP50 ($P = 0.01$). It should be noted that our observation of a slower wild-type developmental rate on HB101 at 25°C differs from a previous study carried out at 18°C [17], which suggests that temperature can alter the growth-influencing factors of some food sources. (**E-F**) Total progeny and temporal profiles of reproduction on different bacteria. *nmur-1* mutants have less total progeny (**E**) than wild type on OP50 ($P < 0.01$), and wild type has more progeny on OP50 than on HT115 ($P = 0.03$). The larger progeny number of *nmur-1* mutants on HB101 ($P = 0.04$) is a

consequence of censoring (see Materials and Methods). *nmur-1* mutants reproduce more slowly (**F**) than wild type on HT115, but behave more similarly to wild type on OP50 and HB101.

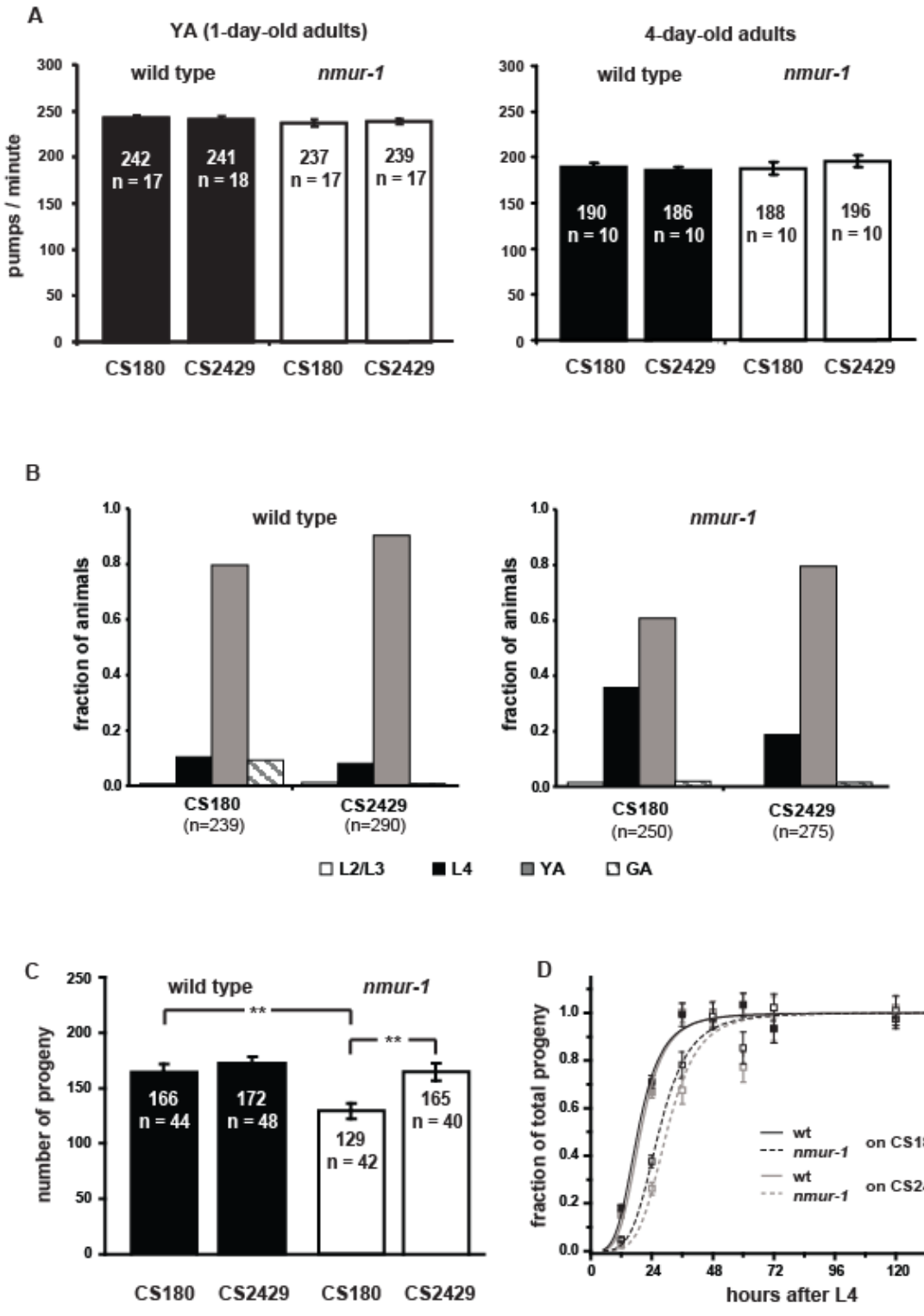

A
YA (1-day-old adults)
4-day-old adults
wild type
nmur-1
pumps / minute
242 n = 17
241 n = 18
237 n = 17
239 n = 17
190 n = 10
186 n = 10
188 n = 10
196 n = 10
CS180
CS2429
B
wild type
nmur-1
fraction of animals
CS180 (n=239)
CS2429 (n=290)
CS180 (n=250)
CS2429 (n=275)
L2/L3
L4
YA
GA
C
number of progeny
166 n = 44
172 n = 48
129 n = 42
165 n = 40
**
D
fraction of total progeny
hours after L4
wt
nmur-1
on CS180
on CS2429

Figure S3. The influence of the LPS structure on feeding, development and reproduction of wild-type and *nmur-1* mutant worms. (**A**) Wild-type and mutant worms have similar pharyngeal pumping rates on both the CS2429 LPS truncation mutant and the CS180 parent strain. (**B**) *nmur-1* mutant worms develop faster on the *E. coli* LPS mutant strain than on the *E. coli* parent strain ($P < 0.001$), but slower than wild-type worms on both *E. coli* strains ($P < 0.001$ for each case). *nmur-1* mutants also (**C**) produce more offspring on the *E. coli* truncation mutant than on the *E. coli* parent strain (**, $P < 0.001$) and (**D**) reproduce at a similar rate, though slower than wild type, on both strains. Together our findings suggest that the *nmur-1* regulation of lifespan, feeding rate, development and reproduction involve more than one pathway and several food-derived factors.

