Behavioral States

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ABSTRACT *Caenorhabditis elegans*' behavioral states, like those of other animals, are shaped by its immediate environment, its past experiences, and by internal factors. We here review the literature on *C. elegans* behavioral states and their regulation. We discuss dwelling and roaming, local and global search, mate finding, sleep, and the interaction between internal metabolic states and behavior.

KEYWORDS neuromodulation; behavioral states; sleep; foraging; satiety; WormBook

AS animals explore their environments, their nervous systems transition between behavioral states that influence how sensory information is processed and how actions are generated. Among the most familiar of these behavioral states are easily observable arousal states like sleep and wakefulness, as well as feeding states controlled by hunger and satiety. Animals also exhibit emotional states, like states of heightened anxiety or depression, as well as

cognitive states, for example, in the contexts of attention or working memory. Understanding the neural mechanisms that give rise to behavioral states is a critical goal of neuroscience: these states are central to the functioning of the brain and are disrupted in human sleep, mood, and cognitive disorders. In this chapter, we describe our current understanding of *C. elegans* behavioral states.

Over recent decades, there have been major advances in our understanding of the mechanisms that underlie behavioral states. Many studies suggest that these persistent states are often controlled by neuromodulatory systems. For example, the neuropeptides orexin and pigment-dispersing factor (PDF) promote wake states in mammals and flies, respectively (Saper *et al.* 2010; Taghert and Nitabach 2012). Dopamine signals reward in the context of motivational states (Schultz *et al.* 1997), and noradrenaline regulates vigilance and attention (Aston-Jones and Cohen 2005; Carter *et al.* 2010). In mammals, most of the neuromodulatory centers receive bottom-up (*i.e.*, sensory) as well as top-down (*i.e.*, from higher brain areas) inputs, such that they receive a complex mixture of information about internal and external cues (Weissbourd *et al.* 2014). Neuromodulatory systems typically influence the activity of neurons distributed throughout many brain regions, a feature that likely relates to their ability to control global behavioral states, but also poses challenges for understanding their mechanism of action. Despite major progress in this area, our understanding of how internal and external cues are integrated by neural circuits to give rise to behavioral states remains limited.

Caenorhabditis elegans has emerged as a premiere model system for the study of behavioral states. Due to its simple, well-defined nervous system and excellent set of genetic and imaging tools, mechanistic studies of behavioral states in the worm currently span from molecular genetic analyses to whole-brain scale studies. In this chapter, we review progress in this exciting research area and highlight challenges that lie ahead. We focus on locomotion states, sleep states, and feeding states. The organization of behavior into long-lasting states is also important for aspects of egg-laying behavior and dauer formation, but for these topics we refer the reader to other WormBook chapters (Schafer 2005; Hu 2007; Baugh and Hu, in press).
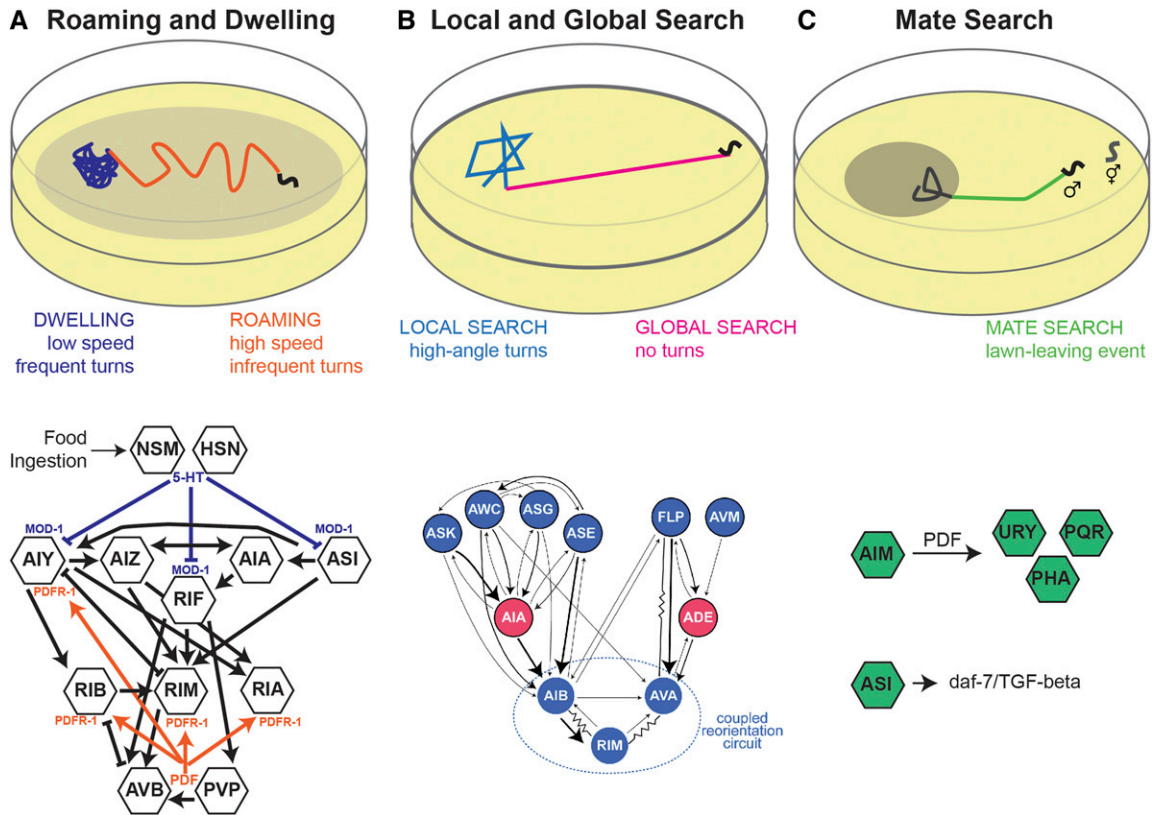
Locomotion States

Like other animals, *C. elegans* exhibit long-lasting behavioral states in which they display different movement patterns. By switching between different locomotion states, animals can alter how they explore their environment, for example searching for food locally *vs.* more globally. As described below, the locomotion states of *C. elegans* have been characterized mostly in the contexts of foraging and search behaviors. A theme that is emerging from these studies is that multi-modal sensory inputs can influence the activity of key interneurons and neuromodulator-producing neurons, which exert long-lasting effects on motor circuits to underlie locomotion states.

In each locomotion state, *C. elegans* animals express a characteristic set of locomotor parameters over a long-lasting, stable time period. *C. elegans* locomotion is comprised of just a few basic building blocks: (1) forward locomotion, (2) brief backward locomotion (aka reversals) and omega turns in which animals change their direction of movement, (3) postural changes such as fine-scale head movements (Von Stetina *et al.* 2006), and (4) locomotion pauses (Steuer Costa *et al.* 2019). These four basic building blocks are present in every locomotion state, but their frequencies and amplitudes can vary considerably. For example, animals in different states can display different forward velocities, altered reversal frequencies, or different head movements. Importantly, each locomotion state that we describe below is reliably observed under specific environmental conditions and consists of a reliable set of locomotion parameters.

Roaming and dwelling states

While animals are feeding on standard *Escherichia coli* food sources, they display a bistable behavioral state structure consisting of roaming and dwelling (Figure 1A) (Fujiwara *et al.* 2002). The roaming state consists of long bouts of high-velocity forward movement (~0.1 mm/s), punctuated by infrequent reversals (Fujiwara *et al.* 2002; Ben Arous *et al.* 2009; Flavell *et al.* 2013). In contrast, the dwelling state consists of short bouts of low-velocity forward movement (<0.05 mm/s), with a high frequency of short reversals. This basic structure is observed in both larvae (Shtonda and Avery 2006; Stern *et al.* 2017) and adults. While the dwelling state is promoted by many of the same mechanisms that promote behavioral quiescence (see *Sleep as a behavioral state* and *Behavioral states regulated by metabolic status*), it is a distinct behavioral state (Gallagher *et al.* 2013): dwelling animals still move, feed, defecate, and lay eggs.

