A Vision That Challenges Dogma Gives Rise to a New Era in the Environmental Health Sciences

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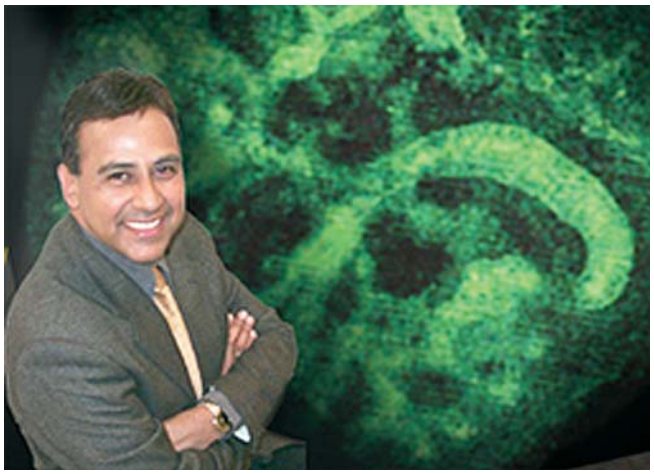

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Reflections

As sequencing of the draft genome of several species is completed and functional genomics studies continue to appear, an exciting new era of biomedical discovery in the environmental health sciences has come upon us. Along with it, technological advances steadily pave the way for a more complete understanding of biology and for realization that integration of molecular information into existing knowledge is essential to unraveling the complexity of biological systems. Diseases with a strong environmental etiology, such as asthma, atherosclerosis, cancer, Parkinson's disease, and sudden cardiac death, can now be conceptualized beyond a select few mRNAs, proteins, or metabolites. And instead of thinking of one gene–one protein–one metabolite at a time, environmental disease can now be evaluated in terms of hundreds or even thousands of molecular targets. With opportunity, however, comes responsibility.

The integration of genomic thinking into environmental health sciences has given rise to the new science of toxicogenomics. The term "toxicogenomics" was broadly defined in the inaugural issue of the Toxicogenomics Section in the National Institute of Environmental Health Sciences (NIEHS) journal *Environmental Health Perspectives* (*EHP*) as the science that combines genetics, genomic-scale mRNA expression (transcriptomics), protein expression (proteomics), metabolite profiling (metabolomics), and bioinformatics with toxicology to understand the role of gene–environment interactions in disease (Ramos 2003). Several other definitions have been offered, and debate continues over what toxicogenomics is and is not and whether toxicogenomics is the dawn of a new science or a fad that will someday go away.

The contributions of NIEHS to advancement of the toxicogenomics research agenda signify the arduous efforts of many, their shared commitment to a goal, and the implementation of a vision that will revolutionize the field of environmental health sciences.

The real value of this debate resides not in the expectation that it will lead to consensus, but rather in the ability to effectively deal with conflict and to increase the level of entropy by leaving the door open for further debate. Meanwhile, the number of papers focusing on genomes and their response to environmental stressors and the number of research programs addressing the application of genomics in drug development, environmental and ecological risk assessment, and molecular investigations continue to soar. The results of the debate will only be known and felt in years to come. For now, I believe, the task at hand is to nurture the debate and to continue to venture into unknown territory.

The complex regulatory code that defines the boundaries of human health and disease lies within the genome. As such, environmental health researchers interested in understanding the pathogenesis of environmental disease, in defining mechanisms of environmental and drug toxicity, in classifying susceptible versus nonsusceptible individuals, and in predicting toxicity outcomes, must decipher the code. Growing evidence indicates that toxicogenomics approaches can in fact be used to define global patterns of gene expression in response to chemical and physical injuries (Aardema and MacGregor 2002), to understand how genes are expressed or inhibited and what their functions are (Kultima et al. 2004) and how the biological instruction manual that prescribes normal function can be altered by the presence of a foreign chemical (Johnson et al. 2004). And during the course of this journey of discovery, toxicogenomics will change the face of environmental health sciences and toxicology.

Transcriptomics

DNA microarrays have been successfully used to address a wide range of scientific questions such as chemical classification, biomarker identification, and phenotypic profiling. Early toxicology studies focused on whether classes of toxicants could be distinguished on the basis of gene expression signatures (Hamadeh et al. 2002a, 2002b). Since then, the numbers of papers using transcriptomics to evaluate chemical toxicity have continued to escalate, and today a significant number of studies can be identified that focus on molecular mechanisms of action (Johnson et al. 2003), classification (Steiner et al. 2004), and biomarkers of exposure and response (Moggs et al. 2004).

