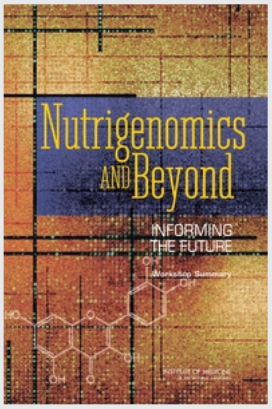

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Nutrigenomics AND Beyond

INFORMING THE FUTURE

Workshop Summary

Ann L. Yaktine and Robert Pool, Rapporteurs

Food and Nutrition Board

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"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the report before its release. The review of this report was overseen by **Jim E. Riviere**, Journal of Veterinary Pharmacology and Therapeutics and College of

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Summary

The integration of biology, genomics, and health has opened the possibility of applying genomics technology to nutrition. In 2001, scientists associated with the Human Genome Project announced the successful mapping of the reference sequence of the human genome. The implications of this achievement to science have been enormous. Since then, a body of information has emerged, including genetic and genomic sequence data, further evidence of nutrient-gene and gene-environment interactions, and the gene expression patterns associated with many chronic diseases. The utility of this information cannot be underestimated. Genomics and related areas of research have contributed greatly to efforts to understand the cellular and molecular mechanisms underlying diet-disease relationships.

Integration and application of genetic and genomics technology into nutrition research is, therefore, needed to develop nutrition research programs that are aimed at the prevention and control of chronic disease through genomics-based nutritional interventions. Of interest is the integration of relevant computational methods into nutritional genomics research; the enhancement of tools applicable to systems biology; and the effective dissemination of genomics-derived information to scientists, policy makers, and the interested public. In short, new knowledge, produced from the interface of genetics, genomics, and nutrition science, is key to further developing research to characterize genetic susceptibility to diet-related chronic diseases and molecular responses to dietary factors.

To address these issues, a workshop was held on June 1 and 2, 2006, at the National Academy of Sciences. The workshop included a keynote presentation to provide policy context and challenges, and presentations that were structured around three focus sessions: human genetic variation, epigenetics, and systems biology (see Chapters 1 to 3, respectively). A fourth session (Chapter 4) presented discussions on the implications of nutrigenomics for the future of nutrition science research. The workshop agenda is contained in Appendix A, and Appendix B lists the names and affiliations of the workshop presenters.

Numerous themes emerged from the workshop presentations. First, nutrigenomics is a complex field because it addresses issues related to multigenetic traits that can be modified by a number of nutritional and other environmental factors. For example, more than 25,000 bioactive food components have been identified, although their modes of interaction and duration of activity are among many questions about these compounds that are still unanswered. Such complexity presents a challenge to the field; and the ensuing research opportunities will require cooperative work among scientific disciplines and across government, academic, and industrial centers, as well as adequate funding, to be realized.

Additionally, the ability to stretch the limits of conventional research methodologies afforded by new genetic and genomic applications at the level of the individual opens the door to a wealth of potential benefits to areas such as disease prevention and wellness, bearing in mind the necessity of ethical safeguards. This potential, however, must be wisely exploited to avoid the pitfalls of overpromising research results and prematurely setting unrealistic expectations for beneficial outcomes. Finally, careful and rigorous research must be employed to optimize outcomes and assure acceptance by the scientific community. In summary, nutrition science is uniquely poised to serve as the crossroads for many disciplines and, using genomics tools, can bring this knowledge together to better understand and address diet-related chronic diseases and molecular responses to dietary factors.

The Promise of Nutrigenomics

INTRODUCTION

The tools of modern molecular biology have transformed many areas of science, from searching for a cure for cancer to understanding the evolutionary relationships among species. Few areas, however, stand to see more of a transformation than the science of nutrition. In the coming years a confluence of factors is promising to make nutrition and, in particular, nutrigenomics, an area in which some of the most exciting and cutting-edge research in biology will take place, as well as offer a wealth of benefits to human health.

In light of this, the Institute of Medicine held a 2-day workshop, Nutrigenomics and Beyond: Informing the Future. Its purpose was to explore the state of the science, examine its potential, and discuss how that potential might best be realized. The following is a summary and synthesis of the presentations and the discussions from the workshop.

KEYNOTE ADDRESS

Presented by Bernadine Healy, Health Editor for U.S. News & World Report

Why Nutrition and Genomics Are Important

Bernadine Healy, former director of the National Institutes of Health (NIH) and now health editor at *U.S. News & World Report*, opened the workshop. She remarked that nutrition research has always had the

potential to benefit human health in important ways. In the 1920s, for example, one of the early successes of the fledgling NIH was the discovery by Joseph Goldberger that he could cure pellagra, a deficiency disease characterized by dermatitis, diarrhea, dementia, and eventual death, by the simple expedient of adding baker's yeast to the diet. (It would take another decade to identify niacin as the ingredient in baker's yeast that was the factor that prevented pellagra.)

Unfortunately, Healy said, the field of nutrition has been woefully neglected by the biomedical community over the years. Medical schools, for example, spend little time teaching nutrition to medical students; and academic researchers find it difficult to get funding for nutrition research. Part of the reason for that neglect, historically, was the perception in the first half of the 20th century that nutrition was "women's work" that was best left to home economics departments; and, indeed, many of the early leaders in the science of nutrition were women, including Ellen Swallow Richards, the first woman admitted to the Massachusetts Institute of Technology and its first female instructor, and Lenna F. Cooper and Lulu C. Graves, who founded the American Dietetic Association.

Healy experienced that neglect firsthand in the early 1990s when, as NIH director, she pushed for a bionutrition initiative to bring different fields together to do integrated systemswide research on food and nutrition. According to the document outlining the proposed initiative, "Bionutrition research employs molecular and genetic techniques to study the metabolic and behavioral consequences of food and nutrients and explores the fundamental role nutrition plays in health maintenance and disease treatment. It encompasses studies on nutrients at the cellular level, the metabolic functioning of nutrients in living organisms including humans, blessedly, and studies on gene-nutrition-environment interactions."

The initiative failed to gain support, in part because of the difficulty of convincing scientists from different areas to come together in a cross-disciplinary project, but also because it failed to capture the interest of either the politicians who would be funding it or the broader public. It was, in Healy's words, "dull, totally and utterly dull."

That is not a problem with nutrigenomics today, as the field has already captured the public's imagination. A number of commercial nutrigenomics websites, for example, offer to analyze a person's DNA and provide advice on what foods to eat and what foods to avoid. If anything, the problem with nutrigenomics could be raising expectations too high. "This is a young field," Healy said, "and you could kill it off very quickly by overpromising."

Nevertheless, the promise is real. For the first time, researchers have the tools to understand how genes and nutrients interact on the molecular level—to drive hard science into nutrition, as Healy put it. The human

genome has been sequenced, and DNA microarrays allow researchers to scan quickly thousands of genes to see which ones are being expressed and at what levels, or to pinpoint genetic variations that differ from one person to the next. The different "-omics" disciplines (e.g., genomics, proteomics, and metabolomics) offer a variety of powerful ways to understand what is going on inside the cell in response to nutrients and to see how those responses differ from person to person. Advances in nanotechnology also promise to offer entirely new ways of observing and manipulating cellular functions.

Nutrigenomics offers a tremendous opportunity for biomedical scientists. For more than a century, scientists have worked to understand nutrition, but it has never been approached in a rigorous scientific way. Now the tools exist to do the kind of cutting-edge research that can push the field in an entirely new direction, one full of novel scientific insights and valuable applications.

It is more than just a matter of having the right tools, however. A number of different factors are coming together that offer the chance to make nutrigenomics a major new area of research. The Women's Health Initiative has shown, for example, that it is possible to do large-scale clinical trials on specific topics in the field of nutrition, which will be important in nutrigenomics research. Over the past decade, a great deal of work has been done in understanding the precise chemical profiles of foods, so that investigators now have a good idea, for example, of which antioxidants are found in which foods and at what levels.

Furthermore, public opinion is now favorable to the approach to medicine embodied by nutrigenomics. People are very interested in functional foods[1] and in the idea that foods can have health-promoting or disease-preventing properties beyond the basic nutritional value that they provide. The field of medicine is also showing growing interest in the idea of personalized, targeted treatments and is moving away from the idea that "one size fits all." In addition, the obesity epidemic has caused many people to be more interested in the general subject of food and how it interacts with their bodies. This convergence of factors offers a rare

[1]Functional foods contain nonnutrient compounds (bioactive food components) that confer a beneficial physiologic effect that delays or prevents the onset of chronic disease. These bioactive components do not work in isolation. Furthermore, it is apparent that not all individuals respond to bioactive components in the same way; interactions at the genetic level result in a great deal of variability in response. There are at least 8 million single-nucleotide polymorphisms that may contribute to individual variation in genetic responses to nutrients and bioactive components. Conversely, nutrients can also modify epigenetic events. DNA methylation, for example, is an epigenetic event that occurs in response to nutrient stimuli.

opportunity, Healy said. "This is the time for the science of nutrition. This is the moment, and we have to seize it."

INTRODUCTORY COMMENTS TO THE SCIENTIFIC SESSIONS

Presented by John Milner, Chief, Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute

There is, today, an unprecedented opportunity to use foods and food components to aid in achieving the genetic potential of humans, improve the overall performance of humans, and reduce the risk for chronic disease. The field of nutrigenomics holds promise for understanding genetic variability and identifying individuals who will or will not respond to a specific dietary change. A key part of understanding how individual variability affects the response to dietary change lies in data coming from research on bioactive food components. Nutrigenomics is the field of research that serves as the bridge to understanding individual variability in the responses to bioactive foods that are consumed and the expression of nutrient-responsive genes.

The current expenditure on nutrition research from federal sources in the United States is approximately $1.2 billion per year. Because this amount of money is relatively small in comparison with the research dollars spent in other areas, it is important that they be spent wisely and in a way that will advance nutritional science. This means finding ways to integrate nutritional science with other basic sciences and with preclinical and clinical models. The Women's Health Initiative was an important step in setting the stage for nutrigenomics research. The science presented in this workshop is furthering that initiative and moving nutrition science forward toward understanding the role of nutrients and bioactive food components in gene expression.