The percent of time that animals spend in the roaming *vs.* dwelling state depends on the food environment. These states are influenced by (i) sensory cues like food odors and oxygen levels, (ii) food ingestion and presence of food in the alimentary canal, and (iii) satiety levels. Chemosensory cues are detected by a set of CNG/TAX-4-expressing olfactory and gustatory neurons. Mutants with impaired chemosensation, like *che-2* and *tax-4*, show increased dwelling, while *egl-4* loss-of-function mutants, which have attenuated sensory adaptation (L'Etoile *et al.* 2002), display increased roaming (Fujiwara *et al.* 2002; McCloskey *et al.* 2017). Roaming/dwelling analysis of mutants with impairments in specific sensory neurons suggests a particularly important role for AWC neurons in the detection of food odors (Ben Arous *et al.* 2009). Consistent with these genetic studies, transitions between roaming and dwelling are influenced by the animal's detection of food odors. When animals detect an increase in the concentration of food odors, they prolong their roaming state to navigate to the food source. But when the concentration of food odors decreases, they transition to dwelling states (Ji *et al.* 2020). Detection of additional chemosensory cues also impacts these states: pheromones that signal a

Figure 1 Locomotion states in *C. elegans*. (A) Top: roaming and dwelling locomotion states can be observed in animals exploring a bacterial food source. Bottom: neural circuitry implicated in the control of roaming and dwelling states. (B) Top: Immediately upon food removal, *C. elegans* animals display a 10–20 min bout of local search, followed by global search. Bottom: neural circuitry implicated in local *vs.* global search. (C) Top: male animals will leave a food source in search of hermaphrodite mates. Bottom: neurons and molecules implicated in mate search behaviors. Figure 1B (bottom) reprinted with permission from Neuron (Lopez-Cruz et al. 2019).

higher density of animals inhibit roaming (Greene *et al.* 2016), and aversive stimuli that signal potential harm can drive a high-speed state reminiscent of roaming (Ardiel *et al.* 2017; Chew *et al.* 2018). These studies suggest that regulation of roaming and dwelling states depends on an integration of diverse chemosensory cues.

The successful ingestion of bacterial food increases the amount of time that animals spend dwelling. This effect appears to be independent of olfactory and gustatory cues external to the animal: treatment of bacteria with a drug (aztreonam) that makes them too large to ingest dramatically decreases dwelling, even though the odorants and tastants produced by the aztreonam-treated bacteria are presumably very similar to those produced by untreated bacteria (Ben Arous 2009). The presence of bacterial food in the pharyngeal and intestinal lumens appears to promote dwelling behavior. Bacteria in the pharyngeal lumen are sensed by the pair of serotonergic NSM neurons, which extend a sensory dendrite into the lumen (Rhoades *et al.* 2019). The DEL-3 and DEL-7 ASIC channels on this sensory dendrite are required for NSM's sensation of bacterial food ingestion, which drives dwelling through serotonin release. Food presence in the intestinal lumen impacts Rictor/TORC2 signaling, which also functions to promote dwelling (O'Donnell *et al.*

2018). It is likely that additional mechanisms for alimentary tract lumen food sensation remain to be identified.

Satiety levels also exert a strong influence on roaming and dwelling behaviors. Animals that have been fasted display an increased level of dwelling when they are re-exposed to food, compared to well-fed animals (Sawin *et al.* 2000; Ben Arous *et al.* 2009). The molecular pathways and neural circuits that mediate these effects are starting to be clarified. The ETS-5 transcription factor functions in ASG and BAG sensory neurons to promote roaming and intestinal fat mobilization (Juozaityte *et al.* 2017). Genetic perturbations to fat metabolism pathways show that changes in fat storage can feed back to the nervous system to influence roaming (Juozaityte *et al.* 2017). In fact, multiple lines of evidence suggest bidirectional communication between sensory neurons and peripheral fat stores. In addition to ASG and BAG, the URX and ASI sensory neurons can also influence intestinal fat storage via neuroendocrine signaling (Noble *et al.* 2013; Palamiuc *et al.* 2017). Of these, URX has notably been shown to detect mobilization of peripheral fat stores, suggesting that it may be a hub for nervous system-intestine interactions (Noble *et al.* 2013). Many of these pathways that couple the animal's satiety to roaming and dwelling states also impact quiescence states (see *Behavioral states regulated*

by metabolic status), suggesting a great deal of overlap in the mechanisms that regulate distinct states.

The above studies provide a view that roaming and dwelling states are influenced by chemosensory cues, food ingestion, and satiety levels. But what are the central circuits that encode the roaming and dwelling states? These states appear to be controlled by diffuse neuromodulatory systems whose receptors are expressed on a diverse set of neurons that control locomotion. In particular, the serotonergic system promotes the dwelling state (Horvitz *et al.* 1982; Sawin *et al.* 2000; Flavell *et al.* 2013; Churgin *et al.* 2017). Both the NSM and HSN classes of serotonergic neurons promote dwelling, and optogenetic activation of these neurons can switch roaming animals into dwelling states. Serotonin likely acts through multiple receptors to mediate these effects, though of these receptors the serotonin-gated chloride channel MOD-1 has the strongest effect (Ranganathan *et al.* 2000; Flavell *et al.* 2013, Churgin *et al.* 2017). The serotonergic system acts in opposition to the pigment dispersing factor (PDF) neuropeptide system that drives roaming. There are two PDF neuropeptide genes in *C. elegans* (*pdf-1* and *pdf-2*), and the PDF-1 neuropeptide released by AVB, PVP, and SIA neurons has the strongest impact on roaming. Optogenetically activating the PDF system drives dwelling animals into long-lasting roaming states. MOD-1 and PDFR-1 are each expressed in interneurons that impact locomotion, including AIY, RIF, RIM, RID, and others (Ranganathan *et al.* 2000; Wenick and Hobert 2004; Janssen *et al.* 2008; Flavell *et al.* 2013).

Each of these neurons receives additional inputs and releases neuropeptides that also influence movement. For example, the RID premotor neuron that drives forward locomotion (Lim *et al.* 2016) receives FLP-20 peptidergic inputs from sensory neurons that drive high-speed locomotion (Chew *et al.* 2018). The AIY interneuron releases multiple neuropeptides, including FLP-1, which modulates locomotion (Buntschuh *et al.* 2018). The ability of the biogenic amines and neuropeptides to exert a strong effect on roaming and dwelling is likely related to the architecture of these systems: diffuse neuromodulators can broadly impact multiple nodes in the *C. elegans* nervous system. The abilities of these neuromodulators to drive long consolidated roaming or dwelling states suggests that a winner-takes-all architecture must be present in the neural circuitry that drives these states. Indeed, ensemble calcium imaging during roaming and dwelling states confirms the presence of a winner-takes-all mutual inhibitory loop between the serotonergic neuron NSM, which promotes dwelling, and the MOD-1- and PDFR-1-expressing neurons that promote roaming (Ji *et al.* 2020). The activities of these two opposing groups of neurons are mutually exclusive in wild-type animals, but mutants lacking PDF signaling display miscoordinated circuit activity where both cell populations can be simultaneously active. Chemosensory inputs acting through AIA interneurons modulate this mutual inhibitory loop, allowing animals to switch between states based on dynamic changes in food odors.

Additional neuromodulators implicated in food detection impact the roaming and dwelling states. Octopamine promotes the roaming state via SER-3 and SER-6 receptors expressed on cholinergic SIA neurons (Churgin *et al.* 2017). Interestingly, octopamine release is thought to be elevated in the absence of food, suggesting that the levels of this neuromodulator may depend on the feeding state of the animal (Horvitz *et al.* 1982). In contrast, dopaminergic neurons are thought to be activated by the presence of bacterial food and the release of dopamine controls the animal's roaming speed (Sawin *et al.* 2000; Stern *et al.* 2017). In addition, dopamine release during roaming increases the animal's egg-laying rate, so that animals display higher egg-laying rates during roaming *vs.* dwelling, which allows them to disperse their eggs across a food source (Cermak *et al.* 2020). The fact that multiple biogenic amines and neuropeptides are required for the proper expression of roaming and dwelling states suggests that these states are specified by the combinatorial action of many neuromodulators.