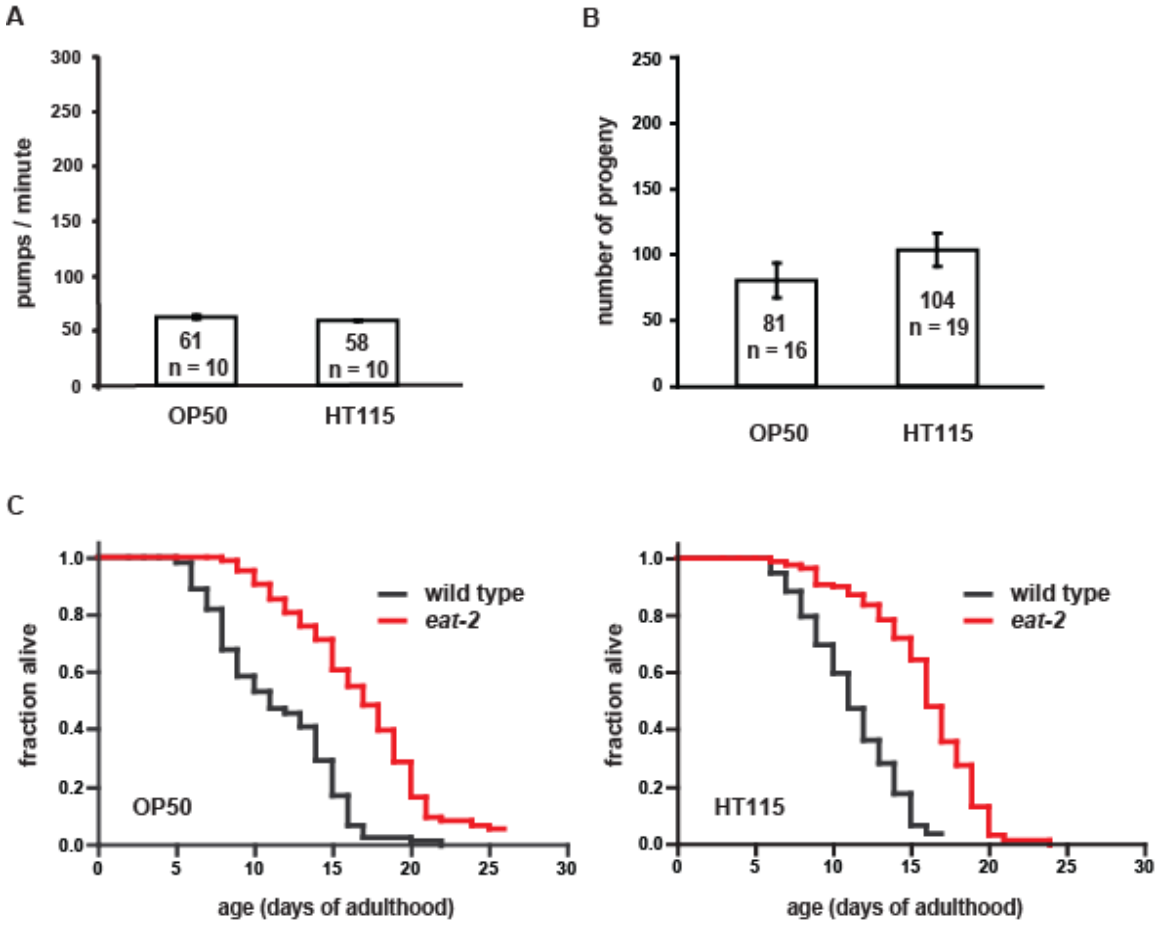


Figure S4. The effect of a genetic model of food-level restriction on feeding, reproduction and lifespan. Worms carrying the mutation *eat-2(ad1116)* display a reduced pharyngeal pumping rate (**A**), a smaller number of progeny (**B**) and increased lifespan (**C**) independent of their food source. Mean lifespan of *eat-2* mutants: 16.8 days (+ 47%, $P < 0.0001$) on OP50, 15.9 days (+ 41%, $P < 0.0001$) on HT115.

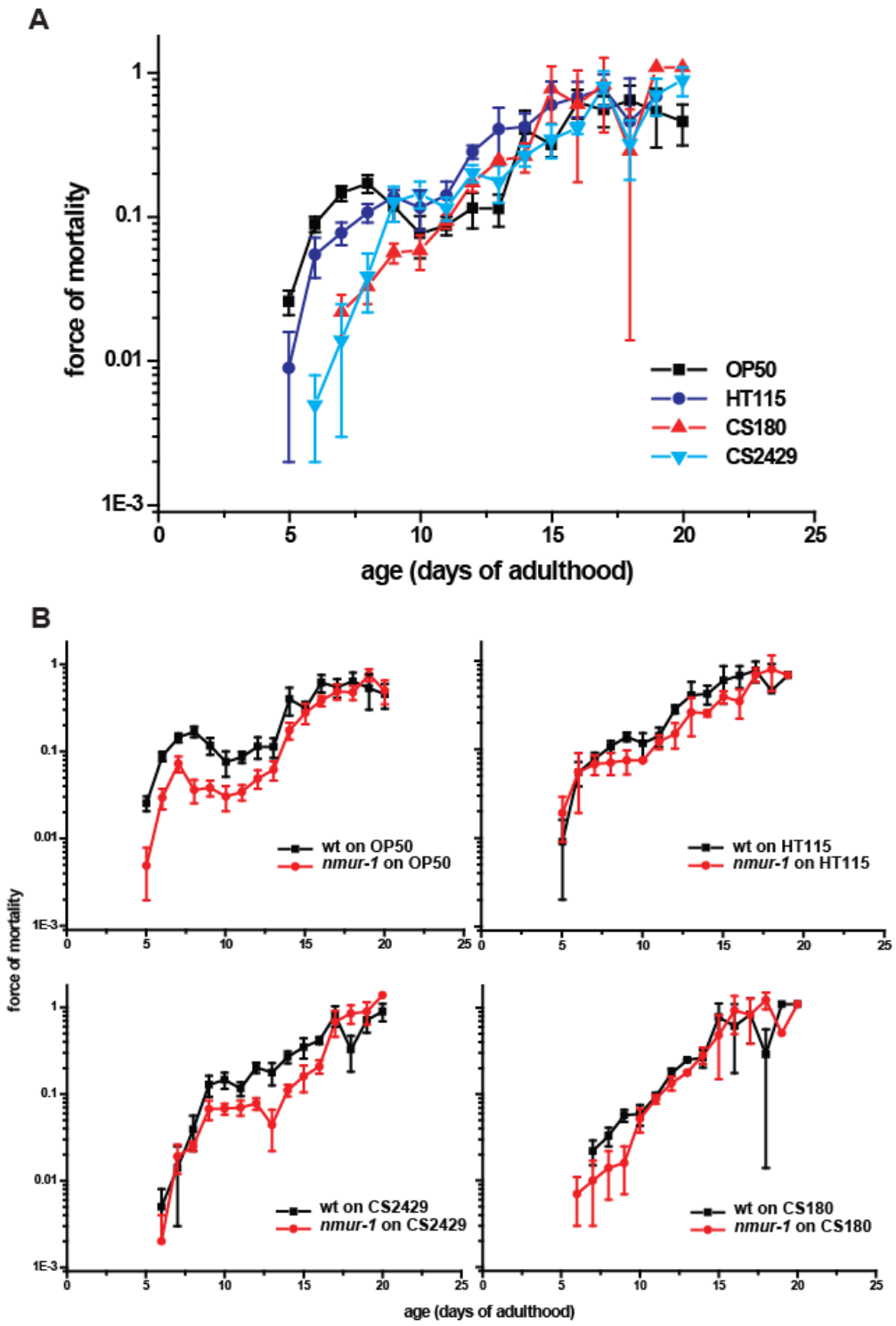


Figure S5. Food source-dependent effects on age-specific rates of mortality. (**A**) Mortality plot of wild type on four different strains of *E. coli*. (**B**) Individual comparisons of wild type and *nmur-1* mutants on the four food sources.