1

Scientific Session I: Human Genetic Variation and Nutrition

Moderated by Nicholas J. Schork,
Director of Research, Scripps Genomic Medicine, and
Professor, Department of Molecular and Experimental Medicine,
The Scripps Research Institute, La Jolla, CA

NEW TOOLS FOR UNDERSTANDING THE ROLE OF GENETIC VARIATION IN HEALTH AND DISEASE

Presented by Francis S. Collins, M.D., Ph.D., Director,
National Human Genome Research Institute,
National Institutes of Health

Over the past decade the field of genomics has seen spectacular and exciting progress. The human genome has been sequenced; and a host of new fields, including genomics, proteomics, and metabolomics, have been developed to use the information made available from the identification of genes and related products. The tools developed in these areas have wide applicability, from medicine to evolutionary biology. However, as Francis Collins noted in the opening session of the workshop, few fields stand to gain more from the application of genomics than the study of human nutrition.

The paradigm for the field of nutrigenomics (see Box 1-1) was established by earlier experiences with nutrient-related diseases, such as phenylketonuria (PKU) and other metabolic disorders. Children with PKU, for example, are unable to metabolize the amino acid phenylalanine into tyrosine, and, if they are left untreated, can develop mental retardation. The discovery that PKU resulted from a genetic mutation led to an effective treatment: a diet that eliminates foods high in phenylalanine, such as dairy products, meats, nuts, and starchy foods. Today infants are routinely

BOX 1-1
Nutrigenomics Defined

"Nutritional genomics or nutrigenomics is the application of high-throughput genomics tools in nutrition research. Applied wisely, it will promote an increased understanding of how nutrition influences metabolic pathways and how this regulation is disturbed in the early phase of a diet-related disease and to what extent individual genotypes contribute to such diseases."

SOURCE: Nature Review Genetics, 4:241 (April 1, 2003).

screened for PKU, and those identified as having the disease-causing variant gene are able to live a normal life with dietary modification.

Nevertheless, Collins remarked, "The field of nutrition has not always come across to us hard-nosed scientists as being based on a very rigorous set of scientific findings. The evidence has not impressed us as being as solid as it might be." The tools of genomics and related fields thus offer the promise of putting the field of nutrition on a firmer scientific footing, supported by both experimental evidence and theoretical understanding. Ultimately, genomics has the potential to dramatically expand comprehension of how nutrients affect the human body and to personalize nutrition, making possible individualized nutritional recommendations. Although nutrigenomics is still an emerging field, many scientists already believe that it will revolutionize the science of nutrition.

The Search for Nutrition-Related Genes

Nutrigenomics can be used in two different ways. The first way is to provide a better understanding of nutrition as it applies to the general population. The second way is to provide an understanding of nutrition at the level of the individual, exploring how nutrients affect people differently, depending on genetic variation.

The first step toward realizing that vision is finding the genetic variants involved in human disease, or what Collins termed "those ticking time bombs that are lurking within our genomes." The problem is that until recently researchers have not had at their disposal efficient and cost-effective tools for finding the genetic variants that increase the risk of common diseases or identifying the environmental triggers, such as diet, that may set them off.

"We have done very well with Mendelian conditions (for example, PKU) and not so well with the genetic variants that contribute to things

like diabetes or heart disease." The reason is that unlike Mendelian disorders, which are caused by mutations in a single gene, most common diseases arise from the interaction of many different genetic variants, each of which contributes only a small amount to the total effect. For example, heart disease generally results from a subtle interplay between many different genes and a variety of environmental components, such as diet, none of which has a major influence by itself.

The Genomics Revolution

There is comparatively little variation in genetic makeup between individuals. When the genetic sequences of two individuals are aligned and compared, only about 1 of every 1,000 base pairs of the nucleotide sequence of human DNA exhibits variance. Many of the variations occur as differences in just a single base pair, or "letter" in the DNA code, for example, a cytosine (C) in place of a guanine (G). Scientists refer to a variation involving a single base pair as a single-nucleotide polymorphism (SNP) (Figure 1-1).

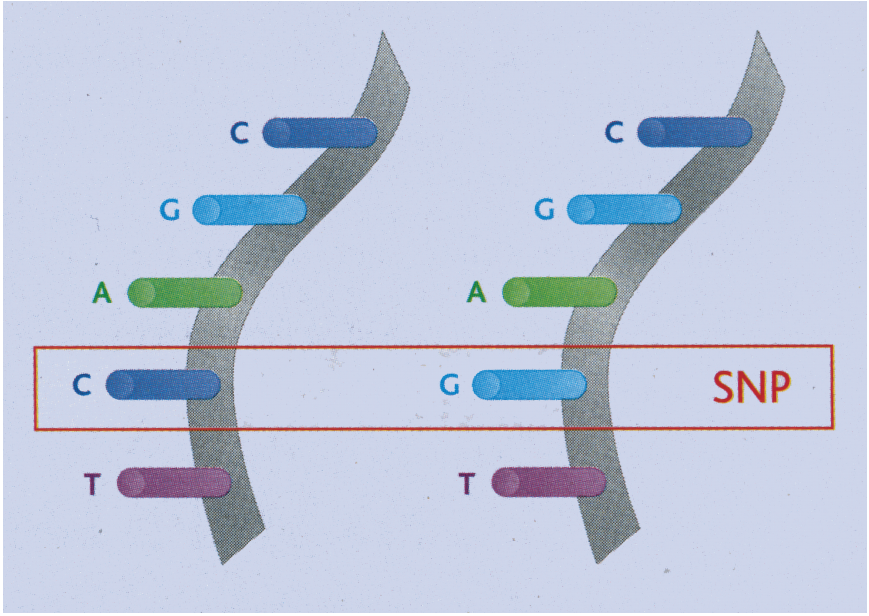

FIGURE 1-1 An SNP is a variant in the genetic code that consists of a single-letter difference in the nucleotide sequence that makes up, for example, DNA.

"Still, when you consider that the size of the human genome is 3 billion base pairs, even a 0.1 percent difference between two people adds up to a lot of genetic variation; some 3 million base pairs" Collins explained. "Most SNPs occur in parts of the genome that aren't doing very much and therefore don't have a lot of consequences. But, some of those differences, probably a couple hundred thousand or so, do have important phenotypic effects. It is those that we are most interested in learning about, even though their effects are likely to be subtle."

To understand a complex disease, particularly one for which nutrition is important, researchers must identify which of the approximately 10 million common SNPs in the human genome are associated with an increased risk of that disease. However, such studies, called "whole-genome association studies," have been a practical impossibility to conduct, until recently. Because the effects that one is looking for are small, such studies need to look at large numbers of people, ideally, at least 1,000 case patients and 1,000 control subjects, for a total of 2,000 subjects. If one were to analyze, or genotype, all 10 million SNPs in each study participant, a total of 20 billion SNPs would have to be genotyped in such a study. Just a few years ago the cost of genotyping was about 50 cents per SNP, which meant a whole-genome association study involving 2,000 subjects would have carried a staggering price of about $10 billion.

"But within the space of just four years, that has all changed," said Collins, noting that two factors have come together to make whole-genome association studies a reality. The first is the completion of the International HapMap Project. That effort relied on understanding how DNA behaves during the process of meiosis (division of the cell nucleus) and recombination. When the strands of DNA break during this process, they tend to break only at certain "hot spots" along the DNA, which means that long stretches of DNA move from generation to generation without ever being broken. These stretches, called haplotypes, generally contain many SNPs that travel together in a neighborhood. Consequently, by identifying just one SNP on a haplotype, it is often possible to predict the other SNPs that reside on that stretch of DNA.

On average, haplotypes span about 20 kilobases (20,000 base pairs) of DNA and contain about 30 to 40 SNPs. "The trick is that these haplotypes are not all 20 kilobases," Collins said. "Some of them are only 1 or 2 kilobases and some are 100 or 150 kilobases. The only way to figure out the boundaries of these neighborhoods is to do the experiment on a certain number of DNA samples."

That is what the International HapMap Project did. It examined 270 samples from four different populations of people whose ancestors were from northern and western Europe; Yoruba in Ibadan, Nigeria; Tokyo, Japan; and Beijing, China (Han Chinese). The International HapMap

Consortium (2003)[1] reported that the Phase I map, a public database of more than 1 million SNP genotypes from the four populations, had been successfully completed and that an even denser Phase II map is now available.

"So, instead of genotyping all 10 million SNPs in each study participant's genome, researchers can pick a carefully chosen set of about 300,000 marker SNPs for European ancestry or Asian populations, and these serve as very effective proxies for the rest," said Collins, adding that African populations require slightly more SNPs because their longer population histories have led to somewhat less linkage between the SNPs.

Thanks to the data generated by the HapMap Project, a whole-genome study of 2,000 subjects today requires a total of only 600 million genotypes instead of 20 billion. Yet, the costs of such a study would still be too expensive, except for the fact that over the past 4 years the cost of genotyping has plummeted from about 50 cents per genotype to about one-third of a cent. Thanks to these dramatic improvements in tools and technologies, researchers today can genotype 2,000 subjects for about $2 million, a relatively affordable cost that has made possible a wide range of whole-genome association studies focused on common complex diseases.

This new genomics capability holds tremendous promise for a number of fields but perhaps none more than nutrigenomics. Researchers would begin by collecting information about an individual's genetic makeup, along with information about what the person eats. They would then compare those data with various health outcomes, for example, blood pressure, cholesterol levels, and rates of cancer and other diseases. With a solid base of research on how genes and diet interact to influence the risk of disease, it should eventually be possible to develop a personalized science of nutrition that would provide an understanding of why individuals respond differently to the same diets and that would allow nutritional recommendations to be tailored to individuals on the basis of their genetic inheritance.

