Care more for the individual patient than for the special features of the disease.
Sir William Osler, Canadian physician/anatomist (1849–1919)

PROTEOMICS

Fingerprinting Cancer Development

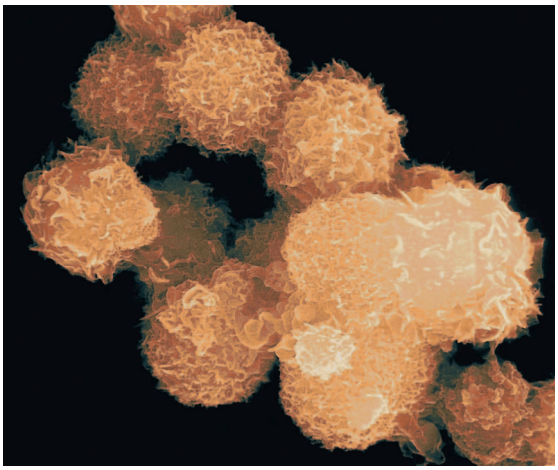
In the world of cancer, early detection is critical for successful treatment, but early diagnostic tools are in woefully short supply. Among ovarian cancer patients, for example, 80% of cancers are diagnosed at a late stage, and, as a result, only 35% of patients live past five years. Now the Clinical Proteomics Program is blending proteomics (the study of proteins in living cells) and clinical cancer research, with the goal of diagnosing cancer earlier, then tailoring drugs to precisely attack tumors with the fewest side effects for patients.

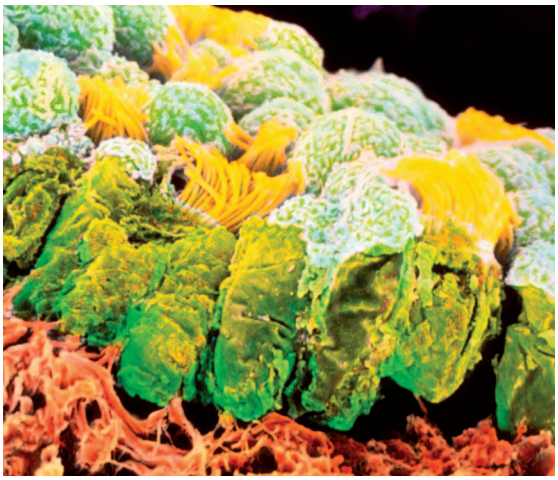
The effort, begun two years ago, is a joint effort of the U.S. Food and Drug Administration and the National Cancer Institute. It is codirected by Emanuel Petricoin, a biochemist at the Food and Drug Administration Center for Biologics Evaulation and Research in Rockville, Maryland, and Lance Liotta, chief of the Laboratory of Pathology at the National Cancer Institute Center for Cancer Research in Bethesda, Maryland.

Whereas genomics deciphers information stored in DNA, proteomics looks at proteins, which carry out the body's complex functions. Program scientists focus on how proteins signal each other and interact in so-called cellular circuits to find key "nodes" to target with drugs. These nodes are critical intervention points in the pathways involved in cancer, spots at which a drug could be used to block a key protein to prevent unwanted events (such as abnormal cell growth) from happening downstream.

The team designs special protein microarrays to detect specific phosphoproteins, which regulate early steps in cancer progression. These protein microarrays detect 50–100 key proteins at a time. "The only way to know if a pathway is [activated or not] is to measure proteomic levels," says Petricoin. Through this proteomics approach, program scientists hope to pinpoint key phosphoproteins that could, for instance, be controlled with a specific drug to block tumor growth with few side effects, as opposed to poisoning both tumors and healthy cells with the harsh chemotherapy drugs used now.

Protein microarrays have helped the team answer a central question in prostate cancer biology: Are rapidly growing tumor cells caused by an increase in growth rate or by a decrease in cell death rate (apoptosis)? In a study published in the September 2002 issue of *Nature Reviews Drug Discovery*, cell growth and apoptosis pathways were compared in normal, premalignant, and invasive carcinoma cells in prostate tumor samples. The researchers found that apoptosis signals were primarily suppressed in early carcinogenesis. "This gives clues about how to treat early lesions," says Petricoin. For example, depending on the proteins found to be important, drugs could be tailored to stimulate apoptosis to remove abnormal cells, or to block cell proliferation to prevent tumor growth, or to impede the growth of blood vessels that feed tumors.