Perhaps the short-term scientific impact of toxicogenomics is best exemplified by the ongoing dialog between academia, industry, and regulatory agencies about the role of toxicogenomics in the practice of risk assessment. Gene expression profiling is now used alongside conventional toxicological assays to assess the safety of drugs and chemicals. Although the standards of practice continue to be defined and refined, toxicogenomics is predicated as a hopeful means of continuing to address the growing needs of regulatory agencies and the private sector. Toxicogenomics experiments are now being used to evaluate safety on the basis of similarities, or lack thereof, to reference standards (Kramer et al. 2004), and to gain in-depth understanding of the molecular basis of toxic action (Waring et al. 2002). The maturation of the field of toxicogenomics is evidenced by scientific reports that follow up on mechanistic associations initially derived from large-scale toxicogenomics experiments using focused knockout or phenotypic rescue experiments (Falahatpisheh and Ramos 2003). In class discovery studies, large samples are now being routinely compared to evaluate modes

of toxic action (Hamadeh et al. 2002b) or identify tumor types (Sorlie et al. 2001).

Proteomics

The integration of proteomics and risk assessment has been identified as a powerful tool for examining global chemical effects on biological systems (Fountoulakis et al. 2000). However, the use of proteomics in environmental health and toxicology is not as common as is transcriptomics. This is partly explained by the fact that proteomics is not high-throughput and that the identification of targets remains a substantial challenge.

Proteomics approaches can be used to study covalent posttranslational modifications that are directly or indirectly associated with chemical toxicity (Mason and Liebler 2003). Studies that attempt to integrate the findings of global mRNA with protein measurements are beginning to appear (Andrew et al. 2003; Ideker et al. 2001), giving credence to the expectation that a more comprehensive view of molecular targets of toxic injury will soon be realized. Proteomics analyses afford several advantages in the context of toxicology investigations, most notable of which is the fact that proteins, not DNA, are often the primary target of chemical toxicity. A significant achievement in the field has been the integration of data across the proteomics and transcriptomics domains (Hogstrand et al. 2002; Ruepp et al. 2002). This level of integration will likely continue to drive us closer to understanding the biological response at a more holistic level.

Metabolomics

Recent technological advances in nuclear magnetic resonance spectroscopy and mass spectrometry have aided the conceptual development of a new field of investigation referred to as metabolomics. The application of metabolomic technologies allows simultaneous measurement of cellular metabolites (Fan 1996), biochemical pathways and fluxes (Lu et al. 2002), enzyme kinetics (Gadian 1995), and metabolic/signaling processes (Pillai et al. 2003). These advances are highly significant, as they allow comprehensive assessment of the response to environmental stressors and changes in the cellular microenvironment caused by the presence of toxic chemicals. Within the framework of toxicogenomics, metabolomics is still in its infancy. Evidence of synergistic interactions between genomics and metabolomics is beginning to appear (Coen et al. 2004), suggesting that such associations will nurture global linkages in the analysis of regulatory molecular networks.

Bioinformatics

Without a doubt, bioinformatics remains the most significant challenge to further development and maturation of the field of toxicogenomics. Although environmental health scientists and toxicologists receive considerable quantitative training that spans biological modeling and biostatistics, the gap between toxicologists and applied mathematicians remains considerable. This is best appreciated by the paucity of environmental health and toxicity studies that use sophisticated bioinformatics tools to evaluate "omics" data. If one focuses on studies that address the interdependency of genes and their products in determining adverse biological outcomes, the picture becomes even bleaker.

On these bases, an imminent need in the field of environmental health sciences and toxicogenomics is the development of research and training programs in environmental systems biology. The discovery of gene/protein/metabolite networks is an absolute requirement for a clear understanding of environmental injuries at their most fundamental levels. This goal becomes highly elusive when considering that knowledge of all the network components, particularly in terms of proteins and metabolites, is seriously lagging behind, as is our understanding of the interactions between components within the framework of space and time. Furthermore, the nonlinearity of interactions within a biological network is a critical factor that must be properly considered in view of the fact that environmental health researchers are classically trained to think in linear terms. As such, full realization of the concept of environmental systems biology will likely take decades to develop fully.

Predictive Toxicology

An important goal in environmental health sciences and toxicology is the prediction of toxicity outcomes in the face of limited, or nonexistent experimental data, and using less than optimal experimental models. Many have proposed that omics technologies will make the goal of predictive toxicology a reality. However, realization of this goal will require development and refinement of models that reliably identify hazardous

substances, and the creation of yet more expansive data sets that link gene, protein, and metabolite profiles with pathology and pharmacokinetics to define a specific biological response. The ultimate goal, of course, is to take full advantage of *in silico* technologies to minimize testing time and costs—the most significant obstacles in risk assessment today. Although it is still premature to determine if predictive toxicology is possible, it helps to consider that the cause is indeed far more important than the effort. In the end, after the dust settles and the weight of the evidence mounts, the real winners in the debate will be the consumers, the patients, and society at large.

