



The FMRFamide Neuropeptide FLP-20 Acts as a Systemic Signal for Starvation Responses in *Caenorhabditis elegans*

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Most animals face frequent periods of starvation throughout their entire life and thus need to appropriately adjust their behavior and metabolism during starvation for their survival. Such adaptive responses are regulated by a complex set of systemic signals, including hormones and neuropeptides. While much progress has been made in identifying pathways that regulate nutrient-excessive states, it is still incompletely understood how animals systemically signal their nutrient-deficient states. Here, we showed that the FMRFamide neuropeptide FLP-20 modulates a systemic starvation response in *Caenorhabditis elegans*. We found that mutation of *flp-20* rescued the starvation hypersensitivity of the G protein β -subunit *gpb-2* mutants by suppressing excessive autophagy. FLP-20 acted in AIB neurons, where the metabotropic glutamate receptor MGL-2 also functions to modulate a systemic starvation response. Furthermore, FLP-20 modulated starvation-induced fat degradation in a manner dependent on the receptor-type guanylate cyclase GCY-28. Collectively, our results reveal a circuit that senses and signals nutrient-deficient states to modulate a systemic starvation response in multicellular organisms.

Keywords: autophagy, metabotropic glutamate receptor, neuropeptide, nutrient-deficient states, receptor-type guanylate cyclase, starvation response, systemic regulation

INTRODUCTION

Since nutrient availability in the environment dramatically fluctuates over time, most animals face frequent periods of starvation throughout their entire life. Animals have evolved a complex layer of regulation in response to starvation, adjusting their behavior and metabolism for survival. Behavioral adjustments include changes in foraging behaviors, which increase the chance of finding new food sources (Douglas et al., 2005; Kniazeva et al., 2015; Wang et al., 2006). Metabolic adjustments are mainly related to the conservation and generation of metabolic substrates and energy, thereby maintaining essential cellular activity for survival (Finn and Dice, 2006; Mizushima, 2007; Wang et al., 2006). These adaptive responses are regulated in both cell-autonomous and non-cell-autonomous manners. The individual cell decreases anabolic pathways, such as general transcription and translation, as well as increasing catabolic pathways, such as protein degradation through the ubiquitin-proteasome system and lysosomal degradation pathways, preserving intracellular metabolites to support its basic functions (Anding and Baehrecke, 2017; Kim et al., 2018; Lee et al., 2020; Miller and Thorburn, 2021; Sebastian and Zorzano, 2020; Shin and Zoncu, 2020). Animals decrease basal metabolic rate by lowering energy-demanding processes, such as reproduction (Gerisch et al., 2020). Furthermore, animals allocate their

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stored energy to several tissues by increasing lipolysis, which in turn generates internal energy sources to maintain their viability (Olsen et al., 2021; Shin, 2020; Texada et al., 2019). These starvation responses can be coordinated systemically through the highly complex endocrine system which consists of various hormones and neuropeptides (Douglas et al., 2005; Wang et al., 2006).

During the past decade, the molecular mechanisms by which hormones and neuropeptides regulate behavioral and metabolic responses to internal nutrient and energy availability have been extensively studied (Berthoud and Morrison, 2008). For example, the insulin-PI3K and leptin pathways are among the best-characterized endocrine mechanisms by which animals sense and signal their nutrient-excessive states (Davis et al., 2010; Konner and Bruning, 2012). By contrast, no particular hormones or neuropeptides were assigned for signaling nutrient-deficient states until recently. Instead, reductions in the levels of hormones and neuropeptides that modulate energy-excessive states were considered a starvation cue, as best exemplified by the leptin-dependent regulation of feeding during starvation (Chan and Mantzoros, 2005). However, starvation responses might be regulated in a more directed manner by specific hormones or neuropeptides whose expression is increased in response to nutrient-deficient states. Consistent with this notion, recent studies suggest a role for the fibroblast growth factor 21 (FGF21), a hormone secreted from the liver upon starvation, in systemically coordinating starvation responses, including gluconeogenesis, fatty acid oxidation, ketogenesis, lipid degradation, and autophagy (Badman et al., 2007; Byun et al., 2020; Fazeli et al., 2015; Inagaki et al., 2008). However, it is still incompletely understood whether additional factors can act as a systemic signal for starvation and, if so, how they work.

The neuropeptide FMRFamides are the first member of the family of RFamide neuropeptides to be recognized and have been shown to regulate various biological processes, such as cardiovascular function, muscle contraction, locomotor activity, neuroendocrine and neuromodulatory activities (Bechtold and Luckman, 2007; Chen et al., 2016; Cohen et al., 2009; Park et al., 2019). In addition, FMRFamide neuropeptides were suggested to act as part of the nutrient-sensing mechanism in worms, birds, and mice. For example, starvation increases the expression of the FMRFamide-related neuropeptide QRFP in mice, which in turn modulates feeding behavior and energy expenditure (Bechtold and Luckman, 2007; Takayasu et al., 2006). The *Caenorhabditis elegans* FMRFamide neuropeptide FLP-18 activates two G protein-coupled receptors of the NPY/RFamide family, NPR-4 and NPR-5 to modulate fat metabolism and foraging behavior, suggesting that FLP-18 acts as a nutrient-excessive signal (Cohen et al., 2009). However, it is currently unclear whether other FMRFamide neuropeptides are also involved in the nutrient-sensing mechanism and, if so, whether they can act as a starvation signal in a systemic manner.

