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Hahn Lab at WHOI: Current Research Projects (2014)

Epigenetic Mechanisms of Toxicity after Developmental Exposure to Marine Toxins and Toxicants

(Please see also Project 3 in the Woods Hole Center for Oceans and Human Health.)

The overall objective of the proposed research is to elucidate the molecular mechanisms by which short-term exposure to harmful algal bloom (HAB) toxins and marine toxicants during development causes physiological and neurological abnormalities later in life. It is now well known that the early life environment can have a profound effect on the health of adults (*the developmental origins of health and disease*). However, the mechanisms by which developmental exposure elicits effects later in life are not understood. The central hypothesis of this research is that embryonic exposure to certain marine toxins or toxicants alters epigenetic programming, leading to long-term effects on gene expression in adult tissues and ultimately contributing to altered neurobehavioral function in adults. We are conducting studies to identify a core set of genes that show long-term transcriptional changes due to changes in the early life chemical environment and identify their epigenetic signature to determine the mechanistic link between adult phenotype and early life exposures. These studies will be conducted using zebrafish, a powerful model organism for research on developmental mechanisms. In Aim 1, we are testing the hypothesis that developmental exposure to HAB toxins (saxitoxin, domoic acid) and toxicants (PCB126, PCB153) causes later life changes in gene expression and behavior. In Aim 2, we are testing the hypothesis that adult effects resulting from developmental exposure to HAB toxins and toxicants are caused by epigenetic reprogramming of gene expression, focusing on altered DNA methylation and microRNA expression. In Aim 3, we will determine whether the proximal mechanisms involving receptors and ion channels known to be responsible for the acute effects of these chemicals are also involved in the delayed effects seen in adults exposed to chemicals during development. This research will identify molecular bases for adult effects occurring after developmental exposure to important HAB toxins and marine toxicants, and determine whether there are similar or convergent epigenetic mechanisms involved.

Mechanisms and Impacts of Dioxin Resistance in Fish

The overall objective of the basic research proposed here is to understand the effects of long-term, multigenerational exposure to high levels of contaminants on natural populations of animals inhabiting Superfund sites. In one set of studies, we are using a fish model species, the Atlantic killifish Fundulus heteroclitus, populations of which have evolved resistance to dioxin-like compounds that act through the aryl hydrocarbon receptor (AHR) at numerous sites. Killifish inhabiting New Bedford Harbor (NBH), MA, a polychlorinated biphenyl (PCB)-contaminated Superfund site, exhibit heritable resistance to altered gene expression and toxicity of 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) and other AHR agonists as com pared to fish from a refe rence site, Scorto n Creek, MA (SC). We have identified and cloned two distinct AHRs (AHR1 and AHR2), ARNT2, hypoxia-inducible factors (HIFs), and an AHR repressor (AHRR) in killifish. The killifish AHR1 gene is highly polymorphic and AHR1 allele frequencies differ between populations of dioxin-sensitive (SC) and dioxin-resistant (NBH) fish. These studies seek to understand mechanisms underlying differential sensitivity to the developmental toxicity of HAHs and PAHs that act through AHR-dependent signaling, and to determine the impact of evolved HAH/PAH resistance on the sensitivity to other environmental stressors.

We also are collaborating with Dr. Isaac Wirgin at New York University to understand the mechanism of resistance to PCBs in Atlantic tomcod (*Microgadus tomcod*) inhabiting the Hudson River. Those studies have revealed that Hudson River tomcod possess AHR2 variants with impaired ability to bind TCDD. WHOI Press release. Science article.

Recent papers:

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Much of our funding comes from the National Institute of Environmental Health Sciences (NIEHS), part of the U.S. National Institutes of Health. We are grateful to NIEHS and the American taxpayers for supporting this research.

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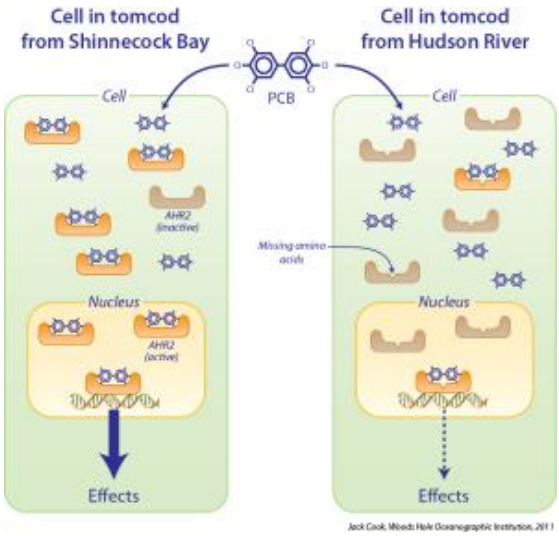
The Superfund Research Program (formerly known as Superfund Basic Research Program) is part of NIEHS and supports our basic research on PCB- and dioxin-resistant fish. The long-term support provided by this program has allowed us to investigate the molecular components of the cellular pathways involved in the response to PCB exposure and how they differ in fish with evolved PCB resistance.

