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The G protein regulator AGS-3 allows *C. elegans* to alter behaviors in response to food deprivation

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Behavioral responses to food deprivation are a fundamental aspect of nervous system function in all animals. In humans, these behavioral responses prevent dieting from being an effective remedy for obesity. Several signaling molecules in the mammalian brain act through G proteins of the $G\alpha_{i/o}$ family to mediate responses to food restriction. The mechanisms for neural response to food deprivation may be conserved across species, allowing the power of genetic model organisms to generate insights relevant to the problem of human obesity. In a recent study, we found that *C. elegans* uses $G\alpha_o$ signaling to mediate a number of behavioral changes that occur after food deprivation. Food deprivation causes biochemical changes in the G Protein Regulator (GPR) domain protein AGS-3 and AGS-3, together with the guanine nucleotide exchange factor RIC-8, activates $G\alpha_o$ signaling to alter food-seeking behavior. These proteins are all conserved in the human brain. Thus the study of behavioral responses to food deprivation in *C. elegans* may reveal the details of conserved molecular mechanisms underlying neural responses to food deprivation.

Keywords: AGS-3, G protein, $G\alpha_o$, GOA-1, RIC-8, food deprivation, octanol avoidance, *C. elegans*, ASH neuron, G protein regulator (GPR) domain

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Introduction

A central function of the nervous system in all animals is to control food seeking and feeding behavior. The need to regulate feeding may have indeed been a primary factor driving the initial evolution of the nervous system. This reasoning suggests the hypothesis that conserved molecular and cellular mechanisms underlie behavioral responses to food deprivation in all animals. The current epidemic of obesity in developed countries can be viewed as essentially a problem of behavior in which the strong drive to seek food that was evolutionarily programmed into animal behavior has become a health liability in societies in which food is no longer scarce. Understanding the neural mechanisms that underlie food-seeking behavior could facilitate the development of therapies to allow individuals to effectively control their weight.

Studies in mammals have shown that homeostatic maintenance of food intake, metabolism and energy expenditure requires a number of signaling molecules that communicate between the periphery and the brain, as well as within the brain, by activating G protein coupled receptors and G proteins of the $G\alpha_{i/o}$ family. The peptide ghrelin signals from the stomach to the hypothalamus in the brain to indicate hunger,[1] while the peptides leptin and insulin signal from adipose tissue and the pancreas, respectively, to the hypothalamus to indicate satiety.[2] Within the brain, neuropeptide Y and melanin-concentrating hormone signal from the hypothalamus to centers of dopamine signaling to increase feeding behavior.[2,3] Dopamine signaling generally promotes feeding behavior by acting in the dorsal striatum (a decision-making center), the nucleus accumbens and prefrontal cortex (reward centers), and in the pituitary gland.[4] Finally, additional neurotransmitters such as serotonin and norepinephrine signal from the brain stem to the hypothalamus and dopaminergic reward centers to alter dopamine signaling and ultimately modulate feeding behavior.[5,6]

The integration of all of these signaling pathways leads to alterations in feeding behavior, energy expenditure and metabolism. All of the signaling molecules described above, with the exception of insulin, act via G protein-coupled receptors that in most cases activate G proteins of the $G\alpha_{i/o}$ family. Thus, many responses to food deprivation in mammals ultimately depend on $G\alpha_{i/o}$ signaling.

Past Studies of Response to Food Deprivation in *C. elegans*

The conserved molecular mechanisms by which animals alter their behavior in response to food deprivation can potentially be studied by taking advantage of the powerful genetics and simple, well-characterized nervous system of *C. elegans*. Past studies of *C. elegans*' response to food deprivation focused on the effects of long-term starvation on a developmental switch that causes formation of dauer larvae.[7] There has been no systematic analysis of how food deprivation alters nematode behavior overall. Nevertheless, a number of studies have identified changes in individual behaviors upon food deprivation. Presumably, these behavioral changes evolved to act together to increase the rate of survival of the animal and its future progeny in conditions when food is scarce. As described below, the molecular mechanisms of these simple behavioral changes involve some of the same signaling molecules and G proteins that alter feeding behavior and metabolism in humans. Thus studies of the simple behavioral responses to food deprivation in *C. elegans* may provide insight into the mechanisms of regulation of feeding behavior in mammals.

