Expression and Immunity

New Findings on *Ahr* Interactions

The transcription factor aryl hydrocarbon receptor (*Ahr*) can activate a multitude of genes that regulate the growth and division of cells, including processes leading to cancer. To date, little is known about the biochemical processes that mediate the activation of *Ahr* receptors in the body, or about the endogenous ligands that bind to and activate these receptors. This month, researchers from the University of Louisville, Texas A&M University, and the University of New Mexico, under the leadership of Charles D. Johnson, report on microarray analyses that illuminate the complexity of *Ahr* interactions and thus provide a focus for future experiments [***EHP* 112:403–412**]. These results include a previously unknown relationship between *Ahr* and genes involved in the activation of the immune system.

The researchers modeled the identification of relevant components of the biological response to *Ahr* ligands using transcriptional profiles of cells from murine embryonic heart, kidney, and vascular smooth muscle cells. The data were analyzed using methods developed at the Texas A&M University Genomic Signal Processing Laboratory to decipher multivariate, nonlinear relationships among genes.

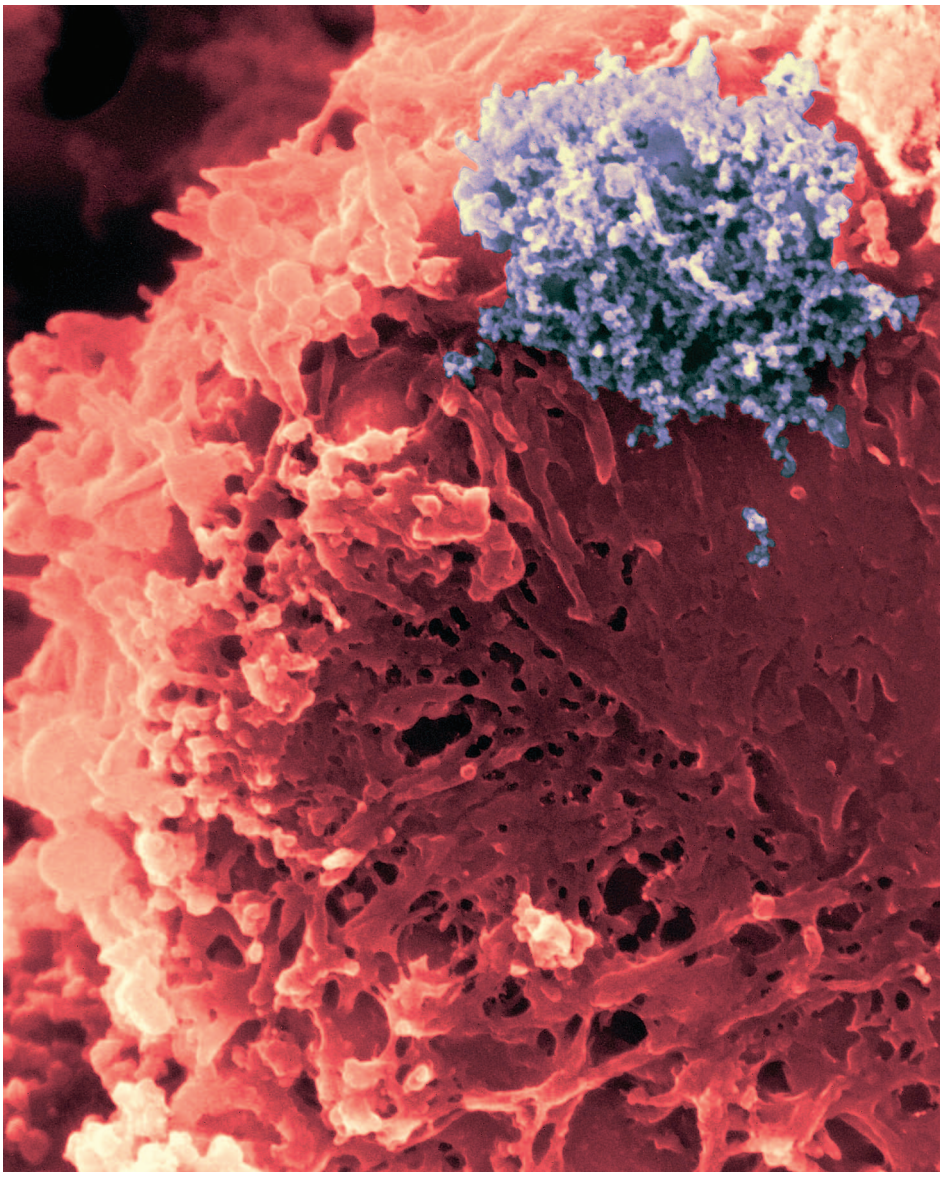
The analysis focused on 200 of the 12,000 clones on the microarrays that showed the greatest changes in response to hydrocarbon exposures. The changes in each of these genes were quantified in relationship to five target genes, selected because they are known to respond to ligands of *Ahr*. The first target gene was *Ahr* itself. The second was *Cyp1b1*, which is involved in the synthesis of steroids.The third was *Igfbp-5*, which regulates the functions of the bones, kidneys, and mammary glands. The fourth was *Lox*, which transcribes a copper-containing enzyme involved in the creation of connective tissue. And the fifth was *Opn*, which transcribes osteopontin, a protein involved in the mineralization of bone and a component of human atherosclerotic plaques.

