Elucidating the Molecular Mechanisms Resulting from Acute Lead Exposure in *Saccharomyces cerevisiae*

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My research began with a news article about PCB pollution in the Hudson River and its effects on a small bottom feeding fish called the Atlantic tomcod. Although this article was geared more toward evolutionary adaptations as a result of environmental pollution, I was drawn to its subtle elements of studying chemical exposure at the molecular level, and I continued to read additional articles and papers concerning toxicology and genetics. I was inspired to study the effects of environmental pollution, specifically lead pollution, on genetics. As an inexperienced yet eager sophomore, I encountered many experiments on my quest for a project but I knew I had to narrow down my project's focus to a question that was feasible to conduct in my school's laboratory. I finally came across the ideal project, one that allowed me to combine my specific variable of interest, lead, to a simple yet efficient yeast model to test this variable's effect on gene expression.

For this project, I conducted my research and lab work in my school laboratory. I decided to conduct my research at my school because I was then able to pursue a project that was modeled on my interests and was executed by me, from start to finish. Rather than starting in an established laboratory where background research and a project had already been developed, I decided to pursue my own interests with a project driven by my curiosity, and therefore had to do extensive background research before I could start in the lab. My inventiveness came in play as I had to develop an experimental design for incorporating a variable by trial and error and literature sources rather than using an established method or procedure. Throughout the course of the experiment, it was up to me to anticipate the next step and it was necessary for me to set internal goals and deadlines, a situation I had never experienced in a high school classroom setting.

For my project, I also had to implement the discipline of mathematics into my procedure. First of all, I learned math and chemistry based equations to solve solubility problems and calculate concentrations of my variable of interest, lead, and also of my nucleic acids and reagents during procedures such as RNA extraction, and Polymerase Chain Reaction. More importantly, one of the most integral parts of my project was the background research to investigate genes that would be expected to be altered under the influence of my variable. In order to find these genes, I had to conduct extensive literature reviews and then learn to operate an online search tool that is uses a mathematical algorithm called BLAST (Basic Local Alignment Search Tool) to compare genes sequences and analyze their similarity.

This research helped me realize the complexity of processes on a molecular level as my research only elucidates the very first step in a long and largely unknown pathway. I also discovered that I was interested in the methodology and instrumentation used in molecular biology experiments. When performing written procedures for common techniques such as RNA extraction and RT-PCR, I found myself spending time learning how each of these techniques was actually created and what components were used. I was especially interested in this aspect of research when I was designing primers. I designed the sequences on my own based on specified parameters, but I found myself interested in the process used in the actual synthesis of the primer by the company. This experience resulted in my interest in the bio-molecular discipline of biomedical engineering, which I also intend to further study. Research helped in making science more alive for me, because it showed that there are many unknown and undiscovered areas of science, and an unlimited amount of questions to be asked, rather than just a definite set of facts in our textbooks.

My advice for other high school students striving to undertake a project combining science and mathematics is that in research, mathematics is one of the greatest tools, as using a defined discipline such as mathematics to explore a scientific question which delves into the unknown is a great advantage for standards, analysis, and interpretation.

Abstract

Lead pollution, a persistent environmental hazard, has been shown to adversely affect humans by damaging the nervous and reproductive systems. My research examines the effect of lead exposure on gene expression in yeast, a model organism that shares thirty-one percent of its genome with that of humans. Ten genes previously associated with differences in regulation by lead exposure were chosen to study. Using RNA extraction, RT-PCR, and gel electrophoresis, six genes were generally found to be up-regulated as expected based on previous literature, one gene was down-regulated as expected, and three genes require further investigation as no clear pattern can be discerned from the results. Differences in gene expression in yeast elucidate the molecular mechanisms behind the effects of lead in humans and are relevant in developing therapies targeting response pathways affected by lead exposure.

Introduction

Lead toxicity is a major public health concern affecting humans, especially children, and is one of the leading environmental hazards. Lead, a heavy metal naturally occurring in the earth's crust, is classified as a priority pollutant by the United States Environmental Protection Agency that due to its highly useful properties has been readily utilized in pipes, storage batteries for cars, ammunition, paints, ceramic glazes, and gasoline (11). According to the Agency for Toxic

Substances and Disease Registry, over the past three hundred years, environmental lead levels have increased more than one thousand fold due to human activities (1). The most prominent source of lead pollution comes from the increased usage of leaded gasoline in the latter twentieth century because of lead's ability to reduce engine knocking, which is uncontrolled combustion, and raise octane rating of gasoline. It is estimated that in 1979, cars in the United States released 94.6 million kilograms (208.1 million pounds) of lead into the atmosphere. Although the EPA has banned the use of leaded gasoline since 1996 and the amount of lead released into the air has been reduced, lead pollution is still a prevailing issue due to the fact that this heavy metal does not degrade to other compounds and adheres strongly to soil particles, remaining in the soil for many years.