We previously showed that MGL-1 and MGL-2, *C. elegans* homologs of metabotropic glutamate receptors, coordinate a systemic starvation response, such as the lysosomal degradation pathway autophagy, by modulating the activity of AIY

and AIB interneurons, respectively (Kang and Avery, 2009a; 2009b). While AIY neurons inhibit a systemic starvation response, AIB neurons stimulate it, suggesting that AIB neurons might secrete a systemic signal, potentially FMRFamide neuropeptides, to modulate starvation responses. However, the identity and *in vivo* relevance of such a systemic signal, possibly hormones or neuropeptides, were not characterized. Here, using the starvation-hypersensitive *gpb-2* mutants in which heterotrimeric Gq protein activity is constitutively active (You et al., 2006), we show that the FMRFamide neuropeptide FLP-20 systemically regulates starvation responses, including starvation survival, autophagy, and starvation-induced fat degradation. FLP-20 is released by AIB neurons and acts on the receptor-type guanylate cyclase GCY-28 to modulate fat degradation. Together, these findings suggest that FLP-20 functions as a systemic signal that directly modulates starvation responses.

MATERIALS AND METHODS

Strains

Strains were maintained as described (Brenner, 1974) at 19°C. All worms were maintained and grown on *Escherichia coli* HB101 bacteria. The following strains were generated using standard genetic procedures: *gpb-2(ad541) l*, *flp-20(pk1596) X*, *gcy-28(ky713) l*, *gcy-28(tm2411) l*, *mgl-2(tm355) l*, *flp-18(db99) X*, *gpb-2(ad541) l*; *mgl-1(tm1811) X*, *gpb-2(ad541) mgl-2(tm355) l*, *gpb-2(ad541) l*; *flp-20(pk1596) X*, *gcy-28(ky713)gpb-2(ad541) l*, *gcy-28(tm2411) gpb-2(ad541) l*, *gpb-2(ad541) l*; *flp-18(db99) X*, *gcy-28(tm2411) gpb-2(ad541) l*; *flp-20(pk1596) X*, *gcy-28(tm2411) gpb-2(ad541) l*; *mgl-1(tm1811)*, *gcy-28(tm2411) gpb-2(ad541) mgl-2(tm355) l*, *gpb-2(ad541) mgl-2(tm355) l*; *flp-20(pk1596) X*, *gpb-2(ad541) l*; *flp-18(db99) mgl-1(tm1811) X*, *gpb-2(ad541) l*; *adls2122[GFP::lgg-1 rol-6(d)]*, *gpb-2(ad541) l*; *flp-20(pk1596)*; *adls2122[GFP::lgg-1 rol-6(d)]*, *gcy-28(tm2411) gpb-2(ad541) l*; *adls2122[GFP::lgg-1 rol-6(d)]*, *gpb-2(ad541) mgl-2(tm355) l*; *flp-20(pk1596) X*; *adls2122[GFP::lgg-1 rol-6(d)]*, *gpb-2(ad541) l*; *flp-20(pk1596) X*; *[podr-2b::flp-20 rol-6(d)]*, *gpb-2(ad541) l*; *flp-20(pk1596) X*; *[pnmr-2::flp-20 rol-6(d)]*.

Molecular biology

cDNA corresponding to the entire coding sequence of *flp-20* was amplified and cloned under cell-specific promoters as indicated. Expression in AIB was achieved using the *odr-2b* promoters, from Cornelia Bargmann (The Rockefeller University, USA).

Polymerase chain reaction (PCR) construction of *flp-20* driven by the *odr-2b* promoter was achieved as follows: the *odr-2b* promoter region was amplified from a *Podr-2b::mod-1* cDNA::GFP plasmid (from Cornelia Bargmann, The Rockefeller University) using primers 5'GTCTAGTCAG-CATTTACCCTG' and 5'TTGAGTGTAACCCAACATATTCT-GTCTGAAATATAAATGT' (PCR#1). cDNA corresponding to the entire coding sequence of *flp-20* was amplified from *yk782e04* (from Yuji Kohara, National Institute of Genetics, Japan) using primers 5'TTATATTCAGACAGAATATGTTG-

GGTTACTCAATCTC' and 5'AGGGAAGGAAAGTTATAATCTCTAG3' (PCR#2). PCR#1 and #2 were fused using primers 5'GTCTAGTCAGCATTTCACCCTG' and 5'AGGGAAGGAAAGTTATAATCTCTAG3'.

PCR construction of *flp-20* driven by the *nmr-2* promoter was achieved as follows: the *nmr-2* promoter region was amplified from Pka155 (from Kaveh Ashrafi, University of California, USA) using primers 5'GAGCCATCAGAATTTATTTGAATTTTC' and 5'TTGAGTGTAACCCAACATTGATTTTTCGAATAACTTCCTT' (PCR#1). cDNA corresponding to the entire coding sequence of *flp-20* was amplified from *yk782e04* (from Yuji Kohara, National Institute of Genetics) using primers 5'AAGTTATTCGAAAAATCAATGTTGGGTTACTCAATCTC' and 5'AGGGAAGGAAAGTTATAATCTCTAG3' (PCR#2). PCR#1 and #2 were fused using primers 5'GAGCCATCAGAATTTATTTGAATTTTC' and 5'AGGGAAGGAAAGTTATAATCTCTAG3'.

