Investigating the molecular mechanisms underlying Kombucha-induced lipid utilization in *C. elegans*

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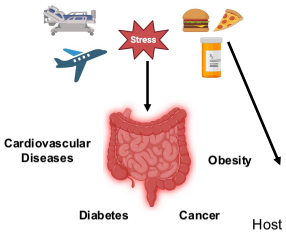
Background

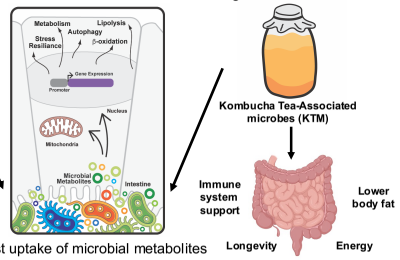
Gut Microbiome

The gut microbiome is involved in the regulation of lipid homeostasis. Unhealthy changes in the gut microbiome, dysbiosis, has been linked to a variety of metabolic diseases.



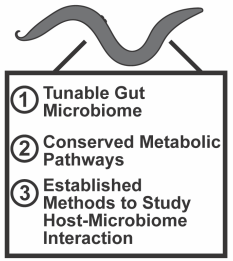
Probiotics

Probiotics, which can be introduced through supplements or by fermented foods such as Kombucha tea, can help restore the gut microbiome.

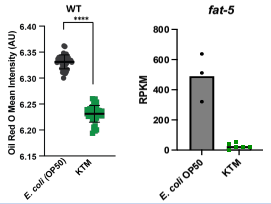


Host uptake of microbial metabolites can alter gene expression resulting in modulation in cellular processes.

Caenorhabditis elegans as a model system



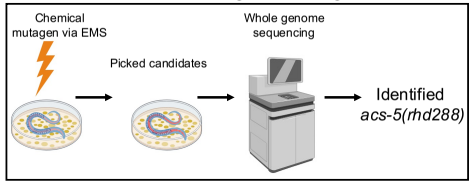
Objectives



1. Identify regulators of the desaturase, *fat-5/SCD*, as it is downregulated on KTM-fed worms and plays a role in mediating fatty acid synthesis.
2. Elucidate the genetic mechanism responsible for *acs-5* mediated *fat-5* regulation.

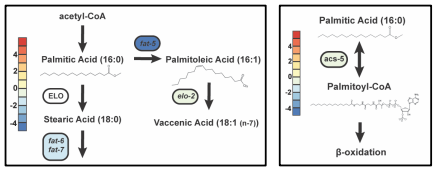
acs-5 is a regulator of *fat-5* expression independent of food source

A. Identification of *acs-5* through a forward genetic screen

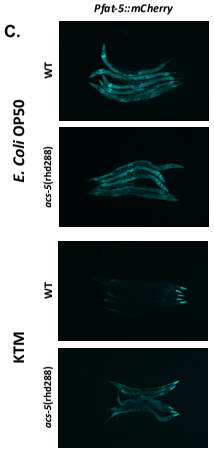


B. FAT-5 desaturates palmitic acid to palmitoleic acid

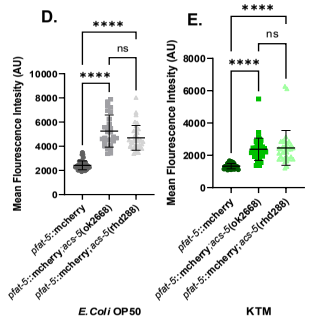
ACS-5 involved in tagging palmitic acid for ß-oxidation



C.



