TOXICANT INDUCED DIFFERENTIAL GENE EXPRESSION AND PRODUCTION OF AN AQUATIC GENE ARRAY

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Most toxic substances released into the environment either accidentally or by deliberate discharge eventually become aquatic contaminants. A concerted effort has thus been made to develop toxicity assays that precisely reflect the impact of chemical exposure on aquatic organisms, aquatic ecosystems and ultimately human health. Recent advances in toxicogenomics have resulted in the development of high throughput gene analysis technology that could literally revolutionize toxicity monitoring. We propose to take advantage of these developments to create DNA arrays for high throughput aquatic exposure assessment. The fundamental basis of these assays is to monitor altered gene expression in aquatic organisms following exposure to environmental toxicants. Because gene expression is a sensitive end point, DNA array technology is ideally suited to identify toxicity following acute and chronic exposures. DNA arrays can screen thousands of genes simultaneously, eliminate the time and cost of traditional biomarker analysis and aid in determining chemical mechanisms of action. Two specific objectives were identified for the development and characterization of these arrays: (1) obtain differentially expressed, toxicant responsive genes following exposure of medaka fish (*Oryzias latipes*), to prototypic classes of environmental toxicants including poly-halogenated hydrocarbons (2,3,7,8-tetrachlorodibenzo-p-dioxin) and environmental estrogens (17-α-ethinylestradiol); (2) array differentially expressed genes and identify unique gene markers of toxicity and characteristic gene expression profiles. Ideal gene markers will be quantifiable, display a high degree of sensitivity (i.e. detectable at low concentrations), have a reasonable degree of accuracy (i.e. responsive over a range of concentrations), and exhibit limited antagonism by secondary stressors. These studies were initiated to test the validity of using DNA arrays to rapidly screen changes in gene expression in response to aquatic toxicant exposure.

The major goal of this project is to develop the use of DNA-arrays as a quantitative tool to assess exposure and effect of environmental toxicants in the aquatic environment. To accomplish this aim, toxicant-induced gene markers for prototypic classes of environmental pollutants were to be identified by suppressive subtractive hybridization. This is a highly sensitive method with a proven track record for isolating novel and rare differentially expressed transcripts. Differentially expressed genes are represented as a library of subtracted clones, which are subsequently arrayed on nylon membranes and characterized for usefulness as biomarkers of toxicity.

Young adult, three month old, sexually mature, medaka were exposed to a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin, or 17-α-ethinylestradiol by i.p. injection for 48 hrs. In this phase, concentrations of each toxicant were chosen that are sub-lethal, yet sufficient to initiate transcription of responsive genes. Environmental conditions were rigorously maintained by partial submersion of exposure containers into a tabletop tank system with a 16 hr light/8 hr dark photoperiod and constant temperature of 25°C. Exposure duration was determined by testing a subset of treated fish for induction of well characterized biomarkers for dioxin, for EE_2 exposure.

Livers were collected form a subset of exposed and control fish at 0, 12, and 48 hrs and western blot analysis was conducted for induction of CYP1A1 and vitellogenin proteins. Each analysis revealed a single, time dependent, protein band of appropriate molecular weight in exposed animals. Total RNA was subsequently isolated from the remaining fish (48 hrs after dosing), cDNA was synthesized, and libraries were constructed for each exposure and control population. Forward and reverse subtractive hybridization was performed from exposure condition. Initially, cDNAs from treated fish were used as tester DNA and non-treated cDNA as driver to generate subtracted libraries that are enriched for genes that have been induced (forward subtraction). A second round of SSH was performed using cDNA from control fish as tester DNA and cDNA from treated fish as driver to generate a subtracted library enriched for genes that are repressed (reverse subtraction). The resulting cDNA libraries were subsequently cloned into a T/A cloning vector and transformed in to *E. coli* DH5α cells. Individual colonies were plated on LB agar plates, selected, and inoculated into replicate 96 well plates for growth, storage and preservation. Significant progress was made on aim 1 of this research project resulting in the identification of several differentially expressed genes from both estrogen and dioxin treated animals. Gene sequences are currently being sequenced and compared to existing data bases for identity. Once sequencing is complete, aim two will be addressed.

Small aquarium fish species including medaka are increasingly being used as vertebrate models in various fields of biology including toxicology, genetics, and developmental biology. These organisms are suitable for use in genomic studies due to their small genome size, and ease of application in forward genetics. The medaka fish have become a standard model organism in aquatic toxicity studies. Their transparent chorions, reproductive capacity, external gender characteristics, and sensitivity to toxicants are but a few of the characteristics that have made them an excellent model for toxicity and toxicogenomic studies. With the emerging technology in gene expression analysis, biomarker applications in applied toxicology can be greatly improved. Genome expression technologies are becoming increasingly reliable and accessible and it is now feasible to generate global mRNA expression profiles for whole genomes following stimuli such as exposure to toxicants. Measurement of global mRNA expression is of particular importance in disciplines like toxicology, where toxic phenotypes are produced by altered expression of multiple genes. Interest in the application of DNA- array analysis for toxicity studies is rapidly growing and has proven useful for defining mechanisms of toxicity and classifying toxicants based on characteristic gene markers and expression profiles. Compared to contemporary standardized bioassays, it is expected that DNA-array assessment of global mRNA expression will greatly improve the accuracy and efficiency of toxicity screening. It is believed that this technology will have a broad base of applications pertaining to many aspects of toxicology including assessment of toxicity in drug development, elucidation of mechanism of action, prediction of disease status, determining occupational exposure and monitoring pollutant exposure in the environment. This work was funded in part by the North Carolina Biotechnology Center NCBC#2001-ARG-0030.