Local search and global search

When *C. elegans* animals are removed from food, they display a stereotyped sequence of two consecutive locomotion states (Figure 1B). For the first ∼15 min, they engage in a local search state (also called "area-restricted search") (Hills *et al.* 2004) consisting of a high frequency of high-angle turns and omega bends (Wakabayashi *et al.* 2004; Gray *et al.* 2005). Then, they transition to a global search state (also referred to as dispersal); Gray *et al.* 2005) where they suppress their turning rates. This behavioral sequence allows them to perform an area-restricted search of the environment immediately after their food source has vanished, but then to broaden their search to a wider area if the local search is unsuccessful. Theoretical studies have suggested these search states likely comprise an effective foraging strategy (Calhoun *et al.* 2014; Salvador *et al.* 2014). Unlike roaming and dwelling, where animals stochastically switch between the two states, the timing of the switch from local search to dispersal reliably occurs ∼15 min after food removal, suggesting that the mechanisms underlying these state transitions are different (Calhoun *et al.* 2014; López-Cruz *et al.* 2019).

The initiation of the local search state is strongly dependent on sensory inputs, including both chemosensory and mechanosensory cues. The food odor-sensing glutamatergic neurons ASK and AWC are particularly important for local search behavior (Gray *et al.* 2005). Glutamate released from ASK and AWC, together with glutamate released from other chemo- and mechanosensory neurons, is required for animals to execute the local search state. This glutamate release is detected by AIA and ADE neurons via the metabotropic glutamate receptor MGL-1 (López-Cruz *et al.* 2019). Activation of the ASK neuron can drive high-angle turns during local search, but has an attenuated effect when animals transition to the global search state (López-Cruz *et al.* 2019). AIA likely acts together with AIB and AIY interneurons to regulate

reversal frequencies during local and global search states. Interestingly, the energy-sensitive enzyme AMPK/AAK-2 regulates MGL-1 expression levels in AIY, as well as GLR-1 levels in AIB, to promote the dispersal state (Ahmadi and Roy 2016). Such a mechanism might allow starvation to tune the levels of glutamatergic signaling from sensory neurons onto these key interneurons. Indeed, the turning frequency during local search is increased when animals are exposed to a higher density of food prior to food removal (López-Cruz et al. 2019). In addition, one study has suggested that the level of variability in the food environment influences turning rates during the subsequent local search (Calhoun et al. 2015). These latter effects require dopaminergic signaling, which has been implicated in sensing the food environment prior to food removal (Hills et al. 2004).

Mate searching in males

When male *C. elegans* animals are positioned on a bacterial food lawn without potential mating partners (Figure 1C), they exhibit lawn-leaving events at a much higher frequency than do hermaphrodites (Lipton et al. 2004). Like hermaphroditic locomotion states discussed above, these lawn-leaving events reflect all-or-none behavioral switches, where there is a sharp increase in the probability of the animal persisting in a forward run when it encounters the edge of the bacterial food lawn.

Male lawn-leaving depends on several sensory inputs that reflect the animal balancing its reproductive drive with its need to consume food. Male leaving rates are dramatically reduced by physical contact with hermaphrodites on the food lawn (Barrios et al. 2008). This effect may be mediated by the ray sensory neurons in the male tail, which mediate responses to hermaphrodite contact. Loss of the ray neurons reduces leaving rates in mate-deprived males, but increases leaving in males exposed to hermaphrodites, suggesting that the function of the ray neurons changes depending on the sensory environment. The food signals that influence male leaving decisions are detected by amphid sensory neurons, as shown by the finding that mutants lacking the OSM-9, OCR-2, or TAX-2 sensory transduction ion channels have higher leaving rates in the absence of mates (Barrios et al. 2008). However, the presence of mates fully suppresses these mutant phenotypes, suggesting that mate detection can overcome reduced detection of food signals. The high propensity of males to leave the food may be partially explained by reduced expression of the food-sensing *odr-10* chemoreceptor (Ryan et al. 2014). Consistent with the notion that animals balance their mate search and food seeking, males that have been starved for three or more hours reduce their lawn-leaving rates (Lipton et al. 2004). These effects may be mediated by DAF-2 insulin receptor signaling to regulate chemoreceptor expression (Lipton et al. 2004; Wexler et al. 2020).

As is the case for other behavioral states, neuromodulation plays a pivotal role in mate searching behavior. PDF neuropeptide signaling promotes the drive to search for mates when animals are deprived of potential mates (Barrios et al. 2012). However, PDF effects are attenuated when mates are present. This function for PDF in promoting a search state is reminiscent of its role in promoting roaming, though, interestingly, PDF-1, as well as its receptor PDFR-1, functions in different neurons to control these two different behavioral states. PDF-1 functions in AIM to promote mate search (Barrios et al. 2012), but in AVB, SIA, and PVP to promote roaming (Flavell et al. 2013). PDFR-1 functions in URY, PQR, and PHA to promote mate search (Barrios et al. 2012), but in RIA, RIM, and AIY to promote roaming (Flavell et al. 2013). This suggests that while PDF may generally promote high-locomotion search states, the neural circuits that utilize this signal may be different for cases where sensory inputs need to be weighed differently. One other prominent target of PDF signaling in the male is the ASJ neuron: PDF-1 promotes the male-specific expression of DAF-7/TGF-beta in the ASJ neurons (Hilbert and Kim 2018). This expression drives increased mate searching and can be repressed by starvation to allow animals to balance their mate search with feeding needs. The nematode oxytocin-like peptide nematocin promotes mate searching (Garrison et al. 2012) and also organizes other steps of the mating behavior, suggesting a broader role in reproductive behaviors.

Sleep as a Behavioral State

The behavioral state whose appearance contrasts most conspicuously with that of other overt behaviors is the sleep state. While electrical recordings can act as a proxy to sleep in mammals and birds, ultimately sleep is a behavioral state, which can be easily distinguished from wake in all animals. The most obvious behavioral feature of sleep is the absence of movement. Sleeping animals are not only behaviorally quiescent, but also less responsive. Rapid reversibility of this quiescent state by strong sensory stimuli distinguishes sleep from other nonresponsive states such as torpor, hibernation, coma/obtundation, and general anesthesia. The last property of sleep, which speaks to its important physiological function, is its homeostatic regulation: following sleep deprivation, animals sleep more deeply or at inappropriate times.

Using these behavioral criteria, *C. elegans* has been shown to sleep during a larval transition phase known as lethargus (Raizen et al. 2008). Lethargus occurs four times in the animal's life cycle, once at transitions between larval stages L1→L2, L2→L3, and L3→L4, and once between L4 and adulthood. Periodic display of sleep during lethargus is likely one manifestation of oscillation of physiology during larval development (George-Raizen et al. 2014; Hendriks et al. 2014; Turek and Bringmann 2014). This oscillation, which has a larval periodicity, is reminiscent of circadian oscillation of physiology and sleep/wake cycles in other animals. LIN-42, the *C. elegans* ortholog of the core circadian regulator PERIOD, oscillates its expression (Jeon et al. 1999) and affects timing of lethargus quiescence (Monsalve et al. 2011), thereby showing molecular parallels to circadian sleep/wake

cycles in other animals. Because lethargus occurs only during larval development, this sleep state is sometimes referred to as developmentally timed sleep (DTS; Trojanowski and Raizen 2016). Most *C. elegans* sleep research has been performed on the L1→L2 and L4→adult lethargus periods. Thus far, the genetic and neural regulation of sleep appears to be the same during these two stages, and it is therefore reasonable to assume that it will be the same during L2→L3 and L3→L4 lethargus periods.

Outside of lethargus, some aspects of sleep behavior including reversible behavioral quiescence and reduced responsiveness have been demonstrated in *C. elegans* during prolonged starvation (Skora *et al.* 2018; Wu *et al.* 2018) and in response to environmental exposures that make animals sick (Hill *et al.* 2014; DeBardeleben *et al.* 2017). In addition, feeding worms display brief spontaneous quiescent bouts (Wu *et al.* 2018), and, after a prolonged fast and full refeeding, display prolonged episodes of movement and feeding quiescence (You *et al.* 2008), consistent with the behavioral sequence for satiety observed in other animals. However, homeostatic regulation of these quiescent sleep-like behaviors has been reported only for starvation quiescence (Skora *et al.* 2018; Wu *et al.* 2018). Satiety quiescence is discussed further in *Behavioral states regulated by metabolic status*.