Looking to the Future

If nutrigenomics is to fulfill this promise, the effort will require a multidisciplinary approach involving scientists from a number of areas other than genomics. Research will be needed, for instance, to develop better ways of determining food and nutrient intakes. Collins observed, "We have to get beyond some of the questionnaire-based methods of assessing intake that have been the mainstay of epidemiologists for a long time." These food-frequency questionnaires are now known to be "hopelessly

[1]The International HapMap Project Nature. 2003 (December 18). 426(6968):789-796.

inaccurate," and nutrition researchers need methods of assessing food intake that will measure what people actually eat instead of what they remember eating.

Fortunately, a number of new high-tech methods of assessing dietary intake are now being developed. One method, for example, depends on a network of cell phones that people in a survey would use, at no cost to themselves, to report what they are eating on a real-time basis, perhaps even using the camera feature of the cell phone to take a picture of the meal.

According to Collins, researchers interested in applying nutrigenomics will face another, more familiar hurdle: finding funding. Even though the cost of whole-genome association studies has dropped by a factor of 5,000 over the past several years, nutrigenomics studies will still require significant resources. Fortunately, though, funding sources are appearing. One of them is a public-private partnership of the Foundation for the National Institutes of Health called the Genetic Association Information Network (GAIN). The partnership is making funds available for whole-genome association studies that will use data from existing case-control studies of patients with common diseases. GAIN has funds on hand for six studies with 1,000 case patients and 1,000 control subjects each. The deadline for submitting proposals for that round of funding was May 9, 2006. However, other rounds will be announced as funding becomes available.

A second possibility is the Genes and Environment Initiative, which was in President George W. Bush's fiscal year 2007 proposed budget and which has been strongly promoted by the secretary of the U.S. Department of Health and Human Services. The initiative's aim is to increase the understanding of the complex interplay of genes and the environment in the development of common diseases, and diet is one of the major environmental factors under consideration. The initiative has two main components. "One is to encourage yet more genotyping of case-controlled studies of common disease. In this initiative, we are particularly looking for studies where good data is available on diet, physical activity, and environmental exposures in order to be able to draw conclusions about interactions," Collins said. "There is also $14 million a year for the next 4 years to develop innovative technologies to measure those environmental exposures, including dietary intake and physical activity, in a more rigorous fashion than what has often been the case in these kinds of studies."

Even as scientists are beginning to appreciate the potential of nutrigenomics, the public is already fascinated. For instance, a number of Internet sites offer products based on the idea that one's diet should be dictated by one's DNA. In general, companies analyze a few genes that are known to be involved in metabolism or that are influenced by certain nutrients,

determine which variants of these genes that a person has, and then make dietary recommendations based on that information. The concern, according to Collins, is that most of these recommendations are based on very slim evidence. The gene variants analyzed may have been found to be associated with particular outcomes, for example, higher cholesterol levels in people who also eat a high-fat diet, but there have been no prospective studies showing that people taking such dietary advice actually benefit from it.

"So, we already have a problem here," Collins said. "Before the field of nutrigenomics has barely gotten itself defined, we have a lot of activity out there marketing products to the public that are, at best, sketchy in terms of their scientific basis. "We need to move quickly. The easiest way to kill a field is to overpromise, and there is a lot of overpromising going on."

Nutrigenomics has tremendous potential to revolutionize the understanding of nutrition, particularly nutrition on the individual level, and to help move the focus of medicine from treatment to prevention. There is also a risk, however, that nutrigenomics could come to be seen as just one more food fad in a long line of food fads. To avoid that fate, Collins said, "We need to give it some good science to grow on."

IMPLEMENTATION OF THE HUMAN HAPMAP INITIATIVE AND LARGE-SCALE POLYMORPHISM STUDIES

Presented by David Cox, Chief Scientific Officer, Perlegen Sciences, Inc.

Over the past 20 years researchers have learned that most complex traits involve probably 20 or more individual genetic changes, according to David Cox. Each of those changes is responsible for only a small contribution on its own, so to understand the genetics of a particular complex trait, it is necessary to identify at least 20 or so individual genes. Many more than 20 may influence the trait, Cox said, but generally 20 or so genes in aggregate will account for enough of the variation in the trait to be practically useful.

According to Cox, once the various genes that influence a trait have been identified, it is generally possible to estimate a person's risk for disease by adding the risks from the individual genes. In statistical terms, that property is called "additive variance." "What we are doing," Cox said, "is just slamming them all together and saying that it doesn't really matter which one does what. You can just add them together without understanding the interactions at all, without understanding the mechanisms, and you can use it in a very useful predictive way."

The key is to identify the individual genes that influence a particular trait, and the smaller the contribution is, the more difficult it is to pinpoint the gene. A hypothetical example can be used to describe the situation: a gene may have two variants, referred to as "Variant A" and "Variant B," and a person with two copies of Variant B has a 50 percent greater chance of developing a trait such as heart disease than a person with two copies of Variant A. The gene would then explain a relatively small part, for example, 2 percent, of the total variation in the population, that is, which people get heart disease versus which people do not; but the risk would still be significant enough to pay attention to the gene, particularly in combination with other genes with similar contributions.

For a gene with this level of effect, one would need several hundreds of subjects of each variant type, that is, those with two copies of Variant A and those with two copies of Variant B, to achieve sufficient statistical power to identify the gene's contribution with a reasonable level of certainty and accuracy. If the contribution of the gene were smaller, a larger subject pool would be needed to determine significance.

The traditional way to identify genes that influence a particular trait has been to use the candidate gene approach. Genes that are likely to be involved, based on previously identified traits, are selected and subjects, both carriers of the trait and noncarrier controls, are tested. The problem with this approach is that it requires that one be able to identify ahead of time most of the candidate genes, and that is seldom the case.

As an example, Cox described a study performed to identify the genes involved in determining the level of high-density lipoprotein (HDL) production, an indicator of risk for cardiovascular disease. Individuals with high HDL levels are at lower risk for coronary heart disease than those with low HDL levels. Although a number of candidate genes that potentially explain a large percentage of the variation in HDL levels among individuals were selected for genetic study, only one of these genes was found to be significantly associated with variations in HDL levels. Individuals with one copy of Variant A and one copy of Variant B of the gene were twice as likely to have high levels of HDL than people with two copies of Variant B. Nevertheless, the gene accounted for relatively little of the total variation among people with regard to their HDL levels. The findings suggest that a large fraction of the genetic variation that leads to differences in HDL levels is in genes other than the "traditional" HDL candidate genes.

Cox referred to the example of "looking under the lamppost," because "that is where the light is. So, we clearly have to light up the street, the entire genome, if we are going to have the power to do this more systematically; and the good news is we have arrived at that ability."

CONTEMPORARY NUTRIGENETICS STUDIES

Presented by Jose M. Ordovas, Senior Scientist and Director of the Nutrition and Genomics Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University

Jose Ordovas offered an example of how nutrigenomics can be used to make sense of what otherwise seem to be confusing and contradictory findings in the field of nutrition. As Ordovas pointed out, a variety of large-scale studies have failed to find a consistent effect of diet on health, despite expectations to the contrary. A study published in the *Journal of the American Medical Association*[2] found no evidence that eating fruits and vegetables lowered the risk of breast cancer, for example. Another study[3] found no effect of either vitamin E or aspirin on the incidence of breast cancer in women. Still others have raised questions about the usefulness of low-fat diets in preventing heart disease.

To show what those studies may be missing, Ordovas focused on a particular question: how levels of HDL, the "good cholesterol," are affected by having a particular version of the gene for apolipoprotein A-1 (*APOA1*). In analyzing data from the large-scale Framingham Heart Study, Ordovas said, it would appear at first glance that the different versions of *APOA1* make no difference to the levels of HDL in a person's bloodstream, as the average HDL levels are the same among the groups with the different genes. He said that the analysis, however, does not take diet into account and that when diet is taken into account, a totally different picture emerges.

The Ordovas laboratory reanalyzed the data and further divided the groups according to how much polyunsaturated fat that they had in their diet. The *APOA1* gene has either the nucleotide guanine (G) or adenine (A) in their DNA at a particular locus, or position, in the *APOA1* gene sequence, depending on which version of the gene a person inherits, so a person's two copies of the gene can be GG, GA, or AA. Ordovas found that for people with the GG genotype, the more polyunsaturated fat that they had in their diets, the lower their levels of HDL were; but the pattern was exactly the opposite for the groups with GA and AA: their HDL levels went up with higher levels of consumption of polyunsaturated fat.

[2]Prentice et al. 2006. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *Journal of the American Medical Association*, 295:629-642.

[3]Cook et al. 2005. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *Journal of the American Medical Association*, 294:47-55.

Ordovas summarized by indicating that "it is particularly important for people of GG type to minimize their consumption of polyunsaturated fat in order to keep their levels of the good cholesterol as high as possible." Conversely, people with the GA or the AA form of the gene can achieve increases in good cholesterol levels by increasing their dietary intake of polyunsaturated fat. It is thus important for both doctors and researchers to take into account the genetic makeups of their patients when they try to understand and predict the effects of diet on health.

2

Scientific Session II: Epigenetics

Moderated by Rowena Matthews,
G. Robert Greenburg Professor of Biological Chemistry,
Research Professor in the Life Sciences Institute, Research Professor in the Biophysics Research Division, and Professor in the Department of Chemistry, University of Michigan

CRITICAL EVENTS: GENOMIC PROGRAMMING AND REPROGRAMMING

Presented by Rudolf Jaenisch, Professor of Biology, Massachusetts Institute of Technology

Genomics studies can identify the specific genes involved in the body's response to nutrients and pinpoint the genetic variations responsible for differences in a given response among individuals, but such studies have little to say about how nutrient molecules interact with genes to modify their expression and why nutrients can affect genetic expression long after the interaction has occurred. To address these questions, a different approach is needed, one that delves into the molecular mechanisms underlying the genetic response to nutrients.