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Chapter III. Results

The neuropeptide *nlp-44* gene encodes neuromedin U peptides that affect lifespan in a food source-dependent manner

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*These authors contributed equally to this work.

Abstract

Sensory neurons recognize food types to affect *C. elegans* lifespan and the *nmur-1* neuromedin U receptor signaling pathway is involved in this process. To determine the precise mechanism by which *nmur-1* mediates the sensory influence on lifespan, we have characterized a neuropeptide gene, *nlp-44*, which is expressed in two pairs of *C. elegans* sensory neurons and encodes three candidate worm neuromedin U (NMU) peptide ligands. Like *nmur-1*, we find that *nlp-44* also affects lifespan in a food source-dependent manner; however, unlike *nmur-1*, *nlp-44* promotes longevity. We show that a deletion mutation in *nlp-44* shortens lifespan and can suppress the long-life phenotype of *nmur-1* loss-of-function (lof) mutants. On the other hand, we observe that the overexpression of *nlp-44* extends lifespan in a wild-type background, but that it does not further extend the lifespan of *nmur-1(lof)* mutants. Thus, our genetic data suggest that at least one of the ligands encoded by *nlp-44* acts downstream of or in parallel to *nmur-1* in its regulation of lifespan. At present, it remains unclear which of the *nlp-44* peptides acts to lengthen lifespan.

Introduction

The sensory system influences lifespan through two distinct mechanisms, the recognition of food levels versus the recognition of food quality (see Chapter II). The effect on lifespan through sensory perception of food quality involves the activity of a neuromedin U neuropeptide receptor, known as *nmur-1* (see Chapter II). *nmur-1* is expressed not only in sensory neurons but also in interneurons, which are known to receive inputs from sensory neurons or to modulate sensory neuron activity [(see Chapter II; (White et al., 1986)]. The effect of *nmur-1* on *C. elegans* lifespan depends on the recognition of the nature of the lipopolysaccharide structure of its *E. coli* bacterial food source (see Chapter II). However, at present, it remains unclear how bacterial food sources regulate the function of *nmur-1*.

Since *nmur-1* is predicted to encode a receptor for neuromedin U-like neuropeptides, it is possible that specific food sources regulate the release of neuromedin U-like ligands that could then act on NMUR-1. In this study, we tested the gene *nlp-44*, which encodes a precursor for neuromedin U-like neuropeptides (Lindemans et al., 2009), for a role in regulating lifespan in a food source-dependent and *nmur-1*-dependent manner.

Results and Discussion

Through a bioinformatic approach, we identified the gene locus Y23B4A.2 (see www.wormbase.org; version WS207) as a neuromedin U neuropeptide precursor that can give rise to three different peptides (Figure 1) that have motifs resembling the bioactive PRX amide motifs of *Drosophila* and mammalian neuromedin U-like ligands (Park et al., 2002). Although *C. elegans* has one other neuropeptide precursor gene, *nlp-39*, that can yield a

peptide with the PRX amide motif, we focused on Y23B4A.2, also known as *nlp-44* (Lindemans et al., 2009), since its gene and protein architecture resemble that of a *Drosophila* neuromedin U precursor gene known as *capability* (Park et al., 2002). Moreover, the third of the *nlp-44* peptides (labeled as peptide 3 in Figure 1) has recently been shown to activate a member of the *C. elegans* neuromedin U receptor family, NMUR-2 (Lindemans et al., 2009). Since the other two peptides that are predicted to arise from the *nlp-44* precursor failed to activate NMUR-2 (Lindemans et al., 2009), these peptides may serve as ligands for NMUR-1.

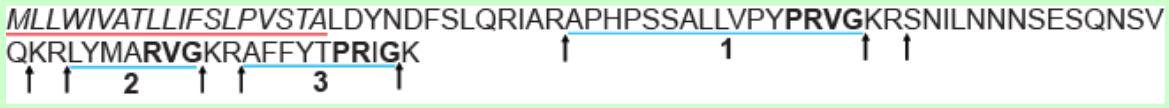

Figure 1. Protein structure of the NLP-44 neuropeptide precursor. The peptides are labeled as 1-3 and underlined in blue. The signal peptide is italicized and underlined in red. The arrows indicate the sites that are presumably cleaved by peptidases, and the bioactive motifs within the peptides are in bold-face.

To test whether *nlp-44* affects lifespan, we analyzed the lifespan phenotype of *nlp-44* loss-of-function or overexpression mutants. We found that removal of *nlp-44* shortened wild-type lifespan (Figure 2A; Table 1), while its overexpression extended lifespan on one food source, *E. coli* OP50, but not on another, *E. coli* HT115 (Figure 3; Table 1). Since the food source-dependent lifespan phenotype of the *nlp-44* overexpression mutant (Figure 3; Table 1) resembles that of the *nmur-1* loss-of-function mutant (see Chapter II), we next tested how loss or overexpression of *nlp-44* affected the lifespan of *nmur-1* deletion mutants. We observed that loss of *nlp-44* can partly suppress the long-life phenotype of the *nmur-1* mutants (Figures 2B and 2C; Table 1), whereas the overexpression of *nlp-44* does not further

increase the lifespan of *nmur-1* mutant animals (Figure 4; Table 1). Thus, our genetic data suggest that at least one of the ligands encoded by *nlp-44* acts downstream of or in parallel to NMUR-1 in its regulation of lifespan.