We will refer to the behavioral quiescence that is a component of sickness behavior as sickness (or cell stress)-induced sleep (SIS).

C. elegans research into the regulation of sleep can be divided into components focused on particular aspects of sleep behavior: behavioral quiescence, reduced responsiveness, and homeostatic regulation.

Behavioral quiescence during developmentally timed sleep

Studies of behavioral quiescence during sleep have benefited from separately quantifying each of the specific components of the quiescence program. These include feeding quiescence, head movement quiescence, body movement quiescence, defecation quiescence, and egg-laying quiescence (Figure 2A). In addition, while there are commonalities, the regulation of quiescence during DTS is in some ways distinct from that observed during SIS (Trojanowski *et al.* 2015; Iannacone *et al.* 2017).

RIS, a GABAergic and peptidergic interneuron, is a chief neuron regulating movement quiescence during DTS. RIS is activated coincidentally with bouts of quiescence; genetic or laser ablation of RIS impairs quiescence; and optogenetic activation of RIS causes quiescence of movement and of feeding during the adult stage (Turek *et al.* 2013). Among about a third of the animal's nervous system RIS alone (and perhaps also the GABAergic RME neurons) has increased calcium activity during DTS (Nichols *et al.* 2017).

Mutations in either *unc-25*, which is required for GABA synthesis, or in *unc-47*, which is required for GABA packaging into synaptic vesicles, do not eliminate RIS induced quiescence during DTS, but cause a delay in the onset of quiescence (Turek *et al.* 2013; Steuer Costa *et al.* 2019). In contrast, removal of *egl-3*, which encodes a proprotein convertase required for maturation of most *C. elegans* neuropeptides, impairs quiescence during DTS, suggesting that neuropeptides processed by EGL-3 promote quiescence. The relevant neuropeptides released from RIS are those encoded by *flp-11* as *flp-11* mutants have little quiescence during lethargus or during RIS activation outside of lethargus (Turek *et al.* 2013; Steuer Costa *et al.* 2019).

Mechanistic details of RIS activation are beginning to emerge. RIS, like ALA (see below), responds to the epidermal growth factor EGF (Konietzka *et al.* 2020), but is also activated by excitatory input from several other neurons (Maluck *et al.* 2020). These include the forward command interneuron PVC, suggesting a mechanism by which locomotion circuit activity during wake behavior can influence sleep (Maluck *et al.* 2020).

In contrast to the role of RIS as a sleep-promoting neuron, wake-and arousal-promoting neurons are not yet well-delineated. Here, the field has used the term "wake" and "arousal" somewhat interchangeably, though different degrees of arousal during wake behavior have been reported (Jee *et al.* 2013; Laurent *et al.* 2015; Chew *et al.* 2018). For example, strains with reduced function of *npr-1* show aroused (enhanced) locomotion under certain conditions (de Bono and Bargmann 1998). In addition to the *dmsr-1*-expressing neurons discussed below, multiple classes of sensory neurons including nociceptive (ASH), touch sensitive (ALM and PLM) and stretch sensitive (DVA) appear to be important since they are required for the aroused locomotion of *npr-1* reduced function mutants (Choi *et al.* 2015). Arousal promoting neuropeptides include PDF-1 and PDF-2 (Chen *et al.* 2016), the *C. elegans* homologs of PDF, which is wake-promoting in *Drosophila* (Parisky *et al.* 2008), as well as FLP-2 (Chen *et al.* 2016). Dopamine signaling, which is wake-promoting both in flies (Andretic *et al.* 2005) and mammals (Wisor *et al.* 2001) may play a role in worm arousal since loss of the dopamine transporter DAT-1 is associated with reduced quiescence and loss of the dopamine receptor DOP-1 is associated with increased quiescence (Singh *et al.* 2014). Activation of dopamine neurons causes increased sensory acuity (Ezcurra *et al.* 2011).

Behavioral quiescence during SIS

SIS (Figure 2B) requires the second-order interneuron ALA as well as RIS (the role of RIS in SIS is described below). Removal of ALA either by laser ablation (DeBardeleben *et al.* 2017) or by mutations that disrupt its development (Hill *et al.* 2014; Nelson *et al.* 2014, 2016) results in continued movements of the pharynx and of the body during SIS. The body movements consist of sinusoidal movements similar to those observed when the animals are awake (Robinson *et al.* 2019). ALA shows elevated calcium upon activation by heat stress (Konietzka *et al.* 2020) and reducing the excitability of ALA neurons via chemogenetics attenuates the feeding and movement quiescence responses to cellular stress (Nelson *et al.*
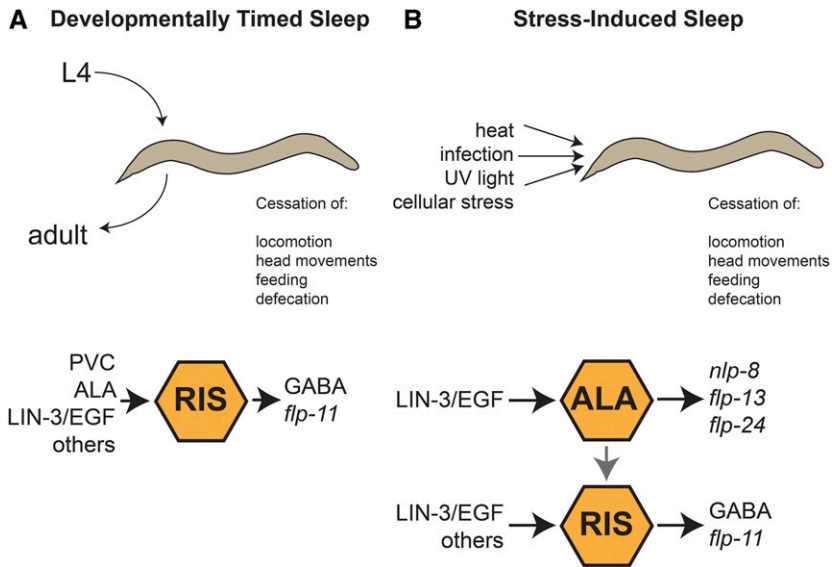
Figure 2 Sleep states in *C. elegans*. (A) Top: Developmentally timed sleep is observed during each larval transition. Studies have focused on the L4 to adult transition (as shown), as well as the L1 to L2 transition. Bottom: The RIS neuron is critical in the control of developmentally timed sleep. (B) Top: Stress-induced sleep is observed in response to a wide range of cellular stressors. Bottom: The ALA and RIS neurons play central roles in the control of stress-induced sleep. The neuropeptide genes *flp-11* (RIS), *flp-13* (ALA), *flp-24* (ALA), and *nlp-8* (ALA and RIS) promote quiescence.

2014). In contrast, optogenetic stimulation of ALA results in slowing of feeding and body movements (Nelson *et al.* 2014). Therefore, depolarization of the ALA neuron is necessary for movement cessation during SIS.

The mechanism by which ALA becomes activated in response to sickness is poorly understood. One mechanism involves the epidermal growth factor LIN-3, which stimulates the EGF receptor LET-23 expressed in ALA (Van Buskirk and Sternberg 2007). However, the source of LIN-3 and the mechanism by which it is released to affect ALA remains unclear. Because *lin-3* null mutants are lethal (Hill and Sternberg 1992), deciphering this mechanism of EGF signaling may require conditional mutants. Downstream of EGF function, the GEF for Rho-family GTPase VAV-1 plays an important role in ALA (Fry *et al.* 2016), but how EGF receptor signaling results in membrane depolarization remains unknown. Another mechanism of ALA regulation involves the CEP sheath glial cells, since Glia-ablated animals display prolonged quiescence bouts, which are suppressed by concurrent ablation of ALA (Katz *et al.* 2018). Finally, ALA is also activated by harsh mechanical stimulation to the body (Sanders *et al.* 2013).

