DISCOVERY OF C. ELEGANS CHEMICAL ECOLOGY

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Introduction

The small soil nematode *Caenorhabditis elegans* is one of the best studied animals. It was the first metazoan to have its genome sequenced. Its entire cell lineage from a single fertilized egg to an adult is known and has been related to the animal's anatomy, and its anatomy has been comprehensively described by ultra high resolution electron microscopy. *C. elegans* is particularly tractable for genetic studies, and as a result many complicated genetic pathways have been identified. The importance of these studies was recognized by the 2002 Nobel Prize in Medicine to three pioneers in the field, Sydney Brenner, Robert Horvitz, and John Sulston and more recently the 2006 Nobel Prize to Andrew Fire and Craig Mellow for their discovery of RNAi in the worm.

Despite this wealth of genetic, cellular, and anatomical information, almost nothing is known about the chemistry that *C. elegans* uses to communicate between individuals and its environment. We hypothesize that *C. elegans* utilizes an extensive chemical "language" that allows individuals to communicate and regulates basic behaviors such as feeding, mating, and population density control. We have formed an international team of collaborators with expertise in *C. elegans* behaviors, chemical isolation, and chemical identification. Our goal is to identify several different molecules produced by the worm in order to begin to decipher its chemical language. This information will not only add to the extensive scientific knowledge of *C. elegans* but may provide important new clues for the biological control of other nematode parasites of plants or animals.

Experimental

High densities of worms were grown in liquid culture using standard protocols and, at specific developmental stages, transferred to water for 1 hour. The worms were removed from the water, and the sample was lyophylized to dryness. The powder was resuspended in a small volume of D_2O, and the 1-mm HTS probe in AMRIS was used to identify small molecules secreted into the water. Each sample is also analyzed by liquid chromatography-mass spectrometry and by behavioral assays.

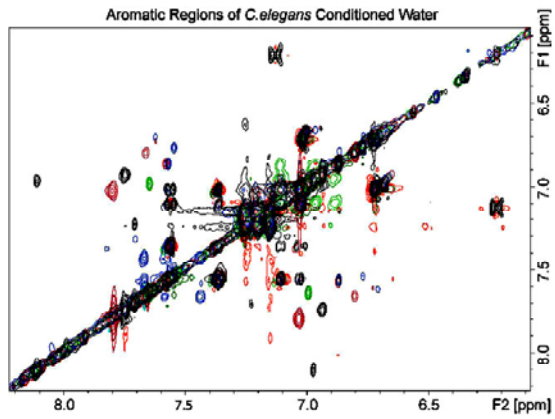

Figure 1) Aromatic expansions of TOCSY spectra of worm-conditioned water from larval stage 2 (green), 3 (blue), 4 (red), and young adult (black). All spectra were obtained on the AMRIS/NHMFL 1-mm HTS probe using 8 μL volumes.

Results and Discussion

We have collected TOCSY and COSY data on "worm water" from all developmental life stages and adult animals (Fig 1). There are several resonances common to all life-stages and several others that are only in specific developmental stages. We soon will analyze these mixtures with covariance NMR to try to sort out specific components for identification.

Conclusions

The 1-mm triple resonance HTS probe has allowed about 200x less sample to be used than in recent work in this area from other labs. This has allowed us to separately examine specific life stages in order to gain chemical insights through biology.

Acknowledgements

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