Our Superfund research is part of the Superfund Research Program at Boston University, in its 16th year.



Genetic Variation at Aryl Hydrocarbon Receptor (AHR) Loci in populations of Atlantic Killifish (*Fundulus heteroclitus*) inhabiting Polluted and Reference Habitats.
Reitzel, A. M., Karchner, S. I., Franks, D. G., Evans, B. R., Nacci, D. E., Champlin, D., Vieira, V. M., and Hahn, M. E. (2013).
BMC Evolutionary Biology, 2014, 14:6.
WHOI Press release

Differential sensitivity to pro-oxidant exposure in two populations of killifish (*Fundulus heteroclitus*).
Harbeitner, R. C., Hahn, M. E., and Timme-Laragy, A. R. (2013).
Ecotoxicology, 22, 387–401 (doi:10.1007/s10646-10012-11033-x)

Transcriptomic assessment of resistance to effects of an aryl hydrocarbon receptor (AHR) agonist in embryos of Atlantic Killifish (*Fundulus heteroclitus*) from a Marine Superfund Site. Oleksiak, M. F., Karchner, S. I., Jenny, M. J., Franks, D. G., Mark Welch, D. B., and Hahn, M. E. (2011) *BMC Genomics* 12, 263.

Mechanistic Basis of Resistance to PCBs in Atlantic Tomcod from the Hudson River.
Wirgin, I., Roy, N. K., Loftus, M., Chambers, R. C., Franks, D. G., and Hahn, M. E. (2011) *Science* 331, 1322-1325

Funded by the National Institute of Environmental Health Sciences (NIEHS) through the Superfund Basic Research Program at Boston University.

Superfund Research Program web site for M. Hahn.

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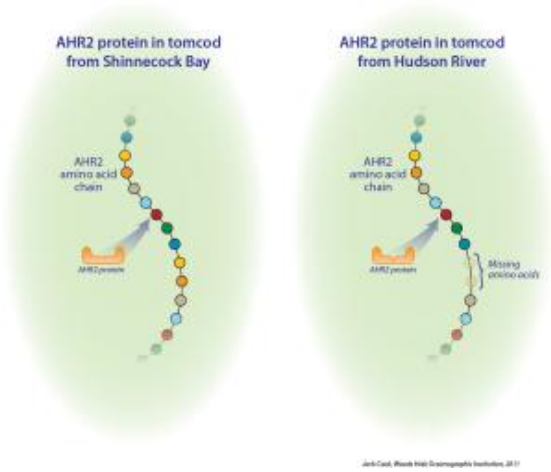

Tomcod from the Hudson River have a variant protein that makes them less sensitive to the toxic effects of PCBs. The effects of PCBs occur through their interaction with a protein called Aryl Hydrocarbon Receptor 2 (AHR2). AHR2 is normally inactive, but when PCB molecules bind to it, AHR2 becomes activated and acts as a molecular switch to turn on other genes that lead to toxicity ("Effects" in the figure). Tomcod such as those from Shinnecock Bay, Long Island, NY (left panel) have a normal version of the AHR2 protein, which has a high affinity for PCBs. Tomcod from the Hudson River (right panel) have a variant AHR2 protein that is missing two amino acids (building blocks of proteins). Without these two amino acids, the AHR2 from Hudson River fish has a reduced ability to bind PCBs as compared to the normal AHR2 protein. This makes the Hudson River fish less sensitive to the effects of PCBs. Killifish from New Bedford Harbor also variants of AHR2, but the functional differences have not yet been identified. For additional details, see *Mechanisms and Impacts of Dioxin Resistance in Fish*. (figure drawn by Jack Cook (WHOI))

AHR signaling in Mammalian and Nonmammalian Models

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The difference between AHR2 proteins in tomcod from Hudson River (right panel) and Shinnecock Bay (left panel) is the loss of two amino acids out of the 1,104 amino acids that normally make up the AHR protein. (figure drawn by Jack Cook (WHOI))

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and polynuclear aromatic hydrocarbons are ubiquitous environmental contaminants implicated in adverse effects on human health. These compounds cause toxicity by activating the aryl hydrocarbon receptor (AHR). The AHR also has physiological roles regulating vascular development, immune function, and cell growth, suggesting a role in human disease. To understand these diverse functions and the possible role of AHR in human disease, it is important to determine how AHR signaling is regulated. The negative regulation of AHR signaling is poorly understood. An inhibitor of AHR transcriptional activation function, AHR repressor (AHRR), has been identified, but its role in regulating AHR signaling remains enigmatic, and possible functions beyond the AHR pathway have been virtually ignored. Recent epidemiological studies have linked AHRR Pr o185 and Ala185 polymorphisms to human reproductive disorders and AHRR has been identified as a likely tumo r suppressor gene in humans. However, fundamental questions concerning the biochemical and functional characteristics of the AHRR and its variants remain unresolved, preventing a full understanding of its roles in human disease.