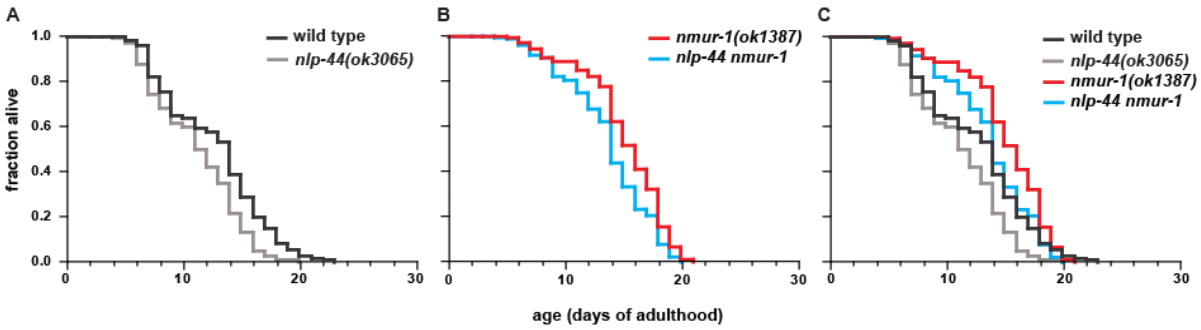

Figure 2. *nlp-44* promotes *C. elegans* longevity. The effect on lifespan upon deletion of *nlp-44* in a wild-type (**A**) or *nmur-1* loss-of-function mutant (**B**) background. (**C**) The lifespan curves from panels **A** and **B** are shown together. The curves in these and subsequent panels represent cumulative data. The detailed statistical analyses on these and subsequent survival assays can be found in Table 1.

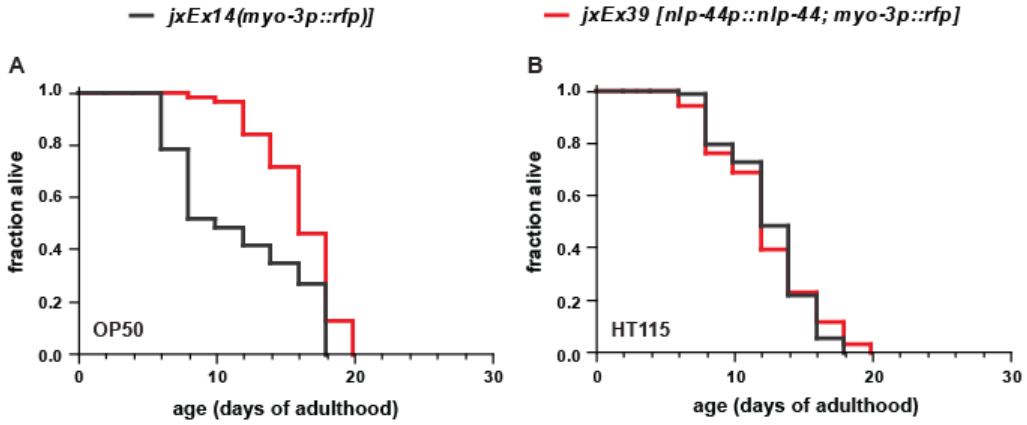

Figure 3. The effect of *nlp-44* on lifespan is food source-dependent. (**A**) The effect of *nlp-44* overexpression on the lifespan of worms grown on *E. coli* OP50. (**B**) The effect of *nlp-44* overexpression on the lifespan of worms grown on *E. coli* HT115. The lifespan of the *nlp-44* overexpression line, *jxEx39*, is compared to that of wild-type worms that carry the *myo-3p::rfp* coinjection marker, *jxEx14*, alone.

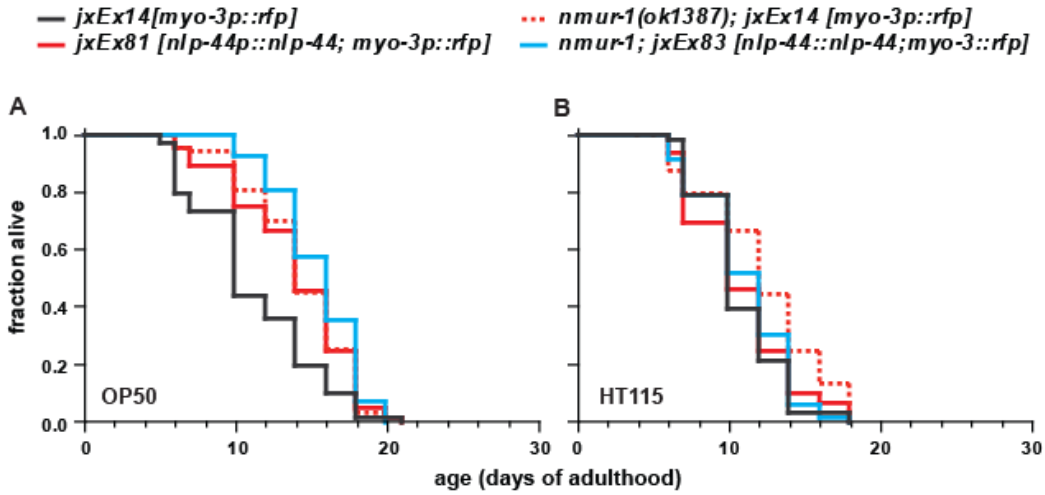

Figure 4. Overexpression of *nlp-44* does not further extend the lifespan of *nmur-1* loss-of-function mutants. The effects of *nlp-44* overexpression on the lifespan of *nmur-1* mutants grown on OP50 (**A**) versus *nmur-1* mutants grown on HT115 (**B**) are shown. The lifespan of worms that overexpress *nlp-44* in the absence of *nmur-1* function, *jxEx83*, is compared to those of wild-type and *nmur-1* mutant worms that carry the *myo-3p::rfp* coinjection marker, *jxEx14*, alone or to worms overexpressing *nlp-44* in the presence of wild-type *nmur-1* activity, *jxEx81*.

As mentioned previously, NMUR-2 has been shown to transduce the signal of one of the peptide ligands (Lindemans et al., 2009). To test the possibility that NMUR-2 also acts downstream of the NLP-44 peptide(s) that promote longevity, we determined whether loss of *nmur-2*, which alone has no effect on lifespan (Figure 5; Table 1; see Chapter II), can suppress the long life phenotype of *nlp-44* overexpression mutants. However, we found that overexpression of *nlp-44* can still extend lifespan even in the absence of *nmur-2* (Figure 5; Table 1), which suggests that *nlp-44* promotes longevity independently of *nmur-2*. Not surprisingly, we also found that the lack of *nmur-2* activity does not affect the increased lifespan of *nmur-1* mutants (Table 1).