Starvation survival analyses

Starvation survival analyses were performed as described (You et al., 2006) with a few modifications. After collection of L1 worms from synchronization by egg preparation, we incubated them in 3 ml of sterilized M9 buffer for the time indicated in the figures at 19°C. At each time point, an aliquot from each sample tube was placed on a plate seeded with *E. coli* HB101. The number of worms surviving to L4 or adulthood was determined after 3 days of further growth at 19°C. The number from day 1 of starvation was used as control and as the denominator to calculate the percentage of worms recovering after starvation. Since starvation survival was influenced by assay conditions, all relevant experimental data were examined and compared within the same experiment.

C. elegans autophagy analysis

Autophagy analysis was performed as described (Kang et al., 2007). For light microscopic analysis of autophagy, starved L1 animals carrying an integrated transgene that expresses a GFP::LGG-1 fusion were collected at 3 days of starvation. GFP-positive punctate regions were visualized in the pharyngeal muscles of L1 animals using a Zeiss Axioplan 2 compound microscope (Zeiss, Germany).

Body fat assessment

Body fat analysis using Oil-Red-O staining was performed as described (Soukas et al., 2009) with a few modifications. Briefly, synchronized animals were collected and washed three times with M9 buffer. We incubated them in M9 buffer for the time indicated in the figures at 19°C. At each time point, using an aliquot from each sample tube, Oil-Red-O staining was conducted. The worms were resuspended and washed twice with phosphate-buffered saline (PBS) and then suspended in PBS to which an equal volume of 2× MRWB buffer containing 2% paraformaldehyde was added. The worms were taken through three freeze-thaw cycles between liquid nitrogen and warm water, followed by spinning at 14,000g, washing once in PBS, resuspension in 60% isopropanol, and addition of 60% Oil-Red-O stain for 1 h. To quantify the amount of Oil-Red-O staining, Adobe Photo-

shop CS5 software was used. For approximate quantification of the levels of Oil-Red-O staining, we categorized stained worms into 3 groups: 1) Full – worms showing strong staining throughout the intestine, 2) Partial – worms showing only staining around the tail region, and 3) None – worms showing no staining.

Statistical analysis was performed using the GraphPad PRISM 9 (GraphPad Software, USA).

RESULTS

To search for the systemic signal secreted by AIB neurons to modulate a systemic starvation response, we checked the expression pattern of neuropeptides with a focus on FMRFamide-related peptides (Kim and Li, 2004; Li and Kim, 2008). FLP-20 is the only one reported to be expressed in AIB neurons. To test whether FLP-20 was involved in the modulation of starvation responses, we examined whether mutation of *flp-20* could suppress the starvation-hypersensitivity of *gpb-2* mutants in which starvation responses, such as autophagy, are overactivated (Kang et al., 2007; You et al., 2006). Suppression of AIB activity by mutation of *mgl-2* suppressed the starvation-induced death of *gpb-2* mutants, as previously demonstrated (Kang and Avery, 2009b). Mutation of *flp-20* also rescued the death of *gpb-2* mutants during starvation, yet did not further suppress the starvation-induced death of *gpb-2 mgl-2* mutants (Fig. 1A). These data suggest that FLP-20 acts in the same pathway as MGL-2 to modulate starvation responses. Consistent with this interpretation, AIB-specific expression of *flp-20* driven by the promoter of *ord-2b*, but not AVA, AVB, AVG, RIM, PVC, AVD, and AVE expression of *flp-20* (driven by the promoter of *nmr-2*), restored the starvation sensitivity of *gpb-2; flp-20* mutants (Fig. 1B). Mutation of *flp-18*, which is expressed in AIY neurons that have been also shown to modulate a systemic starvation response (Kang and Avery, 2009b), did not rescue the starvation-survival of *gpb-2* mutants, indicating the specificity of FLP-20 as a starvation-regulating FMRFamide-related peptide (Fig. 1C). Together, these data suggest that FLP-20 may act as a systemic signal from AIB neurons downstream of MGL-2 to positively regulate starvation responses.

MGL-1 and MGL-2 sense several amino acids, including leucine, as a food signal to modulate AIY and AIB neurons, respectively. Treatment with leucine suppresses MGL-2, which in turn reduces the activity of AIB neurons and thus decreases a systemic starvation response, including autophagy in the pharyngeal muscles (Kang and Avery, 2009b). While searching for additional regulators of these amino acid responses, we found that mutation of *gcy-28*, a receptor-type guanylate cyclase (Tsunozaki et al., 2008), abolished the positive effect of leucine on the starvation survival of *gpb-2* mutants (Fig. 1D). GCY-28 is known to be expressed in many head neurons, including AWC and AIA neurons that are upstream of AIB neurons, as well as in peripheral tissues, including body-wall muscles, hypodermis, and intestine, which are potential downstream targets of AIB neurons during starvation responses (Ortiz et al., 2006). Thus, we hypothesized that GCY-28 is involved in starvation responses, and may act either upstream or downstream of the MGL-2 and FLP-20

