

Transcriptional control of satiety in *Caenorhabditis elegans*

Ava Handley and Roger Pocock 

Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria, Australia

ABSTRACT

Obesity is an enormous worldwide health concern. Chronic illnesses associated with obesity include type-2 diabetes, hypertension, atherosclerosis and certain cancers. Communication between fat storage organs and the brain is essential for regulating feeding, metabolism and organismal activity—and hence obesity control. Model organism research provides opportunities to decipher conserved molecular mechanisms that regulate fat storage and activity levels, which is fundamental to understanding this disorder. We recently identified a transcription factor (ETS-5) that acts in specific neurons of the nematode *Caenorhabditis elegans* to control intestinal fat levels. Furthermore, we discovered a feedback mechanism where intestinal fat controls feeding and motor programs, similar to humans, where a sated stomach can inhibit feeding and induce lethargy. The precise molecular signals and neuronal circuitry underpinning brain-intestinal communication in *C. elegans* are however yet to be discovered. As most animals store surplus energy as fat, communication mechanisms that relay external information regarding food availability and quality, and internal energy reserves are likely conserved. Therefore, our identification of a neuronally-expressed transcriptional regulator that controls intestinal fat levels opens up new avenues of investigation for the control of metabolic disease and obesity.

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Maintaining metabolic homeostasis requires a balance between eating, storing and using energy. The nervous system integrates information about internal nutrient stores, external food quality and availability, and previous food experience to determine the correct behavioral and physiological response to the current environment. The brain not only modulates food intake to maintain metabolic homeostasis, it can communicate with the intestine directly to induce changes in metabolic-gene expression. An important component of brain-intestine communication is transcriptional regulation. For example, changes to the external environment or internal metabolic state can alter gene expression of neuronal signaling factors and nuclear hormone receptors in the intestine.¹⁻³ These transcriptional changes are key to the communication networks between neurons, the intestine and activity programs, since they ensure the correct signaling molecules and receptors are present at the right time, and in the right place. Inducing changes in gene expression is a far slower process than acute calcium-based signaling. As such, transcriptional regulation potentiates longer-lasting responses to environmental

cues that complement fast-acting synaptic responses.¹ We recently identified a member of the E twenty-six transcription factor family in *C. elegans*, named ETS-5, which acts in a specific set of neurons to modulate fat storage in the intestine.⁴ Here we further expand and explain our findings, and discuss their significance in relation to the most recent work in this field.

ETS-5 is a transcription factor expressed in multiple sensory neurons in *C. elegans*.⁵ We previously showed that ETS-5 is important for specifying CO₂ sensing fate of the BAG neurons, and have recently discovered that ETS-5 acts in the ASG and BAG neurons to regulate behavioral state switching in the presence of food.^{4,5} In the laboratory, *C. elegans* is fed a uniform *Escherichia coli* food source, on which they spontaneously transition between 3 behavioral states - roaming (active), dwelling (inactive) and quiescence (sleep-like state).⁶⁻⁸ Switching between these behavioral states occurs in the absence of external stimuli, suggesting that internally-derived, as well as external food cues, can generate these behaviors. Roaming animals move rapidly and turn infrequently to explore their habitat. Dwelling animals are characterized

CONTACT Roger Pocock  roger.pocock@monash.edu  Monash Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash University, Level 3, Building 75, 15 Innovation Walk, Clayton VIC 3800, Australia.

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by slow movement and frequent turning to enable exploitation of the local environment. In contrast, quiescent animals are immotile and do not feed. Quiescence is induced by satiety and closely resembles postprandial somnolence, or “food coma” in mammals.⁹ Quiescent behavior in the laboratory is rarely observed (5% of the time) in wild type strains because worms are typically cultured on low quality food (the OP50 *E. coli* strain), thus laboratory-raised nematodes rarely reach the level of satiety that triggers a quiescent state.⁶ *C. elegans* strains that are unable to synthesize or store fat are defective in quiescent behaviors,³ arguing that fat levels in the intestine are an important determinant in behavioral state switching.