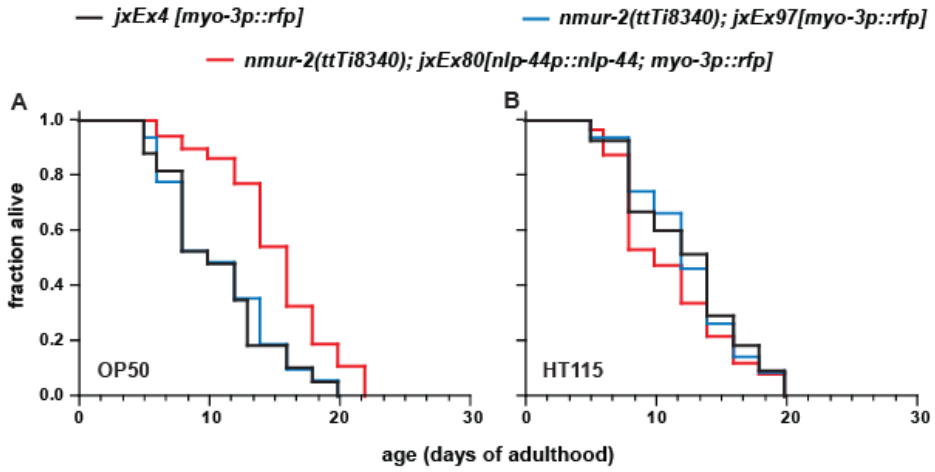

Figure 5. Loss of *nmur-2* has no effect on the long-life phenotype of *nlp-44*-overexpressing mutants. The effects of *nlp-44* overexpression on the lifespan of *nmur-2* mutants grown on OP50 (**A**) versus *nmur-2* mutants grown on HT115 (**B**) are shown. The lifespan of worms that overexpress *nlp-44* in the absence of *nmur-2* function, *jxEx80*, is compared to those of wild-type and *nmur-2* mutant worms that carry only the *myo-3p::rfp* coinjection marker, *jxEx14* and *jxEx97*, respectively.

However, the *C. elegans* NMUR family has at least two other members, NMUR-3 and NMUR-4 (see Chapter II), which can serve as receptors for the other peptide ligands. Thus, it is possible that *nmur-1* can act together with these other receptors to affect lifespan and other processes.

The fact that *nlp-44* can recognize differences in food sources raises the possibility that specific food cues regulate the release and function of the NLP-44 processed peptides to affect lifespan. Consistent with this hypothesis, we found expression of a *gfp* reporter for *nlp-44* in two pairs of sensory neurons: ASG, which is located in the head and has a role in chemosensation (White et al., 1986; Bargmann and Horvitz, 1991b), as well as lifespan regulation (Alcedo and Kenyon, 2004); and PHC, which is located in the tail and has a

Table 1. Adult lifespans at 25°C

Strain	Mean Lifespan ± SEM (Days)	75th Percentile (Days)	No. Animals Observed / Total Initial Animals	% Wild Type	*P* Value Against Control (Wilcoxon)	*P* Value Against Control (Logrank)	% Specified Group	*P* Value Against Specified Group (Wilcoxon)	*P* Value Against Specified Group (Logrank)
nlp-44 loss of function									
OP50: wild type	12.7 ± 0.4	16	131/160 (2)						
OP50: *nlp-44(ok3065)*	11.2 ± 0.3	14	132/160 (2)	**- 12**	0.002	< 0.0001			
OP50: *nmur-1(ok1387)*	15.1 ± 0.3	18	124/160 (2)	**+ 19**	< 0.0001	0.0004			
OP50: *nlp-44 nmur-1*	13.7 ± 0.4	16	110/160 (2)	+ 8	0.08	0.42	**- 9**	0.002[a]	0.002[a]
nlp-44 overexpression									
OP50: *jxEx14 [myo-3p::rfp]*	11.6 ± 0.6	18	61/70 (1)						
OP50: *jxEx39 [nlp-44p::nlp-44; myo-3p::rfp]*	16.1 ± 0.3	18	63/70 (1)	**+ 39**	< 0.0001	<0.0001			
HT115: *jxEx14*	12.5 ± 0.3	14	77/80 (1)						
HT115: *jxEx39*	12.3 ± 0.4	14	64/80 (1)	- 2	0.49	0.97			
OP50: *jxEx4 [myo-3p::rfp]*	13.2 ± 0.5	18	74/80 (1)						
OP50: *jxEx39 [nlp-44p::nlp-44; myo-3p::rfp]*	16.1 ± 0.4	18	71/80 (1)	**+ 22**	0.0001	0.0005			
OP50: *jxEx14*	11.2 ± 0.5	14	63/70 (1)						
OP50: *jxEx81 [nlp-44p::nlp-44; myo-3p::rfp]*	14.0 ± 0.5	16	62/70 (1)	**+ 25**	< 0.0001	0.0006			
OP50: *nmur-1*; *jxEx14*	14.3 ± 0.4	18	67/70 (1)	**+ 28**	< 0.0001	0.0002			
OP50: *nmur-1*; *jxEx83[nlp-44p::nlp-44; myo-3p::rfp]*	15.5 ± 0.3	18	68/70 (1)	**+ 38**	< 0.0001	< 0.0001	+ 8	0.05[b]	0.07[b]
HT115: *jxEx14*	10.7 ± 0.3	12	65/70 (1)						
HT115: *jxEx81*	10.8 ± 0.4	12	61/70 (1)	+ 0.9	0.91	0.72			
HT115: *nmur-1*; *jxEx14*	12.2 ± 0.5	14	62/70 (1)	**+ 14**	0.01	0.002			
HT115: *nmur-1*; *jxEx83*	11.1 ± 0.4	14	66/70 (1)	+ 4	0.37	0.38	- 9	0.07[b]	0.01[b]
OP50: *jxEx4*	10.7 ± 0.5	13	63/70 (1)						
OP50: *nmur-1*; *jxEx4*	15.8 ± 0.5	18	61/70 (1)	**+ 48**	< 0.0001	< 0.0001			