Microwonders. Protein microarrays are being more widely used to uncover the mechanisms of cancers such as prostate cancer (top) and ovarian cancer (bottom), in hopes of targeting intervention in disease development.

Target cells for protein microarrays are gathered using laser-capture microdissection, a technology developed in the late 1990s to tease out normal, premalignant, and tumor cells from tissue samples. In this type of microscopy, a plastic film is suspended a few microns from the microscope slide holding stained cells. When activated by a laser, the plastic film melts, forming a bulge that drops and plucks cells out of the tissue section, explains Petricoin. The cells on the slide are viewed on a computer monitor, and scientists home in on them with a joystick "like in a computer game," he says.

Protein microarrays show patterns, or fingerprints, that identify early-stage cancer or monitor toxic effects of drugs. Petricoin and his colleagues are searching for fingerprints in blood that reflect cancerous processes before tumors are visible. In a study published in the 16 February 2002 issue of *The Lancet*, they used protein microarrays to analyze blood samples from 50 ovarian cancer patients and 66 healthy controls, and correctly singled out all 50 cancer patients and 95% of the controls.

Proteomic fingerprints also detect heart toxicity, a common side effect of the cancer drug doxorubicin. In collaboration with Frank Sistare, acting director of the Office of Testing and Research at the Food and Drug Administration Center for Drug Evaluation and Research, the team obtained preliminary results from a rat model showing that cardiotoxicity was detected 90% of the time before clinical symptoms occurred. "We're trying to find a fingerprint in a drop of blood that identifies early [heart] damage," says Petricoin. Such a fingerprint could identify susceptible patients, who could then be switched to another treatment.

Brian Leyland-Jones, a professor of oncology at McGill University in Montréal, Québec, agrees that sampling blood would be a much preferable means for early detection of cancer. Right now, he says, all known biomarkers must be obtained by the far more invasive sampling of tumor tissue. He adds that new tests based on blood samples "offer the ability to find tumors early on and choose the best customized treatment for each patient." **–Carol Potera**

Top to bottom: Microworks/Phototake; Professor P.M. Motta, Professor S. Makabespl/Custom Medical Stock Photo

ENVIRONMENTAL DISEASE

The EGP at Five Years

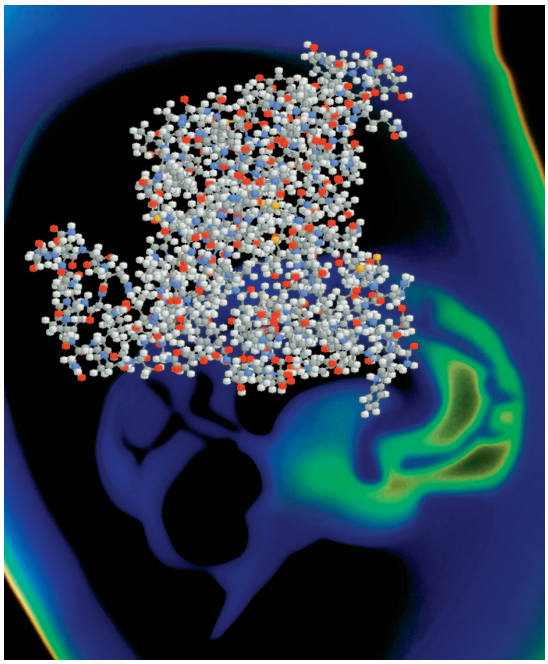
More than 100 scientists, public health professionals, and physicians gathered in Boston 7–9 June 2003 for a symposium, Genes, Environment, and Disease, that provided research updates on the NIEHS Environmental Genome Project (EGP) and its associated Comparative Mouse Genomics Centers Consortium (CMGCC). The EGP was initiated in 1998 with a dual goal: providing information on how individual genetic differences affect disease risk from environmental agents, and proposing appropriate environmental policies in response to that information. In embarking on that quest, the NIEHS divided the work into a number of subject areas ranging from the identification of variations in environmental response genes to the broad examination of the ethical, legal, and social implications of the findings of the EGP.

