The genomics of stress-induced life cycle decisions in nematodes

Thesis by

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Nowadays, I find myself struggling to remember the people I wish I could the most. So, this section is here so that I will never forget the people who helped me along my PhD. (Part of me thinks I would otherwise, because it all seems so unreal.) Animals make decisions based on their environment, and my environment in Caltech was a very good one:

I would like to thank my classmates Alicia Rogers, Benji Uy, Greg Donaldson, and Katie Schretter, who are my role models in life and science; my friends Christopher Gilson, Eugene Shvarts, Haley Stepp, Jennifer Chen, Millie Grimes, and Sarah Gao, whose visits were the cure for my struggles; my mentors Amir Sapir, Mihoko Kato, Ryoji Shinya, and Tsui-Fen Chou, who helped me find myself as a scientist; my committee members Bruce Hay, Ellen Rothenberg, Eric Davidson, and Joseph Parker, who embody everything that I hope to be; my advisor Paul Sternberg, the Wormy Wonka to this wild, one of a kind laboratory, and who taught me everything my family didn't; my family, who taught me everything Paul didn't; my lab: the post-docs who showed me the way, and the grad students with whom we made fun of the post-docs; and my partner Pei-Yin Shih, who is the kindest person I know. I am incredibly lucky to have her support.

Finally, I dedicate this thesis to my grandfather, Jin Sung Koo. He saw the connections that tied every living thing together—he saw the picture in ways geneticists dream of.

ABSTRACT

Organisms including bacteria, insects, and mammals make decisions to alter aspects of their development based on signals from the environment. The roundworm *Caenorhabditis elegans* can escape environmental collapse by halting reproductive growth and entering the stress-resistant dauer larval stage. Dauer larvae are spore-like and have specialized behaviors for finding and stowing onto carrier animals for dispersal. The decision to enter dauer is an anticipatory decision that is based on the inputs of food, pheromone, and temperature.

Here, I show that touch is an overlooked input into the dauer entry decision. Using quantitative dauer entry assays on CRISPR knock-ins and existing mutants in mechanosensation, I demonstrate that gentle, harsh, and piezo touch promote dauer entry. By measuring pheromone sensation and signal tranmission in mechnanosensation-defective mutants, I show that mechanosensation likely inputs into the decision in parallel with pheromone. Further confirmation that touch promotes dauer entry is provided using direct mechanical stimulation of *C. elegans*, and I provide a plausible role for touch in sensing dauer-promoting weather and crowding conditions.

Using RNA-seq, I also show that 8,042 genes are differentially expressed between dauer and reproductive development. Within this dataset, we observed the striking up-regulation of 64 neuropeptide genes (encoding 215 peptides) during dauer. By comparison, the entire human genome contains 97 neuropeptide genes (encoding 270 peptides). In particular, we observed coordinated up-regulation of

the FMRFamide-like neuropeptides (FLPs). Using *sbt-1* mutants to knock down neuropeptide processing, we demonstrate that peptidergic signaling promotes the dauer entry decision, promotes vigorous waving during the dauer-specific nictation behavior (carrier animal-hitchhiking), and is necessary for switching from repulsion to CO_2 (a carrier animal cue) in non-dauers to CO_2 attraction in dauers. By testing individual neuropeptides using CRISPR knockouts and existing strains, we show that 7 FLPs promote dauer entry while 4 FLPs inhibit. I therefore propose plausible roles for these FLPs in acting downstream of and/or modulating the sensation of food, pheromone, temperature, and touch inputs. We also demonstrate that FLP-10/FLP-17, which are expressed in the CO_2-sensing BAG neuron, promote CO_2 chemotaxis and nictation in dauers. These findings reveal that neuropeptides can alter decision-making and behavior during *C. elegans* dauer entry. Through a meta-analysis, we discovered similar up-regulation of FLPs in the dauer-like infective juveniles of diverse parasitic nematodes, suggesting that this may be an ancient mechanism for expanding the behavioral repertoire of nematodes.

Further utilizing our RNA-seq dataset, I identified several markers for conveniently tracking and manipulating the dauer entry decision. These include *col-183* (which tracks dauer fate in the hypodermis), *ets-10* (neurons and intestine), *nhr-246* (intestine and muscle), and *led-1* (reproductive fate in hypodermis). Using condition shift experiments, we demonstrate that the dauer markers label animals during dauer-commitment. We show that these markers can be used to manipulate the entry decision by driving the reproduction-promoting

gene *daf-9*/Cytochrome P450 under the control of the dauer-commitment markers. We further demonstrate that the markers can be used to track tissue coordination and its breakdown in partial dauer mutants, and propose strategies for using the markers to identify the intercellular signals that coordinate the dauer entry decision.

I have discovered that the *C. elegans* dauer entry decision is more complex than previously realized, I have shown that *C. elegans* dauers obtain new behaviors through FLP signaling, and I have engineered tools for conveniently tracking and manipulating the dauer entry decision. My findings may illuminate how animals make robust decisions in uncertain environments, and have implications for how densely information and behaviors can be packed into a nervous system.

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J.S.L. participated in designing research, performing experiments, contributing new reagents, analyzing data, and writing the manuscript.