In *C. elegans,* insulin signals the presence of food, regulates fat storage, inhibits feeding, and activates a quiescence behavior that has been equated to satiety.[8,9] Changes in insulin signaling that occur upon food deprivation alter several additional *C. elegans* behaviors in the absence of food, including avoidance of high levels of CO_2, allowing animals to search for food in areas with high CO_2[10] and male mating behavior, shifting the behavioral focus from mating to food seeking.[11] Thus in both mammals and *C. elegans*, high levels of insulin signaling indicate food satiety, decrease feeding behavior, and alter other behaviors and metabolism appropriately.

Several peptides regulate behavioral responses to food in *C. elegans*. FMRFamide neuropeptides directly alter the activity of the pharyngeal muscles to modulate the rate food intake.[12] In the presence of food, pharyngeal muscle contractions are increased, while in the absence of food, pharyngeal muscle contractions are decreased. Neuropeptides also activate G protein coupled receptors to alter social feeding behavior, a phenomenon in which animals aggregate at the edge of the bacterial lawn where the concentration of food is higher.[13] Neuropeptide activation of $G\alpha_{i/o}$ family proteins plays roles in regulating odorant avoidance responses[14,15] and egg-laying behavior.[16,17] *C. elegans* backs away from noxious chemical odors, but this avoidance response is down-regulated after food deprivation presumably to allow animals to explore for food in areas they would otherwise avoid.[18] Egg-laying is strongly inhibited upon food deprivation,[19] presumably so that adults deposit their eggs preferentially in areas where their progeny will have access to food.

Serotonin and other neurotransmitters, including octopamine, the invertebrate form of noradrenaline, also signal through G proteins and modulate behavior upon food satiety and food deprivation in *C. elegans*. As in mammals, serotonin generally inhibits food seeking behaviors upon satiety, while octopamine does the opposite, stimulating food-seeking behaviors upon food deprivation.[16] In the absence of food, dopamine signaling is required to alter the avoidance response to the noxious stimulus octanol and to switch from the area-restricted food seeking strategy, in which animals turn frequently to remain near a food source, to the wide-area food seeking strategy, in which animals move in straight paths to explore further for food.[20,21] In addition, dopamine is released upon the mechanosensation of food and causes the animals to slow locomotion,[22] further helping them to remain near food.

The simple behavioral changes performed by *C. elegans* after food deprivation appear quite different from those performed by humans upon food deprivation. None the less, the signaling molecules and G protein signaling pathways that allow food availability to regulate feeding behavior, metabolism, and energy expenditure appear to be well conserved from mammals to *C. elegans.* Thus the powerful genetic tools and simple, highly-defined nervous system of *C. elegans* behaviors can be used as tools for understanding, in general, how nervous systems respond to food deprivation.

The G Protein Regulator AGS-3 Alters *C. elegans* Behavior after Food Deprivation

Because most of the signaling molecules that result in behavioral changes upon food deprivation activate receptors coupled to G proteins of the $G\alpha_{i/o}$ family, we investigated how regulators of $G\alpha_o$ affect responses to food deprivation in *C. elegans*. One potential regulator of $G\alpha_o$ in mammals is AGS3, a protein that binds mammalian $G\alpha_o$ in vitro via a set of four G protein regulator (GPR) domains. The in vivo functions of AGS3 are not yet well-understood in mammals, but a knockout of the mouse AGS3 gene results in a lean phenotype, apparently due to an increase in metabolism.[23] We found that, like their mammalian orthologs, *C. elegans* AGS-3 and $G\alpha_o$ bind each other in vitro via the GPR domains of AGS-3.[24]