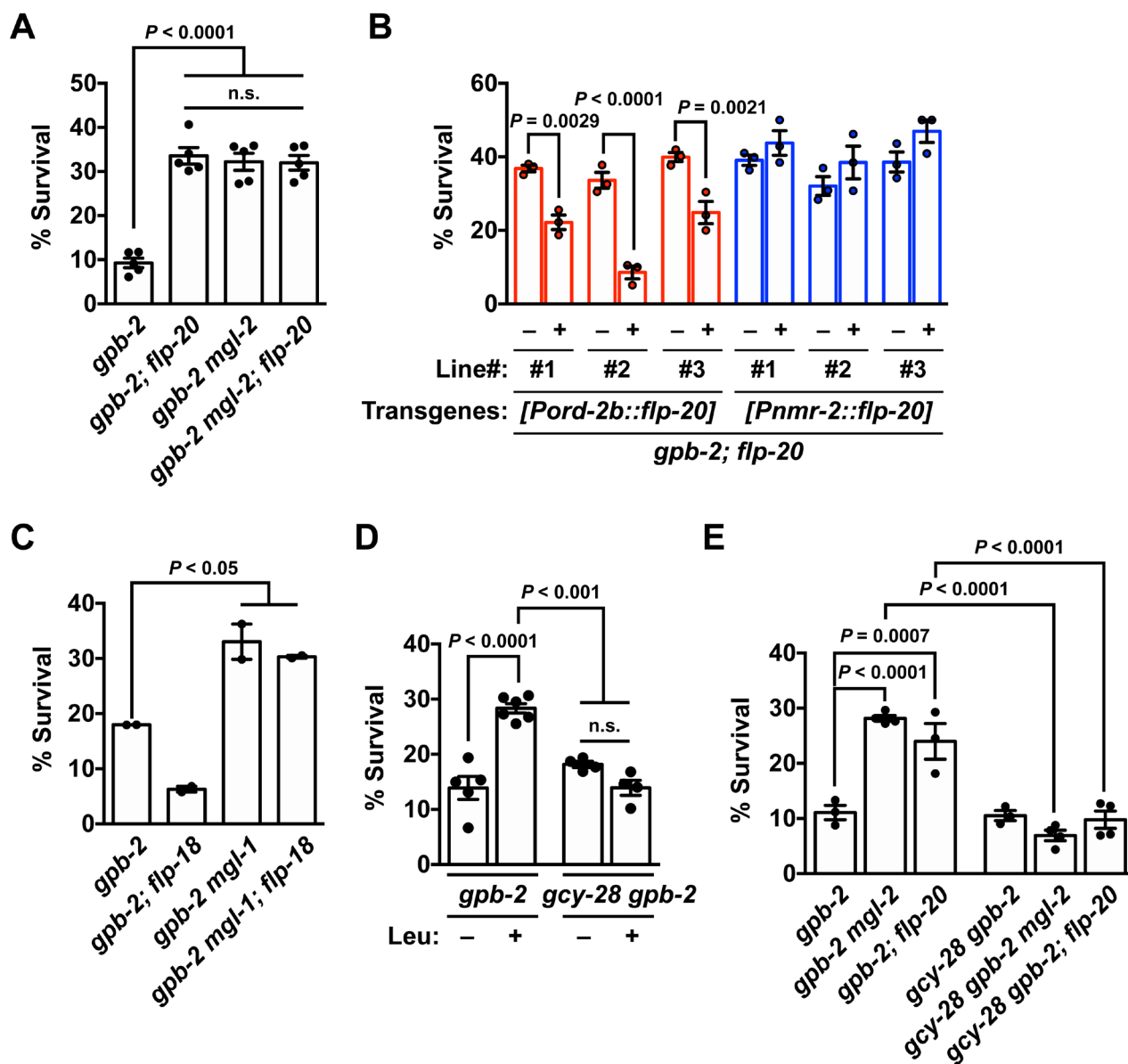


Fig. 1. FLP-20 and GCY-28 modulate the starvation hypersensitivity of *gpb-2* mutants. (A) FLP-20 and MGL-2 modulate starvation responses in the same pathway. Mutation of *flp-20* and *mgl-2* rescued the starvation-induced death of *gpb-2* mutants ($n = 3$, mean \pm SEM, one-way ANOVA test). n.s., not significant. (B) FLP-20 mainly acts in AIB neurons. AIB specific expression of *flp-20* with the promoter of *ord-2b* restored the starvation-sensitivity of *gpb-2; flp-20* ($n = 3$, mean \pm SEM, two-way ANOVA test). (C) Mutation of *flp-18* did not rescue the starvation hypersensitivity of *gpb-2* and *gpb-2; mgl-1* mutants ($n = 2$, mean \pm SEM, one-way ANOVA test). (D) GCY-28 is involved in the amino acid sensing pathway. Mutation of *gcy-28* abolished the rescue effect of leucine on the starvation hypersensitivity of *gpb-2* mutants ($n = 4-6$, mean \pm SEM, two-way ANOVA test). (E) GCY-28 acts downstream of MGL-2 and FLP-20 to modulate starvation responses. Mutation of *gcy-28* restored the starvation sensitivity of *gpb-2 mgl-2* and *gpb-2; flp-20* mutants ($n = 3-4$, mean \pm SEM, one-way ANOVA test).

signaling pathway. To test this, we generated *gcy-28 gpb-2 mgl-2* and *gcy-28 gpb-2; flp-20* mutants. Mutation of *gcy-28* restored the starvation sensitivity of *gpb-2 mgl-2* and *gpb-2; flp-20* mutants, suggesting that GCY-28 acts downstream, but not upstream, of MGL-2 and FLP-20 to modulate starvation responses (Fig. 1E).