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Chapter 1

INTRODUCTION

1.1 Thesis overview

Most of life undergoes developmental decisions

Most of the life on the planet undergoes developmental decisions. By altering aspects of their development, organisms such as bacteria, fungi, nematodes, insects, plants, and mammals can adapt their metabolism, physiology, and reproductive strategy to meet resource availability (1-6). Several examples can be found from various taxa:

- Bacteria can sporulate, become competent to uptake DNA, or transcriptionally respond to predicted changes in environment (1, 7).
- Saprophytic *Arthrobotrys oligospora* fungi can develop carnivorous traps when they are starved for nitrogen (5, 8).
- Plants can change their growth and competition strategies in response to being blocked out from sunlight by neighboring plants (3, 9).
- Insects can switch from solitary to social forms in response to crowding (2). Eusocial insects can also generate queen and worker castes based on nutrition input (2, 10).
- Fish and reptiles can change their sex based on environmental temperature (11-13) or mate availability (14).
- Mammals can change their fur color (15) and immune system (16, 17) to deal with predators and pathogens. In addition, the embryos of various species can suspend development when environmental conditions are unsuitable for pregnancy (4, 18).

Developmental decisions can be stochastic (19), driven by internal cues (20, 21), or determined by environmental signals (22). However, even if the individual is not responding to the environment *per se* during stochastic or internally-driven decisions, the structure of the decision (including its dynamic range, bias, and rate of switching) faces selection from the environment (1, 23). In other words, even these decisions are responses to and anticipations of the environment that are conditioned by evolution (1). (This has been demonstrated using yeast engineered to switch stochastically between two states facing antagonistic selection. Yeast that were engineered as fast-switchers outgrew slow-switchers in fluctuating environments, while slow-switchers dominated in stable environments (24).) Therefore, understanding how the environment inputs into an organism's developmental decision is key to fully understanding the decision. In my thesis, I have taken an ethological approach to studying the *Caenorhabditis elegans* dauer entry life cycle decision, using genomics, quantitative genetics, and behavioral studies.

The enduring larva

One of the best-studied life cycle decisions is the *Caenorhabditis elegans* dauer entry decision (25, 26). Under favorable conditions, *C. elegans* roundworms develop through four larval stages—L1, L2, L3, and L4—to become a reproductive adult. However, declining food, temperature, and crowding conditions promote L1 larvae to enter the pre-dauer L2d stage. If conditions improve, L2d animals can

decide to resume reproductive development, while un-improved conditions cause L2d to enter the dauer larval stage (**Figure 1.1**).

Dauers are spore-like larvae that cease feeding and aging (27). This is accomplished in part by halting their reproductive growth and shifting their metabolism to favor long-term utilization of lipids (28, 29). Dauers have a stress-resistant, impermeable cuticle and can survive dessication for several days—even surviving loses of up to 98% of their body water (6, 25, 30). These factors contribute to dauers having a lifespan of approximately 8 months, which is 10 times longer than that of non-dauers (31).

Half a year to make a decision

L2d larvae make the dauer entry decision based on the inputs of food, pheromone, and temperature (32). The pheromones consist of small-molecule ascarosides (based on the sugar ascarylose) that are constitutively secreted, and can therefore be used to measure population density (33). Food, pheromone, and temperature are sensed by seven amphid sensory neurons, which convert these inputs into insulin and TGF-β signals (34). Specifically, food promotes the release of insulin and TGF-β, while pheromone and temperature inhibit. These signals are integrated in at least one cell—the neuroendocrine XXX cell (35). When insulin and TGF-β levels are high, the XXX initiates amplification of dafachronic acid (DA) growth hormone across the animal body, thus ensuring the decision to resume reproductive development (dauer bypass). How the XXX cell promotes dauer entry

when insulin and TGF-β levels are low is not well understood. Therefore, some questions that remain are:

- Is the XXX cell the only point of integration? XXX was identified as a site of integration because of its expression of *daf-9*/Cytochrome P450, which contributes to the synthesis of DA growth hormone (36, 37). However, it is likely not the sole site of integration since laser ablation of XXX during L1 produces a weak dauer entry phenotype, especially compared to *daf-2*/insulin receptor and *daf-7*/TGF-β mutants (38). In addition, the steps of DA production are distributed among various tissues, with intestine (*daf-36*/Rieske oxygenase), pharynx and head neurons (*dhs-16*/3-hydroxysteroid dehydrogenase), and hypodermis (*dhs-16* and *daf-9*) expressing components of the synthesis pathway (39). Therefore, integration of insulin and TGF-β signals may occur in these tissues as well.
- Do any signals instruct the decision to enter dauer other than the reduction of insulin and TGF-β? The field has mostly focused on insulin and TGF-β (as well as the inputs of food, pheromone, and temperature) because these components of the decision architecture were discovered using forward genetic screens and genetic interaction tests for strong dauer-consitutive and dauer-defective phenotypes (36, 37, 40-43). However, while these screens were performed to saturation, they did not reveal components of the decision that were redundant or modulatory. For

instance, the pheromone receptors *daf-37*, *daf-38*, *srbc-64*, *srbc-66*, *srg-36*, and *srg-37* were not identified in the initial screens because they redundantly sense the ascaroside pheromones (44-46). In addition, in **Chapter 2** I demonstrate that touch is an overlooked input into the dauer entry decision, likely because it modulates the decision. Therefore, it is possible that other environmental inputs and intercellular signals play a role in the dauer entry decision. In **Chapter 4**, several genetic markers for conveniently tracking the dauer entry decision are described, and strategies for using these markers for identifying additional intercellular signals are discussed.

