ETHANOL RESPONSIVE GENES IDENTIFIED BY ANALYSIS OF CDNA MICROARRAY

INTRODUCTION:

Ethanol is a widely used drug; yet, its mechanism and direct effects to the brain are still undetermined. Ethanol studies have been performed using many different model organisms, one being the multicellular nematode, *Caenorhabditis elegans* (Davies et al. 2003). *Caenorhabditis elegans* was the first multicellular organism to have its genome completely sequenced (Consortium TCeGS 1998) and each cell mapped out in a cell lineage (Sulston et al. 1983). With all of this information available, there is a great advantage to using *C. elegans* as a model organism to study genes and proteins that may be involved in the ethanol response pathway.

Since ethanol exposure creates behavioral responses in mammals and invertebrates (Moore et al. 1998; Davies 2003), one can study the behavior and find genes that mediate that behavior. In humans, the behavioral response to ethanol is dependent on the dose. At a low dose of ethanol one may experience a sense of euphoria, whereas at a higher dose motor uncoordination may occur (Moore et al. 1998). Similar to humans, *C. elegans* behavioral responses are also dependent on dose. In fact, the intoxication of both humans and *C. elegans* occurs at similar concentrations of ethanol (Davies et al. 2003). Some behavioral responses to acute ethanol exposure in *C. elegans* include effects on locomotion, speed of movement, and frequency of egg-laying (Davies et al. 2003). As the ethanol concentration increases, the body-bend amplitude during locomotion decreases and by 400mM and 500mM of an exogenous ethanol concentration the body becomes completely flattened (Davies et al. 2003). The flattening and decrease in body-bends of the worms significantly decreases the speed of movement as well. In addition, the egg-laying frequency also significantly decreases by the 400mM and 500mM exogenous concentrations. These two high doses of exogenous ethanol concentrations correlate to 22mM and 29mM internal concentration of ethanol (19mM equals 0.1% blood alcohol in humans) (Davies et al. 2003). On a broader scale, this acute exposure of ethanol caused behavioral effects that are controlled by separate neuromuscular networks, locomotion and egg-laying. This may be an indication that ethanol affects more than one cell type (Davies et al. 2003).

In past experiments, studies have been performed using mutants identified and isolated based on their behavioral resistance to ethanol effects through the use of genetic screening

(Davies et al. 2003). However, with advanced technology and the newer technique of analyzing whole genome expression using DNA microarrays, the expression of genes at specific time points in an experiment could provide very important nonbiased information about the up-regulation and down-regulation of specific genes during transcription (Thibault et al. 2001).

The goal is to identify genes that change expression levels upon exposure to ethanol in hopes of understanding more about ethanol response in *C. elegans*. Previous data includes a cDNA microarray experiment performed by Dr. Jill Bettinger from Virginia Commonwealth University, Pharmacology & Toxicology Department, in conjunction with Dr. Stuart Kim from Stanford University. Within the experiment there were 2 treatments, ethanol-treated *C. elegans* (500mM ethanol) and non-ethanol-treated *C. elegans* (control; 0mM ethanol). There were 3 replicates of each treatment and RNA collection was taken at two time points, 1 hour and 4 hours, from both treatments. The microarray data of this experiment has yet to be vigorously analyzed and interpreted. The analysis of this data set will provide the identification of some up-regulated and down-regulated expressing genes in response to ethanol exposure. These results will also be compared to other microarray data publicly available to help filter out genes involved in other systems such as the shock response. The ethanol-treated *C. elegans* does experience shock when exposed to high concentrations of ethanol (Kwan et al. 2004). Some of this shock response is mediated by the heat shock genes, but as to date there is no evidence that ethanol behavioral response has any relationship with heat shock. Therefore, comparing the changes in heat shock expression genes during a shock response to the ethanol microarray data, we can filter out the overlapping genes and concentrate on genes more closely linked to ethanol response. This is not to say that induction or suppression of heat shock genes does not mediate an ethanol response, but for the sake of simplicity these genes will be excluded.

After the ethanol responsive genes are filtered, I will pick three to five of the genes based on a personal prioritized filter scheme and perform wet lab analysis to confirm their relationship to ethanol response. If these genes are proven to have a direct connection to ethanol exposure, further experimental analysis will be performed.

METHODS:

Using the Stanford MicroArray Database (SMD) online, the raw data of the cDNA microarray experiment performed by Dr. Jill Bettinger will be filtered and sorted. This data will

also be compared to other ethanol microarray experiments performed by others as well as microarray data involving heat shock.

In efforts to narrow the genes of interest, genes will go through a set of requirements before selection. Beginning with about 20,000 genes, the SMD default filters will be used to select reliable spots from the arrays, spots that have been changed 4X in response to ethanol, and appear in at least 3 out of 6 arrays. However, modifications to these filters will be made to make the filters more and/or less stringent. For example, the filter will be changed to alter the number of consistent spots out of the total 6 arrays and allow spots that have been changed 1.5X or 2X in response to ethanol. From the list of genes retrieved after the filtering, another list will be made through prioritization. The first priority in ranking the genes will be that the basic functions have to already be known. Next, the genes will be ranked on the level of change in response to ethanol, but only those that show an up-regulation of gene expression will be selected. This preference for up-regulated genes is based on future experiments that will be performed, which includes labeling the specific gene with a GFP marker and confirming up-regulation in living animals in response to ethanol through visualization in a fluorescence microscope. The down-regulation of genes is equally important as up-regulated genes; however, I will not concern myself with those genes during this project. Any genes remaining will be ranked based on its relationship to anoxia, aging, and insulin pathways.

The top three to five genes will be selected and reverse transcriptase polymerase chain reaction (RT-PCR) will be performed to confirm each microarray result. If the selected genes function as predicted, further testing will be performed, such as labeling the gene with GFP and determining other information like which concentration of ethanol is needed to begin up-regulation.

I may also use BioBIKE to look for any common up-stream regulatory regions that may be responsive to ethanol within the genes identified by the microarray analysis.

RESULTS:

Through the filter of the raw microarray data and the prioritization of genes, the analysis will lead to the selection of a few specific genes of interest. However, some of the raw microarray data may be faulty due to PCR failure or contamination (Sherlock et al. 2001) and this may result in the loss of some important genes within *C. elegans* that respond to ethanol. In

addition, one of the arrays has high background noise and will probably not give very much useful data, but it will be examined nonetheless. After performing RT-PCR and labeling the gene with a GFP marker, visualization of the GFP using the fluorescence microscope will help suggest if the genes are involved in the ethanol pathway.

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