Rudolf Jaenisch, who is a founding member of the Whitehead Institute, offered three definitions of epigenetics that provide different but complementary ways of viewing the same event. First, epigenetics is "the transmission of information through meiosis or mitosis that is not based on the DNA sequence." That is, during cell division (which accompanies meiosis or mitosis) information encoded in the DNA sequence is passed from one stage of cell division to the next; epigenetics concerns itself with the remainder of that information. Second, epigenetics is "a mechanism for the stable maintenance of gene expression states that involve physically marking the DNA or its associated proteins." Because gene expression depends not just on the state of the DNA but also on the state of the

entire chromatin complex, including both the DNA and its protein scaffolding, epigenetics takes all of this into consideration. Third, epigenetics is "mitotically or meiotically heritable changes in gene expression that are not coded in DNA itself." Gene expression states can be passed on from one cell generation to the next, which happens, for example, during embryonic development, when cells differentiate and then reproduce to form lines of specialized cells (e.g., nerve cells and muscle cells), each of which is defined by specific genes. These genes can be either activated or silent. Gene expression states are heritable, making it possible to express traits that are not dependent on the expression of a given gene per se.

In short, epigenetics describes the way in which cells store and pass on information that is not coded in the DNA sequence itself but rather in various modifications made to the DNA and, more generally, to the chromatin complex containing it.

Epigenetics is thus an important tool for nutrigenomics because it offers a way of understanding how nutrients interact at the molecular level with the genome to create long-lasting effects. "Epigenetic regulation is a mechanism that allows the genome to integrate intrinsic signals and environmental signals. It is a way the genome interacts with the environment. So, what you ate for lunch has found its way to change in some very subtle way the epigenetic state of your DNA." That, in turn, is related to how diet can affect health and, in particular, the risk of certain diseases. When gene-environment interactions alter the epigenetic state of the genome, they may affect the incidence of diseases with long latencies or late-stage onset, such as cancer and neurodegenerative diseases.

Relatively little research that has applied the tools of epigenetics to nutrigenomics has been conducted to date; but epigenetics is a rapidly developing field, one that has been invigorated by the sequencing of the human genome. The growing arsenal of tools and techniques available from the study of epigenetics thus offers new and revolutionary ways of studying how nutrients interact with the genome.

The Agouti Mouse Paradigm

The agouti mouse can express a number of different phenotypes. It can be yellow and obese or brown and slim. It can have a mottled yellow or a brown coat. These differences, however, are not genetic in origin. These mice are genetically identical. The differences arise from variations in the expression of the agouti gene; and coat color expression can be controlled by varying the mother's diet before, during, and after pregnancy. The agouti allele is normally expressed only in a mouse's skin, creating a yellow fur wherever it is expressed, but in agouti mice the gene is expressed throughout the body. In the mouse's brain, for example,

BOX 2-1
Methylation

Methylation is one of the primary mechanisms of regulating gene expression. Methylation occurs when a methyl group is attached to a CpG site in the DNA strand. DNA methyltransferase attaches the methyl group to the cytosine, converting it to 5-methylcytosine. CpG sites are relatively rare in human and vertebrate genomes, and they are most frequently found in promoter regions. Methylation can be associated with increased or decreased gene expression, although, in general, when a promoter is hypomethylated the gene can be expressed, whereas when it is hypermethylated gene expression is repressed or turned off.

the agouti protein blocks a feeding control center, leading the animal to overeat and become fat. The reason for the ubiquitous expression is that the gene has an IAP (Intracisternal A-particle) proximal enhancer (IPE) element inserted next to the gene. The IPE element acts as a promoter and switches on the gene everywhere, not just in the skin.

Agouti gene expression can be silenced, however, by methylation (see Box 2-1) of the IPE element at a cytosine-phosphate-guanine (CpG) site. This methylation shuts down the promotion effects of the IPE element. Methylation can inhibit expression of the agouti gene altogether, resulting in a mouse with brown fur and normal weight.

A key point is that the methylation state can be passed from one replicative generation to the next or from parent to offspring, as in the case of the agouti mouse, causing the differences in phenotypic expression. In a 2003 experiment performed by Waterland and Jirtle at Duke University,[1] female mice were fed one of two different diets before, during, and after their pregnancies. Those mice on the normal (control) diet produced pups that had a yellow coat, developed obesity, and were susceptible to a number of cancers. Those given a diet high in vitamin B12, folic acid, and other supplements that promote methylation produced pups with a brown coat that maintained a normal weight and had no increased cancer risk.

This experiment demonstrated that the diet modulated methylation of the IPE element in the mouse genome, which repressed expression of the agouti gene for the lifetime of the offspring. "I think this is a very interesting experiment," Jaenisch said. "It tells us that the environmental effect at a certain short stage early in life affects the gene expression pattern throughout life and has an enormous effect on phenotype." It is, in short, a prototype for the sort of epigenetic actions important to nutrig-

[1]Waterland RA and Jirtle RL. 2003. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. *Molecular and Cellular Biology,* 23:5293–5300.

enomics: diet, acting through the mechanism of methylation, affects gene expression in a stable, lasting way with clear consequences for the health of the individual.

Epigenetics and Cancer

One of the most exciting areas in which epigenetics has been applied is the study of cancer. The development of cancer depends upon a complex series of events. Some of these events are genetic, involving genetic mutations, whereas others are epigenetic, involving changes in DNA methylation or in the chromatin state, such as histone modifications. Epigenetic events are particularly interesting because they can, in principle, be reversed by therapeutic intervention. Genetic events, by contrast, cannot be reversed and thus offer limited potential for intervention.

One of the best experimental models of cancer is the APC mouse, which has a mutation in the APC (adenomatous polyposis coli) tumor suppressor gene. Mice with this mutation develop colon polyps that become tumors in a progression that parallels the development of colon cancer in humans with familial adenomatous polyposis. In the 1980s, researchers studying the APC mouse discovered that the DNA in colon polyps was hypomethylated, which led to the hypothesis that such hypomethylation might be a necessary step in the development of colon cancer.[2] To investigate the role that methylation plays in the development of cancer in the APC mouse, Jaenisch and colleagues manipulated the level of methylation in various ways and observed its effects. If hypomethylation was indeed a necessary step in the development of colon cancer, decreasing the level of methylation should increase the number of polyps. However, the opposite effect was found.

Typically, an APC mouse develops about 130 polyps by 6 months of age. When Jaenisch and colleagues knocked out one allele of the methyltransferase gene, *DNMT1*, in these mice, it reduced the level of methylation activity by half, and the mice in which the *DNMT1* gene was knocked out developed only a third as many polyps as expected. When the APC mice were treated with the anticancer drug 5-aza-D-cytosine, it reduced the levels of *DNMT1* in the mice and reduced the number of polyps by nearly 90 percent. When the knockout of one *DNMT1* allele was combined with the 5-aza-D-cytosine treatment, the number of polyps dropped by two orders of magnitude. "So this is the opposite of what was thought," Jaenisch said. "In this case *DNMT1* acts like an oncogene: the more you have, the more tumors you have."

[2]Reviewed in: Bodmer et al. 1989. Genetic analysis of colorectal cancer. *Princess Takamatsu Symposium,* 20:49-59.

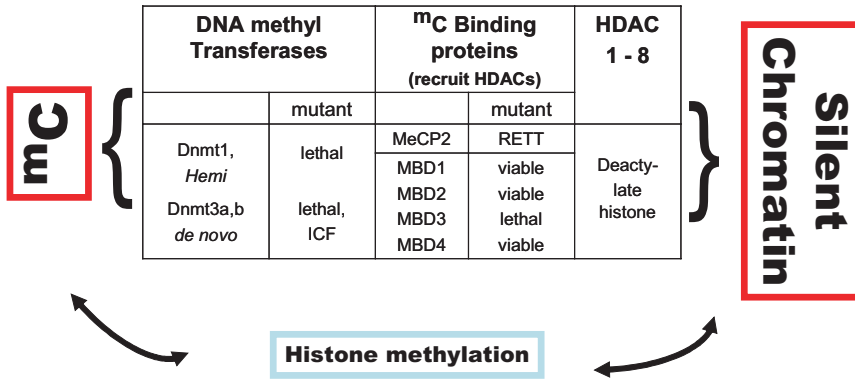

FIGURE 2-1 Epigenetic regulation: DNA methylation, chromatin conformation, and gene expression. MC–methylated cytosine; HDAC–histone deactelyase; MeCP2–methyl-CpG-binding protein; RETT–Rett syndrome phenotype; Hemi –hemi-methylated DNA; ICF–Immunedeficiency, Centromeric region instability, and Facial abnormalities syndrome.

This is just one piece of a very large and complex puzzle. Methylation has various effects on the development of tumors, depending on the tissue and the situation. In the thymus and soft tissue, for example, decreasing the level of methylation enhances tumor formation by increasing genome instability, increasing the probability that mutations that repress the expression of tumor suppressor genes will occur. In short, there is no simple way to characterize the role of methylation in tumorigenesis. Rather, it is important to understand the steps involved in tumorigenesis in specific tissue types. Figure 2-1 shows the interrelationships between methylation, chromatin conformation, and gene expression.

In another set of experiments, the Jaenisch laboratory examined the relationship between genomic imprinting and tumorigenesis. In imprinting, offspring inherit two active copies of a gene from each parent; for a small subset of genes, however, only one copy is active. Imprinting is an epigenetic event, commonly mediated by methylation of either the gene or its regulatory sequence. The loss of imprinting is associated with the development of various cancers, including leukemia, lymphomas, and liver cancer, so the Jaenisch laboratory examined whether the loss of imprinting was a cause or a consequence of the cancer. The strategy was to remove all imprints from a mouse model and watch the development of cancer in the imprint-free mice. This required manipulation of the *DNMT1* gene, which codes for the methyltransferase responsible for maintenance of the existing methylation pattern. (Other methyltransfer-

ase genes, such as *DNMT3a* and *DNMT3b*, are responsible for de novo methylation, such as that which occurs during early embryonic development.) Without *DNMT1*, the imprints would disappear.

