Dietary bacteria fattens up the worm

preview for “Xie *et al.* (2020) Dietary *S. maltophilia* promotes fat storage by enhancing lipogenesis and ER-LD contacts in *C. elegans.* bioRxiv”

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Introduction

Lipid droplets (LDs) have long been thought as inactive passive fat storage centers, however studies in the last three decades that emerged with the discovery of perilipin - an LD resident protein - revolutionized our view about these cellular organelles (Farese and Walther, 2009; Greenberg et al., 1991). They are now recognized as highly dynamic organelles that are capable of expanding and shrinking in response to altered metabolic demands and interact with other cellular structures, including but not limited to mitochondria, peroxisomes and endoplasmic reticulum (ER). Therefore, LDs act as an essential hub for cellular metabolism while mediating inter-organellar communication.

LDs can be found in most cells, from yeast to humans. Although LDs of distinct cells can be different in size and composition, their structural organization and biogenesis mechanisms are similar. The first step in LD biogenesis is the synthesis of neutral lipids - triacylglycerols (TAGs) and sterol esters - via the function of acyltransferases (DGAT1/DGAT2 for TAGs and ACAT1/ACAT2 for sterol esters). As the concentration of these neutral lipids between the ER bilayer leaflet increases, they coalesce forming a "lens" that eventually buds from the confined regions of the ER membrane, where FIT2 and other ER-resident proteins are localized. After budding, LDs expand, which can occur through three different mechanisms: LD-LD fusion, ER membrane contact sites or direct TAG synthesis on the LD surface (Olzmann and Carvalho, 2019). In addition, LDs form extensive contacts with all other organelles. In yeast, the LDs remain continuous with the ER membrane, whereas in mammalian cells a population of LDs is reported to detach from the ER through unclear mechanisms, but often remain tethered to the ER membrane by forming transient "lipidic bridges" (Schuldiner and Bohnert, 2017). Known effectors of the ER-LD contact sites include the SEIPIN complex, DGAT2 that pairs with FATP1, RAB18 that pairs with the NRZ complex and it’s SNAREs (Olzmann and Carvalho, 2019). Despite the extensive research, our understanding of the molecular details of LD biogenesis, as well as the mechanism and function of LD membrane contact sites is still premature and open to many questions.

The nematode *Caenorhabditis elegans* has been widely employed as an important model organism for studying lipid metabolic regulation and LD homeostasis. Owing to their transparency and genetic tractability, worms have been especially instrumental in the discoveries

of novel regulators of lipid metabolism (Srinivasan, 2015) and the dissection of molecular and cellular mechanisms of LD physiology (Mak, 2012; Na et al., 2013; Xu et al., 2012). Previous studies found that dietary choice has significant impacts on *C. elegans*' lipid metabolism. Worms raised on *E. coli* OP50 strain have LDs that are larger in size and number compared to worms raised on *E. coli* HB101 strain (Brooks et al., 2009). In contrast, worms exposed to the pathogenic bacteria, *Pseudomonas aeruginosa*, have decreased number of lipid droplets that are also smaller in size (Ding et al., 2015). In this preprint, Xie *et al.* discovered that pathogenic bacteria, *Stenotrophomonas maltophilia*, induce LD expansion in size and number through enhanced ER-LD interaction.

Results

To study how *C. elegans* lipid metabolism response to different bacterial diets, the authors captured and isolate environmental bacteria and cultivated the worms with transgenic reporter labeling the LD membrane in the individual single bacterial-species lawns, among which a Gram-negative bacterium, *S. maltophilia*, gathered the researchers' attention.

S. maltophilia induced a robust and striking increase in LD diameter in the worm fat storage tissues, intestine and hypodermis, as well as the embryos. As LD enlargement is commonly associated with fat content increase, the authors measured the TAG level directly in the worms fed with *S. maltophilia* and indeed observed a 3.7 fold increase in TAG compared with the common diet OP50-fed worms. As *S. maltophilia* can potently induce fat accumulation, they naturally question the origin of this metabolomic change. A straightforward answer is that differences in lipid composition between bacterial food sources lead to the differences in fat absorption and incorporation. Notwithstanding the substantial differences in lipid composition between *S. maltophilia* and *OP50 E. coli*, they also noticed mutant *S. maltophilia* strain with no difference in fatty acid composition to the wild-type cannot induce the LD size increase, suggesting that unknown factors participate in the microbe-host interaction. Moreover, the authors ruled out the possibility that the lipid difference comes from food intake difference as pharyngeal pumping remains unchanged and mutant with reduced pumping rate, *eat-4* shows comparable LD size with wild-type(WT) worms at *S. maltophilia* diet.
Then how does the worm fed with *S. maltophilia* accumulate more fat? The authors hypothesize that signals from the bacteria lead to alteration in lipid metabolic pathways. Indeed, they detected transcriptional activation as well as protein level increase of lipid synthesis master regulator SBP-1, as well as its downstream FAT-6 and FAT-7, two acyl-CoA desaturases required for lipid synthesis. These components are necessary for the lipid accumulation, as disruption of these genes abolishes the LD size increase in the *S. maltophilia* diet. In order to systematically study the *C. elegans* host factors that mediate the *S. maltophilia* effect on LDs, the authors conduct a forward genetic screen to identify genes required for the LD size increase. After screening 7,000 haploid genomes they isolate 7 mutant strains from which three genes, *acs-13, dpy-9*, and *cyp-*