- Is the dauer entry decision simply made by the absense of DA? And are dauer programs driven in all tissues when DA levels are low? *daf-9* mutants form partial dauers that develop incomplete cuticles, which lack the wild type resistance to SDS detergent, so there appear to be some programs that require non-DA input (47). How these tissue-specific programs can be teased apart is discussed in **Chapter 4**.
- How is the decision integrated? For instance, are dauer-promoting and dauer-inhibiting signals from the environment summed up over time, and if so, how is the information stored? Are environmental signals weighted differently based on their frequency and strength (perhaps to filter out spurious signals)? These are likely the

questions that will require the most work in the future to resolve. One strategy that would be effective in addressing these questions is to use convenient markers of the decision (**Chapter 4**) as high-throughput read-outs to test the dynamics of the decision. Since mechanical stimuli input into the decision (**Chapter 2**), and because its delivery (*e.g.* via vibrations or acoustic speakers) can be dynamically controlled (48), these inputs can be applied in bursts or as a stable signal, pulsed early during integration or late, and so forth in order to test how the resulting decision rates change.

Notably, L2d larvae spend 17 hours integrating environmental inputs to make the dauer entry decision (35). If the 3 week lifespan of *C. elegans* is scaled to the lifespan of humans, then L2d larvae spend 2.7 worm years making the decision. Or, if we consider that a 3 hour pulse of favorable conditions can trigger dauer bypass (35), then the decision-making period scales to half a year. In other words, the dauer entry decision can occupy a considerable proportion of the *C. elegans* lifespan. This is likely because the natural environment of *C. elegans* is noisy, consisting of a complex mix of microbes, invertebrates, and predators that can add to, alter, and corrupt the signals that *C. elegans* uses to assess its surroundings (8, 49, 50). When decisions need to be made in uncertain environments, trends in incoming signals must be integrated over time to average out the noise and to make an informed decision (51). (An everyday example of this occurs when one begins to suspect that it has started to rain: The first drop could be from anything (air conditioning unit, guttation from trees, drain pipe), but five

drops later and rain may be looking likely. By collecting trends in the data (*e.g.* frequency between drops) and integrating against a threshold (*e.g.* “five drops means rain is likely”), an appropriate response can be made despite uncertainty in the environment.)

In addition to dealing with uncertainty, the dauer entry decision likely aims to predict whether environmental conditions will continue to support growth. Entry into L2d, which stores more fat and has a longer intermolt than L2, allows *C. elegans* to anticipate an unfavorable environment, and provides the animal with developmental flexibility in case the environment does or does not collapse (52) (**Figure 1.1**). In this way, dauer entry may be similar to diapause in insects such as the mosquito *Culex pipiens* and the monarch butterfly *Danaus plexippus*, where diapause is triggered by stimuli (photoperiod and temperature) that signal the advent of an unfavorable condition (winter) (22, 53-55).

Based on current observations, the dauer entry decision can likely be conceptualized in terms of a drift diffusion model. Drift diffusion models of a decision assume that the decision is made by accumulating noisy evidence towards a decision-triggering threshold, and describe the accumulation of the evidence as a diffusion process (56, 57). Since these models resemble the algorithm that broke the Enigma code in World War II, they represent computationally fast and effective methods for dealing with uncertain information (51, 56, 57). In addition these models have been used to successfully describe decision-making in various animals. The dauer entry decision, therefore, likely fits a drift diffusion model with a reproductive development-triggering threshold that

can be reached by accumulating favorable stimuli, and which defaults to dauer entry when the decision times out at the end of the L2d integration period (**Figure 1.2**). This is because it appears that entry into dauer cannot be locked in before the end of L2d integration (at 33 hours post hatch), whereas a 3 hour pulse of favorable conditions at any time during integration can initiate dauer bypass (35). In this framework, the timed-out dauer entry decision relies on low levels of insulin and TGF-β (though other intercellular signals may be revealed; see above), and is locked-in by the absense of DA but also some non-DA inputs.

Touch is an overlooked input into the dauer entry decision

Touch is an important sensory modality that is present in every organism that has been observed (58-60). In humans, touch is the first sense that develops, and it can be used to assess social as well as physical aspects of the environment through distinct nerve fibers (61, 62). Social touch plays an important role during human development, affecting infant feeding behavior, stress response, weight gain, and even word detection during the early stages of vocabulary assembly (61, 62). Interpersonal touch can also affect human behaviors, including compliance, social participation, and resource sharing (*e.g.* tipping of waitstaff) (63). Remarkably, touch alone can communicate emotions such as anger, fear, disgust, sympathy, happiness, love, and sadness (64, 65).

(Correspondingly, perhaps, art and literature has depicted touch as a fundamental aspect of humanity. Touch is shown to literally impart humanity in

Michelangelo's *The Creation of Adam*, whose visual motif is repeated in such films as *E.T.* Other works visit this idea of touch as an inextricable part of humanity, such as Alfonso Cuaron's space-locked *Gravity* and Denis Villeneuve's existential *Blade Runner 2049*. We see this idea inverted in the common symbolism of gloves being used to hide or deny one's true self, such as in Disney's *Frozen* or Nicolas Winding Refn's *Drive*. It is likely no mistake that the phrase "human touch" combines the two words to describe things that are authentically human, and that things that move us are said to be "touching.")

