The Biosynthesis of Ascarosides in *Caenorhabditis elegans*

Thesis by

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ABSTRACT

Ascarosides comprise a family of small signaling molecules that have been shown to regulate important events and behaviors in the life history of the nematode *Caenorhabditis elegans*. Although the different roles of individual ascarosides appear to be determined by the variances in chemical structure, the mechanisms by which ascarosides are synthesized as well as the locations in which ascarosides are produced within the worm are largely unknown. In this thesis, we examined ascaroside production in the intestine, hypodermis, and body wall muscle of the worm by driving the expression of the protein DAF-22 under different tissue-specific gene promoters. While the body wall muscle and hypodermis are capable of synthesizing ascarosides, the intestine appears to be the major site of pheromone production. Additionally, we found through transgenic rescue and HPLC-MS analysis, that the acyl-CoA synthetase ACS-7 plays a significant role in the addition of moieties derived from primary metabolic pathways to the 4'-position of the ascarylose sugar core of ascr#9.

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The work presented in this chapter represent a collaboration between the Sternberg Lab (California Institute of Technology, Pasadena, CA) and the Schroeder Lab (Cornell University, Ithaca, NY). Allison Akagi (AA), Joshua Judkins (JJ), and Oishika Panda (OP) designed the mutant screens, cultured strains, and generated exometabolome extracts. JJ and OP acquired all HPLC-MS data. The data was analyzed by AA, OP, and JJ. Tiglic supplementation feeding assays were conducted by JJ and OP. AA performed *oac-50* mutant outcrossing. Genetic rescue of *acs-7* was performed by AA and ACS-7 localization studies were performed by AA and OP.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS AND/OR TABLES

Figure *Page*

Figure *Page*

Table *Page*

NOMENCLATURE

Ascaroside. (ascr) A glycoside of the dideoxysugar ascarylose.

> **icas.** A simple ascaroside modified at the 4'-position with an indole-3-carbonyl head group
>
> **hbas.** A simple ascaroside modified at the 4'-position with a *p*-hydroxybenzoyl head group
>
> **mbas.** A simple ascaroside modified at the 4'-position with an (*E*)-2-methyl-2-butenoic acid head group.
>
> **osas.** A simple ascaroside modified at the 4'-position with an *N*-succinylated octopamine head group.
>
> **tsas.** A simple ascaroside modified at the 4'-position with an *N*-succinylated tyramine head group.

Dauer. A stress-resistant alternative L3 larval stage induced during times of low food, high population density, and high temperatures.

Excretome. The collection of metabolites and molecules excreted by *C. elegans*.

Gut granules. Lysosome-related organelles found in the intestine of *C. elegans* necessary for the production of 4'-modification of ascarosides.

High performance liquid chromatography tandem mass spectrometry. (HPLC-MS) An analytical chemistry technique that first separates chemical components in mixtures using liquid chromatography and then subjects them to mass spectrometry for identification.

Peroxisomal β-oxidation. A four-step process that iteratively truncates the lipid carbon chain by two carbons in the form of acetyl-CoA within the peroxisomes.