Identification of putative endocannabinoid N-acyltransferases in *C. elegans*

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Introduction

- N-acylethanolamines (NAEs) are an important class of endocannabinoid signaling lipids that are involved in a variety of physiological processes including pain-sensation, appetite, inflammation, and mood[1].
- The rate limiting enzyme N-acyltransferase (NAT) catalyzes the synthesis of N-acyl phospatidylethanolamine (NAPE) which is the first step in the synthesis of NAEs as seen in Figure 1.

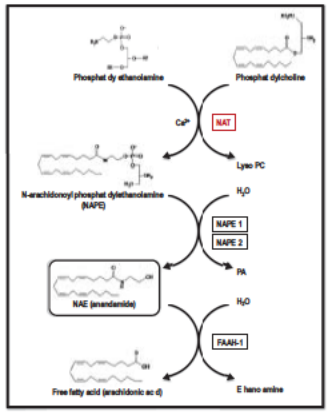

Figure 1. A schematic for the synthesis and degradation pathway of NAEs. Shown is the metabolic pathway for anandamide, the major NAE of mammals (adapted from Ueda and Wang[2]).

- Both calcium dependent and independent activities have been identified, with the Ca^{2+} dependent enzyme thought to be the predominant form expressed in neuronal tissues.
- The calcium dependent NAT enzyme has been characterized biochemically but has not been cloned.
- If identified, the NAT enzyme could be targeted pharmacologically to control levels of NAEs. This would have important implications in controlling appetite and therapeutically treating pain and inflammation.

C. elegans provides a tractable genetic model system to screen for the NAT enzyme.

- The nematode *C. elegans* is a model organism that is easy to manipulate genetically.
- Recently, it has been shown that the worm produces various NAEs and NAPEs and that it has functionally conserved homologs of NAPE-PLD and FAAH[3].
- We hypothesize that *C. elegans* has an N-acyltransferase enzyme capable of synthesizing NAPE and that the discovery of the worm enzyme will help identify the mammalian NAT enzyme.

Strategy for finding an NAT enzyme

Bioinformatic analysis of the *C. elegans* genome for identification of candidate genes.

- Searching *C. elegans* genome with bioinformatic tools, we identified 35 protein candidates with conserved acyltransferase domains and orthologs of human genes.

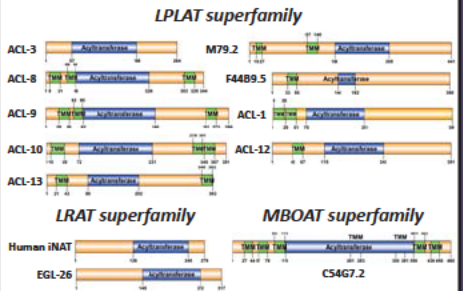

Figure 2. Conserved protein sequence domains of select candidates. The NAT enzyme should possess acyltransferase activity and be membrane bound. The acyltransferase domains are shown in blue and the transmembrane domains are shown in green.

Candidate genes were knocked down with RNA interference to identify the NAT enzyme.

- These 35 candidate genes were then screened in a mutant worm strain which overexpresses *nape-1* and a deletion in *faah-1*, and exhibits delayed growth as a result of high NAE levels.
- We hypothesized that knockdown of the NAT enzyme in this strain should reduce the levels of NAEs and restore normal growth.
- Each gene was selectively knocked down by feeding the worms with *E. coli* expressing the double stranded RNA specific to that gene, which inhibits translation through RNA interference (RNAi) in a mechanism outlined in Figure 3.

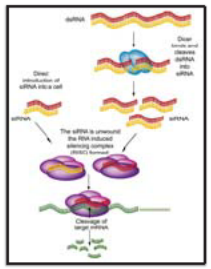

Figure 3. RNAi silencing of translation[4]. Genes of interest can be knocked down by introducing a double stranded RNA molecule into the cell that is complementary to the target mRNA.

Results

RNAi screening identifies 10 acyltransferases that rescues growth delay associated with high NAE levels.

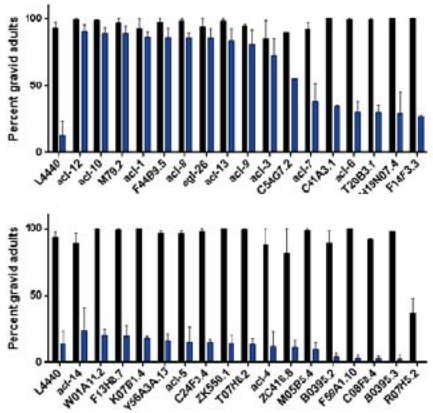

Figure 4. RNAi screen of 35 acyltransferase candidates. Each gene was knocked down in the wild type N2 strain shown in black, and in a mutant strain which has extra copies of *nape-1* and a deletion in *faah-1* shown in blue. The screen identified 10 genes that when knocked down rescued the growth delay phenotype of the mutant strain.

qRT-PCR identifies 4 acyltransferases that are upregulated at mRNA level in response to low NAE levels.

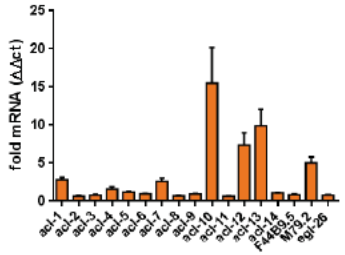

Figure 5. mRNA expression level changes in *faah-1* overexpressor mutant. Levels of mRNA of several candidate genes were measured using qRT-PCR in a strain that has multiple copies of the *faah-1* and as a result has reduced levels of NAEs. It is predicted that the expression of the NAT enzyme will be increased in order to compensate for these decreased levels of NAEs. *acl-10, acl-12, acl-13,* and *M79.2* were found to be expressed at significantly higher levels in the mutant strain when compared to the wild type.

Discussion

- From the original 35 candidates, the RNAi screen was successful in identifying 10 genes that restored normal growth in a strain with high NAE levels, suggesting that knockdown of these genes reduces NAEs in worms.
- Of these 10 genes, *acl-10, acl-12, acl-13,* and *M79.2* were upregulated at the transcriptional level in the *faah-1* overexpressor mutant, a behavior that is consistent with that expected of the NAT gene as its expression is predicted to increase when levels of NAEs decrease in the above worm background.
- Together, these data suggest that these four genes could play a role in controlling NAE levels, making them attractive candidates for further biochemical studies to determine if they are the NAT enzyme.

Future Directions

- Test whether supplementing plates with NAEs prevents the rescue of growth delay caused by the RNAi knockdown of the 10 candidate genes in the strain which has extra copies of *nape-1* and a deletion in *faah-1.*
- *In vitro* activity assays for *acl-10, acl-12, acl-13,* and *M79.2* will be performed from proteins expressed and purified from bacteria to see whether they catalyze the synthesis of NAPE. The reaction will be carried out with phospatidylethanolamine and phosphatidylcholine containing radiolabeled fatty acid at the SN1 position as substrates. Generation of radiolabeled NAPEs will be monitored by thin layer chromatography.

References

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Acknowledgements

A special thank you to Pedro Reis-Rodrigues for his guidance and help on this work. I'd like to thank Matthew Gill for his support and for giving me the opportunity to work on this project. I'd also like to thank Museer Lone for his advice. This work is funded by a grant from Florida Atlantic University's Office of Undergraduate Research and Inquiry and by the National Institutes of Health (R01 AG036992).