Table 1 continued

Strain	Mean Lifespan ± SEM (Days)	75th Percentile (Days)	No. Animals Observed / Total Initial Animals	% Wild Type	*P* Value Against Control (Wilcoxon)	*P* Value Against Control (Logrank)	% Specified Group	*P* Value Against Specified Group (Wilcoxon)	*P* Value Against Specified Group (Logrank)
OP50: *nmur-1*; *jxEx87[nlp-44p::nlp-44; myo-3p::rfp]*	15.8 ± 0.4	18	65/70 (1)	**+ 48**	< 0.0001	< 0.0001	0	0.78[c]	0.80[c]
HT115: *jxEx4*	12.4 ± 0.6	16	47/70 (1)						
HT115: *nmur-1*; *jxEx4*	11.8 ± 0.5	14	61/70 (1)	- 5	0.60	0.45			
HT115: *nmur-1*; *jxEx87*	11.7 ± 0.5	14	67/70 (1)	- 6	0.55	0.32	- 1	0.94[c]	0.8[c]
OP50: *jxEx4*	10.7 ± 0.5	13	63/70 (1)						
OP50: *nmur-2(ttTi8340)*; *jxEx97[myo-3p::rfp]*	10.9 ± 0.6	14	56/70 (1)	+ 2	0.85	0.74			
OP50: *nmur-2*; *jxEx80[nlp-44p::nlp-44; myo-3p::rfp]*	15.3 ± 0.5	18	65/70 (1)	**+ 43**	< 0.0001	< 0.0001	**+ 40**	< 0.0001[d]	< 0.0001[d]
HT115: *jxEx4*	12.4 ± 0.6	16	47/70 (1)						
HT115: *nmur-2*; *jxEx97*	12.5 ± 0.6	16	51/70 (1)	+ 1	0.88	0.88			
HT115: *nmur-2*; *jxEx80*	11.2 ± 0.6	14	51/60 (1)	- 10	0.24	0.26	- 10	0.11[d]	0.26[d]
OP50: *jxEx4*	10.7 ± 0.5	13	63/70 (1)						
OP50: *nmur-2*; *jxEx97*	10.9 ± 0.6	14	56/70 (1)	+ 2	0.85	0.74			
OP50: *nmur-2*; *jxEx88[nlp-44p::nlp-44; myo-3p::rfp]*	14.6 ± 0.5	18	62/70 (1)	**+ 37**	< 0.0001	< 0.0001	**+ 34**	< 0.0001[d]	< 0.0001[d]
HT115: *jxEx4*	12.4 ± 0.6	16	47/70 (1)						
HT115: *nmur-2*; *jxEx97*	12.5 ± 0.6	16	51/70 (1)	+ 1	0.88	0.88			
HT115: *nmur-2*; *jxEx88*	11.8 ± 0.5	14	62/70 (1)	- 5	0.62	0.42	- 6	0.37[d]	0.44[d]
CS180: *jxEx14*	11.3 ± 0.2	12	65/70 (1)						
CS180: *jxEx81 [nlp-44p::nlp-44; myo-3p::rfp]*	12.0 ± 0.3	14	65/70 (1)	+ 6	0.14	0.02			
CS2429: *jxEx14*	11.1 ± 0.2	12	57/68 (1)						
CS2429: *jxEx81*	11.4 ± 0.3	14	59/70 (1)	+ 3	0.53	0.17			
nmur family (*nmur-1* and *nmur-2*) on OP50									
wild type	11.5 ± 0.3	15	187/210 (3)						
nmur-1	15.1 ± 0.3	18	187/220 (3)	**+ 31**	< 0.0001	< 0.0001			

Table 1 continued

Strain	Mean Lifespan ± SEM (Days)	75th Percentile (Days)	No. Animals Observed / Total Initial Animals	% Wild Type	*P* Value Against Control (Wilcoxon)	*P* Value Against Control (Logrank)	% Specified Group	*P* Value Against Specified Group (Wilcoxon)	*P* Value Against Specified Group (Logrank)
nmur-2	12.5 ± 0.3	16	197/216 (3)	+ 9	0.02	0.04			
nmur-2; *nmur-1*	15.1 ± 0.3	17	113/140 (2)	**+ 31**	< 0.0001	< 0.0001	0	0.65[a]	0.27[a]
							+ 21	< 0.0001[e]	< 0.0001[e]

Table 1. Adult lifespan at 25°C. We assayed wild type and mutant worms in parallel in independent trials and we show statistics from cumulative experiments on different *E. coli* strains. The 75th percentile is the age when the fraction of worms alive in each group falls below 0.25. The first number in the fourth column is the number of worms observed as having died, while the second number gives the total number of worms in each experiment, including worms that were censored during the course of the assay. The numbers in parentheses in the fourth column indicate the number of trials performed. Worms that crawled off the plate, exploded or bagged were censored at the time of the event, allowing these worms to be incorporated into the data set until the censor date and to avoid loss of information. Differences that are significant ($P \leq 0.01$) according to the Wilcoxon test, which in most cases are also significant according to the logrank test, are underlined and in boldface type. Differences that are significant only according to the logrank test are italicized. The % difference between the the mutants and the wild-type control that does or does not carry co-injection markers is indicated in the fifth column. The % difference between certain groups of worms that are specified by the superscripted symbols is shown in the eighth column. The superscripted symbols indicate the following: [a], compared to the cumulative data for *nmur-1(ok1387)*; [b], compared to *nmur-1* mutants that carry the co-injection marker alone, *jxEx14[myo-3p::rfp]*, on the same food source; [c], compared to *nmur-1* mutants that carry the co-injection marker alone, *jxEx4[myo-3p::rfp]*, on the same food source; [d], compared to *nmur-2(ttTi8340)* mutants that carry the co-injection marker alone, *jxEx97[myo-3p::rfp]*, on the same food source; and [e], compared to the cumulative data for *nmur-2* mutants.

putative chemosensory function (White et al., 1986). Interestingly, however, the lifespan effect of *nlp-44* is independent of the *E. coli* lipopolysaccharide (LPS) structure, since *nlp-44* overexpression has no effect on isogenic *E. coli* strains, whose main difference is the nature of their LPS structure: CS180, the parental strain with long LPS (Pradel et al., 1992), versus CS2429 (Zhang et al., 2006), the LPS truncation mutant derived from CS180 (Table 1; see Chapter II). Thus, these data suggest that *nlp-44* recognizes other food-derived factors in OP50 versus HT115 to affect lifespan.

At present, it remains unclear whether *nlp-44* encodes any peptide ligands for NMUR-1. Our current data is consistent with the model that *nlp-44* acts in parallel to *nmur-1* in affecting lifespan in a food source-dependent manner. However, our data also does not preclude the alternative likelihood that *nlp-44* can encode peptide ligands for NMUR-1 and the other NMUR receptors, which may act in parallel or even downstream of NMUR-1. We have shown here and in the preceding chapter that the LPS structure is only of the factors that elicit the food source-dependent effects on lifespan. Thus, an intriguing possibility that certainly needs to be tested is whether *nlp-44*, in response to specific food cues, gives rise to different ligands that could then act on different NMUR receptors or combinations of these receptors. Indeed, future studies on the possible genetic interactions among the different NMUR receptor family members, as well as ligand-receptor interactions, should yield insight into how this neuromedin U signaling pathway affects lifespan in response to food quality.

Materials and Methods

All worm mutant strains used in this study were backcrossed 6 times to our lab wild-type (N2) strain, before generation of different mutant combinations and any phenotypic analysis. The different worm mutant strains used are indicated within the figures and their legends. Worms were grown for at least two generations at 25^{o}C on the same food source used in a given phenotypic analysis. The bacterial culture, assay plate preparation, lifespan assays and statistical analyses were as described in Chapter II.

