Octopamine promotes lipid mobilization in *C. elegans*: a new solution for obesity?

Preview for "Tao *et al.* Octopamine connects nutrient cues to lipid metabolism upon nutrient deprivation. Science Advances 2016"

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Introduction

Food availability fluctuates over time and animals have to diligently evaluate the changes to adjust accordingly when times of famine will occur. *C. elegans* responds to the change of food availability by adjusting its physiology and behavior to cope with limited nutrients and to enhance survival. During starvation, *C. elegans* decreases anabolism and increases catabolism to provide enough ATP to maintain basal level of cellular activities. Accordingly, worms decrease energy-consuming behaviors including growth, reproduction and foraging for resource conservation.

In *C. elegans*, the nuclear receptor DAF-12 senses nutrient depletion and switches partners from dafachronic acids (DAs) to DAF-12 interacting protein 1 (DIN-1). The insulin/transforming growth factor-β (TGF-β) induces DAF-9 to catalyze the synthesis of DAs. Favorable conditions activate insulin/IGF-1 and TGF-β pathways to elicit DA/DAF-12 downstream reproductive growth signaling. Under unfavorable environments, the insulin/TGF- pathways stay inactive, allowing DAF-12/DIN-1 to interact and promote dauer formation.

Fat storage is the last reserve for energy production under starvation when glucose is no longer available. One major group of enzymes that liberate fatty acids from triglyceride (TAG) molecules is represented by lipases. ATGL-1, the homolog of mammalian adipocyte triglyceride lipase, is the main lipase that mobilizes fatty acids from TAGs during fasting[1]. Transcription of *atgl-1* is repressed by the bHLH (Helix Loop Helix) homolog HLH-11 in the presence of food and activated by nuclear hormone receptor-76 (NHR-76) in response to serotonin signaling[2]. ATGL-1 stability is augmented by the energy sensor AMP activated protein kinase (AMPK) in response to fasting resulting in increased lipolysis[1]. Lysosome plays an important role in the catabolic degradation of lipids during fasting. Three lipases have been discovered in the lysosome including LIPL-1, LIPL-3 and LIPL-4[2], among which *lipl-1* and *lipl-3* are repressed or activated by the transcription factors MXL-3 or HLH-30 under well-fed or starved conditions, respectively[3]. In the endoplasmic reticulum, two lipase genes *fil-1* and *fil-2* are activated by IRE-1 and HSP-4 under starvation[4]. Like many other complex biological processes in evolutionarily more advanced organisms, the perception of food and the metabolic responses are coordinated across different organelles, cells and tissues. Any unbalanced or uncoordinated starvation/stress response can be detrimental to the organism. Lipolysis is such a complex catabolic process that requires integrated orchestration across different cellular compartments and tissues.

Bioamines have been shown to be important for the starvation response as first exemplified by the administration of octopamine in worms to elicit similar behavioral changes observed during

starvation[5]. A pair of octopaminergic RIC neurons can release octopamine in response to food scarcity[6]. Regulatory mechanisms including inhibitory dopamine and downstream targets including the cAMP response element binding protein (CREB) have been proposed to work in the perception of food by octopamine[6]. However, the precise regulatory connection to upstream stimuli, downstream tissue specific molecular and metabolic outcome and communication across different tissues, have yet to be elucidated. The study by Tao *et al.* explored the link between DAF-12, octopamine and lipolysis in the perspective of tissue specificity and tissue communication under starvation challenge.

Results

Because the role of octopamine in nutrient deprivation is poorly characterized, the authors examined whether octopamine production is upregulated in *C. elegans* upon starvation. Using quantitative liquid chromatography-tandem mass spectrometry, they demonstrated that levels of octopamine in starved worms were two times higher than the well-fed counterpart. However, where does this increase arise from? Not surprisingly, they found that the enzyme responsible for producing octopamine biogenic amine, *tbh-1*, was also transcriptionally upregulated in starved worms. This induction is also functionally important during starvation, as the *tbh-1* mutants showed significantly lower survival compared with wild-type (WT) worms after 3 days of starvation.

Why does octopamine production lead to starvation resistance? A straightforward explanation would be the better mobilization of the stored nutrient in the organism. In *C. elegans*, lipid droplets are stored in the intestine and can be measured either by Oil Red staining or biochemical extraction and gas chromatography-mass spectrometry (GC-MS) quantification. Both methods revealed that the consumption of lipids during starvation was completely abolished in *tbh-1* mutants: wild type worms showed only 40% of the original fat level after one-day starvation, while the fat level in *tbh-1* mutants remained unchanged. Accordingly, supplementation of octopamine can promote the lipid hydrolysis in WT as well as *tbh-1* mutants where its synthesis is perturbed. As *tbh-1* is known to mainly express in the RIC neurons, they further showed that overexpression of *tbh-1* in the RIC neurons specifically can enhance lipid hydrolysis in WT worms.