It is impossible to create mice that are free of *DNMT1*, however, as DNMT protein deficiency is lethal in somatic cells, and embryos without *DNMT1* die after the gastrulation stage of development. Embryonic stem cells can survive without DNMT, and so embryonic stem cells were generated with two alleles of *DNMT1*, both of which were conditional—that is, they could be turned on or off experimentally. One of the alleles was made conditional with CRE-Lox technology and the other was made conditional with FLP-FRT. Therefore, if the stem cells were exposed to CRE, it would deactivate the first *DNMT1* copy, which was otherwise active, and if they were exposed to FLP, it would activate the second *DNMT1* copy, which was otherwise inactive. The approach, then, was to expose the stem cells first to CRE to deactivate *DNMT1* and cause the entire genome to be demethylated. They would then be exposed to FLP, which would turn on the other *DNMT1* copy and remethylate everything except the imprinted genes. Control cells were exposed first to FLP and then to CRE, so that at least one copy of *DNMT1* was always active and the cells never went through demethylation.

When microarrays were used to compare the imprint-free cells with control cells, the pattern of gene expression was as expected. Some genes in the imprint-free cells had double the expected level of expression; these were the genes in which imprinting involved methylation that silenced one of the copies. Others genes were not expressed at all in the imprint-free cells; thus, the imprinted genes were inactive unless they were methylated. The imprint-free stem cells grew well in culture and were immortal, so they could be injected into developing mouse embryos to produce chimeras. In these chimeras some of the tissue would descend from the imprint-free stem cells, whereas the rest would develop from the cells of the original embryo.

The animals were aged, and once they reached 12 months of age, every animal developed multiple tumors. Tumor types included hepatic and intestinal cancer, one seminoma, and leukemia and lymphomas. The conclusion was that the loss of imprinting played a causal role in the formation of multiple tumor types.

The last question addressed was the possibility of reversing those cancer states that are epigenetic in origin. A series of experiments were conducted in which somatic cell nuclear transfer techniques were used to remove the nuclei from tumor cells and inject them into enucleated eggs. The eggs were then allowed to develop to the blastocyst stage, which were then explanted in culture to derive cloned embryonic stem cells. The cells were injected into normal blastocysts to generate chimeric animals.

In these animals the embryonic stem cells cloned from the original tumor cells contributed to all tissues, suggesting that the cancer genome had been "reprogrammed" to a pluripotent epigenetic state. Thus, by monitoring the development of cancer in the chimeras, it was possible to determine whether transplantation of the cancer nucleus into the normal eggs would influence tumor developmental potency and rate of development.

When these experiments are done with different types of cancer, some prove to be reversible and others do not, depending on the tumor type. Thus, there appears to be two extremes in tumorigenesis: "one where the phenotype is largely determined by epigenetic changes which are reversible and another where it is all genetic. Most tumors are probably somewhere in between, and one of the issues is to determine what is epigenetic and what is genetic."

Finally, Jaenisch spoke of some of the issues facing those who would apply epigenetics tools to nutrigenomics. One of the key questions to be answered, he said, is how diet affects long-latency diseases. "I think diet strongly affects cancer incidence, but does it have an effect on neurodegenerative diseases, such as Alzheimer's disease or Parkinson's?" To be able to answer these sorts of questions, he said, one of the key tools will be the ability to determine the methylation state of individual CpG sites across the entire genome. "We don't have these methods; that is a major issue. We can struggle to do it for a few genes, but we need it for the whole genome." Similarly, he said, tools are needed to determine other sorts of chromatin modifications across the entire genome as well, because methylation of CpG sites is only one type of modification that is important in epigenetics. There are a number of others, and they may all play a role in how nutrients interact with the genome.

FOLATE METABOLISM AND THE FETAL ORIGINS OF ADULT DISEASE

Presented by Patrick Stover, Professor and Director of the Division of Nutritional Sciences, Cornell University

In 1986, David Barker of the University of Southampton proposed what would come to be known as the fetal origins hypothesis (Figure 2-2). Noticing that coronary heart disease was the most common cause of death among a group of men who had none of the usual risk factors, such as obesity or smoking, he noticed a pattern of low birth weight and suggested that the increased risk for heart disease might have its origins in nutritional deprivation in the womb decades earlier. Since then not only has Barker's hypothesis been borne out, but epidemiological evidence

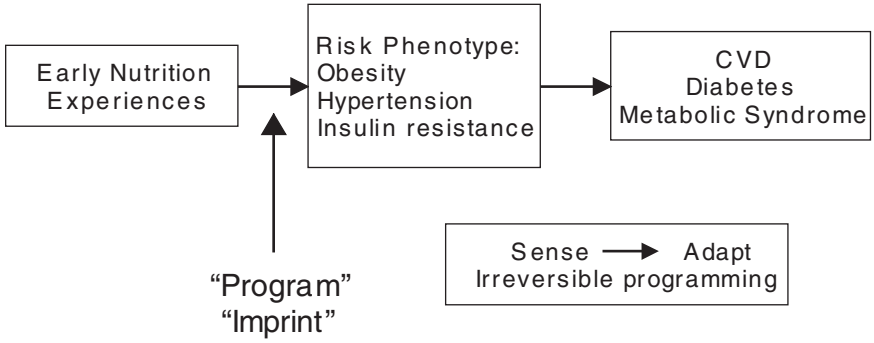

FIGURE 2-2 The Barker hypothesis of the fetal origins of adult disease. Fetal environmental exposures, especially nutrition, act in early life to program risk for adult health outcomes.

has linked low birth weight to a host of other diseases, from breast and prostate cancer to diabetes and depression.

As Patrick Stover explained, the underlying concept is that the nutritional environment in utero must somehow program a risk phenotype that later manifests itself in an increased risk for various diseases. "Implicit in this hypothesis is the idea that the fetal genome can sense its environment [and] make the necessary adaptations to survive in that environment and that those adaptations would be irreversible." Unfortunately, the programming events that increase the chances of the fetus surviving its time in the womb prove deleterious later in life.

The question that arises is how the nutritional environment in utero programs the genome epigenetically and, in particular, how a nutritionally deficient environment results in programming that increases disease risk later in life. "We know that programming is associated with chromatin modification," Stover noted, "whether it be DNA methylation or demethylation, acetylation, ADP ribosylation, or biotinylation of histone proteins. We also know that nutrients can influence some of these processes. There is increasing evidence, for instance, that both nutrients and metabolism can modify the probability of certain chemical modifications occurring, such as methylation." These nutrient-driven changes can be transient, but according to the Barker hypothesis, if they occur within a critical window in development, the result may be a meta-stable (irreversible) modification that persists throughout life.

Research from the Stover laboratory focuses on one particular aspect of this issue: folate metabolism and its role in epigenetic modification of the genome. Folate, which refers to a family of chemically related com-

pounds and which is one of the basic B vitamins, has a variety of metabolic roles, particularly in DNA replication and genome maintenance, but the role that is most relevant to genome programming is as the source of the methyl groups that are used to modify chromatin and, hence, gene expression.

The end points of folate-mediated one-carbon metabolism include purines and thymidylate, which are necessary for DNA synthesis, and methionine, which can be adenylated and function as a cofactor in methylation reactions, including the methylation of DNA and histone proteins in chromatin. Once these reactions occur, the spent cofactor, *S*-adenosylhomocysteine, can be reused in additional methylation cycles. "This methylation cycle is key because it is a segue, if you will, between metabolism and chromatin modifications."

A number of different nutritional deficiencies can affect the metabolic pathway, for example, a deficiency in folate or vitamin B12, since the enzymatic activity of methionine synthase is dependent upon both of these vitamins. When the pathway is disrupted, all of the intermediate steps and cross-pathways will also be disrupted because they all compete for a limited pool of folate. Repercussions can occur, such as a decrease in the amount of thymidylate available to the nucleus, which then causes DNA polymerase to mistakenly incorporate uracil into replicating strands. Individuals who are marginally deficient in folate can have up to 10 times as much uracil as normal in their DNA. A decrease in the amount of 5-methyltetrahydrofolate will also decrease methionine synthesis, in turn reducing the rate of DNA methylation.

A common mutation that can negatively affect the folate pathway is present in the gene for methylenetetrahydrofolate reductase (MTHFR); carriers of this mutation have an increased risk for neural tube defects, miscarriage, and other fetal disorders. This common polymorphism in the gene for MTHFR causes the encoded proteins to have reduced activity and stability and, in particular, lessens the cell's ability to accumulate methyltetrahydrofolate. This in turn results in a decreased capacity for methylation reactions, but because the methylation pathway is impaired, the competing thymidylate pathway actually becomes more efficient. This change in biochemistry confers both risks and benefits. The decreased methylation rate increases the risk for neural tube defects in the developing fetus, but in adults the increased level of thymidylate synthesis leads to a lower risk of colon cancer.

The Stover laboratory has investigated the enzyme serine hydroxymethyltransferase (SHMT), which is active only in certain tissues. SHMT converts serine and tetrahydrofolate to glycine and methylenetetrahydrofolate. In those tissues in which it appears, it has the effect of inhibiting the methylation cycle and increasing flux through the thymidylate pathway;

therefore, modifying the activity of the gene will affect both methylation status and DNA stability. Experiments with knockout mice have shown that if one of two copies of the gene is knocked out, which reduces the amount of SHMT in half, then the amount of uracil incorporated into DNA increases by as much as 10-fold. At the same time, the increased methylation affects the level of expression of some 200 other genes in the cell. Interestingly, the gene for SHMT functions as a "nutrient sensor," in that it is highly responsive to zinc, retinoic acid, and ferritin. This exemplifies how the levels of several different nutrients can affect gene expression and DNA stability through a common pathway.