We found that both AGS-3 and $G\alpha_o$ are absolutely or partially required for three different behavioral responses of *C. elegans* to short-term food deprivation: animals lacking either AGS-3 or $G\alpha_o$ fail to slow egg-laying rates, alter food-seeking strategy to a wide-area search, or delay their aversive response to dilute octanol.[24] We also found that food deprivation causes a physical change in the state of the AGS-3 protein. Using AGS-3 antibodies to analyze the fractionation behavior of the protein in whole-animals lysates after various periods of food deprivation, we found that AGS-3 protein from whole-animal lysates of well-fed animals cannot be solubilized by the detergent Triton X-100, but that the protein progressively moves into a Triton X-100 solubilizable state over a period of several hours of food deprivation. AGS-3 is expressed in most or all neurons of *C. elegans*, and since the

change in AGS-3 solubility occurs in whole-animal lysates, AGS-3 is apparently affected by food deprivation in many neurons. Further, the behavioral changes that require AGS-3 and $G\alpha_o$ activity depend on a variety of neurotransmitters and neural circuits.[18-20,25] Thus, it appears that AGS-3 mediates a wide variety of responses to food deprivation.

We analyzed in detail one behavioral response to food deprivation, the avoidance of dilute octanol. When well-fed *C. elegans* are presented with dilute octanol, the chemosensory ASH neurons release glutamate onto interneurons, causing the animals to quickly back away.[18] However, when food-deprived, several neurotransmitters signal onto the ASHs to activate G proteins, including $G\alpha_o$, reducing glutamate release to delay the aversive response octanol.[15,26-28] We showed that AGS-3 is expressed in and functions in the ASH neurons to allow food-deprived animals to delay the octanol avoidance response.

AGS-3 Activates the G Protein $G\alpha_o$ via the Nucleotide Exchange Factor RIC-8

A variety of mechanisms by which GPR domain proteins might activate or inhibit $G\alpha_{i/o}$ signaling have been proposed, based principally on in vitro biochemical studies.[29-34] Previous genetic studies of the Drosophila GPR protein Pins and the redundant *C. elegans* protein GPR-1 and GPR-2 showed they function with the G protein $G\alpha_o$ and the nucleotide exchange factor RIC-8 to regulate mitotic spindle positioning during asymmetric cell division.[35-37] However, the sequence of interactions among these proteins, and even the basic issue of whether the GPR proteins activate or inhibit the G protein, has remained unsettled. Thus we used both biochemical and genetic methods to investigate how AGS-3, RIC-8 and $G\alpha_o$ control octanol avoidance in the ASH neurons.

In vitro, purified AGS-3 protein can bind via its GPR domains to both the inactive GDP-bound and the active GTP-bound states of purified $G\alpha_o$.[24] RIC-8 is a nucleotide exchange factor that, like an activated G protein coupled receptor, can activate $G\alpha_o$ by catalyzing the exchange of GDP for GTP on $G\alpha_o$.[38] Work from Tall and colleagues, showed that mammalian Ric-8A can also catalyze nucleotide exchange on a complex of AGS-3 bound to $G\alpha_o$ -GDP, resulting in dissociation of the complex and release of active $G\alpha_o$-GTP.[32-34] We found that the purified *C. elegans* proteins can carry out the same process.[24]

We used genetic epistasis and cell-specific expression experiments to show that in vivo, AGS-3 and RIC-8 indeed activate $G\alpha_o$ in the ASH neurons of *C. elegans* to delay octanol avoidance. Overexpression of RIC-8 specifically in the ASH neurons resulted in a delay in octanol avoidance response in the presence of food, and this effect was dependent on the presence of AGS-3 and $G\alpha_o$. In addition, overexpressing AGS-3 in the ASH neurons caused a similar effect that was dependent on the presence of RIC-8 and $G\alpha_o$.[24] These genetic epistasis results suggest that AGS-3 and RIC-8 act together to activate $G\alpha_o$ signaling and alter behavior after food deprivation.