If touch is fundamental to humans, there is evidence that it plays a similarly large role in other organisms as well. Touch can be used to convey social information (*e.g.* population density) in bacteria, plants, and insects (2, 9, 66), and is important for the growth and development of invertebrates and vertebrates (61). For instance, the development of nurturing behavior in rats has been shown to depend on mechanical stimuli received during early growth (67), and mating behaviors are dependent on mechanosensation in *C. elegans* and *Drosophila melanogaster* (68, 69). Importantly, the molecular mechanisms of touch are conserved, and the same mechanotransducers are present in the genomes of invertebrates and mammals:

- **Degenerin/epithelial Na+ channel (DEG/ENaC) family**: Degenerin/epithelial Na+ channels and their accessory proteins are involved in *C. elegans* gentle touch (*mec-2*, *mec-4*, *mec-6*, *mec-10*), harsh touch (*degt-1*), and nose touch (*deg-1*, *delm-1*, *delm-2*); *D. melanogaster* nociception (*pickpocket*); and texture discrimination in

mice (SLP3) (60, 70).

- **Transient receptor potential channel (TRP) family**: TRPs are a diverse family, consisting of seven subfamilies: TRPA (ankyrin), TRPC (canonical), TRPML (mucolipin), TRPM (melastatin), TRPN (NOMPC-like), TRPP (polycystin), and TRPV (vanilloid) (71).
    - TRPA: The TRPA homologs *trpa-1* in *C. elegans*, *painless* in *D. melanogaster*, and TRPA1 in mammals share roles in touch and nociception (60).
    - TRPN: The TRPN1 homolog *trp-4* in *C. elegans* is required for nociception (72), and shares roles with its *D. melanogaster* homolog *nompC* in touch and proprioception (70, 73, 74). Homologs of TRPN1 are found in zebrafish and amphibians, but not in mammals (60).
    - TRPV: In *C. elegans* the TRPV channels *ocr-2* and *osm-9* are involved in nose touch. TRPV4 in mammals has modest effects on touch sensitivity (60).
- **Transmembrane channel-like (TMC) family:** TMCs are multipass membrane channels, and *Tmc1* and *Tmc2* in mice are necessary for hair cell mechanosensation (75). In *C. elegans tmc-2* is expressed in PVD harsh touch mechanosensory neurons, and is therefore a putative mechanoreceptor channel (76).
- **Piezo family:** Piezos are large ion channels (over 2,000 amino acids long) that are involved in touch sensing in flies and mammals (77, 78).

Its role in *C. elegans* is unknown.

In the wild, *C. elegans* can use olfaction (79) and mechanosensation to navigate the complex environments it is found in (usually rotting vegetation and fruits) (80). In these habitats, *C. elegans* can encounter bacteria, fungus, insects, carriers, predators, and other nematodes. *C. elegans* can sense several types of touch including gentle touch, harsh touch, nose touch, and food texture (70), and these have been shown to affect ethologically relevant behaviors such as dwelling on food (81) and predator-avoidance (82).

Gentle touch is sensed in *C. elegans* by six touch receptor neurons (ALML, ALMR, AVM, PLML, PLMR, PVM) whose processes extend along the length of the animal, and whose activities resemble the Pacinian corpuscles in human skin that detect the onset and offset of light force (83). Gentle touch is therefore likely analogous to low-threshold, discriminative touch in humans, which detects light touch, hair movements, vibrations, quivering, and social touch (60, 84, 85). On the other hand, harsh touch is sensed by nine neurons in *C. elegans* (ADE, AQR, BDU, FLP, PDE, PHA, PHB, PVD, SDQR) and is likely analogous to high-threshold nociception, which detects physically damaging forces (60, 72, 86). Nose touch and texture discrimination likely represent harsh touch that is modulated by other neurons that respond to context. (*e.g.* (87)).

In **Chapter 2**, I demonstrate that gentle and harsh touch are used to modulate the dauer entry decision in *C. elegans*. I also provide a plausible role for mechanosensation in assessing weather and crowding conditions that promote

dauer entry. My findings reveal that the decision is more complex than previously recognized, and raises the intriguing possibility that other cues such as light, O_2/CO_2, pH, and osmotic stress may input into the decision as well. Furthermore, I discuss evidence that suggests that touch may be a common modulator of developmental decisions in organisms across biology. Due to noise in the environment, it is conceivable that multiple inputs are necessary for accurately assessing the environment in order to make appropriate developmental decisions.

Acquiring new behaviors with a constrained nervous system

The dauer is the most commonly observed stage of *C. elegans* in nature, since *C. elegans* feeds on transient microbial communities that collapse approximately every three of their generations (88, 89). In other words, *C. elegans* growth can be characterized by short periods of boom followed by potentially long periods of bust, during which time dauers must migrate to find improved conditions. It has been noted that soil nematodes can cover a distance of 15 cm on their own (90), but aided by vectors such as wind and carrier animals (*e.g.* isopods and slugs), *C. elegans* dauers are able to effectively disperse to dramatically different environments (91, 92). In fact, *C. elegans* has even been shown to migrate between continents, likely aided by large vectors such as humans (6, 91).