How does ALA promote behavioral quiescence? Electron micrographs of ALA show vesicles with dense cores (White *et al.* 1986), suggesting that ALA secretes neuropeptides. Further supporting a neurosecretory role for ALA in quiescence is its requirement for UNC-31/CAPS (Van Buskirk and Sternberg 2007), which functions in dense core vesicle release (Speese *et al.* 2007). Although ALA stains weakly for GABA and expresses both the GABA uptake transporter SNF-11 and the GABA vesicular transporter UNC-47 (Gendrel *et al.* 2016), a function for GABA or other clear vesicle neurotransmitters in ALA has not been reported. Finally, genetic axotomy does not eliminate ALA function (Van Buskirk and Sternberg 2007), indicating that ALA likely can signal via volume transmission [it likely also signals synaptically, by inhibiting activity of the command interneuron AVE (Fry *et al.* 2014; Katz *et al.* 2018)]. These data suggest that ALA promotes sleep via neuropeptide release.

Peptidomic analysis of ALA in *Ascaris suum* (Jarecki *et al.* 2010) as well as transcriptomic analysis of ALA in *C. elegans* (Nath *et al.* 2016) suggested that *flp-7*, *flp-13*, and *flp-24* are expressed in ALA. Nath and colleagues also found numerous other neuropeptide-encoding genes including *nlp-8* to be enriched in ALA [*nlp-8* is also enriched in the RIS neuron (Konietzka *et al.* 2020)]. *flp-7* null mutants have no apparent SIS phenotype (Van Buskirk and Sternberg 2007; Nath *et al.* 2016), and *flp-13* mutants have only a small impairment in feeding quiescence, locomotion quiescence, and head movement quiescence during SIS (Nelson *et al.* 2014, 2016). However, *flp-13*; *nlp-8* as well as *flp-24*; *flp-13* double mutants show strong defects in quiescent behaviors (Nath *et al.* 2016). In contrast to the weak loss-of-function single gene effects, inducible over-expression of *flp-13*, *flp-24*, or *nlp-8* alone each results in strong quiescent phenotypes (Nath *et al.* 2016). The observation of weak or no SIS phenotype in single gene mutants yet strong phenotypes in multi-gene mutants suggests a high degree of neuropeptide degeneracy in the regulation of sleep during SIS. Such degeneracy has also been observed in a vertebrate system (Chiu *et al.* 2016; Lee *et al.* 2017), pointing to a common theme across phylogeny.

Elucidation of the signaling pathways downstream of *flp-13*, *flp-24*, and *nlp-8* remains in its nascent stages. The G-protein coupled receptor DMSR-1 is potently activated *in vitro* by peptides encoded by *flp-13*, is required for *flp-13* overexpression-induced quiescence, and is partially required for SIS (Iannacone *et al.* 2017). *dmsr-1* is expressed in ~10 neuron types including the roaming-promoting neurons RID and AIY (Iannacone *et al.* 2017). It is not detected in the AVA or AVE neurons connected directly with ALA, supporting the notion that FLP-13 signals through volume transmission. Silencing *dmsr-1*-expressing neurons increases quiescence

during SIS suggesting that these neurons are, in sum, wake promoting (Iannacone *et al.* 2017). FLP-13, in addition to its role in ALA-regulated quiescence, also plays a role in quiescence mediated by the BAG neuron [see *Satiety quiescence: fat storage* (Figure 3B)]. Receptors for FLP-24 or for NLP-8 have yet to be reported but their sites of action should shed light on the circuit downstream of ALA mediating behavioral quiescence.

RIS, in addition to its role in DTS, also functions in SIS. RIS is activated by exposure to heat (Kotera *et al.* 2016; Konietzka *et al.* 2020), which causes cellular stress. Optogenetic activation of ALA or over-expression of the ALA neuropeptide FLP-24 results in RIS activation, suggesting that RIS acts downstream of ALA in the quiescence program (Konietzka *et al.* 2020). *aptf-1* mutants, in which RIS development is defective (Turek *et al.* 2013), are deficient in SIS (Robinson *et al.* 2019; Grubbs *et al.* 2020; Konietzka *et al.* 2020). However, there are reported differences in the type of movements made by RIS-defective mutants, such as *aptf-1* and *flp-11*, compared to those made by ALA mutants, such as *ceh-14* and *ceh-17* (Robinson *et al.* 2019). RIS depolarization causes complete movement quiescence with elongation of the head (Steuer Costa *et al.* 2019) whereas ALA depolarization slows but does not fully stop behavior (Nelson *et al.* 2014). Details of the circuit downstream of ALA and RIS and connecting these two neurons remain to be worked out. Since feeding and body movement quiescence caused by *flp-13* overexpression can be reversed by stimulation of cholinergic motor neurons (Trojanowski *et al.* 2015), some effects of ALA activation (at least those mediated by *flp-13*) are likely mediated at the level of motor neuron inhibition (Fry *et al.* 2014).

Reduced responsiveness during sleep

Arguably the most mysterious property of sleep is the reduction of responsiveness to sensory stimuli since it would seem to be maladaptive from an evolutionary standpoint. The reduction in responsiveness is not absolute: strong stimulation will wake up even a deeply sleeping animal. This property of sleep is often referred to as "sensory gating"—there is a barrier to the registration of sensory information but that barrier can be overcome if the sensory input is sufficiently strong. The mechanism of sensory gating in mammals is poorly understood.

Research to date in *C. elegans* has implicated sensory neuron responsiveness as well as connectivity between interneurons as sites of sensory gating during sleep. Mechanosensory receptor neurons as well as the multimodal sensory ASH neurons show reduced sensitivity to stimuli during sleep states (Schwarz *et al.* 2011; Cho and Sternberg 2014). In addition to gating at the level of primary sensory neurons, synchrony between interneurons downstream of ASH is reduced during sleep and restoring this synchrony can arouse the animal from sleep (Cho and Sternberg 2014). The molecular mechanisms of reduced sensory neuron sensitivity and of interneuron desynchrony are currently unclear. The cGMP-dependent protein kinase EGL-4 plays a role in the former (Raizen *et al.* 2008), by acting in primary sensory neurons to promote sensory adaptation (L'Etoile *et al.* 2002). Neuromodulators may play a role in the latter but the mechanism remains to be worked out.

The neuropeptides encoded by *flp-18* and *flp-21* to activate the neuropeptide Y-like receptor NPR-1 to promote sensory gating, as *npr-1* loss-of-function mutants show elevated responsiveness and reduced quiescence under conditions of mild sensory stimulation (Choi *et al.* 2013) or under conditions of normoxia (Nichols *et al.* 2017). NPR-1 signaling occurs at least partially in the highly connected hub interneuron RMG (Choi *et al.* 2013; Nichols *et al.* 2017). The elevated arousal under normoxia that results from loss of *npr-1* function is suppressed by genetic ablation of oxygen sensing neurons (Nichols *et al.* 2017).

Homeostatic regulation of sleep

Following sleep deprivation, animals display homeostatic regulation in different fashions. In some animals, like *Drosophila*, the main manifestation of homeostasis is sleeping at a time of day when the animals are usually awake. In mammals, the chief manifestation of sleep homeostasis is a deepening of sleep following sleep curtailment; sleeping mice or humans are less likely to be awakened from sleep following a period of sleep curtailment. In other words, their sensory gating is stronger. Mechanisms of sleep homeostasis remain largely a mystery. In early conceptual models of sleep regulation, natural unperturbed sleep and sleep after deprivation were considered to be controlled by the same biological process. However, in recent years, studies in mice (Halassa *et al.* 2009) and fruit flies (Seidner *et al.* 2015; Dubowy *et al.* 2016; Liu *et al.* 2016) have suggested that these mechanisms appear to be at least partially distinct since genetic manipulations can affect the homeostatic response to sleep deprivation without affecting sleep amounts when the animals are unperturbed. As we will describe below, *C. elegans* too shows a dissociation between regulation of unperturbed sleep and sleep after deprivation.