The symposium occurred only weeks after completion of Phase 1 of the EGP, which called for the resequencing of more than 200 environmentally responsive genes for the purpose of identifying single-nucleotide polymorphisms, or SNPs, that are important in determining disease susceptibility. The mission of Phase 2, which is currently under way and expected to extend to 2004, is to conduct functional analysis studies to characterize the SNP variants discovered in Phase 1. Phase 3 of the EGP, now in its initial planning stages, will include the Molecular Epidemiology Program, in which population-based epidemiologic studies will be initiated to understand the role of gene variations in human genetic susceptibility.

Because animal models are an essential part of Phase 2, the NIEHS created the CMGCC, which consists of five universities. The consortium is charged with developing mouse models containing, among other SNPs, the human polymorphisms identified in Phase 1. The focus to date has been on modeling mice with variants in environmentally responsive cell cycle and DNA repair genes.

At the symposium, NIEHS deputy director Samuel Wilson said that "all of us at the [NIEHS] are very pleased" with the work done so far. But he also suggested that much work lies ahead and warned against succumbing to pressure brought by the public or the media, who may be anxious to see genetically based disease cures. "It's too easy to fall into the trap of 'tell me what it means next week,'" he said. "It's an ongoing activity we're talking about. We're not looking to an end point of five years or even fifty years." He emphasized the importance of sound polymorphic studies, because "otherwise we'll be doing superficial science."

Joan Packenham, the NIEHS program director for the CMGCC, and Kimberly Gray, a health science administrator with the institute's Susceptibility and Population Health Branch, presented overviews of various facets of the EGP. They were followed by CMGCC directors Raju Kucherlapati of Harvard Medical School and Jan Vijg of the University of Texas Health Science Center at San Antonio, who described consortium accomplishments. For example, the CMGCC recently launched the Mouse Federated Database, an integrated bioinformatics tool that allows access to the biological data on CMGCC mouse models. Richard Sharp, an assistant professor of medicine at the Baylor College of Medicine, gave an introductory talk about the ethical, legal, and social implications of the EGP, discussing privacy issues arising from individual participation in research and protection of racial or ethnic groups in which certain genetic variants may be more common.



Uncovering connections. At a recent meeting on the EGP, scientists reported findings on diseases with possible gene–environment interactions, such as spongiform encephalopathies, caused in part by prion proteins.

The balance of the agenda was devoted to presentations by researchers working on a variety of studies related to the scientific interests of the EGP. For example, Jeanne Manson, a research scientist in human genetics and molecular biology at the Children's Hospital of Philadelphia, described her work to identify and evaluate risk factors for hypospadias (an abnormality of the urethral opening on the penis) in 250 families to date. Among her findings, she said, are a relationship between hypospadias and exposure to pesticides, paints and stains, fuels, and solvents. Susan L. Lindquist, director of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported on her research on prion proteins in baker's yeast. Prions self-perpetuate by inducing other proteins to assume their same shape. The research, she said, has advanced understanding of mammalian prions, which cause spongiform encephalopathies that can spread through the environment.

Packenham said the symposium was intended to make the scientific community aware of research being conducted within the EGP and the consortium, and was organized specifically to bring together scientists from two different communities under the EGP: mouse modelers and epidemiologists.

One of those mouse modelers, David Johnson, director of the University of Texas M.D. Anderson Cancer Center Comparative Mouse Genomics Center in Smithville, agreed, pointing out that the utility of the two disciplines meeting with each other went beyond just mutual edification. "We could see where we could help each other," he said. "We [mouse people] needed help identifying which SNPs to model, for instance. And [the epidemiologists] were interested in what we've already found so they can identify the most promising SNPs to do association studies with."

He also noted that there are certain big-picture benefits to meeting with other scientists working within the broad EGP. "I got a much clearer picture of what the overall Environmental Genome Project is and how we fit in," he said. "I also got an idea about how much progress has been made." **–Richard Dahl**

MEETING REPORT

Genes and Environment: A SNPshot

Have you ever heard someone try to dispel concern about their smoking by describing elderly relatives who were lifelong smokers? This gambit usually fails, but there actually is something to the excuse. Increasingly, researchers are uncovering the extent to which genes control susceptibility and vulnerability to environmental health hazards including cigarette smoke, toxic chemicals, alcohol, and more.