Dauers have two behaviors that aid in finding carrier animals. The first is nictation—a hitchhiking behavior where dauers stand on their tails and wave their

bodies (93). Dauers can nictate individually or in large amassed groups that have been termed dauer towers (88, 94). Nictation increases the likelihood of attaching onto a passing animal, and has been shown to affect the rate that *C. elegans* are transported by flies and isopods (93, 95). Conceivably, dauers may even nictate to draw the attention of animals in order to be eaten, as it has been shown that dauers can safely harbor in the intestines of slugs after being consumed by them (80, 96).

The second behavior that dauers use for dispersal is CO_2 chemotaxis. While non-dauers are repelled by CO_2, dauers are attracted, and in other nematode species the CO_2 produced by three mealworms is enough to elicit taxis behavior (97, 98). Non-dauers are likely repelled by CO_2 since it can signal the presence of predators (*e.g.* mites and springtails) or crowding (80). On the other hand, dauers are likely to take the risk in order to find carriers, especially since they can survive (and benefit from) being eaten by some animals (96).

Both nictation and CO_2 chemotaxis are dauer-specific behaviors, indicating that the neural state of dauers and non-dauers are different. However, this acquisition of behaviors is surprising given that *C. elegans* has a numerically simple nervous system of only 302 neurons (99). By comparison, the human eye alone carries over 120 million neurons (100, 101), and that the simple gill withdrawal reflex in *Aplysia* sea slugs requires the activity of around 300 neurons (102). In addition, the *C. elegans* nervous system is densely interconnected—almost any two neurons in *C. elegans* are connected by three degrees of (synaptic) separation (103). In other words, there are no synaptically

compartmentalized circuits that *C. elegans* can switch between during dauer and non-dauer that could explain the differences in their behavior and neural state.

Therefore, one way that *C. elegans* generates a new neural state during dauer is by rewiring its neurons (104). Specifically, the processes of ADE, AFD, ASG, ASI, AWC, and IL2 sensory neurons change their positions and morphologies during dauer. The reconfiguration of IL2, involving dendritic arborization and axonal remodeling, is necessary for the acquisition of nictation behavior (93). The role of rewiring in the other neurons is unknown, but based on the functions of these neurons, it can be presumed that these changes affect sensitivity to temperature (AFD), chemicals (ASG, ASI, AWC), and harsh touch (ADE) (105).

In **Chapter 3**, I demonstrate another method that *C. elegans* use to generate a new neural state in dauer. I show that *C. elegans* neuropeptides are massively up-regulated during dauer entry, and that this peptidergic signaling promotes the dauer entry decision, promotes vigorous waving during nictation, and is necessary for the switch to CO_2 preference in dauers.

Neuropeptides are evolutionarily ancient signaling molecules that likely pre-date the classical neurotransmitters, such as acetylcholine and dopamine (106-108). Neuropeptides are short sequences of amino acids that can act as transmitters, neuromodulators, and hormones. Other than a few instances (*e.g.* insulin-like peptides), neuropeptides bind G-protein coupled receptors to affect their target cells (109). After binding to their receptor, neuropeptides can modulate

the response amplitude, polarity, sensitivity, gene expression, and signaling repertoire of a target neuron (110, 111). Neuropeptides can also diffuse to facilitate signaling between synaptically unconnected neurons (103, 112). Through privileged ligand-receptor communication channels, neuropeptides can shape which circuits are active in the nervous system, the membership of these circuits, and their functions (103).

The *C. elegans* genome encodes for three families of neuropeptides—the insulin-related peptides (40 *ins* genes), the neuropeptide-like proteins (47 *nlp* genes), and the FMRFamide-like peptides (31 *flp* genes) (113):

- **Insulin-like neuropeptides (*ins*):** Insulin neuropeptides have evolutionarily conserved roles in regulating growth and metabolism in Metazoa (106). In *C. elegans*, signaling through DAF-2/insulin-like receptor promotes reproductive growth (113). Perhaps as a result, few of the *ins* genes were up-regulated during dauer entry (**Chapter 3**). In fact, the only *ins* gene that was up-regulated between dauer-commitment and reproductive development was *ins-1*, which likely antagonizes DAF-2 signaling and increases dauer entry (114).
- **Neuropeptide-like proteins (*nlp*):** The NLPs are a miscellaneous group of non-INS, non-FLP neuropeptides (113) that likely function in several independent processes. We observed the up-regulation of 25 of 47 *nlp* genes during dauer entry (Chapter 3). The specific roles of these neuropeptides during dauer remain untested.
- **FMRFamide-related peptides (*flp*):** FLPs are present across the

animal kingdom and have conserved roles in regulating feeding and reproduction in nematodes, arthropods, mollusks, and vertebrates (106, 115-117). The FLP family is especially expanded in the phylum Nematoda (118), and the FLPs in *C. elegans* represent the largest family of neuropeptides yet described (119). Strikingly, we observed that the *flp* neuropeptides are coordinately up-regulated during *C. elegans* dauer entry (**Chapter 3**). In addition, we discovered that *flp-8*, *flp-10*, *flp-11*, *flp-17*, *flp-21*, *flp-25*, and *flp-26* promote dauer entry, while *flp-2*, *flp-6*, *flp-18*, and *flp-34* inhibit dauer entry. Therefore, FLPs act redundantly and with opposed effects to modulate dauer entry. Conceivably, these *flp* neuropeptides could act downstream of and/or modulate the sensation of food, pheromone, temperature, and touch inputs (**Figure 1.3A**). As downstream signals, the FLPs could act as intercellular signals in addition to insulin and TGF-β to instruct the dauer entry decision. As modulators of input sensation, the FLPs could potentially be secreted by the sensory neurons to cross-talk with other modalities (120). For instance, cross-modal communication could allow one modality to compensate for defects or uncertainty in another (by, for instance, increasing sensitivity to mechanical stimuli to help assess crowding when pheromone cannot reliably be measured). Similarly, cross-modal communication could allow evidence to be corroborated or screened from the decision.