Homeostatic regulation of sleep has been studied primarily during DTS. In the absence of perturbation or following weak photic or mechanical stimulation, Nagy and colleagues observed a correlation between the duration of quiescence bouts and the duration of motion bouts that immediately preceded that quiescence. This pairwise correlation was disrupted in mutants for NPR-1 signaling (Nagy *et al.* 2014).

In contrast to this bout-to-bout homeostatic regulation, which they term "microhomeostasis," strong photic or mechanical stimuli result in prolonged active bouts followed by an increased overall quiescence. Moreover, animals that had been stimulated to swim for at least 20 min during lethargus, will subsequently show deeper sleep, as manifested by elevated arousal threshold (Raizen *et al.* 2008). This homeostatic response to prolonged sleep deprivation is independent of NPR-1 signaling but is instead dependent on the stress-responsive FOXO transcription factor DAF-16 (Driver *et al.* 2013; Nagy *et al.* 2014). In contrast to its importance in the
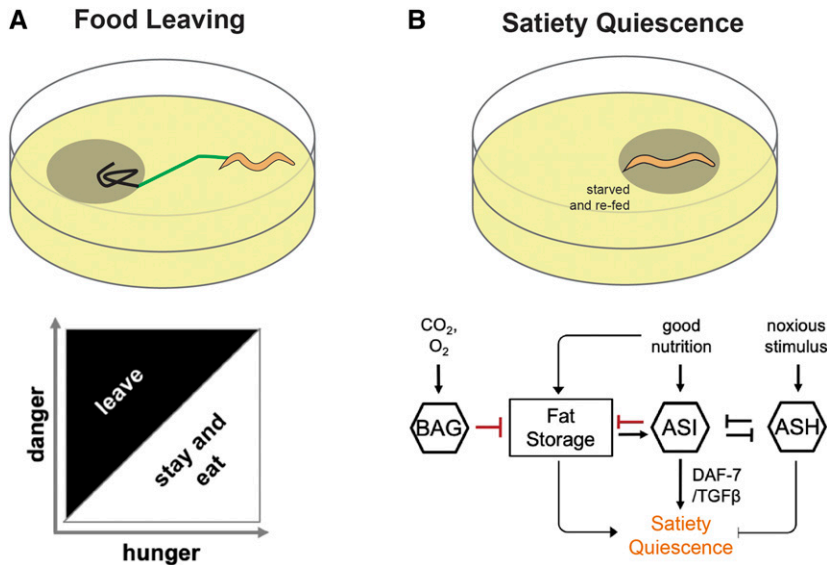
Figure 3 Behavioral states regulated by metabolic status. (A) Top: animals will at times leave their bacterial food source in search of other resources. Bottom: Food leaving rates are influenced by the animal's hunger (past and present feeding conditions), as well as harmful environmental cues. (B) Top: animals can enter a state of satiety quiescence. This is commonly observed when starved animals are refed with a nutritious food source. Bottom: Neural circuits that control fat storage (red connections show negative feedback) and satiety quiescence.

homeostatic response to sleep deprivation, removing *daf-16* function alone minimally affects baseline sleep (Driver *et al.* 2013; Nagy *et al.* 2014; Wu *et al.* 2018) or microhomeostasis (Nagy *et al.* 2014). However, removing *daf-16* together with the AMP kinase-encoding genes *aak-1* and *aak-2* results in a near-complete absence of quiescence in the adult stage (Wu *et al.* 2018). Further evidence for a role of DAF-16 in sleep homeostasis comes from analysis of mutants for the NOTCH ligand LAG-2 or for the DAF-16 regulator JNK-1. The sleep depth in both *lag-2* and *jnk-1* loss-of-function mutants is reduced yet they show an overall increased duration of quiescence, suggesting that longer quiescence is required to discharge homeostatic sleep drive (Bennett *et al.* 2018). Removing the function of *daf-16* in these two mutants does not correct the arousal threshold defects but does restore total quiescence time back to a level similar to that of wild-type controls (Bennett *et al.* 2018).

There remains several unknowns regarding the role of DAF-16 in sleep homeostasis. It is not clear if it acutely activates during sleep deprivation or whether it promotes relevant signaling throughout larval development. It also remains unclear where DAF-16 is acting to promote sleep homeostasis. Expression in neurons restores the enhanced quiescence response to sleep deprivation (Nagy *et al.* 2014) but expression in muscle is sufficient to restore the elevated arousal threshold response to sleep deprivation (Driver *et al.* 2013). The compensatory elevated quiescence of *lag-2* requires *daf-16* in neurons, whereas that of *jnk-1* requires *daf-16* in muscle (Bennett *et al.* 2018). Though sleep is conventionally considered a nervous system state, there is evidence also in mammals for a homeostatic regulation of sleep in muscle, where the β−HLH transcription factor BMAL1 regulates sleep drive (Ehlen *et al.* 2017). Since DAF-16 is a transcription factor, it is likely that one or more of its transcriptional targets is required for sleep homeostasis. These targets have not been identified.

In all animals studied to date, sleep deprivation causes not just behavioral changes (increased sleep pressure) but also cellular stress (Cirelli and Tononi 2000; Cirelli *et al.* 2005; Naidoo *et al.* 2005; Jones *et al.* 2008), and, in some animals, total sleep deprivation is lethal (Rechtschaffen *et al.* 1983; Shaw *et al.* 2002; Vaccaro *et al.* 2020). In *C. elegans* too, sleep deprivation results in cell stress, as manifested by movement of DAF-16 into the nucleus (Driver *et al.* 2013; Sanders *et al.* 2017), and by upregulation of markers for ER and mitochondrial proteostatic stress (Sanders *et al.* 2017). Sleep deprivation by mechanical stimulation of *daf-16* mutants defective for the cellular stress response can be lethal (Driver *et al.* 2013; Bennett *et al.* 2018). However, RIS-defective *aptf-1* mutants that are severely defective in behavioral quiescence during DTS do not die during lethargus, unless mechanically stimulated (Bennett *et al.* 2018). These observations suggest that sleep-deprived animals are hypersensitive to injuries caused by mechanical stimulation.

Behavioral States Regulated by Metabolic Status

A major determinant of an animal's behavioral state is its metabolic status: a hungry animal may explore to seek food and take risks, while a sated animal may rest, sleep, and reduce risk-taking. Because the perception of hunger or satiety can interact with factors such as the degree of danger in the environment, a behavioral state emerges from the integration of multiple cues, both internal and external. Furthermore, an animal's behavioral state is also influenced by its history, since the association of environmental cues such as innocuous chemicals with a past metabolic status can also influence the behavioral state.

This section summarizes our current understanding of the neuro-molecular mechanisms by which the internal metabolic state of the animal, together with external sensory cues, affects behavior.

Metabolism affects behavioral states

Since feeding is essential for survival, it impacts numerous processes and decisions throughout an animal's life. In *C. elegans*, feeding controls not only growth rate, body size, fat accumulation, brood size, and lifespan, but also behaviors and decisions such as various forms of taxis, dauer decision, and egg-laying. In most cases, sensory perception of food is integrated into the worm's metabolic status and influences the animal's behavioral output to maximize its fitness.

The locomotion states described in *Locomotion States* concern food, since the metabolic states of hunger or satiety can influence whether animals stay on a food source or decide to leave. Various aspects of bacteria, such as their smell (Bargmann 2006) and texture (Ranganathan *et al.* 2000; Sawin *et al.* 2000) influence behavioral states. The size of bacteria, which affects how well *C. elegans* can consume them, is closely related to food quality (Shtonda and Avery 2006).

Food quality is operationally defined by how well the bacteria support the growth of *C. elegans* (Shtonda and Avery 2006). Bacterial metabolic and size properties can explain some dietary influences on worm growth rates. For instance, the bacteria *Comamonas* sp., which synthesizes vitamin B12, supports faster *C. elegans* growth than *E. coli* strains (Watson *et al.* 2013). *E. coli* bacteria, whose cell-wall division is blocked by the antibiotic aztreonam, are large and therefore poor quality food (Ben Arous *et al.* 2009). Poor food quality promotes roaming whereas good food quality promotes dwelling and quiescence (You *et al.* 2008; Ben Arous *et al.* 2009).

