ZEBRAFISH CARDIOVASCULAR cDNA MICROARRAYS: EXPRESSION PROFILING AND GENE DISCOVERY IN EMBRYOS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN

by

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submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

and the

WOODS HOLE OCEANOGRAPHIC INSTITUTION

September 2003

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September 20003

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"Success is going from failure to failure without a loss of enthusiasm."

-Winston Churchill

Acknowledgements

I was twelve years old when I first set my heart on a Ph.D. from Woods Hole Oceanographic Institution. So I think I should begin by thanking everyone in Academic Programs, especially John Farrington, Judy McDowell, and Julia Westwater, for helping me make that dream a reality.

I need to thank my thesis supervisor, John Stegeman, for constant intellectual stimulation and scientific guidance. Even more important, though, has been the unflagging support and encouragement he has provided. The hugs and pep talks are uncountable, and kept me from walking away more than once. Thank you, John.

Mark Fishman welcomed me into his lab and, in doing so, really made this thesis work possible. Mark, your vision and dedication are inspiring.

Mark Hahn, Nancy Hopkins, and Iain Drummond have guided and supported my research. Their insightful questions and constructive criticisms are greatly appreciated.

I have led a dual life, jumping back and forth between Boston and Woods Hole, for nearly four years now. Thank you to **all** of the members of both Stegeman and Fishman labs for making both places feel like home. In particular, Bruce Woodin, Margaret Boulos, and Amy Dougherty have made it possible for me to be scientifically productive, even when I barely knew my way around the lab or was altogether absent! They have also become dear friends.

Matthew Grow and I were nearly inseparable through a grueling year of tedious technical work that I absolutely could not have done on my own. Ashok Srinivasan provided the cDNA libraries, without which there would be no microarrays. Tyler Aldredge and the staff at the Harvard Center for Genomics Research provided invaluable technical support. Randy Peterson and Jordan Shin have each taken me under their wing at one time or another. Jordan – your encouragement and your sense of humor have both been greatly appreciated!

Ann Michelle and Ru Morrison, Joanna Wilson, Andrew McArthur, Amy Kukulya, Catherine and Brant Davis, Ann and Ray Jones, Chris Morse, Brian Tivol, Rose Koch

and Brian Litofsky – you are esteemed colleagues and great friends. Thank you for many wonderful meals together. Your support and understanding have meant so much to me.

Mom and dad – I do not think that any parents in the world could have done more to foster curiosity and instill the deep respect and passion for learning that I carry with me. You have always encouraged me to follow my dreams, and your love has given me the strength to do so.

Jed, my best friend, soon to be my husband – You have been beside me from day one of this long, rocky trip called graduate school. Your love and support kept me going when my own enthusiasm failed.

This research was supported in part by the WHOI Academic Programs Office, an NSF Coastal Research Traineeship, NIH Superfund Basic Research Program grant 5-P42-ES07381, EPA grant R827102-01-0, and WHOI Ocean Life Institute and Coastal Ocean Institute grant 39591300.

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent teratogen that impacts the developing cardiovascular system. Hallmarks of embryonic exposure include cardiac malformation, impaired circulation, loss of erythrocytes, pericardial and yolk sac edema, and early life stage mortality. However, the mechanism of TCDD cardiovascular embryotoxicity is poorly understood. The primary goal of this thesis was to identify TCDD-responsive genes likely to be involved in processes of toxicity.

We constructed microarrays using cDNA libraries derived from zebrafish embryonic and adult heart tissue. Embryonic heart arrays were used for protocol development. The resulting workflow was employed in the production of adult heart microarrays containing ~2800 unique cardiovascular genes.

These arrays were used to establish gene expression profiles of zebrafish embryos exposed to 1.84±0.42 or 10.74±0.1.38 ng TCDD/g embryo. Alterations in cardiovascular gene expression were limited; 44 genes or ESTs were significantly differentially expressed ≥1.8-fold (p-values $\leq 5 \times 10^{-4}$), and only CYP1A and CYP1B1 were induced >4-fold. Transcriptional responses to TCDD were highly dose-dependent, and adaptive responses were a prevalent feature of TCDD-modulated gene expression.

Microarray analyses indicated induction of genes in three major functional classes – xenobiotic detoxification, sarcomere structure, and energy transfer. TCDD-modulation of selected genes was verified by RT-PCR. Induction of mitochondrial electron transfer genes was variable and modest; such induction provides a possible pathway to reactive oxygen generation and cardiac pathology. Sarcomere genes were generally robustly induced, but RT-PCR indicated suppression of cardiac troponin T2. The current data suggest that TCDD causes cardiomyopathy in zebrafish embryos.

Investigation of a TCDD-induced EST cluster led to the discovery of a novel retroelement, EZR1. EZR1 elements lack genes necessary for autonomous retrotransposition, but are highly expressed in normal and TCDD-exposed cardiac tissue. Putative regulatory elements in LTR sequences may account for observed expression patterns. The function, if any, of EZR1 remains open to speculation.

Thesis Supervisor: John J. Stegeman, Senior Scientist and Chairman of Biology, WHOI

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