To corroborate our finding that FLP-20 and GCY-28 mod-

ulate a systemic starvation response, we further focused on their roles in modulating autophagy and fat mobilization, two well-described starvation responses. The main cause of death in *gpb-2* mutants during starvation is excessive autophagy in their pharyngeal muscles, and MGL-2 modulates such autophagy in a systemic manner (Kang and Avery, 2008; 2009a; 2009b; Kang et al., 2007). Thus, we asked whether FLP-20

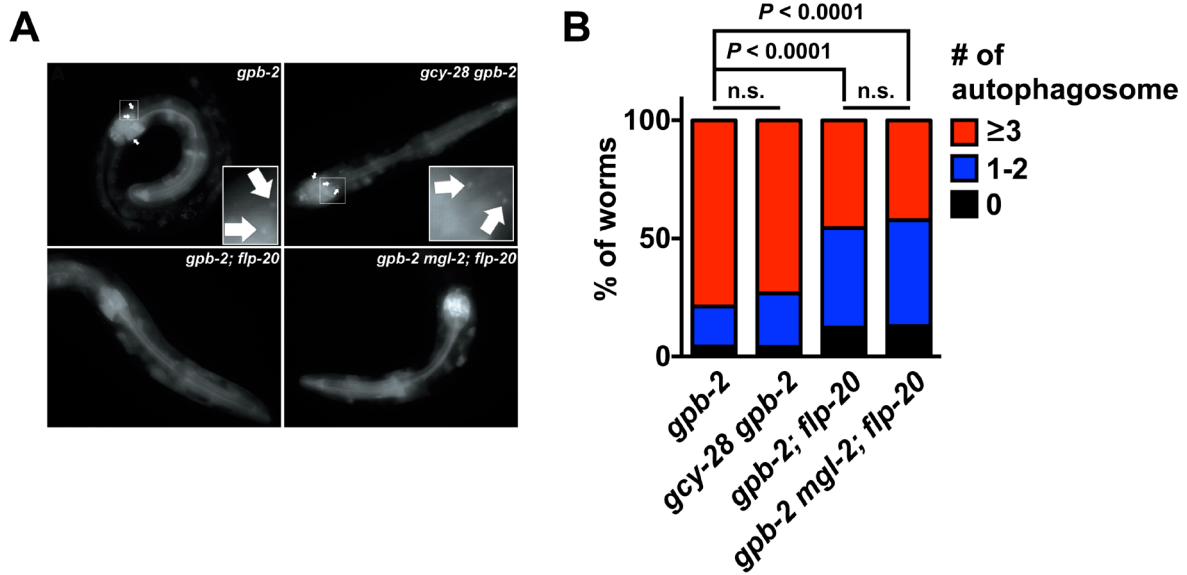


Fig. 2. FLP-20 modulates autophagy in the pharyngeal muscle of *gpb-2* mutants during starvation. (A) Representative images of the indicated genotype after 3 days of starvation. The arrows show representative GFP::LGG-1 positive punctate structures. GFP::LGG-1 labels pre-autophagosomal and autophagosomal structures. In the inset, the area marked by the box is magnified. (B) Quantification of autophagy in the pharyngeal muscle of worms of the indicated genotype after 3 days of starvation ($n = 116-147$, chi-square test). n.s., not significant.