Understanding why individuals react differently to the same chemicals requires analysis of differences in their genetic makeup. Single-nucleotide polymorphisms (SNPs) are the simplest differences to examine on the wide scale, agreed participants at Genetic Variation and Gene–Environment Interaction in Human Health and Disease, a seminar held 16 April 2003 at the NIH campus in Bethesda, Maryland. The NIEHS, the National Human Genome Research Institute, and the National Institute on Alcohol Abuse and Alcoholism sponsored the seminar, which was part of an NIH conference marking the 50th anniversary of the discovery of the chemical structure of DNA and the recently completed sequencing of the human genome.

Pinpointing long-term exposure to cancer-causing agents in the environment can be extremely difficult due to challenges such as the near-impossibility of determining a person's diet or occupational exposures over many years. SNPs, on the other hand, are abundant and traceable, said seminar participant Martyn Smith, a toxicologist at the University of California, Berkeley, School of Public Health and director of the university's NIEHS-sponsored Environmental Health Sciences Center. Smith said functional SNPs are likely to explain the majority of people's susceptibility.

A typical gene of 30,000 base pairs has 150 SNPs, noted Deborah Nickerson, a geneticist at the University of Washington in Seattle. Most SNPs have little or no effect on human health. But some greatly influence disease risk. SNPs near one another in the genome can be related, forming blocks in a gene and potentially making it easier to trace susceptibilities in the general population. Only days prior to the seminar, Nickerson discovered such blocks in the *BRCA1* breast cancer gene, which will make it easier for researchers to understand the role of *BRCA1* in breast cancer development in women who don't have rare inherited mutations in this gene.

Smith and collaborators in Leeds, England, are looking for SNPs that confer leukemia susceptibility. Most cases of leukemia arise from gene–environment interactions, he told seminar participants. In the early 1990s, scientists discovered that a SNP on the *NQO1* gene increases the risk of benzene-induced leukemia. This led Smith and colleagues to propose that chemicals that cause oxidative stress and that are detoxified by *NQO1*, such as benzene and flavonoids in high doses, may increase the risk of myeloid leukemia. They also suggest that low folate intake increases the risk of lymphocytic leukemia in adults and children, whereas certain SNPs in folate-metabolizing genes decrease the risk. They are looking at SNPs in genes involved in apoptosis and DNA repair in relation to leukemia risk, and are further expanding their research to the study of lymphoma.

Small changes, big differences. Single-nucleotide polymorphisms are the starting point for investigating genetic variation in response to environmental exposures.

Clement Furlong, a geneticist at the University of Washington in Seattle, reported that some people are more sensitive to insecticides and possibly nerve agents because of genetic variability in the gene that regulates production of the enzyme paraoxonase-1 (PON1). PON1 oxidizes lipids, metabolizes organophosphates, and activates or inactivates medications including statins, glucocorticoids, and antibiotics.

Furlong cited research from the 15 June 1999 issue of *Toxicology and Applied Pharmacology* showing that veterans who suffered from Gulf War syndrome had low PON1 levels. Other studies have shown that injecting purified PON1 into mice without the *PON1* gene protects them against chemical assault. Furlong is confident that injections of engineered recombinant PON1 will someday be similarly used to detoxify humans who have been exposed to organophosphates.

Major advances in molecular methods now enable researchers to rapidly sequence whole genomes and associate SNPs with specific diseases. "We spent many, many years uncovering about a dozen polymorphisms in the [*PON1*] gene," Furlong said at a press conference following the seminar. But thanks to revolutionary new technologies, in just the last couple of months Nickerson and her group have identified more than 150 additional *PON1* polymorphisms. In a matter of days, she sequenced the entire *PON1* gene from four individuals suspected of having sequence variations and then identified those variations, Furlong said.