We have proposed a model (**Fig. 1**) in which the presence or absence of food affects release of dopamine, neuropeptides, and other neurotransmitters that signal through $G\alpha_o$ to alter behavior (**Fig. 1A**). Food deprivation independently activates the AGS-3 protein (**Fig. 1B**) by somehow releasing it from interaction with a Triton X-100 insoluble cytoskeletal complex, causing a conformational change within AGS-3 that activates this protein. Active AGS-3 can then bind to $G\alpha_o$-GDP (**Fig. 1D**), preventing $G\alpha_o$ from reassociating with $G\beta\gamma$. If $G\alpha_o$-GDP reassociates with $G\beta\gamma$ to form a $G\alpha\beta\gamma$ heterotrimer (**Fig. 1C**), it is no longer a substrate for RIC-8 activation[32] and $G\alpha_o$ signaling is terminated. By preventing heterotrimer formation, active AGS-3 preserves $G\alpha$ in a form (the $G\alpha_o$ -GDP/AGS-3 complex) that can be activated by RIC-8 (**Fig. 1E**). Thus in this model, active AGS-3 and RIC-8 together act to continuously keep $G\alpha_o$ in its active GTP-bound form. Because active $G\alpha_o$ inhibits neurotransmitter release,[15] this could explain how the ASH neuron of *C. elegans* suppresses glutamate release after food deprivation to delay octanol avoidance. More generally, this model is consistent with studies on AGS-3 family proteins in asymmetric cell division in flies,[37] worms[39] and mammalian cells,[40] as wells as studies of mammalian Ric-8A in vitro.[32] Given that AGS-3, $G\alpha_o$, and RIC-8 are highly conserved from *C. elegans* to humans, this model provides a general mechanism for response to food deprivation across many species.

AGS-3 Interacting Proteins may Regulate AGS-3 Activity and Triton X-100 Solubility to Mediate Effects of Food Deprivation

Many details of the mechanisms by which food deprivation activates AGS-3, as well as the specifics of the interactions of AGS-3 with $G\alpha_o$ and RIC-8, remain to be worked out. We saw that, like the GPR domains of Drosophila Pins,[37] the GPR domains of AGS-3 interact with $G\alpha_o$ bound to both GDP and GTP.[24] In Pins, the ability of one of the GPR domains to bind to $G\alpha_o$-GTP is masked by an intramolecular interaction between the TPR repeats and the GPR domains. This same intramolecular interaction has been seen in the mammalian AGS3-like protein LGN, where it is thought to be regulated by LGN-interacting proteins.[40] Thus, the intramolecular association of the TPR and GPR domains seems to be a general feature of AGS-3 family proteins that regulates the ability of these proteins to act on Gao. The physical basis for the Triton X-100 insolubility of AGS-3 remains unclear, but a reasonable model is that it results from association of AGS-3 with an insoluble cytoskeletal structure via its TPR repeats, and that AGS-3 is somehow released from this structure upon food deprivation. Thus one potential mechanism of activating AGS-3 upon food deprivation may be release of AGS-3 TPR repeats from binding a cytoskeletal structure, in turn releasing the TPR repeats from the GPR repeats of AGS-3 and altering the interaction of the GPR repeats with $G\alpha_o$.

What proteins might the AGS-3 TPR repeats bind to anchor AGS-3 to a Triton X-100 insoluble structure? Several interacting proteins were identified by a yeast-two-hybrid screen performed with the