We will discuss two particular behavioral states, leaving and satiety quiescence, to explain how behavior states are modulated by food quality, past experience, and fat storage.

Leaving: food quality and past experience: *C. elegans* modify their behavior depending on their previous experience of food quality and familiarity (Figure 3A). Worms rarely leave high quality food, but will frequently leave poor quality food (Avery and Shtonda 2003; Shtonda and Avery 2006). When offered the choice between two bacterial diets of equal quality, worms prefer familiar food (Song *et al.* 2013).

Feeding-defective mutants show a higher probability of leaving a medium-quality bacterial food lawn (Shtonda and Avery 2006). Reduced food ingestion due to either poor food quality (*i.e.*, difficult to ingest) or mutations that impair pharyngeal function can lead to leaving behavior. These effects may be mediated by reduced adiposity, reduced signaling from pharyngeal neurons that sample the pharyngeal lumen, or both. The observation that leaving probability reaches steady-state values within 10 min (Shtonda and Avery 2006) suggests that food sampling plays an important role. Leaving also depends on prior experience: after being conditioned for 3 hr on high-quality food, worms leave medium-quality food at a higher frequency then worms conditioned on low-quality food. Remarkably, leaving behavior is influenced even by remote experience during larval development.

After experiencing starvation-induced dauer formation in early life, adults do not leave food as frequently as animals that have never experienced starvation (Pradhan *et al.* 2019). This suggests that transient metabolic stress during development forms a long-lasting memory that influences behavior. This plasticity is mediated by *glb-5*, an oxygen sensor expressed in several neurons including BAG and URX, which play important roles in fat storage and satiety (Juozaityte *et al.* 2017; Hussey *et al.* 2018) (discussed below).

Satiety quiescence: fat storage: When satiated, *C. elegans* stop eating and moving and become quiescent (Figure 3B), exhibiting a behavioral sequence of satiety similar to that observed in mammals (Antin *et al.* 1975). Satiety quiescence depends on food quality, intestinal function, past metabolic experiences, and fat storage. Insulin (*daf-2*), TGFβ (*daf-7*), and cGMP (*daf-11* and *egl-4*) pathways, all of which are necessary for reproductive growth in response to favorable environmental conditions, regulate satiety quiescence (You *et al.* 2008). TGFβ (DAF-7), which is produced in the ASI sensory neurons in well-fed animals (Schackwitz *et al.* 1996), binds to its receptor in the RIM and RIC neurons (Greer *et al.* 2008). Activation of the DAF-7 receptor DAF-1 in the tyraminergic RIM neuron and the octopaminergic RIC neuron promotes satiety quiescence (Gallagher *et al.* 2013). How DAF-1 signaling in RIM and RIC promotes behavioral quiescence remains unclear, though it presumably involves RIS activation (Wu *et al.* 2018; Maluck *et al.* 2020).

Satiety quiescence is observed most consistently when animals are fully refed with high quality food after starvation. If the food quality is low, or if the animal has a defect absorbing nutrients from the intestine, satiety quiescence is reduced. The duration of starvation also influences satiety quiescence: the longer the starvation, the deeper the subsequent quiescence, as measured by reduced responsiveness to sensory stimulation. During satiety quiescence, animals respond poorly to the touch of an eyelash, suggesting a change in arousal threshold (You *et al.* 2008).

Several studies suggest that fat storage affects satiety quiescence. Like mammals, *C. elegans* store fat in the form of triacylglycerol (TG) (Watts and Ristow 2017). Pathways for synthesis, storage, and mobilization of fatty acids are highly conserved between *C. elegans* and other animals (Ashrafi 2007; Watts and Ristow 2017). Fatty acids are obtained both from bacterial diet and from synthesis via the SREBP (steroid response element binding protein)—FAS (fatty acid synthase)—SCD (stearoyl CoA decarboxylase)—ACC (acetyl CoA carboxylase) pathways (Brock *et al.* 2007). The SREBP-FAS-SCD-ACC pathways are essential: although 80% of fatty acids are obtained from the bacterial diet, this is insufficient to support larval growth of *sbp-1* mutants (McKay *et al.* 2003; Nomura *et al.* 2010).

Most mutants defective in satiety quiescence, such as mutants of insulin, TGFβ, or cGMP pathways, also misregulate fat storage, suggesting that fat metabolism and satiety quiescence are linked. However, dissociating cause and effect

between satiety and fat storage is complicated. A satiety-defective mutant may constantly eat and therefore accumulate more fat. If a mutant is hypersensitive to a satiety signal, it may reduce food intake and therefore reduce fat accumulation. Selective nutrient manipulations and genetic perturbations can begin to disentangle causality in the relationship between fat storage and satiety. For example, supplementing the worm's diet with the monosaturated fatty acid oleic acid, a product of the enzyme SCD in the SREBP pathway, promotes satiety quiescence (Hyun *et al.* 2016). Also, mutants in the SREBP-FAS-SCD-ACC pathway are defective for satiety quiescence due to reduced fat storage (Hyun *et al.* 2016).

How does fat storage regulate satiety quiescence? Two transcription-based mechanisms have been implicated: one involving the ETS-5 transcriptional factor and the other involving nuclear hormone receptors (NHRs).

ets-5, an ETS (E twenty-six) family transcription factor and an ortholog of mammalian FEV/Pet1, regulates both fat storage and satiety quiescence. When fed with the *E. coli* strain OP50, a mediocre quality food (Avery and You 2012), *ets-5* mutants show reduced roaming and enhanced satiety quiescence. This enhanced quiescence requires excessive fat storage; if fat storage is reduced either by growing worms on poor quality food or by introducing a mutation such as *eat-2* that impairs feeding, the enhanced satiety quiescence of *ets-5* is suppressed. Additionally, knockdown of *atgl-1* (an adipocyte triglyceride lipase), which results in enhanced fat storage, phenocopies the *ets-5* mutation (Juozaityte *et al.* 2017). These results support that increased fat storage promotes satiety quiescence.

NHRs also regulate behavioral states controlled by fat storage. In mammals, NHRs play a critical role in fat metabolism; peroxisome proliferator activated receptor α (PPARα) and hepatic nuclear factor (HNF) mediate the fasting response by regulating the expression of genes involved in fatty acid beta-oxidation, whereas PPARγ is required for adipogenesis. *C. elegans* has about seven times as many NHRs (293) as mammals (48) (Chawla *et al.* 2001; Taubert *et al.* 2011). Their roles in dauer formation (Antebi *et al.* 2000) and molting (Gissendanner and Sluder 2000) suggest that NHRs link the animal's metabolic status to developmental decisions. NHRs also regulate the transcriptional network that coordinates metabolic adaptation to different diets (Watson *et al.* 2013), suggesting that NHRs promote adaptive behavioral states by controlling the expression of specific sets of genes. A total of 11 NHR genes regulate both fat storage and satiety quiescence, supporting the role of NHRs in linking adiposity and behavioral states (Hyun *et al.* 2016).

How do ETS-5 and NHRs link fat storage to satiety quiescence? *ets-5* promotes transcription in BAG neurons of *flp-13* and *flp-19*, which encode neuropeptides partially required for the enhanced satiety quiescence of *ets-5* (Guillermin *et al.* 2011; Brandt *et al.* 2012). Most of the 11 NHRs that regulate satiety quiescence are expressed in the intestine and in neurons, but how and where NHRs function to regulate adiposity related to satiety quiescence is unknown.

The nervous system directly modulates intestinal fat storage. Serotonin stimulates fat loss in the intestine by promoting the release of FLP-7 from ASI neurons (Palamiuc *et al.* 2017). FLP-7 binds to the NPR-22 receptor in the intestine to upregulate *atgl-1* transcription and thus promotes fat loss (Palamiuc *et al.* 2017). Two oxygen-sensing neurons, URX and BAG, antagonize each other to regulate fat storage via FLP-17 and its receptor EGL-6 (Hussey *et al.* 2018). These studies suggest that there is bidirectional communication between the gut and the brain. ASI plays a major role in DAF-7-dependent satiety quiescence (Gallagher *et al.* 2013), whereas BAG plays a major role in FLP-13- and FLP-19-dependent mechanism of satiety quiescence (Guillermin *et al.* 2011; Brandt *et al.* 2012). The neuronal regulation of fat storage by ASI and BAG suggests that satiety quiescence and fat storage can be modulated by the same set of neurons.