These studies are utilizing established vertebrate model systems (human cells and zebrafish embryos) to determine the transcription factor specificity and gene selectivity of AHRR and its polymorphic variants, the mechanism by which AHRR represses AHR and hypoxia inducible factors (HIFs), and the role of AHRR in regulating embryonic development and the response to TCDD and hypoxia in vivo.

Recent papers:

Identification of Cinnabarinic Acid as a Novel Endogenous Aryl Hydrocarbon Receptor Ligand That Drives IL-22 Production.
Lowe, M. M., Mold, J. E., Kanwar, B., Huang, Y., Louie, A., Pollastri, M. P., Wang, C., Franks, D. G., Schlezinger, J., Sherr, D., Silverstone, A. E., Hahn, M. E., and McCune, J. M. (2014).
PLoS ONE 9(2): e87877. doi:10.1371/journal.pone.0087877.

The African coelacanth genome provides insights into tetrapod evolution.
Amemiya, C. T., *et al.* (2013).

Nature 496, 311-316.

Aryl hydrocarbon receptor (AHR) in the cnidarian *Nematostella vectensis*: comparative expression, protein interactions, and ligand binding.
Reitzel, A. M., Passamaneck, Y. J., Karchner, S. I., Franks, D. G., Martindale, M. Q., Tarrant, A. M., and Hahn, M. E. (2013).
Development Genes and Evolution, Nov 29. [Epub ahead of print].

Comparative Analysis of Homology Models of the Ah Receptor Ligand Binding Domain: Verification of Structure-Function Predictions by Site-Directed Mutagenesis of a Non-Functional AHR.

Fraccalvieri, D., Soshilov, A. A., Karchner, S. I., Franks, D. G., Pandini, A., Bonati, L., Hahn, M. E., and Denison, M. S. (2013).
Biochemistry 52(4): 714–725.

Regulation of Constitutive and Inducible AHR Signaling: Complex Interactions Involving the AHR Repressor.
Hahn, M. E., Allan, L. L., and Sherr, D. H. (2009).
Biochemical Pharmacology 77, 485-497.

Distinct roles of two zebrafish AHR repressors (AHRRa and AHRRb) in embryonic development and regulating the response to 2,3,7,8-tetrachlorodibenzo-p-dioxin.
Jenny, M. J., Karchner, S. I., Franks, D. G., Woodin, B. R., Stegeman, J. J., and Hahn, M. E. (2009).
Toxicol Sci 110, 426-441.

The active form of human aryl hydrocarbon receptor repressor lacks exon 8 and its Pro185 and Ala185 variants repress both AHR and HIF.
Karchner, S. I., Jenny, M. J., Tarrant, A. M., Evans, B. R., Kang, H. J., Bae, I., Sherr, D. H., and Hahn, M. E. (2009).
Molecular and Cellular Biology 29, 3465-3477.

Funded by the National Institute of Environmental Health Sciences (NIEHS).

Mechanisms of embryo response to oxidative stress

Oxidative stress resulting from environmental exposures is associated with a variety of human diseases ranging from chemical teratogenesis to cardiovascular and neurodegenerative diseases. Developing animals appear to be especially sensitive to chemicals causing oxidative stress. The expression and inducibility of antioxidant defenses are critical factors affecting susceptibility to oxidants at these early life stages, but the ontogenic development of these responses in embryos is not well understood.

In adult animals, oxidants initiate an anti-oxidant response by activating NF-E2-related factor 2 (NRF2) and related proteins, which bind to the anti-oxidant response element and activate transcription of genes such as glutathione S-transferases, NAD(P)H-quinone oxidoreductase, glutamyl-cysteine ligase, and superoxide dismutase. The overall objective of the research proposed here is to elucidate the mechanisms by which vertebrate embryos respond to oxidative stress during development. These s tudies are being performed *in vivo* using embryos of the zebrafish (*Danio rerio*), a valuable model in which to examine mechanisms of toxicity in developing animals and to screen chemicals for developmental toxicity. The results of these studies will establish the composition and ontogeny of the transcriptional response to oxidative stress in vertebrate embryos, elucidate fundamental mechanisms underlying this response, generate tools for screening chemicals for activity as developmental toxicants or antioxidants, and provide insight into the role of oxidative stress in human disease.

Recent papers:

Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo.
Timme-Laragy, A. R., Goldstone, J. V., Imhoff, B. R., Stegeman, J. J., Hahn, M. E., and Hansen, J. M. (2013).
Free Radical Biology & Medicine 65C, 89-101.