Reality Checks

The usefulness of omics technologies to evaluate environmental disease and chemical toxicity rests on the notion that chemical or physical injuries of environmental origin involve changes in the relative expression of mRNAs, proteins, or metabolites. Consequently, monitoring these changes is likely to provide insight into the biological response. Clearly, the challenge in moving the toxicogenomics research and educational agenda forward requires realistic understanding of the limitations posed by these technologies. Among the most significant obstacles in this realm are the management and analysis of toxicogenomics data, the ability to complete meaningful cross species extrapolations, and the proper interpretation of toxicogenomics data. Coupled to these limitations is the need for proper validation and verification of the biological findings obtained using omics platforms.

In the end, those interested in toxicogenomics must recognize that biology lies not solely in technology but in the interpretation of the data generated and, most important, in the integration of findings into an existing body of knowledge. To achieve this goal, cross-disciplinary training will become an essential element for successful integration of the omics data into environmental health sciences and toxicology. Multidisciplinary and transdisciplinary training programs are needed to foster a new way of thinking, a new way to design and interpret experiments, and a fundamental change in how scientists in the field of environmental health science and toxicology are currently trained. At a minimum, quantitative analysis must re-enter undergraduate and graduate curricula, as should principles of applied mathematics.

Closing Remarks: The Olden Years

In the span of 4 years (1999–2003), NIEHS created a National Center for Toxicogenomics (NCT), released a request for applications for and coordinated the creation of the Toxicogenomics Research Consortium, expanded its extramural portfolio with supplemental grants to support toxicogenomics experiments, and launched the Toxicogenomics section of *EHP*.

The NCT under the leadership of Ray Tennant advanced the intramural research agenda on the elucidation of mechanisms of environmental injury through a series of studies focusing on the interaction of genes with chemicals and the environment. In addition, the NCT helped catalyze fruitful interactions between the NIEHS and the extramural research community that have resulted in several first-rate publications. The NCT provided the necessary infrastructure to advance the toxicogenomics initiative through collaborations with the six academic institutions that comprise the Toxicogenomics Consortium. In the process, the concepts of molecular diagnostics in environmental disease and phenotypic anchoring have been advanced by the NCT. Furthermore, the development of standards for the generation and interpretation of toxicogenomic data has been greatly facilitated by interactions between the NCT and members of the Toxicogenomics Consortium. For *EHP* the NCT provided fertile ground on which to build and nurture the growth of the journal.

The NCT collaborates with the EMBL-EBI European Bioinformatics Institute, International Life Sciences Institute, Microarray Gene Expression Data Society, and National Center for Toxicological Research Center for Toxicoinformatics on the development of data exchange standards for toxicology and toxicogenomics. A significant accomplishment of the NCT in collaboration with external partners has been creation of the Chemical Effects in Biological Systems (CEBS) knowledge base. CEBS was conceptualized to apply a systems biology way of thinking to understand the biological effects of environmental chemicals and stressors (Waters et al. 2003). Although the database is not yet fully populated, its availability has opened the door for more substantive and meaningful collaboration across the agencies and the private sector.

To promote its vision for toxicogenomics on a more global scale, the NIEHS approached the National Academies to solicit the creation of a public forum for communication exchange among government, industry,

environmental groups, and the academic community concerning emerging issues in the environmental health sciences. In 2002, the National Academies constituted the Standing Committee on Emerging Issues and Data on Environmental Contaminants and charged it to examine the impact of emerging technologies, particularly omic technologies, on the environmental health sciences and to articulate a vision for development of a toxicogenomics framework with applications in environmental and pharmaceutical safety assessment, risk communication, and public policy. Through it all, Dr. Samuel Wilson has played a critical and influential role in helping to shape the national agenda and enabling the field of toxicogenomics. This will be a legacy for years to come.

These milestones signify the arduous efforts of many, their shared commitment to a common goal, and the implementation of a vision that will revolutionize the field of environmental health sciences. Whether one agrees that toxicogenomics will eventually live up to its promise or that it will become a mainstay in risk assessment and regulation, this new field of intellectual endeavor has changed the scientific questions we ask, the answers we expect to get, and the way of thinking for an entire generation of scientists.

And in so doing, the NIEHS and Ken Olden have played a major role in shaping our view of the world. What an accomplishment!

Summary

Environmental health researchers interested in understanding the pathogenesis of environmental disease, or defining mechanisms of environmental and drug toxicity, or classifying susceptible versus nonsusceptible individuals, or predicting toxicity outcomes must decipher the codes encrypted by the genome. To meet this challenge, the marriage of transcriptomics, proteomics, metabolomics, and bioinformatics with toxicology in the emerging field of toxicogenomics allows the study of hundreds or even thousands of molecular targets simultaneously. Whether one agrees that toxicogenomics will live up to its promise or will become a mainstay in risk assessment and regulation, the visionary thinking of leaders at the National Institute of Environmental Health Sciences has paved the way for a new field of intellectual endeavor. Toxicogenomics has changed the scientific questions we ask, the answers we get, and the way of thinking for an entire generation of scientists.

doi:10.1289/ehp.7930 available via *http://dx.doi.org/*

Notes

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The author declares he has no competing financial interests.

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