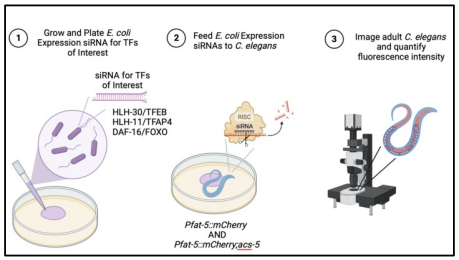
D. **E.**



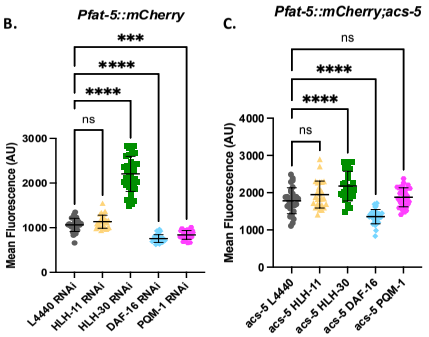
The commercially available mutant allele, *acs-5(ok2668)*, and the EMS-derived mutant allele, *acs-5(rhd288)*, both increase *Pfat-5::mCherry* expression to similar levels relative to wild-type regardless of whether animals consumed a KTM or *E. coli* diet. (A) Schematic of mutagenesis screen. **(B)** Schematic of the biological role of FAT-5 and ACS-5. **(C)** Representative images of *Pfat-5mCherry*, of *Pfat-5mCherry; acs-5 (rhd288)*. Quantifications of the grey values from the mCherry fluorescence on worms consuming **(D)** *E. coli* (OP50) and **(E)** KTMs. One-Way ANOVA, *p>0.05, **p>0.1, ***p>0.01, ****p>0.001.

Transcriptional regulators of *acs-5* induced *fat-5* expression

A. Transcription Factor RNAi Screen



B. *Pfat-5::mCherry* **C.** *Pfat-5::mCherry;acs-5*



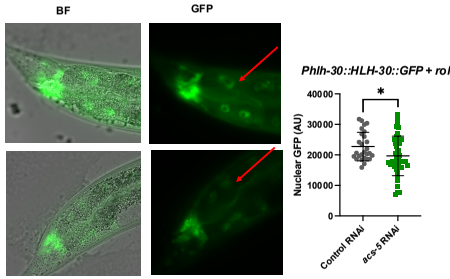
DAF-16/FOXO, HLH-30/TFEB and HLH-11/TFAP4 may coordinate *fat-5* gene expression under basal and *acs-5* mutant conditions. (A) Schematic of RNAi screen workflow. **(B)** Quantification of *Pfat-5::mCherry* fluorescence shows that RNAi knockdown of the known *fat-5* transcriptional regulators *hlh-11/TFAP4* and *hlh-30/TFEB*, but not *daf-16/FOXO*, increases *fat-5* levels. **(C)** Quantification of *Pfat-5::mCherry; acs-5* fluorescence reveals that knockdown of *hlh-11* increases *fat-5* expression while *hlh-30/TFEB* and *daf-16/FOXO* RNAi has modest effects . One-Way ANOVA, *p>0.05, **p>0.1, ***p>0.01, ****p>0.001.

Conclusions

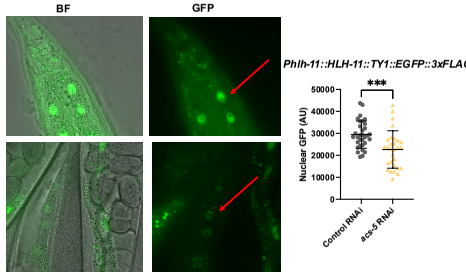
1. *acs-5* is not a KTM specific regulator of *fat-5* expression.
2. HLH-11/TFAP4 and HLH-30/TFEB act to suppress *fat-5*
3. ACS-5 may repress *fat-5* expression by controlling the nuclear localization of the HLH-11 and HLH-30 transcription factors.

acs-5 RNAi reduce nuclear accumulation of HLH-11 and HLH-30

A. Phlh-30::HLH-30::GFP + rol-6



B. Phlh-30::HLH-11::GFP + rol-6



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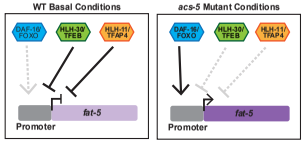

ACS-5 may be inhibiting a key mediator that is sequestering HLH-11 and HLH-30 out of the nucleus. (A) Fluorescent and DIC images of Phlh-30::HLH-30::GFP + rol-6 on control L4440 and ACS-5 RNAi and quantification of intestinal nuclei. Two independent experiments were performed with similar results. **(B).** Fluorescent and DIC images of Phlh-11::HLH-11::GFP + rol-6 on control L4440 and ACS-5 RNAi and quantification of intestinal nuclei. Two independent experiments were performed with similar results. One-Way ANOVA, *p>0.05, **p>0.1, ***p>0.01, ****p>0.001. **(C)** Schematic of TF RNAi knockdown results.

Future Directions

1. Perform a RNAi reverse genetic screen on different long-chain fatty acid-CoA ligases to determine if other regulators of lipolysis modulate lipid metabolism.
2. Perform RNA sequencing on subsequent long-chain fatty acid-CoA ligase candidate knockouts
3. Identify key mediators responsible for the ACS-5 induced repression of *fat-5* gene expression that may be controlling the nuclear localization of the HLH-11 and HLH-30 transcription factors.

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Acknowledgements

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UNC bio RENDER