Transgenic Worms. The overexpression lines were generated according to standard methods. The *nlp-44* overexpression plasmid (injected at 100 ng/ul) contains a 4.97 kb-long fragment of the wild-type *nlp-44* genomic locus, which includes the 2.13-kb sequence upstream of the *nlp-44* start codon and the 2.04-kb sequence downstream of the stop codon (see www.wormbase.org; version WS207). The *nlp-44* genomic locus is inserted into the pPD117.01 vector (gift of Andrew Fire), in which the *gfp* open reading frame was removed and replaced with the *nlp-44* locus. The *myo-3p::rfp* (gift of Cori Bargmann) was used as a co-injection marker (injected at 100 ng/ul). As controls, we also generated wild-type and *nmur-1* or *nmur-2* mutant worms that carry the *myo-3p::rfp* co-injection marker alone. The analyses of these animals were carried out with at least two independent transgenic lines in each genetic background: wild type or in the presence of the *nmur-1* or *nmur-2* mutation.

To elucidate the expression pattern of *nlp-44*, we generated a transcriptional *gfp* reporter construct (*nlp-44p::gfp*), in which the *gfp* in the pPD117.01 vector is flanked by the 2.13-kb sequence upstream of the *nlp-44* start codon and the 2.04-kb sequence downstream of the

stop codon. This construct was also injected into wild type worms at a concentration of 100 ng/ul to generate the strain *jxEx82*.

Chapter IV: General discussion

Food is a highly sophisticated environmental factor that serves not only as a source for energy but also as a source of material for the diverse cellular and extracellular structures found within each animal. Hence, food is a rich supply of many different types of cues that can provide an animal a mixture of complex information, which can have different effects on many of its physiological processes. Not surprisingly, an animal's perception of its environment would include, in large part, its perception of its diverse food sources. Indeed, different subsets of gustatory and olfactory neurons have already been shown to regulate lifespan in a variety of ways (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Libert et al., 2007). For my thesis and in collaboration with others, I set out to understand how these sensory neurons promote their effects on lifespan.

Accordingly, we have discovered a neuropeptide signaling pathway, the neuromedin U pathway, which is implicated in this process. In addition, we have identified one of the food-derived cues that modulate lifespan and elicit the activity of this pathway, which is distinct from that of food-level restriction, also known as calorie restriction.

A. Sensory neurons recognize food quality to influence lifespan.

Sensory neurons act at the interface between an animal's external and internal environments. Their dendritic endings, which are exposed to the external environment, detect external information, which can be transmitted to the animal's internal environment. Sensory neurons produce a number of short-range signals, which can act locally through neuronal circuits, and long-range signals, which can act hormonally. In our investigation of the molecular

mechanisms underlying the sensory influence on lifespan, we have found that sensory neurons recognize different food types to affect lifespan in a variety of ways.

We have identified four sensory genes that have food source-dependent effects on lifespan: the cilia structure genes *osm-3* and *daf-10* (Perkins et al., 1986; Tabish et al., 1995; Snow et al., 2004), the neuropeptide neuromedin U-like receptor *nmur-1* (see Chapter II) and the neuropeptide neuromedin U-like precursor *nlp-44* (see Chapter III). *osm-3* and *nmur-1* act together to shorten lifespan only on some food sources, whereas *daf-10* modulates the food-source effects of *nmur-1* (see Chapter II). However, unlike the effects of the first three sensory genes (see Chapter II), the food source-dependent effect of *nlp-44* is to lengthen lifespan (see Chapter III). All four genes are expressed in sensory neurons (see Chapters II and III). Indeed, the expression patterns of *osm-3* and *nmur-1* overlap, which is consistent with the observed genetic interaction between these two genes in affecting lifespan. On the other hand, the specific expression pattern of *daf-10* remains unknown, but there is evidence that it is expressed in sensory neurons (Perkins et al., 1986) that transmit information to or receive information from interneurons (White et al., 1986) in which *nmur-1* is also expressed. Thus, these expression patterns are again consistent with our findings that these two genes also act together on certain food sources.

It is currently not known whether *nlp-44* acts with *osm-3* or *daf-10*, but *nlp-44* is expressed in a pair of sensory neurons that also express *osm-3* [(Tabish et al., 1995); see Chapter III], which might suggest a possible interaction. At the same time, our genetic studies suggest that *nlp-44* might act downstream of or in parallel to *nmur-1* (see Chapter III). These two

genes do not overlap in their expression patterns, yet *nlp-44* is expressed in a pair of sensory neurons that can send inputs to or receive inputs from *nmur-1*-expressing interneurons (see Chapters II and III). Future studies would be needed to elucidate these interactions further and determine whether *nlp-44* encodes different peptide ligands that could then act on several NMUR proteins, including NMUR-1.

The effects of *nmur-1* and/or different food sources on lifespan might be due to calorie restriction, which is commonly studied as food-level restriction. However, the lifespan effect of calorie restriction is linked to a delay in development and reproduction, a decrease in total progeny and changes in feeding rates (Klass, 1977; Weindruch and Walford, 1988). In contrast, our study shows that the lifespan effects of *nmur-1* and different food sources do not correlate with feeding or developmental rates (see Chapter II). We also find that *nmur-1* can affect lifespan independently of reproduction, while the effect of food sources on lifespan is linked to faster, and not a delay in, reproduction. Moreover, we find that the different food sources and *nmur-1* affect early and middle-age mortality and not late-age mortality (see Chapter II), which again is unlike calorie restriction in its effects on late-age mortality (Lenaerts et al., 2007). Thus, our data suggest that the sensory influence on lifespan through recognition of food quality is distinct from that of calorie restriction. Furthermore, since sensory neurons sense diverse environmental cues, it is not surprising that the sensory system not only mediates the lifespan effects of calorie restriction, as previously described in *Drosophila* (Libert et al., 2007), but also those of different food types, as shown in this work.