Clinical evidence also indicates that diet can affect epigenetic programming. A study in Italy evaluated seven patients with uremia, which resulted in elevated levels of homocysteine, the precursor to methionine in the folate-mediated one-carbon metabolism. The elevated homocysteine levels resulted in a reduction in methylation in the patients. When the patients were tested for the level of expression of a particular imprinted gene, *H19*, one copy of which is normally methylated and is thus inactivated, three of seven patients (who had the highest levels of homocysteine) had a second copy of the *H19* gene that was also being expressed. Thus, the elevated homocysteine levels that resulted from uremia were reversing the imprinting of the gene. An effective treatment was to give the patients a folate supplement. This reduced their homocysteine levels and silenced the expression of the second copy of *H19*. Its imprinting had been restored. "So," Stover concluded, "metabolism can influence reversibly imprinted gene expression once the imprint is already established." This may be a property that is exclusive to stem cells.

Lastly, Stover offered examples of how folate can induce epigenetic changes to compensate for genetic shortcomings. In one group of mice that had been given a *Hox* gene knockout that caused a skeletal defect, putting the mothers on a high-folate diet prevented the skeletal defects. "In fact," he said, "this has been shown for a number of mouse models: that you can reverse a variety of defects by giving high levels of folate or other methyl supplements." In humans, giving folate supplements to mothers sharply reduces the risk of neural tube defects and can also reduce rates of spontaneous abortion.

The evidence, then, is that proper nutritional interventions can be a powerful tool for epigenetic reprogramming. However, a great deal of research must be done before the field begins to reach its potential. "This is an emerging area. There is lots of descriptive work. Mechanistic work is now emerging, and it is going to be absolutely critical in determining how nutrition can modify these epigenetic processes for long-term benefit," Stover indicated. Research is needed to obtain an understanding of the effects of nutrient exposures on genome programming; identifying the

affected pathways, critical timing, and nutrient levels that alter programming; and determining the long-term risks and benefits associated with such nutrition interventions.

THE ROLE OF MATERNAL AND INFANT NUTRITION IN GENETIC PROGRAMMING AND EPIGENETICS

Presented by Cutberto Garza, Provost and Dean of Faculties, Boston College

Public health will respond as nutrigenomics research progressively unravels the bases of associations between early diets and medium and longer-term health outcomes. Cutberto Garza described one particular area in which nutrigenomics may have major public health implications. Several retrospective and prospective studies from multiple countries support the possibility that low birth weight and rapid "catch-up" weight gain (defined by the upward crossing of centiles on growth grids) increase the risk of type II diabetes and, possibly, cardiovascular disease, especially among individuals who experience significant in utero growth retardation. Studies based on the Dutch famine are similarly intriguing. Men exposed to famine conditions in utero during the first trimester experienced a nearly twofold increased risk of obesity. Other studies have reported a U-shaped relationship between birth weight and the risk of diet-related chronic diseases. Low birth weight is also reported to enhance the risk of increased blood pressure. Positive associations between postnatal weight gains and adult systolic blood pressure have also been reported. Some investigations detected this relationship only among those who experienced rapid weight gain in early childhood but not during infancy. It is particularly relevant that many well-controlled studies with animals also report links between early perinatal nutritional experiences and an increased risk of chronic disease.

Human and animal studies such as these raise three questions that are especially relevant to public health: Are the long-term adverse effects of early prenatal nutrition experiences on chronic disease risk reversible? Do increased risks persist across generations? What are the implications of such possibilities to public health?

Animal experiments have examined both the reversibility of phenotypes apparently enabled by early prenatal nutrition experiences and the underlying mechanisms that may explain the putative reversibility of targeted phenotypic characteristics. Investigations with animals of methylation-based epigenetic mechanisms, perinatal experiences, and the functional consequences of adult onset may be particularly instructive. For example, pharmacologic and dietary agents have been used to

investigate the epigenetic methylation patterns associated with specific functional consequences and their reversibility. For the present, the significance of the findings from the available animal studies to providing an understanding of the links between early diets and the later onset of chronic disease remains difficult to assess.

The second question relates to the intergenerational potential of such associations; that is, do adverse effects of harmful nutritional conditions in one generation persist in succeeding generations exposed to improved nutrition? Garza identified two categories of transgenerational mechanisms. The first is limited to maternal transmission. This mechanism requires that dietary exposure somehow alter maternal reproductive structures or functions in a way that affects the mother's progeny. The second enables both paternal and maternal transmission. The latter mechanism requires that exposure somehow alter the epigenetic development of the germ line.

The best human data relevant to the first category come from studies of the impact of maternal size on the progeny's birth weight. Maternal stature, not uncommonly, is a good indicator of past nutritional status but is independent of current nutritional status; yet, an infant's birth weight remains significantly dependent on maternal stature. Thus, associations between the progeny's birth weight and various long-term outcomes often provide evidence for transgenerational effects of maternal in utero or early childhood experiences. These associations, however, provide limited insight into the underlying mechanisms.

Among the more interesting animal models used to examine the second category are those that study epigenetic inheritance in the agouti mouse and those that investigate epigenetic transgenerational effects of environmental toxins. Both may prove relevant to understanding the mechanisms underlying the functional long-term consequences of early diets.

Thus, studies with animals have explored both categories of trangenerational effects and their underlying mechanisms. Nonetheless, knowledge as to how early nutritional environments affect health in later life is limited. Importantly, there is a growing consensus that early nutrition remains relevant throughout life. Enhancing knowledge of early nutrition's impact on longer-term and intergenerational health could play a large role in informing public health policies and thus are relevant to today's research agenda. This relevance is likely to grow as the global population ages and life spans increase.

What are the practical implications of such information to public health, and what influence does such information have on contemporary public health practices? Over the longer term it is likely that such information will be relevant to a wide range of dietary manipulations designed

to enhance short-, medium-, and long-term outcomes, for example, food fortification policies, nutrient supplement use, and population-based chronic disease management. More immediately compelling, however, is the growing relevance of maternal care and growth-monitoring programs. Data from studies with humans and animals that support links between prenatal and early childhood growth patterns and multiple long-term disease risks underscore the need for promoting physiologic growth in utero and throughout childhood and the need for robust tools to enable those assessments.

3

Scientific Session III: Systems Biology

Moderated by Robert Cousins, Boston Family Chair in Nutrition, Director of the Center for Nutritional Sciences, and Affiliate Professor of Biochemistry, University of Florida

Throughout the 20th century, much of biology embraced reductionist goals. That is, researchers broke systems down into their component parts to understand them. This was perhaps most obvious in molecular biology, as scientists explored genes, the products of their expression, and the molecular pathways through which they act. The expectation has always been that an understanding of the individual pieces would eventually allow greater understanding of biological systems on a broader scale.

In the 21st century, biologists will find themselves dealing increasingly with integrated approaches, said Robert Cousins. Systems biology, which is the study of the interactions among the components of a biological system, exemplifies this integrated approach.

The ultimate goal of systems biology is to create a computational model that describes the system of interest. Quoting Leroy Hood, one of the pioneers of systems biology, Cousins remarked that systems biology generally follows a pattern of first defining the elements of a system and then defining a mathematical model for the system, performing simulations with that model, comparing the simulations with what is known about the real system, refining the model based on those comparisons, and repeating those steps again and again to zero in on a model that accurately captures the behavior of the system itself. Cousins recalled Hood's comments from a conference at the National Institutes of Health in 2005: "Dr. Hood was asked: what are the two areas of science that are most amenable to the systems biology approach and his answer was plant molecular biology and nutritional sciences."

GENETIC NETWORKS AND APPLIED SYSTEMS BIOLOGY

Presented by C. Ronald Kahn, President and Director, Joslin Diabetes Center, Harvard University

Diabetes and the Insulin-Signaling Network

Understanding a biological system demands understanding the details of its components, and their interactions. As an example, Ronald Kahn described his research into the pathways that control insulin action.

Kahn began by noting that the current epidemic of diabetes and obesity is having a tremendously deleterious effect on human health in the United States. He added, however, that "diabetes and obesity are really a small part of a much larger problem that we call the metabolic syndrome." The metabolic syndrome includes a constellation of diseases that are linked to insulin resistance and, in many cases, to the effects of overnutrition and underactivity. These diseases include glucose intolerance and type II diabetes; obesity; hypertension; lipid abnormalities; accelerated atherosclerosis; fatty liver; reproductive dysfunction, particularly polycystic ovarian disease in women; and even Alzheimer's disease and neurodegenerative diseases, which are more common in individuals with insulin-resistant states (see Box 3-1).

BOX 3-1
Glucose Metabolism and Insulin Resistance

A stable level of glucose in the blood is necessary to provide energy to the brain, muscles, and organs; and excess energy is stored in fat tissue. When glucose levels in the blood decrease, pancreatic beta cells produce glucagon, which stimulates the liver to mobilize glycogen stores to release glucose into the bloodstream. When glucose levels rise, pancreatic alpha cells produce insulin, which inhibits glucose output from the liver and stimulates muscle and fat tissue to absorb glucose from the blood.

In insulin resistance the liver, muscle, and fat do not respond to the presence of insulin, which in turn leads to elevated blood glucose levels. This increases signaling to tissue receptors, which respond, although at a minimal level, allowing glucose levels to remain elevated. In type II diabetes, however, tissue receptors are desensitized to glucose stimuli and blood sugar levels rise, leading to symptoms of diabetes and, if left uncontrolled, to diabetic complications.

To understand the genesis of the metabolic syndrome and what it will take to address it, Kahn posed three questions:

1. What are the key tissues and pathways of insulin action that are involved in the development of insulin resistance and the metabolic syndrome?
2. What important features of the insulin-signaling network play a role in insulin-resistant states and the metabolic syndrome?
3. How do genes and the environment, especially diet, contribute to gene expression changes that might interact with the development of these syndromes?

To address these questions, Kahn primarily relied on research from the Diabetes Genome Anatomy Project, whose goals are "to use mainly genomics and to some extent proteomics to define normal action of insulin on gene and protein expression in cells, mice, and in humans; to define the abnormalities of gene expression in insulin-resistant states; and to determine the role of genetic variation of insulin-signaling proteins in this." In short, the goals are to understand insulin action on a fundamental level and to use that understanding to pinpoint what goes wrong (and why) when insulin resistance develops.