At the postseminar press conference, NIEHS director Kenneth Olden announced the completion of the first phase of the institute's Environmental Genome Project, which seeks to identify genetic variations among individuals that make them more vulnerable to environmental agents. Research in this phase focused on finding common sequence variations in human genes involved in DNA repair and cell cycle pathways. Future goals involve studying apoptosis, homeostasis, and drug-metabolizing genes, all of which are thought to play a role in vulnerability to environmental exposure. **–Tina Adler**

txgnet

The Pharmacogenetics Research Network and the Pharmacogenetics and Pharmacogenomics Knowledge Base

In the United States, much of the pharmacogenetics research sponsored by the federal government is overseen by the Pharmacogenetics Research Network (PGRN). Formed three years ago, the PGRN is based at the National Institute of General Medical Sciences and counts among its programs research being done at five other NIH institutes and eight universities. The PGRN website, located at **http://www.nigms.nih.gov/pharmacogenetics/**, provides a comprehensive overview of the network's projects and activities.

The Research Network page features a complete listing of PGRN scientists, with links to profiles of their projects. Each profile includes links to the member's website, investigator contact information, a synopsis of the member's work to date, and a link to all submissions the member's group has made to the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB). Also on the Research Network page is information on the governing structure of the PGRN, including network policy statements and links to contact information for its steering and other committees, PGRN interest groups, and the network's industry liaison group. Links to summaries of industry liaison group meetings are also available.

PHARMACOGENETICS RESEARCH NETWORK

The Ethics & Communities page describes the work of the Populations Advisory Group, which was established by the National Institute of General Medical Sciences to consider the possible ethical and legal impact of pharmacogenetics research. The page is stocked with the group's reports to date as well as summaries of ongoing studies in this area and a paper on points to consider when planning genetic research involving a community setting.

The site's News & Events page offers links to information on the PGRN's annual meetings. These one-day meetings, the next of which will be held 8 March 2004 in Los Angeles, California, feature morning keynote sessions followed by an afternoon of presentations by network members on their latest scientific findings. Full meeting reports from previous meetings are available on the site. The News & Events page also features PGRN press releases detailing the growth of the network's membership and offering insight into other significant milestones of the group.

The Related Resources page includes links to two NIH brochures on pharmacogenetics. *Medicines for YOU* describes pharmacogenetics in general and tells how it is used in drug development. *Genes & Populations* explains genetic research for the layperson and tells how this research sometimes focuses on specific population subgroups to study genetic differences. Both brochures are also available from the site in Spanish. Other links lead to the Department of Energy's site on pharmacogenomics, the Pharmaceutical Research and Manufacturers of America's genomics resource guide, the Human Genetic Cell Repository, dbSNP (a database of single-nucleotide polymorphisms), the Protein Data Bank, and PharmGKB.

PharmGKB, an integrated data resource that supports and consolidates the findings of the PGRN, is housed at Stanford University's Department of Genetics; its website is located at **http://www.pharmgkb.org/**. PharmGKB accepts data from scientists both within and outside of the PGRN. Data are organized into searchable categories according to the type of gene–drug relationship studied: clinical outcomes, pharmacodynamics and drug responses, pharmacokinetics, and molecular and cellular functional assays. Visitors to the site can also search for data by other categories including genes with primary data, genes with variant data, drugs with primary data, all diseases, and all pathways, as well as by project.