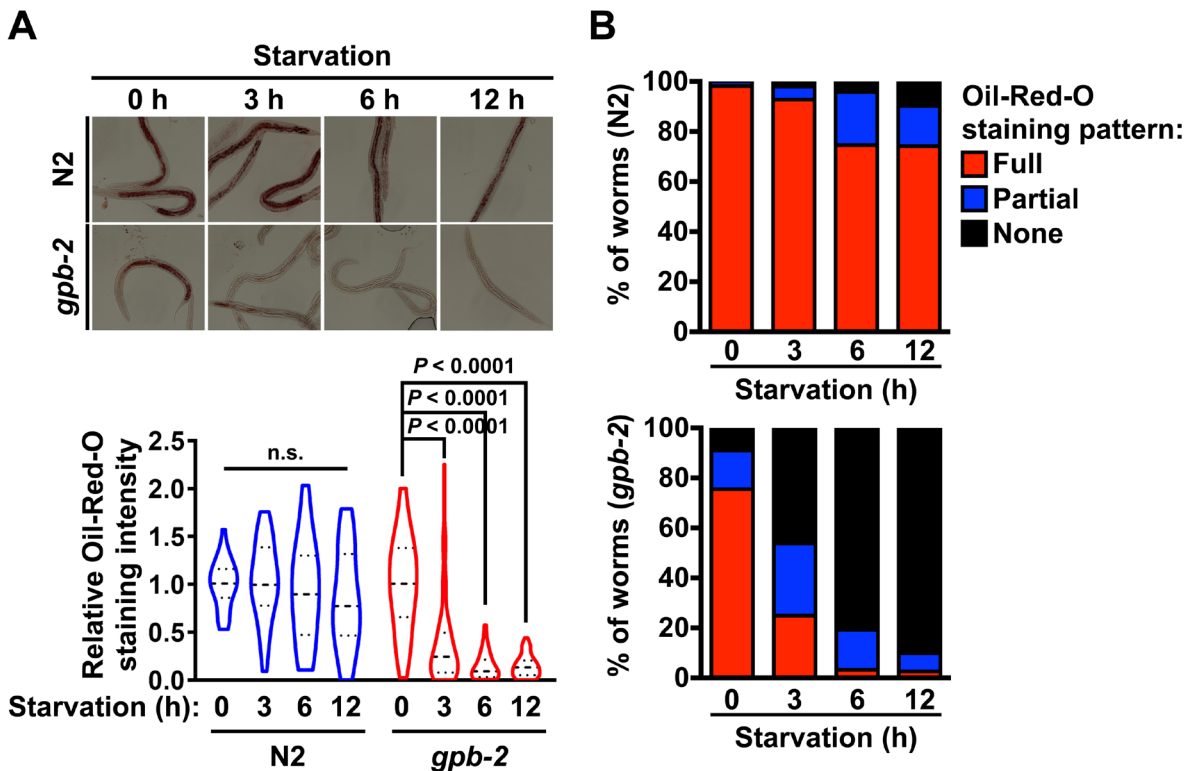


Fig. 3. *gpb-2* mutants exhibit an increased rate of fat degradation during starvation. (A) Representative images of Oil-Red-O staining in N2 (wild-type) worms and *gpb-2* mutants (top). Violin plot analysis of Oil-Red-O staining (bottom, the horizontal solid line shows the median and the horizontal dot lines show the 1st and 3rd quartiles, one-way ANOVA test). n.s., not significant. (B) Quantification of Oil-Red-O staining. (Full) worms showing strong staining throughout the intestine; (Partial) worms showing staining only around the tail region; (None) worms showing no staining ($n = 62-370$).