35B, were mapped. The authors showed that *acs-13*, acyl-CoA synthetase is required for the LD expansion through the peroxisomal β-oxidation pathway. While the function of *dpy-9*, a cuticle collagen gene, and *cyp-35B,* the worm cytochrome P450 enzyme needs further investigation. Aside from the genetic pathways, the authors also take a close look at cell biology changes in *S. maltophilia* fed worms. The ER is the primary site where TAGs are synthesized. Interestingly, with the genetic reporter line labeling the organelle membrane, they identified ER-wrapped LDs in the intestine as well as ring-like ER structures in the hypodermis. Electron microscope images indicate LDs of *S. maltophilia* fed worms have increased connection to the ER through small tubular structures, namely ER-LD bridge. SEIP-1 is the worm homolog for ER-LD contact site structure protein. SEIP-1::GFP positive structures are observed almost exclusively in worms on the *S. maltophilia* diet, further reinforcing the formation of ER-LD bridge in the *S. maltophilia* fed worms. The ER-LD bridge is also functionally relevant, as *seip-1* inactivation partially suppressed the effect of *S. maltophilia* feeding on LDs. Finally, the authors demonstrate that the formation of ER-LD bridge is dependent upon the *dpy-9* and *cyp-35B,* connecting the genetic pathway to the ER remodeling.

Perspectives

Xie et al. set out to find environmental bacteria that infect worms chronically. Most studies use pathogenic bacteria to induce an acute response from the worm and ultimately kill the worm in a short period of time. The authors screened for an isolate that the worm can live on for an extended period of time. *S. maltophilia* does not induce an acute innate response however it causes metabolic burden to worms, increases fat storage, and reduces mobility. *S. maltophilia* can be a good model to study chronic host-pathogen interaction if it is shown to inoculate the worm intestine and be part of the microbiota.

This work opens up more questions in the regulation of lipid metabolism by microbiota. A recently developed resource CeMbio will be a valuable tool to understand the control of lipid regulation by host-microbiota interaction in-depth (Dirksen, 2020). CeMbio provides a natural microbiome of 10 genera for in-depth analysis which *S. maltophilia* is not part of. It is likely that *C. elegans* encounter *S. maltophilia* in the wild as a pathogen and the study of *S. maltophilia* infection in the natural microbiota background can help us understand the potential bacterial interaction.

Lipidomics analysis by gas chromatography-mass spectrometry (GC-MS) shows large differences between OP50 and *S. maltophilia* lipid profiles. 15 carbon lipids (C15iso and C15anteiso) lipids are abundant, while 17 carbon lipids levels were low in *S. maltophilia* compared to OP50. Considering the predominant use of 17 carbon lipids as sphingolipid long chains, decreased 17C lipid levels in *S. maltophilia fed worms* may affect sphingolipid synthesis and function in general. Surprisingly, there was no clear difference in lipid profiles between

obesogenic *S. maltophilia* strain and a strain generated by the authors in a forward genetic screen on *S. maltophilia to* find the strains that do not enlarge LDs. This suggests that there are several non-lipid factors modulating the *S. maltophilia* induced LD enlargement. Authors did not follow up with this mutant in this current work, but it would be an interesting lead to understand the factors contributing to the increased LD size. Future high-throughput mass spectrometric studies can reveal the *S. maltophilia* factors that signal the worm either directly at the fat storage tissues or possibly through cell-non-autonomous neuronal signaling.
LDs are dynamic organelles that interact with other organelles in the cell. The LD position in the cell may reveal its function. The nascent LDs are in contact with or in close proximity to ERs, while LDs interact with peroxisomes and mitochondria during lipid utilizations. The *S. maltophilia* induced supersized LDs are linked to ERs suggesting either a compensatory mechanism to store increased lipid molecules or a defective LD emergence. ER ensheathed LDs are less likely to interact with other organelles functionally explaining low levels of lipid catabolism. Isotope labeled pulse-chase experiments coupled with stimulated Raman scattering microscopy imaging may tell more about the rate of lipid incorporation and catabolism.
The high fat/ high carbohydrate diet can phenocopy Metabolic Syndrome in a variety of model systems. The ectopic accumulation of fat in muscle and nervous systems is involved in pathogenesis. The far-reaching question would be whether there is lipotoxicity with *S. maltophilia* feeding? Lipid imaging in these tissues can reveal another aspect of *S. maltophilia* feeding.

The final question is how *S. maltophilia* impacts the behavior of the worms and whether the changes in fat storage contribute to the alterations in physiology and behavior. For example, would suppressing the LD size induction affect the immune sensitivity of worms to *S. maltophilia*? In addition, the metabolic status of worms influences their feeding behavior. Well-fed worms exhibit a quiescence state analogous to mammalian sleep. Worms with defective fat synthesis genes have decreased quiescence. *S. maltophilia* feeding did not induce increased fat phenotype in *fat-6*; *fat-7* double mutants and *sbp-1* mutant worms. However, the authors did not report the activity and quiescence levels of these animals, leaving the interaction of microbiota-lipid metabolism and sleep open to further investigation.

Taken together, the authors established a novel microbe-host interaction mechanism for lipid metabolism regulation. There are several missing links in the pathway, but the functional importance of microbe regulating host metabolism and the novelty in organelle contact stands prominent.

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