The insulin-signaling network has two major pathways. The Ras-MAP kinase pathway is involved in regulating gene expression and cell growth and differentiation. The PI 3-kinase pathway modulates the metabolic effects of insulin; the ability to stimulate glucose transport into the cell; and initiates glycogen, lipid, and protein synthesis. To study these pathways and their roles in insulin resistance, the Kahn laboratory has produced mouse models with knockouts of single genes in the insulin-signaling pathway; compound knockouts, either homozygous or heterozygous, of the genes in the pathway; various types of tissue-specific knockouts; and the use of RNAi and shRNA to do gene knockdowns in various tissues (Figure 3-1). These mouse models and the cell lines created from them have allowed investigators to examine signaling proteins and their respective pathways and the physiologic effects of their gene-knockout strategies using a systems biology approach.

An important outcome from this approach is the discovery that the insulin-signaling pathways are not redundant. They are complementary pathways of insulin action, which increases their complexity. For example, unlike knockout mice, in humans there is no single flaw that leads to insulin resistance. According to Kahn, "In human Type II diabetes and in all of the precursor states related to the metabolic syndrome, we think we are looking at polygenic diseases created by partial defects in either

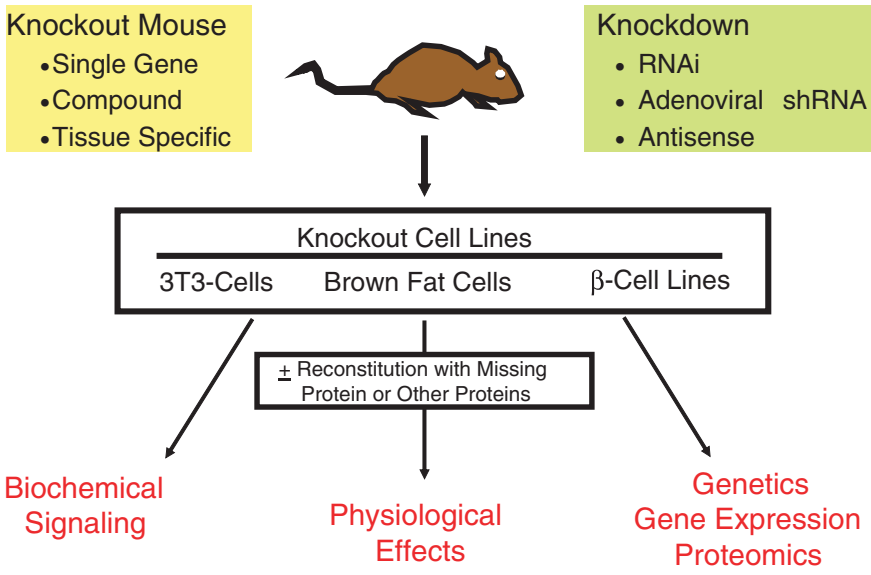

FIGURE 3-1 Knockout and knockdown technology used to study insulin-signaling pathways.
SOURCE: Presentation by C. Ronald Kahn, used with permission, January 9, 2007.

gene expression or gene function and that these lead to more subtle phenotypes."

As a result, the Kahn laboratory created mice that had heterozygous knockouts for both the insulin receptor (IR) gene and the IR substrate 1 (IRS-1) gene, so that mice expressed both genes at only half the normal level. A heterozygous knockout of either gene failed to create a diabetic phenotype in the mice, leading to the question of what would happen if both genes had 50 percent defects.

The answer was somewhat surprising, in that the effect varied greatly depending on the genetic background of the mouse. In strain C57 Bl6 mice, 90 to 100 percent of the animals would develop diabetes within the first 6 months of life, but in strain 129 mice less than 2 percent would develop diabetes even if they were monitored to 2 years of age. "It turns out that this phenotypic difference is not due to differences in insulin secretion," Kahn said, "but is due to additional defects in insulin action." A genomewide scan and gene expression experiments to identify variations that contributed to the phenotypic difference revealed four loci on three chromosomes that play a role. Furthermore, not only did the loci contribute to the difference in how the IR and IRS-1 heterozygous knockouts affect the mice, but they also contributed to differences in how the

two strains developed insulin resistance in response to a high-fat diet. Interestingly, the C57 Black 6 mouse is fatter than the 129 mouse when they are on the same diet, and the C57 Black 6 mouse actually eats less and moves around more; the C57 Bl6 mouse was just more efficient at storing calories.

In looking for an explanation of the phenotypic differences between the mice, investigators identified one locus on chromosome 14 that had a strong correlation with the differences between the mice in their development of insulin resistance; however, there were large differences in the levels of gene expression between the strains of mice. Investigators identified about 250 differences in gene expression overall that could contribute to the phenotype, and 12 of them were located within a specific region on chromosome 14. Many of the genes showed a twofold, fivefold, or eightfold difference in expression between the two strains of mice. The difference in gene expression between these two normal strains of mice was greater in some cases than the difference between heterozygous knockouts or the RNAi and shRNA knockdowns. These experiments demonstrated that gene expression can be modified by disease status; in this case, the diabetic mouse had a gene expression pattern different from that of a healthy mouse of the same strain. "We not only have to consider the background genetics of the animal, but we have to consider all of the extrinsic factors which can be regulating gene expression," Kahn said.

It is important to understand exactly what is causing the change in gene expression. In the case of diabetes, for example, the changes could be the result of a decrease in insulin levels, but they could also result from the metabolic state created by the diabetes. To distinguish between the two, the Kahn laboratory looked at gene expression in mice that were made diabetic by the administration of streptozotocin and the MIRKO (muscle insulin receptor-knockout) mouse, whose muscles cannot respond to insulin, and MIRKO mice made diabetic with streptozotocin. Each type of mouse has a large number of genes whose expression is different from those in the normal mouse, but there is a small overlap between these two sets of genes. The experiments revealed additional changes in gene expression, but they only partially overlapped with the diabetic state in the normal mouse. Therefore, there are genes that can be viewed as being directly regulated by the insulin signal, for example, those that are changed in the MIRKO mouse, those that are changed in the diabetic animal but not in the MIRKO mouse, and those that are discordantly regulated between diabetes and insulin signaling (that is, the absence of insulin signaling causes an increase in gene expression, but the hyperglycemic state represses expression; in effect, the steady-state levels are not changed).

Researchers face a tremendous challenge in understanding the details

of biological systems. Nutrigenomics will be a complex process. Evaluation of the most well-characterized of metabolic disorders like obesity, type II diabetes, and the metabolic syndrome will require rethinking the entire nature of this system because not only can insulin directly regulate metabolism and gene expression in its target tissues, but the action of insulin is also affected tremendously by nutrients, the genetic background of the animal, and the nutritional state at the time of study. Only by dissecting all of these individual components will real understanding of the true control of gene expression in response to hormones and nutrients be achieved.

GENOME-SCALE RECONSTRUCTION OF THE HUMAN METABOLIC NETWORK

Presented by Bernhard Palsson, Professor of Bioengineering and Adjunct Professor Medicine, University of California, San Diego

One of the core goals in systems biology is to construct models of networks, particularly computational models that can be tested and interrogated mathematically. It is only by creating a well-tested, mostly complete model that a network can be truly understood. This process is done in steps, explained Bernhard Palsson. The first step is the acquisition of data, the second is reconstruction of the network and the creation of a mathematical model that captures the reconstruction (Box 3-2), and the third is computation with the model to make predictions about the

BOX 3-2
Network Reconstruction

Bernhard Palsson described network reconstruction as being analogous to a map of Los Angeles, California, highways. There is the physical network, the set of roads itself, but also the functional state, that is, the arrangement of cars and other vehicles on the roads. That functional state at 5 p.m. will be very different from that at 5 a.m. Similarly, a gene network or a metabolic network will have different states, depending on the situation.

It is generally not possible to compute the particular functional state of a network because that would require more information than is practically available. It is possible, however, to compute the allowable functional states on the basis of various constraints. What all the cars on the road are doing may not be known, but it is certain that there is not a line of traffic traveling through downtown at 80 mph. For a reconstruction, according to Palsson, one can determine a “cone” of allowable solutions so that everything inside the cone represents a possible state of the system and everything outside is a state that is not possible.

network's behavior. "The promise of systems biology that these computational models can be used for prospective experimentation is just beginning to be realized."

Network reconstruction (Step 2) can be approached in two different ways: from the top down or from the bottom up. The top-down approach performs various statistical calculations with data from a high-throughput data set and looks for correlations. In a gene network, for example, one would look for pairs of genes that were always expressed at the same time or for genes that were negatively correlated, with one of them on whenever the other was off, and vice versa. The approach is a "very coarse-grained resolution" of how gene networks function; nevertheless, it does have some utility.

The bottom-up approach, on the other hand, requires more human effort: reading the literature, manually evaluating the process step by step, and evaluating the data component by component. Each connection between components stems from experimental evidence and is described mathematically in terms of input and output, addition and subtraction, and starting point and end point. The bottom-up approach aims to be very accurate and has well-defined chemical interactions among cellular components. The results are self-consistent, in which no information is included in the database until it has been checked and rechecked against experimental results and against other items in the database. The contents of the databases are biochemically, genetically, and genomically accurate and represent knowledge in a structured format. By comparison, the top-down approach aims at being comprehensive; but in trying to measure everything simultaneously, the results are often inconsistent and the final conclusions soft or suggestive rather than coherent and solid. These are often called "inference methods," based on observation and correlation.

As a "context for content," such models can bring the top-down and bottom-up approaches together. This is because the most useful models are those that can integrate data derived by the top-down and bottom-up approaches. To date, bottom-up reconstructions have been done for the metabolic networks of a number of microbes, including *Escherichia coli*, *Haemophilus influenzae*, and *Helicobacter pylori,* and have proven to be quite useful. "These models are actually quite good at computing the consequences of gene knockouts. They can also compute the optimal growth rates of cells. Surprisingly, they can predict the outcome of adaptive evolution, which is quite a complicated biological process." Microbial reconstructions have also been used to study horizontal gene transfer and the evolution of a complex bacterial genome to a simple bacterial genome. Importantly, reconstructions have proven to be a valuable tool in filling the gaps in literature knowledge. Once a map is put together, knowledge gaps remain that emphasize areas in research that require further explora-

tion. However, it is now possible to develop automated methods that fill these gaps and generate hypotheses that lead to biochemical experiments and the discovery of unknown functions of organisms.