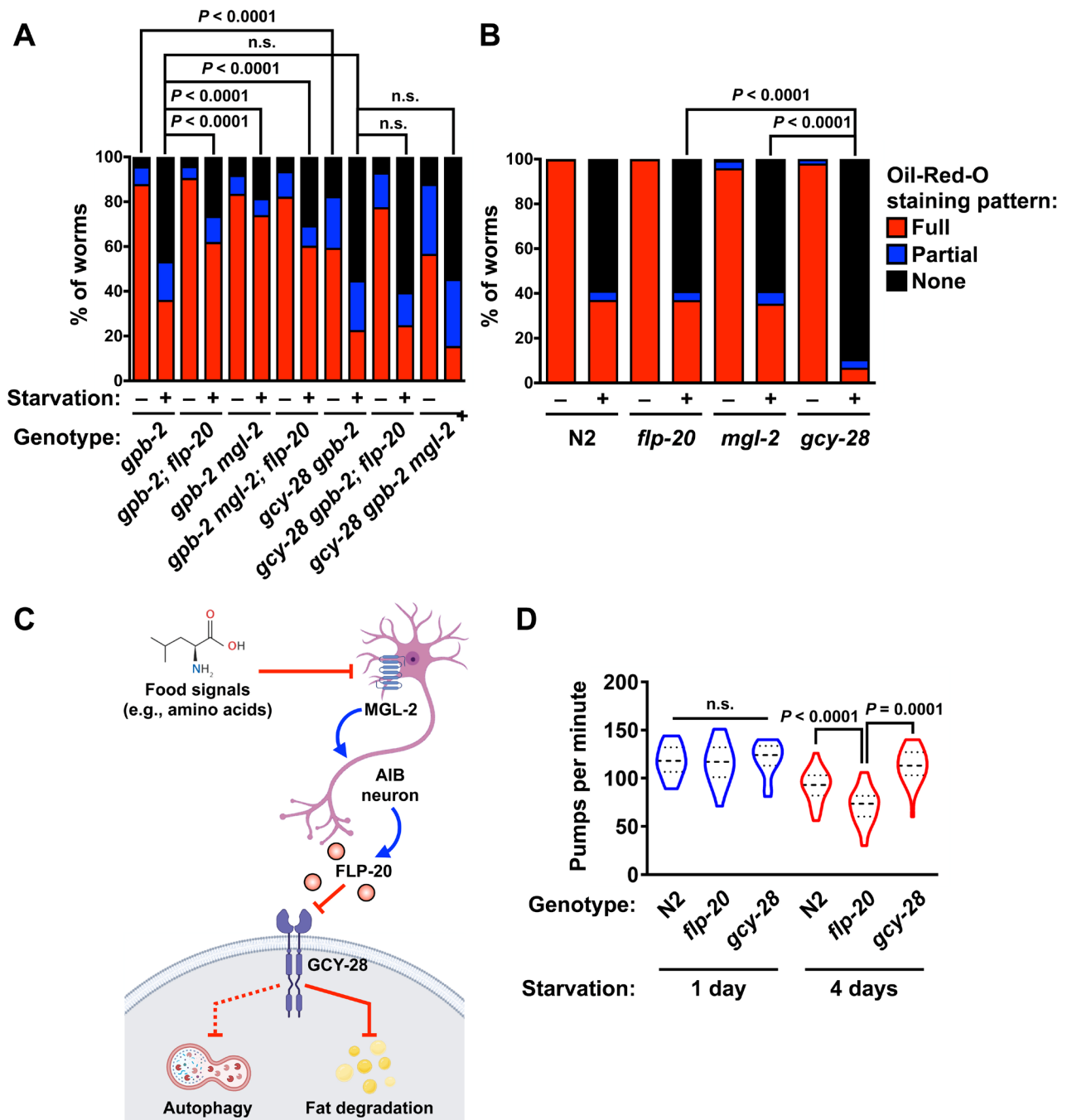


Fig. 4. FLP-20, MGL-2, and GCY-28 modulate starvation-induced fat degradation. (A) Quantification of Oil-Red-O staining of the indicated genotype with or without 4 h starvation. Mutation of either *flp-20* or *mgl-2* decreased the rate of starvation-induced fat degradation, and mutation of *gcy-28* reversed it (left, $n = 214-446$ for *gpb-2*, $n = 243-265$ for *gpb-2; flp-20*, $n = 53-71$ for *gpb-2 mgl-2*, $n = 88-142$ for *gpb-2 mgl-2; flp-20*, $n = 27-39$ for *gcy-28 gpb-2*, $n = 109-156$ for *gcy-28 gpb-2; flp-20*, and $n = 16-20$ for *gcy-28 gpb-2 mgl-2*, chi-square test). n.s., not significant. (B) Quantification of Oil-Red-O staining of the indicated genotype with or without 24 h starvation. *gcy-28* mutants increased the rate of starvation-induced fat degradation (right, $n = 171-407$ for N2, $n = 221-389$ for *flp-20*, $n = 196-229$ for *mgl-2*, and $n = 248-365$ for *gcy-28*, chi-square test). (C) Model of the systemic regulation of starvation responses by the MGL-2-FLP-20-GCY-28 axis. Amino acids or food signals modulates the activity of AIB neurons through MGL-2, which in turn stimulates the secretion of FLP-20. FLP-20 acts on GCY-28 that suppresses starvation responses, including autophagy and fat degradation in the peripheral tissues. (D) Pumping rates of the indicated genotype with or without starvation. Unstarved or 4-day starved L1 animals were examined on food. Each dot represents an individual worm. Horizontal lines represent the average (line) and SEM (error bars).

and GCY-28 are also involved in autophagic regulation of the pharyngeal muscles during starvation. We generated mutant strains carrying an integrated LGG-1::GFP transgene, a specific marker for autophagy in *C. elegans* (Kang et al., 2007; Klionsky et al., 2016; Melendez et al., 2003; Zhang et al., 2015), and found that mutation of *flp-20* decreased excessive levels of autophagy in *gpb-2* mutants during starvation (Fig. 2). Double mutation of *flp-20* and *mgl-2* did not show an additive effect on suppressing excessive autophagy in *gpb-2* mutants, suggesting that FLP-20 and MGL-2 act in the same pathway to modulate autophagy, consistent with their effects on starvation survival. By contrast, mutation of *gcy-28* did not aggravate excessive levels of autophagy (Fig. 2). This could be due to the fact that GCY-28 is not expressed in the pharyngeal muscles (Ortiz et al., 2006). Alternatively, GCY-28 could only abolish the suppressive effect of amino acids on excessive autophagy, as was the case for fat degradation.

Like mammals, *C. elegans* can store surplus energy as fat and consumes stored fat during starvation to maintain energy homeostasis (Jo et al., 2009; McKay et al., 2003; Van Gilst et al., 2005). Indeed, we found that starvation decreased the levels of fat in wild-type worms, and *gpb-2* mutants had an increased rate of fat depletion, as assessed by the Oil-Red-O staining (Fig. 3), a histochemical method for measuring stored fat (Soukas et al., 2009). In addition, *gpb-2* mutants already lower their fat deposition under control conditions, compared to wild-type worms, which is consistent with previous observations that they are in a perceived state of starvation (You et al., 2006). These results are consistent with the finding that starvation signaling is overactivated in *gpb-2* mutants.

Next, we examined whether MGL-2, FLP-20, and GCY-28 are involved in starvation-induced fat degradation. Since *gpb-2* mutation provides a sensitive genetic background to readily observe starvation-induced fat degradation (Fig. 3), we first examine the effect of each mutation on fat degradation during starvation in *gpb-2* mutants. We found that mutation of either *mgl-2* or *flp-20* reduced the rate of fat degradation during starvation, and these effects were not additive (Fig. 4A). These data suggest that MGL-2 and FLP-20 positively regulate starvation-induced fat degradation in the same pathway, as was the case for starvation survival and autophagy regulation (Figs. 1A and Fig. 2). By contrast, mutation of *gcy-28* accelerated the rate of fat degradation, and completely reversed the effects of *mgl-2* and *flp-20* mutations on fat degradation (Fig. 4A). These data suggest that GCY-28 acts downstream of MGL-2 and FLP-20 to modulate starvation-induced fat degradation, consistent with its role in starvation survival (Fig. 1E). Next, we examined whether such regulation also operates in a wild-type genetic background. As wild-type worms are less sensitive to starvation-induced fat degradation, we starved worms for 24 h to assess their fat contents (Fig. 4B). Mutation of *gcy-28* exacerbated starvation-induced fat degradation, suggesting that GCY-28 is generally involved in fat regulation during starvation. Together with our previous studies, these data suggest that MGL-2 positively regulates the activity of AIB neurons, which in turn secretes FLP-20 that acts on GCY-28 in peripheral tissues to modulate a systemic starvation response, including starvation

survival, autophagy, and fat degradation (Fig. 4C).