Our recent study found that *ets-5* mutant animals exhibit increased quiescence behavior and store more intestinal fat than wild type animals.⁴ We hypothesized that *ets-5* mutants likely exhibit increased quiescence because they are fully sated. Consistent with this, when we decreased fat levels in the intestine of the *ets-5* mutant, by malnourishing worms or by reducing the expression of POD-2, an acetyl coxylase, which is crucial for fatty acid synthesis in the intestine, animals explored more.⁴ Thus, decreasing intestinal energy stores overrides the *ets-5* mutant quiescence phenotype. Interestingly, we found that *ets-5* mutant animals revert to wild type exploratory behavior very rapidly when malnourished. This suggests that mechanical stimuli in the pharynx indicating low food intake may predominate over internal satiety cues. Taken together, the *ets-5* mutation leads to increased intestinal fat deposition, such that animals are sated and enter a quiescent or sleep-like state.

The question remained how ETS-5 controls intestinal fat stores from the ASG/BAG neurons? One possibility is that the *ets-5* mutant consumes food more quickly than wild type animals, thus gaining more energy in a shorter space of time, which could lead to quiescence. However, the *ets-5* mutant does not display increased pharyngeal pumping—a key indicator of increased food consumption, making it unlikely that the phenotype is due to increased food intake. ETS-5 is required to specify the BAG neurons,^{5,10} and these neurons enable the nematode to sense O₂ and CO₂. Therefore, it is possible that the gas-sensing function of these neurons may be responsible for the increased intestinal fat storage. However, animals in which the GCY-9 CO₂ sensing and GCY-31/GCY-33 O₂ sensing molecules^{5,11} are removed, and are deficient in O₂ and CO₂ sensing, explore like wild type animals. Therefore, an inability to sense O₂ and CO₂ does not contribute to the exploration phenotype of *ets-5* mutant animals. In addition, the *ets-5* mutant proved invariant from wild type animals in

multiple other gustatory and odortaxis behavioral assays, and exhibited normal locomotion speed.⁴ These data support the idea that the *ets-5* mutants do not gain fat because of altered food sensing or seeking ability, but because the communication between the nervous system and the intestine is dysregulated.

How does the neuronal function of ETS-5 fit into known pathways that govern the brain-intestine communication axis? One of the most well studied regulators of the brain-intestine axis is serotonin (5HT).^{12–14} 5HT is synthesized and secreted from a discrete number of neurons in *C. elegans*,¹⁵ and promotes the breakdown of body fat by inducing ATGL-1 lipase expression in the intestine.¹² 5HT signals to the URX and ASI neurons, which results in secretion of the neuropeptide FLP-7 into the pseudocoelomic fluid.¹⁶ Intestinal cells bind circulating FLP-7 via the NPR-22 receptor, which results in increased ATGL-1 expression.¹⁶ Generally, 5HT is a signal of satiety; promoting dwelling behavior and fat breakdown.^{7,12} We used a mutant for the 5HT receptor MOD-1, which explores more than wild type animals,⁷ to study the relationship between 5HT signaling and ETS-5 function. We found that when both 5HT signaling and ETS-5 are absent, these mutants exhibit normal levels of quiescence, yet still have reduced exploration.⁴ This suggests that ETS-5 acts genetically downstream of 5HT signaling in terms of promoting exploratory behavior, but 5HT is genetically upstream of ETS-5 in terms of promoting quiescence, thus highlighting the exquisite complexity of the neural networks that govern behavioral-state switches. In addition to 5HT, the activity of dopamine-expressing neurons has also been shown to promote dwelling behavior.¹⁷ Therefore, monoaminergic seems to be critical for regulating organismal activity.