We also observed that FLP-10/FLP-17, which are expressed in

the CO_2-sensing BAG neuron, promote CO_2 chemotaxis and nictation in dauers. While the functions of the other FLPs during dauer remain untested, I suspect that they dramatically change the neural state of dauers by altering the composition and function of the active circuits in the nervous system (103). For example, FLP-10 signaling likely produces a dauer-specific circuit where the BAG neuron signals directly to DVA, HSN, and SDQ—which express the FLP-10 receptor EGL-6 (113, 118)—whereas these neurons are not connected in a single circuit in non-dauers (99) (**Figure 1.3B**). Interestingly, this FLP-10 circuit would allow the BAG neuron to signal to the ALM gentle touch neuron, as well as the AQR, FLP, PDE, and SDQR harsh touch neurons. I speculate that the role of this may be to suppress nociception and touch avoidance while the dauer performs CO_2 chemotaxis, so that mechanical contact with a carrier animal does not result in avoidance.

Therefore, the coordinated up-regulation of the FLPs likely functions to switch the neural state of *C. elegans* during dauer. In **Chapter 3**, I demonstrate that this strategy may reflect an ancient mechanism for expanding the behavioral repertoire of nematodes, and, from this framework, attempt to explain the expansion of the *flp* genes in Nematoda.

Conceivably, using neuropeptides to generate new neural states could be a crucial strategy in other organisms that lack highly compartmentalized nervous systems (*e.g.* species in Cnidaria, Ctenophora, and Echinodermata that possess

nerve nets). It is also plausible that this may have been a dominant strategy during early animal life, when complexity in the nervous system was low (121). Furthermore, neuropeptides are likely important for switching neural states within local regions of a compartmentalized brain. Indeed, the neuropeptide NPY (an evolutionary relative of the FLPs (109, 117)) fine-tunes the activity of the retina, perhaps playing a neuroprotective role (122). Because of their wide array of modulatory functions, and their ability to signal beyond the physical connectome, neuropeptides likely underlie many neural state changes, such as in sleep, post-traumatic stress disorder, and depression (123, 124).

The genomics of the dauer entry decision

Forward genetic screens have been useful in studying the dauer entry decision, revealing much of the core components of the decision. Using genomics, I have expanded the study of dauer by analyzing gene families that were prioritized based on our RNA-seq. I then tested the role of these genes in the integration of environmental signals and the acquisition of dispersal behaviors.

I studied how *C. elegans* use mechanosensation to increase the accuracy of the dauer entry decision (**Chapter 2**). Modulation of the decision from senses that assess various aspects of the environment could minimize uncertainty and allow *C. elegans* to make robust developmental decisions. Touch is also an important modality for growth and development in organisms across biology, so it is conceivable that it modulates the developmental decisions of other organisms

as well.

I also discovered how a coordinated class of neuropeptides, the FMRFamides, modulates the entry decision, and allows *C. elegans* to acquire dispersal behaviors after it decides to enter dauer (**Chapter 3**). Cross-modal communication by the FLPs may be an important aspect of the computation of the decision. Behavioral repertoire expansion by the FLPs allows adaptive behaviors to be expressed at the right time, despite lack of compartmentalization in the *C. elegans* nervous system.

Using data from our RNA-seq timecourse, I identified genetic markers that can be used for tracking and manipulating the dauer entry decision (**Chapter 4**). These tools will likely be useful for testing the dynamics of the decision, and for identifying any intercellular signals that work in addition to insulin, TGF-β, and DA.

My findings have revealed that the dauer entry decision is more complex than previously recognized, and may illuminate how animals make robust decisions in uncertain environments. In addition, my findings have revealed how animals acquire new behaviors, even with a physically constrained nervous system. It is remarkable how much *C. elegans* can achieve with a “little brain” of 302 neurons, and it is clear that dauers have much to reveal about how densely information and behaviors can be packed into a nervous system.