Activity in all five target genes was related to some degree; when one was expressed, so were the other four. The analysis was not designed to determine whether activated genes were upstream or downstream of each other—in other words, which of a given set of genes initiated a reaction.

The expression of *Ahr* was most closely linked to that of lymphocyte antigen 6, locus e, which is involved in the activation of T cells. This relationship had not been predicted by previous studies and helps fill a gap in the map of relationships among *Ahr* and retinoids (forms of vitamin A). Lymphocyte antigen 6e responds to the presence of retinoic acid, and recent research has shown that *Ahr* controls the expression of genes that metabolize this compound. Thus, computational strategies allowed the delineation of connections between *Ahr* and retinoic acid that otherwise could not have been predicted in the absence of biological information.

In other relationships found in this study, *Cyp1b1* was best predicted by the gene that codes for spleen tyrosine kinase, which participates in signaling leading to activation of the transcription factor NF-κB. *Igfbp-5* was most frequently predicted by *Opn*; *Lox* was best predicted by lymphocyte antigen 6 complex, locus H; and *Opn* was most often predicted by brain-derived neurotrophic factor, interleukin 6, and proliferin.

The authors write that the computational approach they used allowed them to begin constructing gene networks that define broad-ranging interactive biological relationships. "Although the biological bases for these theoretical relationships must be investigated further," they conclude, "the number of possible combinations is now reduced to a manageable size that can be systematically scrutinized using established molecular methodologies." **–Kris Freeman**



T with a twist. Adding an unexpected new element to the map of relationships between *Ahr* and retinoids, recent microarray analysis reveals a novel link between this gene and T cells (above [red], engaging with a virus [blue]).

Taking Stock of Toxicogenomics

Mini-Monograph Offers Overview

Many early concerns about the utility of genomics technologies have largely been put to rest, but several issues remain to be resolved if toxicogenomics is to live up to its full potential. Chief among these is the concern that, although there is a healthy spirit of scientific collaboration and sharing within the toxicogenomics community, standardized submission of and open access to data has not yet been accomplished. The International Life Sciences Institute Health and Environmental Sciences Institute (HESI) Committee on the Application of Genomics to Mechanism-Based Risk Assessment has been actively engaged in working on these challenges by sharing experience, best operating practices, and data to achieve standardization of toxicogenomics data. In this issue, committee members William Pennie, Syril Pettit, and Peter Lord present an overview of the committee's research program [***EHP* 112:417–419**]. The overview by Pennie and colleagues leads into the mini-monograph appearing in this issue, which explores the issues, challenges, and triumphs of using genomics in mechanism-based risk assessment.

Established in 1999, the committee is a collaborative research effort incorporating the unique perspectives and scientific talents provided by its members from government, academia, and corporate organizations representing the pharmaceutical, agrochemical, chemical, and consumer products industries. The committee has conducted and analyzed toxicogenomics experiments within the broad fields of hepatotoxicity, nephrotoxicity, and genotoxicity with two goals in mind: first, to determine whether known mechanisms of toxicity could be associated with characteristic gene expression profiles; and second, to identify technological and biological sources of variability associated with toxicogenomic experimental protocols. The answers to these questions helped the committee evaluate the usefulness of gene expression technology for the purposes of risk assessment.

There is an ongoing need for standardized submission of and open access to data. Researchers also must have access, through public repository databases, to standardized microarray data formats that are linkable to toxicology data. To address these needs, the committee has developed a database in partnership with the European Bioinformatics Institute. Based on the ArrayExpress database structure and Minimum Information About a Microarray Experiment (or MIAME) data format standards, Tox-MIAMExpress will be available to the public early in 2004, and will contain all of the data generated by the committee's research collaborations.

According to Pennie and coauthors, the committee's experimental programs have in fact shown that toxicogenomics is an inherently valuable tool for assessing toxicity. Specifically, they assert that the value of toxicogenomics is supported by the research, which has shown four things. First, patterns of gene expression relating to biological pathways are robust enough to allow insight into the mechanisms of toxicity. Second, gene expression data can provide strong information on topographic specificity. Third, dose-dependent changes can be observed. Finally, concerns about oversensitivity of the technology may be unfounded.

The writers stress that it is important that microarray data results be considered along with other biologic end points to understand the mechanisms underlying toxicity. Pathway-level results will be much more relevant for meaningful risk assessment than single gene expression data, they write, particularly within the regulatory arena.

Clearly, toxicogenomics has come a long way in the short time since the HESI committee was first established. As the mini-monograph in this issue shows, the committee's research portfolio and collaborative approach have contributed significantly to rapid progress in the field. In all likelihood, this contribution will continue, thanks to what the authors call "this collective experience for the benefit of the regulators and regulated industries as well as for the toxicology community as a whole."

The broad impression imparted by the papers, as expressed by Pennie and colleagues in their overview of the program, is that "genomics, and more specifically toxicogenomics, can no longer be regarded as 'new' technology." With increasing experience has come increasing awareness that toxicogenomics is fast maturing. The field has proven its value with solid research and significant additions to the scientific knowledge base; its utility in mechanism-based risk assessment is less likely to be considered tentative, potential, or pending. **–Ernie Hood**

Chris Reuther/EHP. Rupesh P. Amin, et al. [*EHP* 112:465–479 (2004)]

Working together. Researchers are sharing experience, best operating practices, and data to achieve standardization in toxicogenomics data.