PharmGKB
The Pharmacogenetics and Pharmacogenomics Knowledge Base

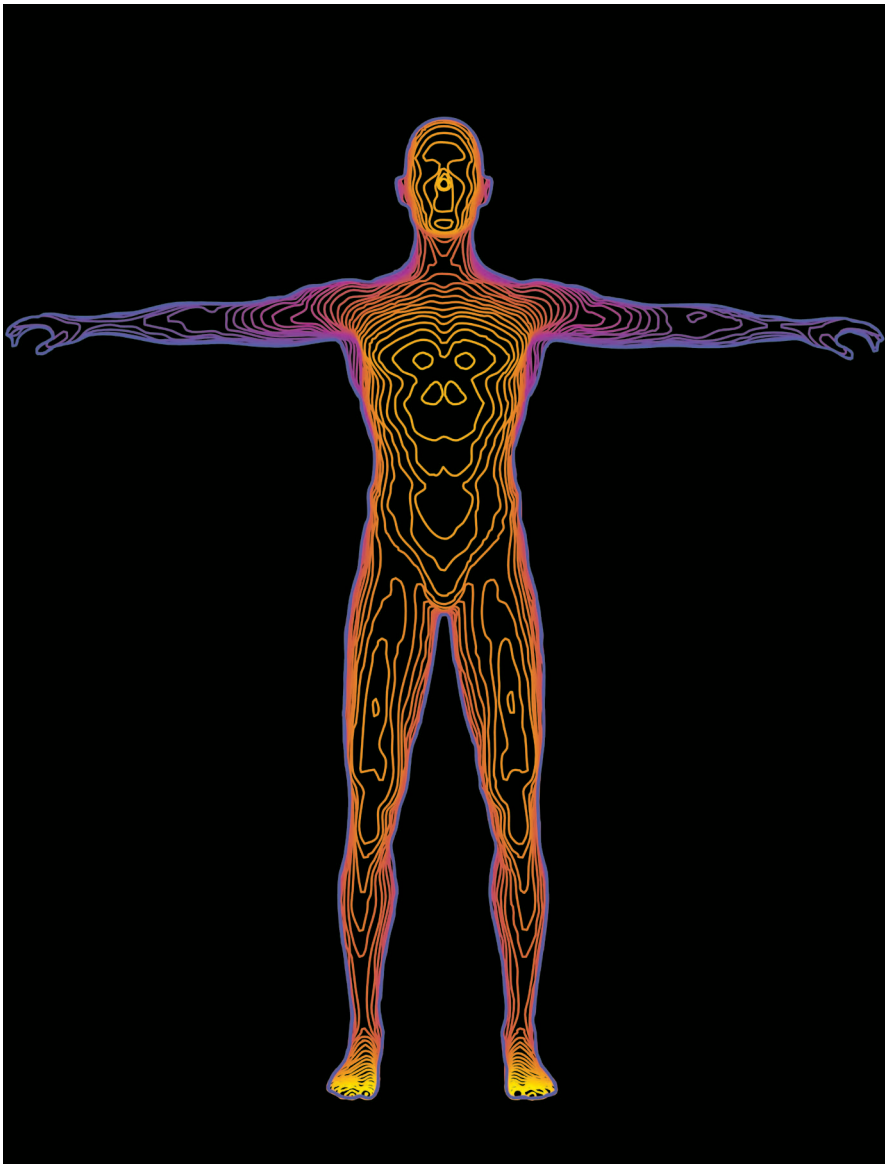
The Overview page provides background information on the project, while annual newsletters, site and citation advisories, and PharmGKB usage policies are available through the bar at the top of the homepage. The Projects page describes two efforts that are ongoing within the group. The PharmGKB Community Project allows scientists to deposit verifiable information on gene–drug relationships that would be classified as phamacogenetic. The Pharmacogenetics Ontology Project is working to develop a standardized mechanism for organizing and annotating pharmacogenetic information.

Researchers and others can find links to a number of useful web-based resources on the Resources page. Among these are the Classification of Expression Array (Cleaver) site, the Environmental Genome Project's GeneSNP site, PHASE (a program used to reconstruct haplotypes), and the Cytochrome P450 Drug Interaction Table. Following the Education link at the top of the Tools page takes visitors to a list of other useful resources, including an overview of pharmacogenetics as illustrated using the Asthma Case Study and the CYP2D6 Study, and a link to the Minority Pharmacogenomics website. **–Erin E. Dooley**

Top to bottom: PGRN; PharmGKB

METABONOMICS

A Big Circuit Model



Systemic perspective. Metabonomics, the analysis of metabolites, offers a new tool for describing the genomic network of the body.

Brand X Pictures

You can call it metabolic profiling, metabonomics, or metabolomics; one speaker at a recent conference called it "biochemistry grown up." Scientists aren't settled on exactly what to call it, but this addition to the growing list of "-omics" subspecialties is rapidly gaining acceptance as a vital link in the chain of knowledge starting at the genome and ending at the whole human body and how it is affected by its environment.