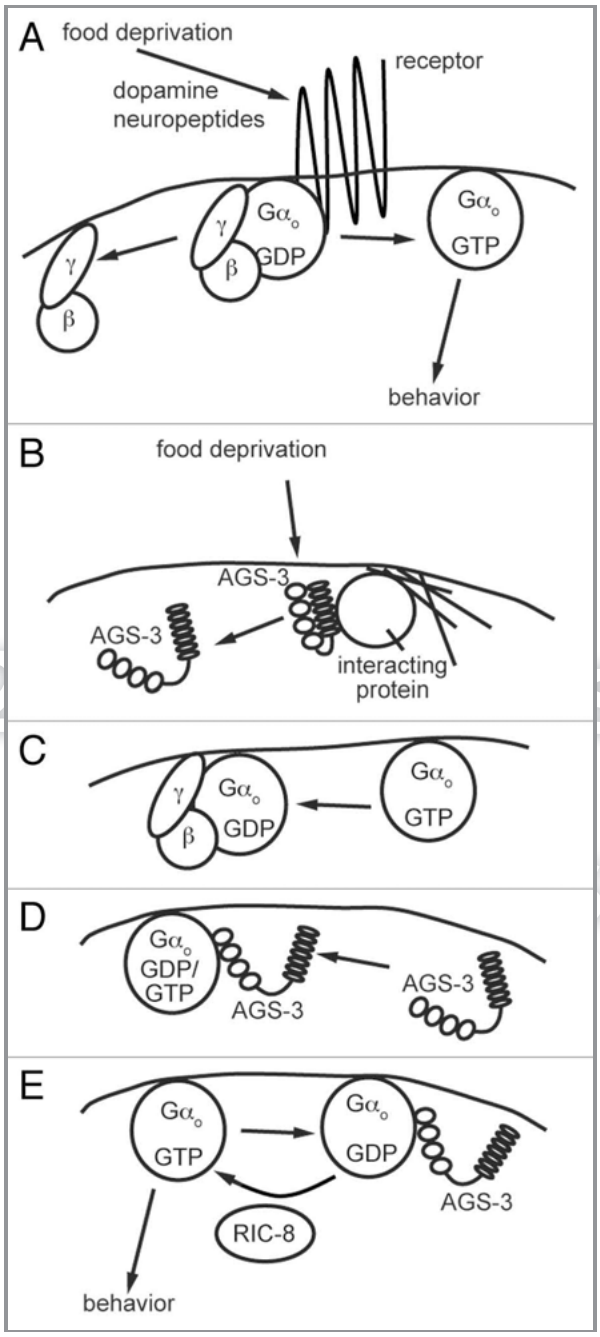

Figure 1. (A) Upon food deprivation, signaling by neurotransmitters such as dopamine and neuropeptides activate G protein coupled receptors on the ASH neurons. The GPCRs activate G protein signaling by causing nucleotide exchange on $G\alpha_o$ and subsequent activation of downstream signaling leads to changes in behavior. (B) Food deprivation also activates AGS-3 by an unknown mechanism. Activation of AGS-3 results in a change in the Triton X-100 solubility of the protein, possibly to due changes in interactions with cytoskeletal-associated proteins. Activation of AGS-3 may also result in the release of the potential intramolecular interaction between the TPR domains and the GPR domains. (C) If $G\alpha_o$ becomes inactivated due to hydrolysis of the GTP back to GDP, it may reassociate with $G\beta\gamma$ and signaling will be terminated. (D) $G\alpha_o$ in both its GTP-bound and GDP-bound states can interact with activated AGS-3. (E) $G\alpha_o$-GDP bound to AGS-3 can be reactivated by RIC-8, prolonging downstream signaling and behavioral changes.

N-terminal TPR motif region of mammalian AGS3.[41] The majority of the interacting proteins identified are conserved in *C. elegans*, including Frmpd1 (FRM-8), MACF (VAB-10), ROBO (SAX-3), and LKB1 (PAR-4). *frm-8* encodes a protein of unknown function containing several conserved protein domains, including a WW domain, a PDZ domain, and a FERM domain, thought to be involved in protein scaffolding and in interactions with the cytoskeleton.[42] *vab-10* encodes two spectraplakins that are required for mechanical resilience of the epidermis during contraction of actin microfilaments.[43] Studies using purified mammalian AGS3 and Frmpd1 suggest that Frmpd1 directly competes for AGS-3 binding with the $G\alpha$ subunit.[41] Since the potential AGS-3 interacting proteins FRM-8 and VAB-10 contain domains that are known to interact with cytoskeletal components, it is possible that FRM-8 and/or VAB-10 act to tether AGS-3 to the cytoskeleton, resulting in the Triton X-100 insolubility of the protein in whole-animal lysates. The interactions between LKB1 and AGS3 have been also been studied and it appears that phosphorylation of the linker region between the TPR motifs and GPR domains of AGS3 by LKB1 prevents AGS3 from interacting with $G\alpha_{i/o}$ subunits.[44] The relationship between AGS3 family proteins and the axon-guidance protein ROBO remains undefined. Studies of the all these proteins that potentially interact with AGS-3 to alter its activity deprivation constitute an exciting avenue for future studies of the mechanism by which the nervous system responds to food deprivation.

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