1.2 Figures

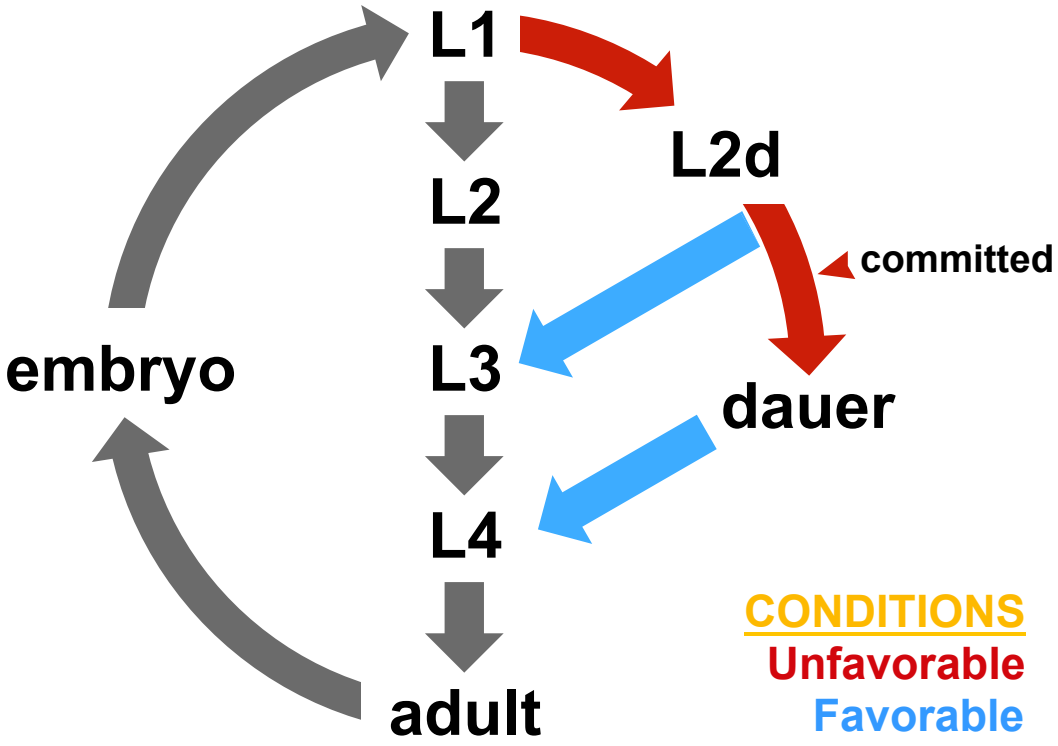


Figure 1.1. Life cycle and dauer entry decision of *C. elegans*. The arrowhead indicates the dauer-commitment time point (approximately halfway between the start of L2d and molt into dauer), after which the decision to enter dauer cannot be reversed. Red indicates dauer development under unfavorable conditions, and blue indicates the two possible paths out of dauer development under favorable conditions. Grey indicates reproductive development.

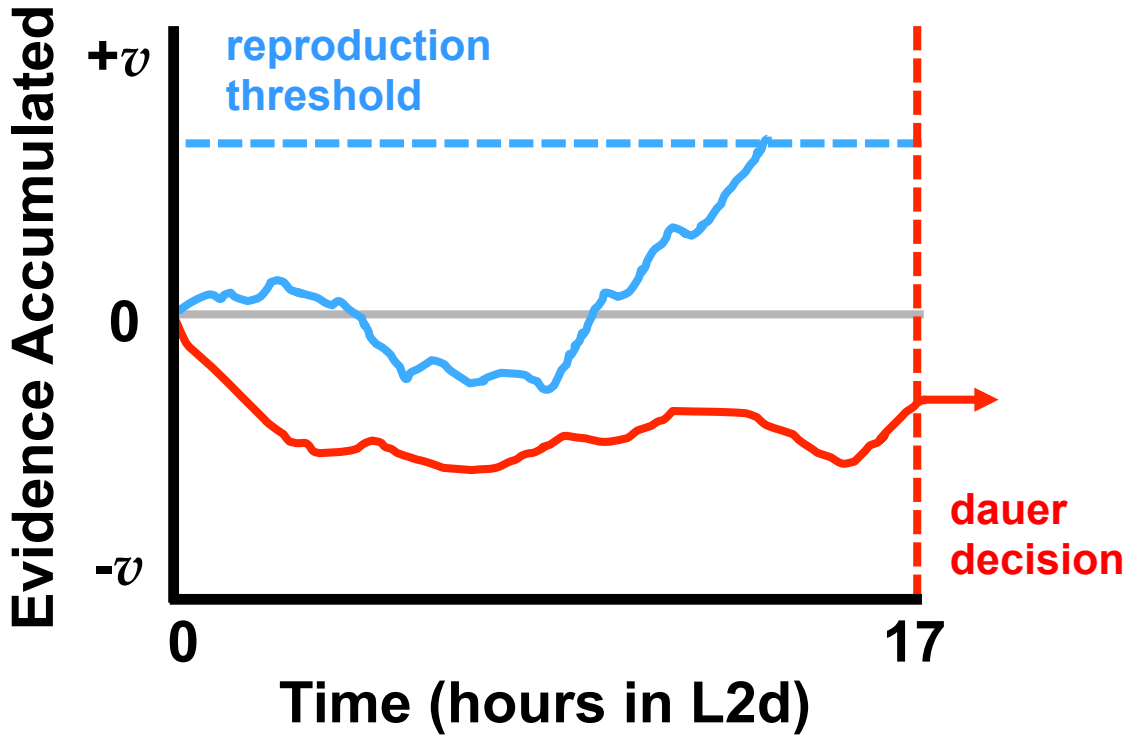
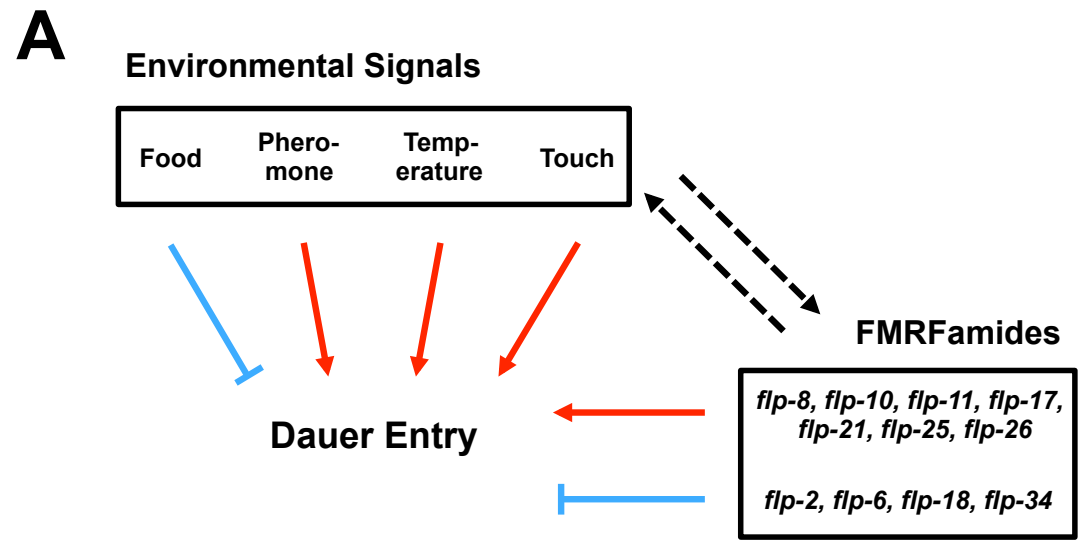