Having established the crucial role of TBH-1/octopamine signaling during nutrient deprivation, the authors aim to bidirectionally map the upstream signaling to induce *tbh-1* expression as well as the downstream signaling events to promote lipid hydrolysis.

How does octopamine activate lipid hydrolysis? Previously microarray data indicated that TAG lipases (*fil-1*, *fil-2*, and *lips-6*) are induced during starvation[4]. However, only *lips-6* was inhibited by mutation in *tbh-1*. Knocking down *lips-6* significantly suppressed lipid hydrolysis during starvation, suggesting that its role in mediating the starvation. As *lips-6* is expressed in the intestine, could there be octopamine receptors expressed in the intestine? Among the three predicted octopamine receptors SER-3, SER-6, and OCTR-1 in *C. elegans*, only *ser-3* is required for the *lips-6* induction during starvation. Moreover, knocking down *ser-3* specifically in the

intestine reduced lipid hydrolysis and the expression of *lips-6* during fasting. Tissue-specific rescue of *ser-3* also demonstrates that it functions in the intestine.

For the upstream cue, a good candidate would be nuclear receptor DAF-12, as it is known to sense nutritional cues[7]. Indeed, the promoter region of *tbh-1* harbors three known DAF-12 AGTACA hexamer binding elements, and the mutation or truncation of the binding elements abolishes the induction of *tbh-1* in the reporter line. Moreover, knocking down *daf-12* or its cofactor *din-1* as well, abolishes the up-regulation of *tbh-1* during starvation in the fluorescent reporter line, mRNA levels as well as the protein levels. The DAF-12/DIN-1 complex is also functionally important, as the disruption of *daf-12* or *din-1* phenocopies both the reduced survival and the reduced induction of *lips-6* during starvation. Overexpression of *daf-12* under the control of the *tbh-1* promoter, however, can rescue all the aforementioned phenotypes, demonstrating that DAF-12/DIN-1 confers resistance to starvation in the same RIC neurons as TBH-1.

Taken together, the authors established this novel cross-tissue endocrine signaling cassette of octopamine during nutrient deprivation sensation in *C. elegans*.

Significance and Future Directions

Because the role of norepinephrine or octopamine in nutrient deprivation is poorly characterized, Tao *et al.* demonstrated that among several genes involved in energy supply, the induction of the lipase *lips-6* through its receptor SER-3 in the intestine, promotes lipid mobilization. In addition, the authors discovered a novel regulation mediated by the nuclear receptor DAF-12 associated with DIN-1, which results in octopamine level induction as a mechanism to adapt to food scarcity.

However, many intriguing questions remain to be addressed for future research investigations. First of all, although *lips*-6 is expressed in the intestine, it would be interesting to identify the subcellular localization of this protein. Lipases might be expressed in lysosomes, lipid droplets or cytoplasm. Determining its localization will open new avenues to further dissect the underlying mechanism of LIPS-6 enzyme activity and test whether its organelle-specific lipid hydrolase is required for fat storage content regulation. Additionally, is there any specificity in which class of TAG will be released by LIPS-6? Are unique fatty acids involved in this organelle-peripheral tissue lipid signaling communication?

Second, environmental changes including nutrient deprivation are usually sensed by distinct sensory neurons. The authors dissected in a very elegant way how upon starvation, the tyramine beta- hydroxylase TBH-1 catalyzes the conversion of tyramine to octopamine in a DAF-12/DIN-1 dependent manner in the interneuron RIC. It was previously established that RIC interneuron receives stimuli and signals from upstream ASI pair of sensory neurons[8]. It would be interesting to test whether in adverse environmental conditions this signaling pathway is still involved or whether additional sensory neurons and key players might act upstream to initiate the cascade of DAF-12/DIN-1 complex activity.

Third, it is unclear how *lips-6* is activated in the intestine downstream of the receptor SER-3, nor how octopamine is released from RIC neurons to directly/indirectly interact with SER-3 in the intestine. In addition, the authors only focused their studies on the fat content reduction upon *lips-6* overexpression. However, it would be fascinating to further characterize the downstream mechanism of peripheral lipid mobilization by performing a whole organism transcriptome analysis using RNA-seq on *lips-6 Tg* animals, as well as performing a screen to identify possible downstream transcription factors or nuclear hormone receptors during food availability and nutrient deprivation.

Lastly, but not least, Tao *et al.* provided evidence showing that increased levels of octopamine might result in lifespan extension. In contrast, Burkewitz et *al.* claimed a putative role of octopamine reduction in longevity extension[9]. Despite this controversy discussed by the authors in the paper, it is possible to speculate that different levels of octopamine as well as the developmental stages of supplementation can result in different effects including a putative hormesis effect beneficial to nutrient deprivation adaptation. Overall, this study reveals that octopamine acts as an endocrine signal linking nutrient availability to energy metabolism, providing conserved mechanistic insights in norepinephrine regulation in mammals.

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