The field's identity crisis did not dampen the enthusiasm of the more than 185 participants at Metabolic Profiling: Applications to Toxicology and Risk Reduction, a conference held 14–15 May 2003 at the NIEHS in Research Triangle Park, North Carolina. The meeting brought together molecular biologists, analytical chemists, toxicologists, clinicians, nutritional scientists, and computational biologists from government, academia, and industry to assess the current state of the science in the emerging area of metabolic profiling, and to define its potential applications to the health sciences. It was organized by the NIEHS, the National Institute of Diabetes and Digestive and Kidney Diseases, the NIH Office of Rare Diseases, the U.S. Food and Drug Administration, Paradigm Genetics, and Waters Corporation.

In metabonomics, biofluids and tissues are analyzed to determine the metabolites present, both in homeostasis and when the organism has been affected by factors such as environmental exposures. "It is both a systems biology and a dynamic approach," said speaker Hector Keun, a postdoctoral researcher at Imperial College of the University of London, "as metabonomics analysis can provide a description of the integrated physiological behavior of an entire living system across time."

Metabolic profiling is a hugely complex undertaking, generating huge amounts of data to be analyzed and mined for nuggets of significant information about metabolic pathways and networks, novel biomarkers, and how metabolites interact not only with genes and proteins, but also with environmental, nutritional, and lifestyle factors.

The integration of this dizzying array of variables holds the potential to tell researchers a great deal about human health and disease etiology, with translational rewards already emerging in diagnostics and drug targeting, development, and safety screening. "Metabolism is phenotype," said Steve Watkins, president of Lipomics Technologies of West Sacramento, California. "It integrates all the factors you'd want to know about, from nutrition to environment to genetics; it's really the only way to assess how you're doing as an individual. If you want to assess your current state of health, you have to [study] the metabolome."

Lipomics Technologies has developed a quantitative assay that measures several hundred lipid metabolites from biosamples. The assay is used to study the role of lipid metabolism in disease and develop diagnostic profiles of drug safety and efficacy. A Research Triangle Park company, Metabolon, has identified a metabolic signature for amyotrophic lateral sclerosis. This profile holds great promise for identifying disease-related biochemical and signaling events, diagnostic markers, and potential therapies.

Metabolic profiling technology is booming in both the analytical and computational realms, but the metabolome is only one element of the entire picture. "If you want to learn about pathways," said Trey Ideker, Pfizer Computational Biology Fellow at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, "you're going to have to characterize metabolites, genes, and proteins, and for each we have interactions

and levels. That's six different things we're going to have to integrate together in a big circuit model, or blueprint, of the cell, and of every cell type, and of every tissue type." The key to meeting this huge challenge, he said, is data mining.

Ideker described work being conducted by his group in developing a computational "scaffold" approach to modeling complex cellular interactions. The modeled scaffolds are mined to reveal a hierarchy of signaling, regulatory, and metabolic pathways. Keun told participants of a system to analyze metabolic profiling data being constructed by the Consortium on Metabonomic Toxicology, a project of scientists at six pharmaceutical companies and Imperial College. The consortium's prototype has already shown promise in elucidating the nature of the relationships between traditional toxicological end points and metabolic descriptors, helping to validate the role of metabonomics data in predictive and mechanistic toxicology.

Scientists expressed excitement at the potential contributions offered by metabolic profiling to toxicology, toxicogenomics, and risk assessment, reduction, and prevention. "There is some incredible technology that can be used to assess risk, link exposure with disease etiology, and reduce the uncertainty of risk in the population," said William Suk, director of the NIEHS Center for Risk and Integrated Sciences. "This is just the beginning."

Kenneth Ramos, chairman of the Department of Biochemistry and Molecular Biology at the University of Louisville Health Sciences Center and toxicogenomics editor for *EHP*, said, "Metabolic profiling can be a much more effective way of communicating risk and of having an impact on risk reduction strategies in the future, because a metabolite is something people relate to and have a better grasp on than genes. And of course, metabolites ultimately being a reflection of the genomic network, it's probably going to be quite significant."

Participants agreed that perhaps the most consequential future direction for the field—where it will ultimately yield its most profound benefits—lies in the integration of metabolic profiling data with genomic and proteomic data. "One of the most important take-home messages from this meeting," said Ramos, "is the recognition that metabonomics is a systems biology–integrated approach that's going to give us meaningful answers to vitally important questions about ecological and human health." **–Ernie Hood**