DISCUSSION

Although physiological and behavioral effects of the starvation response have been widely studied (Douglas et al., 2005; Finn and Dice, 2006; Wang et al., 2006), little is known about the systemic signals that directly modulate starvation responses in multicellular organisms. Previously, it has been proposed that reduction in the levels of leptin, in response to nutrient-excessive states, is a signal of nutrient-deficiency and induces starvation responses, including increased levels of food intake (Ahima et al., 1996; Chan and Mantzoros, 2005; Chan et al., 2003). However, leptin-deficient *ob/ob* mice did not perfectly mimic the starvation state, as exemplified by their normal levels of proteolysis in skeletal muscles (Turpin et al., 2009). Thus, it is reasonable to propose that specific hormones or neuropeptides that increase their expression under nutrient-deficient conditions positively regulate starvation responses, including autophagy that is key in maintaining cellular homeostasis during stress responses including starvation (Cho et al., 2020; Kim et al., 2018; 2021; Kwon et al., 2017; Lee et al., 2021; Molinari, 2021; Sebastian and Zorzano, 2020; Zachari et al., 2019). In fact, recent studies suggest that FGF21 is a starvation-regulating hormone, which is induced under nutrient-deficient conditions and positively regulates a collection of starvation responses, such as growth inhibition, tissue breakdown, autophagy, and lipid degradation (Badman et al., 2007; Byun et al., 2020; Fazeli et al., 2015; Inagaki et al., 2008). We identified the FMRFamide neuropeptide FLP-20 as a systemic starvation signal in *C. elegans* that positively regulates starvation responses, including starvation survival, autophagy, and starvation-induced fat degradation, echoing the discovery of FGF21 in mammals. Since, however, mutation of *flp-20* could not fully rescue starvation-induced responses, it is likely that FLP-20 acts in parallel with other systemic signals, potentially originating from AIY neurons that have been previously shown to modulate starvation responses together with AIB neurons (Kang and Avery, 2009a; 2009b).

GCY-28 is a receptor-type guanylate cyclase, which has been previously shown to modulate chemotaxis (Tsunozaki et al., 2008). GCY-28 is expressed in various tissues, including neurons, body-wall muscles, hypodermis, and intestine, suggesting that its ligand(s) should be a cue of general interest to many tissues. However, the ligand(s) of GCY-28 has not been identified yet (Ortiz et al., 2006; Tsunozaki et al., 2008). Previous studies suggested that *gcy-28* mutants seem to exist in a state of perceived starvation, based on the phenotype of avoidance of AWC^{on}-sensed odors (Tsunozaki et al., 2008). Our results showing that mutation of *gcy-28* is epistatic to mutation of *flp-20* in starvation survival and starvation-induced fat degradation suggest the possibility that FLP-20 may be a ligand for GCY-28, possibly acting as an antagonist. It will be interesting to test this possibility using a heterologous expression system. In addition, further experiments are necessary to elucidate where and how GCY-28 acts to modulate starvation responses. Our starvation hypersensitive model system might be helpful to elucidate such molecular mecha-

nisms.

Starvation also induces behavioral changes in animals (e.g., changes in foraging behavior), which contribute to increasing the chance of locating new food sources (Douglas et al., 2005; Wang et al., 2006). Foraging behaviors are often coordinately regulated with metabolic changes in various organisms, including *C. elegans* (Cohen et al., 2009; Greer et al., 2008; Srinivasan et al., 2008), suggesting the possibility that FLP-20 and GCY-28 may regulate behavioral changes in response to starvation as well. Indeed, we found that mutations of *flp-20* and *gcy-28* oppositely affect feeding rates of worms during prolonged starvation (Fig. 4D). Further experiments are needed to elucidate the circuit regulating this starvation-regulated feeding behavior.

Interestingly, after 24 h starvation, a portion of wild-type worms (~36%; Fig. 4B) still retained the high levels of fat. It was recently reported that starvation induces adult reproductive diapause in approximately 34% of worms (Angelo and Van Gilst, 2009). Given the fact that fat accumulation is important for another developmental diapause, dauer entry in *C. elegans* (Fielenbach and Antebi, 2008), our observation leads to the intriguing possibility that fat retention during starvation is critical for adult reproductive diapause. It would be interesting to test whether *gcy-28* mutants, very few of which retained high levels of fat during starvation (Fig. 4B), show defects in entering adult reproductive diapause.

In summary, our data suggest that the FMRFamide neuropeptide FLP-20, probably secreted from AIB in an MGL-2 dependent manner, acts as a systemic signal for starvation responses through the receptor-type guanylate cyclase GCY-28. Thus, this study strongly indicates a specific neuroendocrine circuit that senses and signals nutrient-deficient states to modulate a systemic starvation response in multicellular organisms.

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AUTHOR CONTRIBUTIONS

C.K. and L.A. conceived and designed the project. C.K. performed all experiments, and C.K. and L.A. analyzed the data. C.K. and L.A. wrote the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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