Figure 1.2. Conceptual framework of the dauer entry decision. Lines do not represent real data, but are drawn to highlight the accumulation of noisy evidence. Evidence in favor of a favorable environment is given a positive value (+*v*) and evidence for an unfavorable environment is negative (-*v*). The blue line describes a possible path for an animal that decided to resume reproductive development after accumulating enough evidence to pass the reproduction threshold. The red line represents an animal that entered dauer as a result of the decision timing out.


A
Environmental Signals
Food
Phero-
mone
Temp-
erature
Touch
FMRFamides
flp-8, flp-10, flp-11, flp-17,
flp-21, flp-25, flp-26
flp-2, flp-6, flp-18, flp-34
Dauer Entry

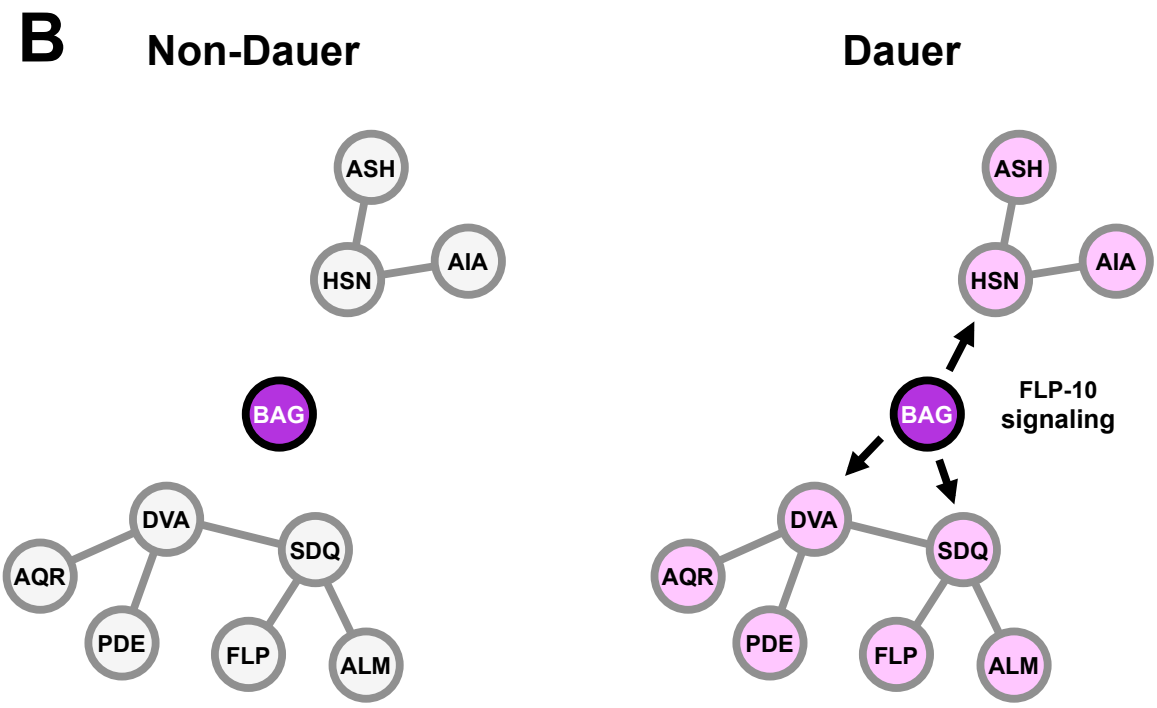

B
Non-Dauer
Dauer
ASH
HSN
AIA
BAG
DVA
SDQ
AQR
PDE
FLP
ALM
FLP-10
signaling

Figure 1.3. Model for signal input during the decision, and for circuit changes during dauer entry. (A) Red and blue indicate dauer-promoting and dauer-inhibiting effects, respectively. Dashed arrows indicate the possibility of flp neuropeptides acting downstream of the environmental inputs, and/or modulating the sensation of the environmental signals. (B) Circuit changes via non-synaptic FLP-10 signaling between the CO_2-sensing BAG neuron and FLP-10 receiving neurons. This figure is a zoomed-in version of Figure 3.12 in Chapter 3.

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