B. A neuropeptide signaling pathway mediates the sensory influence on lifespan

The sensory influence on lifespan that is food source-dependent is mediated by the neuromedin U pathway. Neuromedin U signaling regulates food-dependent behavioral and physiological activities in mammals, birds, fish and insects (Howard et al., 2000; Hanada et al., 2004; Melcher and Pankratz, 2005; Shousha et al., 2005; Melcher et al., 2006; Maruyama et al., 2008). The mammalian neuromedin U receptor NMUR-2 is expressed in the hypothalamus and its peptide ligand NMU-8 has been shown to affect food intake and energy balance (Howard et al., 2000; Hanada et al., 2004). In addition, the intracerebroventricular injection of the neuromedin U ligand has been found to suppress food intake in birds and fish (Shousha et al., 2005; Maruyama et al., 2008). Moreover, the *Drosophila* homolog of neuromedin U, *hugin*, regulates food-searching behavior and feeding rates (Melcher and Pankratz, 2005; Melcher et al., 2006). Interestingly, the effect of *hugin* on adult feeding rates is also dependent on food type (Melcher and Pankratz, 2005). Thus, these effects of neuromedin U signaling in other animals are consistent with the activities of the *C. elegans* neuromedin U receptor, *nmur-1*, and neuromedin U precursor, *nlp-44*, in their food source-dependent effects on lifespan and/or other processes, like feeding rate, development and reproduction.

nmur-1 is expressed in sensory neurons and interneurons, whereas *nlp-44* is expressed in only two pairs of sensory neurons. These observations support the notion that this pathway acts with the sensory system to regulate lifespan. The fact that *nmur-1* is expressed in a large number of neurons, as well as in the somatic gonad, also suggests that *nmur-1* might act in different cells to control different processes, *e.g.*, lifespan, feeding rate, development

and reproduction. Although it remains unclear whether *nlp-44* encodes a ligand for NMUR-1, it is possible that other neuropeptide precursors, like *nlp-39* (see Chapter III), also encode a ligand for this receptor. Indeed, a possibility that needs to be tested later is whether NMUR-1 regulates the different processes in response to a specific ligand acting from a different neuron on a different *nmur-1*-expressing cell.

The observation that the sensory influence on lifespan is mediated by neuropeptide signaling, in response to specific food-derived cues, raises an interesting hypothesis: other neuropeptides might also affect lifespan in response to other types of sensory cues. Some of these neuropeptides might only exert their effects on lifespan under specific conditions, like another food source, in the presence of pheromones (*e.g.*, high concentrations of dauer pheromone) or so on.

C. A food-derived cue, the *E. coli* LPS structure, which elicits the *nmur-1* response

The nature of the environmental cues that are perceived by the sensory system to affect lifespan is of great interest. In our study, we found that the nature of the lipopolysaccharide (LPS) structure of the *E. coli* bacterial food source can have different effects on *C. elegans* lifespan and that these effects are dependent on *nmur-1* function.

The LPS is a major component of the outer membrane of gram-negative bacteria and is recognized by multicellular organisms in the context of defense against pathogens. It has been demonstrated that LPS recognition by the innate immune system involves Toll-like receptors (Takeda et al., 2003). *C. elegans* has a Toll-like receptor, *tol-1* (Pradel et al.,

2007), which regulates the animal's innate immune responses through the p38 MAPK *pmk-1* (Kim et al., 2002). This pathway has also been shown to play a role in *C. elegans* pathogen avoidance behavior (Pradel et al., 2007). *tol-1* is expressed in four sensory neurons and six interneurons (Pujol et al., 2001), none of which expresses *nmur-1*. However, *tol-1*-expressing interneurons can receive inputs from *nmur-1*-expressing sensory neurons (White et al., 1986). Thus, with these observations in mind, it is possible that the effect on lifespan by *nmur-1* recognition of LPS is mediated by the *tol-1* pathway. Nonetheless, we have found that *nmur-1* affects lifespan independent of *pmk-1* (see Chapter II), a downstream effector of TOL-1 (Kim et al., 2002), which suggests that *nmur-1* recognizes LPS to affect lifespan through another pathway.

Besides Toll-like receptors, there is also evidence for an involvement of GPCRs in LPS recognition (Triantafilou et al., 2001; Triantafilou et al., 2008). It has been shown that one of the mammalian GPCR, the CXC chemokine receptor 4 (CXCR4) is part of the LPS "sensing apparatus" and that LPS can directly interact with CXCR4 (Triantafilou et al., 2001; Triantafilou et al., 2008). At present, it is unclear whether *C. elegans* has a CXCR4 homolog or what the expression pattern would be for such a homolog. A BLAST search of possible homologs of CXCR4 in *C. elegans* gives the GPCR gene *tag-49* as the closest candidate, which is expressed in the intestine and nervous system (see www.wormbase.org, version WS210). Future studies could certainly determine whether this receptor, *tag-49*, is involved in the *nmur-1* effect on *C. elegans* physiology and lifespan.

D. Conclusion and perspectives

Our identification of a signaling pathway that affects lifespan depending on the animal's food source provides a genetic framework to elucidate the mechanisms that mediate the effects of different food sources on lifespan, which at present are unknown. Consistent with the *nmur-1* and *nlp-44* expression in sensory neurons and/or interneurons, it is possible that this neuromedin U pathway is involved in the integration of different sensory information with signaling pathways already known to regulate lifespan. At least one such information is generated by the LPS structure of the bacterial food source, which raises the possibility that sensory cues affect lifespan by eliciting stress-related and/or innate immune responses. Thus, future studies on the identification of the ligands and downstream effectors of this neuromedin U pathway should yield further insight into how this pathway mediates the sensory influence on lifespan in response to different types of sensory cues.

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Abbreviations

aak-2	*C. elegans* homolog of the catalytic alpha subunit of AMP-activated protein kinase (AMPK)
BL21	*E. coli* B strain
Bli	Blistered-cuticle phenotype
Daf	dauer formation-defective phenotype
daf-2	*C. elegans* insulin/IGF-1-like receptor
daf-16	*C. elegans* FOXO transcription factor ortholog
DH5α	*E. coli* K-12 strain
DY330	*E. coli* K-12 strain
Egl	Egg laying-defective phenotype
FMRFamide	phenylalanine-methionine-arginine-phenylalanine-NH2
FOXO	Forkhead homeobox type O
GPCR	G protein-coupled receptor
HB101	*E. coli* hybrid strain between B and K-12
HSF	Heat shock factor
hsf-1	*C. elegans* heat shock transcription factor 1
HT115	*E. coli* K-12 strain for bacteria-mediated (feeding) RNAi
IGF	Insulin growth factor
IR	Insulin receptor
IRS	Insulin receptor substrate
kpc	Kex2/subtilisin-like proprotein convertase gene

L1	first larval stage
L2	second larval stage
L2d	predauer larval stage
L3	third larval stage
L4	fourth larval stage
LPS	lipopolysaccharide
nmur-1	first phenotypically characterized *C. elegans* Neuromedin U receptor homolog
OP50	uracil auxotrophic E. coli B strain
PAL	peptidyl-α-hydroxyglycine α-amidating lyase
PHM	peptidylglycine-α-hydroxylating monooxygenase
pmk-1	*C. elegans* homolog of p38 mitogen-activated protein kinase
SCN	suprachiasmatic nucleus
TGF	Transforming growth factor
VNO	vomeronasal organ

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