In an extraordinary forward genetic screen for mouse sleep mutants, Funato *et al.* (2016) discovered that SIK3, a salt-inducible kinase in the AMPK super-family, promotes sleep. Similarly, the lone *C. elegans* ortholog of the three mammalian SIKs, KIN-29, promotes quiescence associated with satiety (van der Linden *et al.* 2008), molting (Funato *et al.* 2016), and recovery from sickness (Grubbs *et al.* 2020). Despite storing excessive fat, *kin-29* mutants behave like starved animals and have reduced ATP levels (Grubbs *et al.* 2020), indicating a defective response to cellular energy deficits. Liberating energy stores by over-expressing ATGL-1 corrects adiposity and sleep defects of *kin-29* mutants, suggesting that free fatty acids or their metabolites are a signal for promoting sleep. KIN-29 functions in nuclei of ciliated sensory neurons to promote both fat stores and sleep, demonstrating an intricate association between neuroendocrine regulation of behavior and metabolism.

Neural circuits integrating metabolic state with sensory responses

ASI is a critical regulator of metabolism-dependent larval development, physiology, and behavior. ASI is required to prevent dauer formation (Bargmann and Horvitz 1991), to extend lifespan caused by calorie restriction (Bishop and Guarente 2007), and to exhibit satiety quiescence (Gallagher *et al.* 2013). Nutrient activation of ASI (Gallagher *et al.* 2013) is enhanced by starvation (Davis *et al.* 2018), showing that ASI sensory responses are modulated by the metabolic status of the animal.

Activation of ASI by nutrients is blunted by simultaneous activation of ASH (Davis *et al.* 2018), a nociceptive neuron that can be activated by high NaCl concentration. Starved animals are more willing than well-fed animals to cross an aversive high osmotic strength barrier to reach food (Ghosh *et al.* 2016). The degree to which animals suppress the aversive response, which is mediated by ASH, increases with the duration of prior fasting, showing that decision-making circuits integrates hunger and harmful sensory cues (Ghosh *et al.* 2016). Indeed, food, serotonin and dopamine sensitize ASH to stimulate aversive response (Harris *et al.* 2010;

Ezcurra *et al.* 2011). Reciprocal inhibition between ASH and ASI via a circuit that includes serotonin and octopamine modulates nociception and avoidance (Guo *et al.* 2015). These studies indicate that internal metabolic conditions are integrated with external sensory cues to influence behavioral states.

The antagonism between danger and hunger is conserved across animals. For instance, in mammals, a risk of predation suppresses roaming when well-fed, but extreme hunger overrides danger perception and results in prioritizing food-seeking behavior (Sternson 2013; Burnett *et al.* 2016).

How does hunger override danger cues? In this circuit, ASI acts to gauge the animal's internal metabolic state, AWA to gauge external nutritional cues, and ASH to sense external danger. The integration of ASI and ASH activities may occur in interneurons such as RIM via PDF-2 (Ghosh *et al.* 2016). Additional elements of the relevant circuit have also been characterized. Serotonin released from ADF and octopamine released from RIC control ASI and ASH antagonism (Guo *et al.* 2015). Another potential mechanism underlying the antagonism is via opioid signaling mediated by NPR-17, a *C. elegans* opioid receptor. Food and serotonin sensitize ASH by increasing release of NLP-3, which activates NPR-17 in ASH (Harris *et al.* 2010). During starvation, the endogenous opioid NLP-24 activates NPR-17 in ASI, which sensitizes ASI to food and results in increased feeding (Cheong *et al.* 2015). Hunger can even fully reverse the valence of certain sensory cues: CO_2 repels well-fed animals but attracts starved animals. This switch is mainly controlled by dopamine, which promotes CO_2 repulsion, and octopamine, which promotes CO_2 attraction, working via antagonism between the interneurons AIY and RIG (Rengarajan *et al.* 2019). Interestingly, CO_2 is sensed by BAG neurons (Hallem and Sternberg 2008), which, as described above, regulate both satiety quiescence and fat storage (Gallagher *et al.* 2013; Cunningham *et al.* 2014).

Methodological Considerations

Studying behavioral states in *C. elegans* can present a number of experimental challenges. These states reflect behavioral modulation and appear to be more sensitive to variation in environmental conditions than studies of more hard-wired aspects of behavior, such as sinusoidal locomotion and pharyngeal contraction.

Studies of locomotion states are impacted by several aspects of the environment that need to be carefully controlled. First, the bacterial food source is a pivotal sensory stimulus for many of these states (Shtonda and Avery 2006, Ben Arous *et al.* 2009). The exact species and strain of bacteria that animals eat during their development and during the behavioral assay can profoundly alter these states. For example, the *E. coli* bacterial strain OP50 (Brenner 1974), which is used in nearly all *C. elegans* laboratories, is considered mediocre quality food, whereas the *E. coli* strain HB101 is considered high quality food (Shtonda and Avery 2006). Conditions for

bacterial growth prior to the experiment also matter, since they can impact production of bacterial metabolites; they too must be standardized.

Animal transfer to the assay plates can stimulate the animals and impact their behavioral state. The method of transfer (picking *vs.* washing) and duration of time between transfer and behavioral analysis need to be standardized for these assays. In addition, these states vary significantly over the course of development and during adulthood (Nagy *et al.* 2013; Stern *et al.* 2017), so precise staging of animals is essential. Finally, many different tracking systems for recording worm locomotion have been developed (Husson *et al.* 2013). While there is no evidence that this impacts the animal's behavioral state, direct comparisons of behavior across systems remain limited.

The nature of the chamber housing the worm during monitoring is important. Variables demonstrated to affect behavioral measurements include oxygen tension (Nichols *et al.* 2017; Soto *et al.* 2019), mechanical pressure on the worm body (Gonzales *et al.* 2019), temperature (Gonzales *et al.* 2019), food availability (McCloskey *et al.* 2017) (Gonzales *et al.* 2019), and liquid *vs.* solid media (Ghosh and Emmons 2008; McCloskey *et al.* 2017).

Three chief approaches have been employed for measuring movement and quiescence. The first is direct visual observations (You *et al.* 2008; Choi *et al.* 2013; Nath *et al.* 2016; Robinson *et al.* 2019), which allow the experimentalist to quantify several behaviors including body bends, nose movements, pharyngeal pumping, and defecation cycles (Nath *et al.* 2016; Robinson *et al.* 2019). The drawbacks of direct observation are reduced throughput, the potential for experimental bias, and the possibility of disturbing the worms with light or mechanical vibration. A variant of direct observation that minimizes perturbation of the worm is to track the position of the nose tip off line after the video recording (Turek *et al.* 2013). In general, nose tip quiescence is associated with body movement quiescence (Iwanir *et al.* 2013) and therefore reliably identifies a quiescent animal. However, this method is labor intensive, and, while it can reliably identify a fully quiescent animal, it does not readily distinguish different modes of behavior in animals that are not quiescent. Some animals may move just the nose while others may make dorso-ventral body bends resulting in translating the position of the worm (Robinson *et al.* 2019). Frame subtraction analysis, in which temporally adjacent video frames are digitally subtracted, has the advantage of higher throughput and of being fairly robust to lighting and animal contrast (Raizen *et al.* 2008; Zimmerman *et al.* 2008; Donelson *et al.* 2012; Nagy *et al.* 2014; Huang *et al.* 2017; Churgin *et al.* 2019). However, as in the case nose tip tracking, frame subtraction analysis does not distinguish the type of movement the worm makes when active (Robinson *et al.* 2019).

Our current understanding of the regulation of behavioral states has emerged from the use of various chambers and analysis methods, and, while most conclusions we discuss appear to be robust to the methods used, it is possible that

some results will in the future be shown to be due to an interaction between the biology and the method used to study it.

Acknowledgments

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