The effects of ethanol on gene expression in *Caenorhabditis elegans*

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Introduction

Alcohol is a widely used drug, though its behavioral effects are not fully understood. Ethanol affects humans and the nematode *C. elegans* similarly: the same doses of alcohol that cause intoxication in humans decrease the speed and amplitude of locomotion in *C. elegans* as well as reduce the rate of egg-laying (Davies et al. 2003). Thus, it follows that by studying the effects of intoxicating doses of ethanol on the behavior of *C. elegans*, we might be able to gain a better understanding of the ways in which alcohol effects human behavior. We intend to use an existing set of microarray data to identify how ethanol affects gene expression in *C. elegans.*

Identifying key mechanisms through which ethanol causes behavioral changes in *C. elegans* brings us a great deal closer to identifying the corresponding pathways in humans. Experimentation by Davies et al. (2003) has already demonstrated the importance of BK potassium channel activation in behavioral responses to ethanol. Davies and Bettinger (2004) identified the role of *npr-1* in the development of acute tolerance to ethanol. Research by Kwon et al. (2004) studied the effect of more concentrated, lethal doses of ethanol on gene expression in *C. elegans* and identified a number of genes that responded to ethanol, specifically several heat shock protein genes as well as two genes that were unique to ethanol responses. The goal of our study is to expand on this knowledge and identify genes responsible for intoxication in *C. elegans*.

Methods

We hope to use microarray data from tests exposing *C. elegans* to intoxicating doses of ethanol for 1 and 4 hours to identify genes that are up- or down-regulated in

response to ethanol. Filters in the Stanford Microarray Database will be used to identify genes that fit parameters concerning magnitude and consistency of change across six microarrays. It is likely that we will need to try a number of different parameters with varying degrees of stringency in order to identify a manageable number of genes for further testing. We will concern ourselves primarily with genes that are up-regulated because such results are easier to confirm in the lab. Ideally, we will be able to locate ethanol response genes that are not part of heat shock response. Priority will also be given to genes with known human homologues. The top candidates resulting from these filters will then be subject to further testing in lab. RT-PCR will be used to confirm the microarray results. This step will further narrow the number of genes, allowing us to discard genes that are not consistent between the microarray data and the RT-PCR. Genes that pass RT-PCR will then be tested by GFP and mutant analysis. For this reason, genes that correspond to readily available mutants will have priority in RT-PCR testing. If mutants are not available for any candidate genes, RNAi will be used to observe the role of the candidate gene in ethanol response.

Possible Results and Implications

It is not possible to be completely certain which genes, or types of genes, will emerge from the filtered microarray data set. The data resulting from our microarrays is highly imperfect; the distribution of points across all six arrays is consistently skewed towards red. Consequently, the nature of our data set might call for leniency in the filtering process, which increases the probability of error in gene selection. RT-PCR will be an essential step in reducing this error, though it is still possible that some selected genes might be unrelated to the behavioral changes caused by ethanol. Through careful confirmation of our results with GFP and mutant and/or RNAi analysis, however, we

hope to accurately identify a gene, or genes, responsible for ethanol-related behavioral changes in *C. elegans*.

References

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