The pigment-dispersing factor (PDF) neuropeptide-signaling pathway, acts antagonistically to 5HT signaling, to promote exploratory behavior.⁷ We found that mutants for the PDF-neuropeptide receptor—PDFR-1, do not roam and have a higher incidence of quiescence than the *ets-5* mutant. Animals in which PDF signaling and ETS-5 is removed exhibit the *pdf-1* mutant phenotype, therefore ETS-5 acts genetically upstream of PDF signaling. Other signaling mechanisms that control food-dependent behavioral state switching in *C. elegans* include the cGMP, TGF- β and insulin pathways.^{6,8,18} How ETS-5 coordinates behavioral state switching through or in conjunction with these signaling pathways will be a major subject for future studies.

The ASG/BAG neurons, whose function in behavioral state switching is specified by ETS-5 activity, play an integral part in the neuronal-intestine axis. As the *C. elegans* intestine is not innervated, signaling between the nervous system and intestine must ultimately be

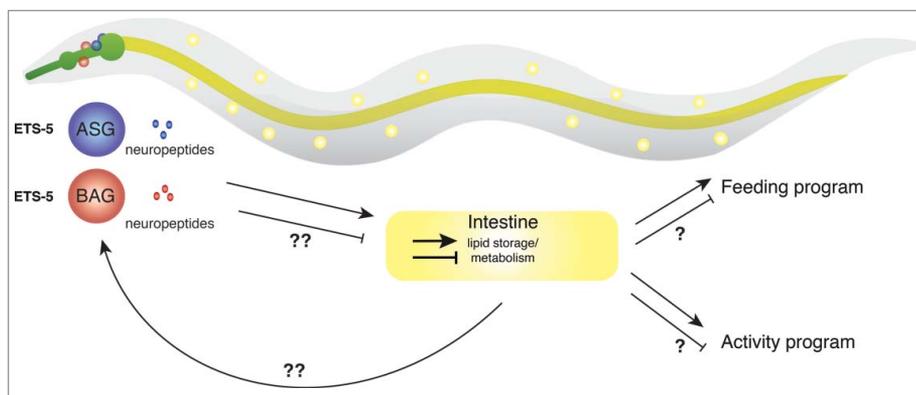


Figure 1. Neuronal regulation of intestinal fat and motor activity in *C. elegans*. ETS-5 expression in the ASG and BAG neurons controls intestinal fat levels through an unknown mechanism.⁴ We hypothesize that intestinal fat levels are interpreted by the nervous system to regulate feeding and activity programs. The question marks denote, as yet, unknown brain-intestinal communication processes.

through the action of neuropeptides. Indeed, we found that neuropeptide secretion from the ASG/BAG neurons is important for them to fulfil this function. We showed that knocking down UNC-31, a protein required for neuropeptide secretion,¹⁹ specifically in the ASG/BAG neurons of wild type animals significantly decreased exploratory behavior.⁴ Which neuropeptides are being regulated by ETS-5 still remains an open question. We showed that deleting 2 known ETS-5-regulated neuropeptides—FLP-13 and FLP-19⁵—caused reduced exploration, but not to the same extent as *ets-5* mutant animals. Thus, ETS-5 may act in the ASG and BAG neurons to control neuropeptide release or may directly control the expression of FLP-13 and FLP-19, and likely other neuropeptides, for signaling to other components of the network.

What may those other components be? As mentioned above, neuropeptides secreted from neurons are capable of circulating in the pseudocoelomic fluid and directly interacting with neuropeptide receptors on the surface of intestinal cells. However, these neuropeptidergic pathways are also likely to participate in networks of neuronal interactions to provide enhanced modulatory capacity for survival in ephemeral habitats. The accessibility of the *C. elegans* wiring diagram will enable a full appreciation of the molecular and cellular requirements for the regulation of food-associated behaviors.

In conclusion, the identification of a transcription factor acting in the nervous system to control fat levels in the intestine provides a platform for the identification and characterization of brain-intestinal communication mechanisms. The *C. elegans* genetic model provides an excellent opportunity to identify such means of communication. As the molecules and pathways under study are conserved in mammals, future work will potentially be of benefit in our understanding of the causes of obesity.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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ORCID

Roger Pocock  <http://orcid.org/0000-0002-5515-3608>

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