Environmental lead negatively affects humans as it can be absorbed into the body from soil, polluted air, or contaminated water (1). In both adults and children, the main target of lead toxicity is the nervous system; however, children are more susceptible to the adverse effects because of their developing brains, higher proportions of lead absorbed into the blood than adults correlated with high calcium uptake and high prevalence of nutrient deficiency, and higher probability of inhaling or ingesting lead-based paints (1, 2, 9). Studies indicate that there is no threshold level of lead that is considered safe for either children or adults (1, 4). One study demonstrated that increased blood lead concentrations are accompanied by overall decreases in arithmetic, reading, nonverbal reasoning, and short term memory test scores (8).

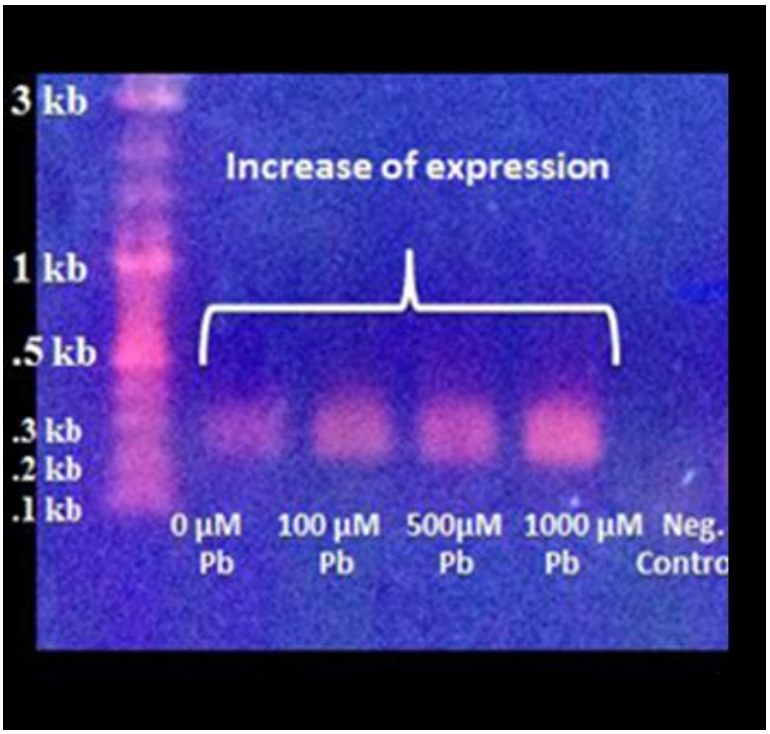
Due to the negative effects of lead toxicity on neuronal processes and the nervous system, several neuronal genes were chosen to be examined in this study; in total, ten genes were chosen to be analyzed and were categorized based on their function in humans.

My research utilizes *Saccharomyces cerevisiae*, commonly known as yeast, to visualize differences in gene expression following exposure to various concentrations of lead. Yeast was chosen as an ideal model organism to study genomic level changes because it is a eukaryotic organism, and it is simple to culture, grow, and control. Most importantly, it shares approximately thirty-one percent (1895 genes out of 6116 genes) of its genome with humans and the fully sequenced yeast genome is readily available (6). Since yeast is the model organism, any changes in gene expression seen in yeast should model what would be expected in humans in the corresponding homologs of the genes analyzed. Changes in gene expression were visualized using RNA extracted from lead-exposed yeast, synthesis of cDNA, PCR, and gel electrophoresis.

In order to identify genes of interest, yeast homologues of genes in rats and humans found to be expressed differently after lead exposure were obtained from previous research and using yeastgenome.org and the Homologene database. Percent homology to human genes, or the percent of the sequence conserved between yeast and human, was determined using the BLAST tool. The expected results after lead exposure were hypothesized using results of previous studies involving genes of interest and their up/down regulation after exposure to lead in other model organisms.

Results

My experimental model allows for an initial screen of multiple genes that can be tested economically to identify the genes most responsible for the negative effects of lead on the nervous system.



My results were obtained in the form of visualization of expression that I have shown here. The expression of each gene was shown under four increasing concentrations of lead, starting with a sample with 0 µM concentration and ending with 1000 µM, the highest concentration. The 0 µM sample serves as a control sample and shows a baseline expression that all normal functioning cells without the treatment should exhibit; it is therefore the sample that the other samples are compared with. The analysis of these figures is qualitative and I therefore visually measured the intensity of brightness of the bands compared to the control. This figure shows the gene expression of the gene THS1, and shows a pattern of increase of expression as compared to the control sample, as each band is brighter than the previous. The ladder in the first column is used as a standard to measure how long the gene samples are as the fragment lengths are already known and labeled. Using the ladder, we can also estimate that the gene product is around 300 base pairs.

The gene THS1, and the other genes in the same category, were hypothesized to be up-regulated in response to lead exposure because of previous research involving homologues of these genes and lead exposure in rat cells. The trend of gene expression for THS1 therefore supports the hypotheses of up-regulation.

Out of the ten genes that I tested in my research, seven were found to be regulated as expected according to the hypotheses made from previous literature, while the other three showed no apparent pattern of expression and should be further investigated. Although this research cannot show how changes in gene expression affect in the nervous system and body, it is a preliminary step in pinpointing which genes and pathways should be further investigated. Differences in gene expression observed in a yeast model elucidate the molecular mechanisms behind the negative neuronal effects of lead in humans. My research is relevant in determining the appropriate therapies targeting response pathways affected by exposure to lead in humans.

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