The effects of ethanol on gene expression in *Caenorhabditis elegans*: An exploration of microarray data filtering and analysis

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Introduction

C. elegans is an important model system in the study of the ways in which ethanol affects human behavior. In addition to the fact that *C. elegans* react to ethanol in much the same way humans do (a dose similar to that which causes intoxication in humans leads to decreased speed and amplitude of locomotion, as well as a reduced rate of egg-laying), *C. elegans* is an ideal model because its genome has been sequenced in full (many of its genes have human homologues), and its cell lineage is well understood. It follows, then, that the study of the ways in which ethanol affects gene expression in *C. elegans* might provide helpful clues as to how human gene expression is affected by this prevalent yet poorly understood drug. Previous studies have shown that ethanol does in fact alter gene expression in *C.* elegans. 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. Furthermore, the use of microarrays to study the effects of more concentrated, lethal doses of ethanol on gene expression in *C. elegans* by Kwon et al. (2004) revealed a number of genes that responded to ethanol treatment, specifically several heat shock protein genes as well as two genes that were unique to ethanol responses.

In a previous study, we attempted to analyze a set of microarray data measuring the effect of 1 and 4 hours of exposure to an intoxicating dose of ethanol on gene expression across the *C. elegans* genome (Bettinger, J. unpublished data). A total of six genes were

determined to be significantly up-regulated in response to ethanol treatment. However, our attempt to confirm these results through RT-PCR was not entirely successful. RT-PCR results were inconclusive, as the gene used for control appeared to be up-regulated by ethanol. In addition to this, we noticed that two separate attempts to filter out significantly up-regulated genes resulted in two different lists of significant genes pulled from the same data set. As a result of these inconsistencies, we would like to begin a study of the process of filtering sets of microarray data, in order to refine our list of significant genes, as well as to locate an acceptable control gene for use in further RT-PCR assays.

Methods

We intend to study the process of microarray filtering by subjecting our data set to different software tools, including the SMD, which was used in our previous study, as well as SAM microarray. Combining and comparing different ways of filtering the data should help us to compile a new an improved list of genes of interest (those that are up-regulated in response ethanol in a meaningful way). We then intend to use our knowledge of scheme programming (attained by completing an introductory course in computer science at Grinnell College) in an attempt to use BIOBIKE to filter genes using our own algorithm, which we hope to develop through a thorough study of microarray methods literature, and a thorough exploration of SAM microarray.

Possible Results and Implications

From this study, we hope to assemble a refined list of up-regulated genes from our original data and use this list to improve our continuing study of ethanol-related gene expression in *C. elegans* in the summer of 2007. Specifically, refining our filtering process to create a good list of up-regulated genes should also give us a good idea of

which genes remain unchanged by ethanol, which should in turn aid us in the selection of a viable control gene for use in RT-PCR. An adequate control gene will contribute a great deal to the success of our study, removing a considerable amount of the ambiguity from our results. A refined list of genes would be useful in that it might help us to identify particular systems or pathways altered by ethanol, as well as providing us with a better selection of genes to study, i.e. a selection of genes that would be more interesting to study through GFP transformation and locomotion analysis.

References

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