Developmental expression of the Nfe2-related factor (Nrf) transcription factor family.
Williams, L. M., Timme-Laragy, A. R., Goldstone, J. V., McArthur, A. G., Stegeman, J. J., Smolowitz, R. M., and Hahn, M. E. (2013).
PLoS ONE 8, e79574.

Nrf2b: a novel zebrafish paralog of the oxidant-responsive transcription factor NF-E2-related factor 2 (Nrf2).
Timme-Laragy, A. R., Karchner, S. I., Franks, D. G., Jenny, M. J., Harbeitner, R. C., Goldstone, J. V., McArthur, A. G., and Hahn, M. E. (2012).
J. Biol. Chem. 287: 4609-4627.

Funded by the National Institute of Environmental Health Sciences (NIEHS).

Molecular Indicators of Dioxin Sensitivity in Birds

Chlorinated dibenzo-p-dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related planar halogenated aromatic hydrocarbons (PHAHs) are highly toxic to most vertebrate animals, including mammals, birds, and fish. However, there are some dramatic species differences in sensitivity to TCDD and in the relative potencies of certain PHAHs, such as mono-ortho-substituted polychlorinated

biphenyls (PCBs). These differences are a major limitation in ecological risk assessment, which often requires extrapolation among species.

Most PHAH effects occur through binding and activation of the aryl hydrocarbon receptor (Ah receptor or AHR), a ligand-activated transcription factor that plays an essential role in the mechanism of PHAH toxicity. We rec ently identified two amino acid differences in the AHR protein, providing a mechanistic explanation for the difference in PHAH sensitivity between common terns (resistant) and chickens (sensitive). Based on these results and on the AHR sequences of other avian species of known sensitivity, we suggested that AHR sequences might serve as indicators of differential PHAH sensitivity among bird species. The overall objective of the research proposed here is to determine whether species differences in AHR structure can explain and predict avian species differences in AHR function and in vivo sensitivity to PHAHs.

Recent papers:

Species-specific relative AHR1 binding affinities of 2,3,4,7,8-pentachlorodibenzofuran explain avian species differences in its relative potency.
Farmahin, R., Jones, S. P., Crump, D., Hahn, M. E., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., and Kennedy, S. W. (2014).
Comp Biochem Physiol C Toxicol Pharmacol 161: 21-25.

Amino Acid Sequence of the Ligand Binding Domain of the Aryl Hydrocarbon Receptor 1 (AHR1) Predicts Sensitivity of Wild Birds to Effects of Dioxin-like Compounds.
Farmahin, R., Manning, G., Crump, D., Wu, D., Mundy, L., Jones, S., Hahn, M. E., Karchner, S., Giesy, J., Bursian, S., Zwiernik, M. J., Fredricks, T., and Kennedy, S. (2013).
Toxicol Sci. 131: 139-152.

Sequence and In Vitro Function of Chicken, Ring-Necked Pheasant, and Japanese Quail AHR1 Predict In Vivo Sensitivity to Dioxins.
Farmahin, R., Wu, D., Crump, D., Herve, J. C., Jones, S. P., Hahn, M. E., Karchner, S. I., Giesy, J. P., Bursian, S. J., Zwiernik, M. J., and Kennedy, S. W. (2012).
Environmental Science & Technology 46, 2967-2975.

The molecular basis for differential dioxin sensitivity in birds: role of the aryl hydrocarbon receptor. Karchner SI, Franks DG, Kennedy SW, Hahn ME. (2006) Proc Natl Acad Sci U S A. 103(16):6252-7.

Funded by NOAA through WHOI Sea Grant.

MicroRNAs in developmental toxicology

Congenital malformations are a major source of human morbidity and mortality. The role of chemical exposure and other environmental factors in the etiology of congenital malformations is not completely understood. However, many xenobiotic chemicals are known to be developmental toxicants or teratogens in experimental animals and several human birth defects are associated with embryonic exposure to xenobiotics. Despite this understanding, the mechanisms by which most developmental toxicants and teratogens disrupt embryonic development are not known. We are investigating a new potential mechanism of developmental toxicity—disruption of microRNA expression—and evaluating the zebrafish embryo as a model for studying the roles of microRNAs in developmental toxicology and teratogenicity.

Recent papers:

Developmental exposure to valproic acid alters the expression of microRNAs involved in neurodevelopment in zebrafish.
Aluru, N., Deak, K. L., Jenny, M. J., and Hahn, M. E. (2013).
Neurotoxicology and Teratology 40, 46-58.

Effects of Short-Term Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin on MicroRNA Expression in Zebrafish Embryos.
Jenny, M. J., Aluru, N., and Hahn, M. E. (2012).
Toxicol Appl Pharmacol, 264: 262–273.

Funded by the National Institute of Environmental Health Sciences (NIEHS).

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