The Palsson laboratory has completed a genome-scale reconstruction of the human metabolic map. "It took 18 months for six people to do this, and the map is now complete and has been published," he said. "This will be of interest to everybody studying nutrigenomics." To create the reconstruction, the group began with data from various databases, such as the human genome sequence and lists of reactions and metabolites. After the assembly of all those data, the group members reviewed the entire assembly, component by component, fitting the components together, followed by comprehensive evaluations of literature to fill in everything that was not found in databases.

According to Palsson, it is relatively easy to go through an annotated genome, identify the metabolic genes, determine the biochemical reactions that certain gene products catalyze and elementally balance them, determine their cellular location, and then review the relevant literature. Finally, functional testing of the network is performed once it has been put into a mathematical framework wherein the network is checked for gaps. The first version of the reconstruction, Recon 1, accounts for 1,496 open reading frames, 2,004 proteins, 2,766 metabolites, and 3,311 metabolic and transport reactions. However, this is not considered a complete map. For example, metabolomic profiling of human blood and tissues and the construction of lipid maps has documented thousands of potential metabolites and lipids. Not all of these identified compounds may be real; some of them will likely be "false peaks," while others could be real. "We now will be able to reconcile metabolic data against this map, and hopefully we will find metabolites that are missing and need to be included in the network," Palsson said. "We still have some things to discover." After the reconstruction was completed, the Palsson group tested it by using their model to carry out 288 functional tests to check for metabolic capabilities known to exist in human physiology.

Another application important to nutrigenomics is computational interrogation of the model. That is, once the model has been validated, it can be used to study aspects of metabolism that are not easily assessed through laboratory experimentation. It can be used, for instance, to study disease states of metabolism and determine how they differ from normal states or to perform computational experiments on the effects of various nutrients on the metabolic network.

Perhaps most importantly, the reconstruction should serve as the basis for what Palsson hopes will become a rapidly expanding resource. "We now have a platform," he said, "which different groups can expand to different cell types based on expression profiling of particular organ-

elles as well as other types of high-throughput data that may be of interest. This will be a community resource that I would like to build on the web and have people contribute to and iteratively build. I may not be the best person to do that, so maybe we can get a group of bioinformatics labs to continue this process."

EMERGING TECHNOLOGIES: NANOTECHNOLOGY

Presented by Martin Philbert,
Professor of Toxicology and Senior Associate Dean for Research,
University of Michigan School of Public Health

Nutrigenomics researchers will need a variety of tools for studying the cell and its contents, including DNA, proteins, and nutrients. As an example of some of the innovative tools that are becoming available, Martin Philbert described how he, Raoul Kopelman, and their coworkers have used nanotechnology to peer into living cells and see their activity in real time and in three dimensions.

"Nano" refers to the scale of a structure, in which at least one relevant dimension is 100 nanometers or less, and "nanotechnology" is the use of nanoscale structures in research and development. At the nanoscale, materials tend to have properties that are very different from those of the same materials at larger scales. Philbert explained that much of the power of nanotechnology comes from a bottom-up approach to constructing nanostructure devices, which makes it possible to create molecular assemblies with unique properties that can be manipulated with an unprecedented degree of control.

An example of a practical nanostructure application is the use of zinc oxide nanoparticles in sunscreen. When these particles are less than 100 nanometers in diameter, they are able to capture high-energy photons and convert them into low-energy photons, which are less harmful to the skin, thus allowing for a sun protection factor (SPF) of 50 or 60 instead of the usual SPF 15 or 20.

A molecular application developed in the Philbert laboratory is the creation of nanostructures that can be used as intracellular sensors. These nanosensors are able to enter a cell and measure metabolic activity with minimal perturbation of cell function. These nanosensors are termed "PEBBLEs" (probes encapsulated by biologically localized embedding). "We can make these within the range of 20 to 600 nanometers in diameter with very fine control of the mean diameter of the sensors," Philbert said. "At 20 nanometers one PEBBLE occupies one part per billion of the neuronal somata of the average anterior horn motor neuron cell body, so

a large number of them can be inserted without interfering significantly with cellular functions.

A key feature of PEBBLE structures is that they can be engineered to fluoresce in the presence of various small molecules or ions, such as oxygen, nitric oxide, calcium, potassium, and zinc, among many others. By flooding a cell with the PEBBLE nanosensors and then monitoring the cell under a microscope, investigators can observe the distribution of oxygen, nitric oxide, or other target molecules throughout the cell and monitor its evolution in time. With a little cleverness combined with a thorough knowledge of physics, chemistry, and cellular biology, it is possible to measure a great number of cellular metabolic activities, including changes in temperature, viscosity, and local magnetic and electric fields. "The beauty of this," Philbert said, "is that we can now begin to add richness to the data sets in local areas in the cell where there may be intracellular electrophoresis contributing to the association of proteins, many of which have very large electrical dipoles."

Another area of research pursued by the Philbert laboratory is the incorporation of Photofrin (porfimer sodium), a light-activated cancer drug, into nanostructures. Normally, Photofrin cannot cross the blood-brain barrier and thus cannot be used to treat brain cancer by conventional means. Because of the size of nanostructures, however, when the drug is incorporated into them it can cross the blood-brain barrier and target the tumor cells. A laser device is then used to activate the Photofrin and destroy the tumor cells. In experiments with rodent models, the treatment has dramatically increased survival rates. Without treatment, the brain tumors kill 100 percent of mice within 10 to 12 days. With treatment, about 60 percent of the mice are still alive after 39 days and about 40 percent are alive after 3 months.

Nanostructures are not expensive to make, costing about a dollar a kilogram for the materials incorporated into the most common ones. Synthesis demands a great deal of expertise, however, and the high cost is acquired through the requisite toxicology screening and federal approval processes. "Still," stated Philbert, "their potential to do so many things that cannot be done in any other way makes them well worth the investment."

4

Implications for the Future

Moderated by John Milner, Chief, Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute

As a field of scientific research, nutrigenomics has tremendous potential. Science is not done in a vacuum, however, and if nutrigenomics is to reach its potential, a number of details in arenas outside of science must be addressed. These include issues involving ethics, economics, industry, and public policy.

ISSUES IN ETHICS

Presented by David Castle, Chair in Science and Society, Department of Philosophy, University of Ottawa, Ontario, Canada

Public Attitudes Toward Nutrigenomics

Acceptance of nutrigenomics by the public will depend not just on the products of scientists and industry but also on attitudes toward this field of research, said David Castle. Castle's work focuses on how science and technology agendas are established, how the products and services that arise from innovations in technology make their way into society, and what determines the success or failure of a new technology such as nutrigenomics. One of the primary factors is how the technology is received by the public.

The general public has a number of concerns about nutrigenomics. For example, how advanced is the state of the science, and what is the evi-

dence that nutrigenomics can make a difference in an individual's health? It is also important to understand the cause-and-effect relationships being studied and the balance of the benefits and the risks involved in making lifestyle and diet changes that may be advised through a nutrigenomics-derived nutritional prescription.

A primary area of concern with the collection of the information to be used to generate a nutritional prescription is what happens to genetic information that is submitted to a company or a clinic for testing. It is important to consumers to know who has access to their information and whether their employers or insurers get access it. Other concerns include the use of personal information in a public database. Will it ever be completely deleted, or will it always be there for others to uncover if they really want to?

Consumers are also concerned with how nutrigenomics technology will be regulated. "They want the tests regulated," Castle said. "They want the health claims regulated." At the same time, consumers recognize that nutrigenomics will demand a type of regulation that is different from the types of regulation for both pharmaceuticals and foods, perhaps a type of regulation that contains elements of both.

"This is actually the principal challenge for regulators," Castle said: "to understand what new innovative regulatory structures have to be developed in order to be able to handle this complex field." The problem is that the government is used to regulating in vertically well-organized, tightly integrated fields, such as pharmaceuticals or foods, and when something like nutrigenomics that cuts across these fields comes along, there is no easy way to develop novel regulations. There are additional concerns about access and equity. Many individuals worry that nutrigenomics will turn out to be a luxury product that is available only to a few and that much of the world's population will not benefit because of a lack of access.

These issues are closely tied to that of how nutrigenomics will be delivered to the public. Castle introduced two basic delivery models: private and public. These models parallel the scientific divide between the analysis of an individual's DNA to provide a personalized dietary prescription and the use of an understanding of the relationship between genes and nutrients to make dietary recommendations that will apply to almost everyone. There are four separate delivery models. The first one is the consumer model, in which individuals pay for and consume nutrigenomics products on their own. An individual consumer might, for example, find a nutrigenomics company on the Internet, send away for a test kit, take a cheek swab, return it to the company, and then use the test results to shape his or her eating habits. The consumer model puts nutrigenomics into the hands of the public. It is convenient, empowers the

consumer, and conveys a sense of control over a part of life and insight into what the future might look like, all within the privacy and comfort of one's own home. However, since such testing is done outside of consultation with a medical doctor or other health care professional, it raises the issue of the standardization and regulation of testing procedures.

The second type of model is the health care practitioner model, in which the testing and counseling are offered by genetic specialists, primary care practitioners, or nutrition specialists. The benefit of this model, according to Castle, is that the patient would be receiving information that is integrated into all of the rest of the health care that he or she is receiving. Unfortunately, there are not enough trained practitioners to provide such care. "Nutrition and genetics are still not high on the medical training curricula," Castle noted, "so most doctors might be unable or unwilling to play such a role."

The third model offers an alternative. This is a blended model, in which a comparatively few nutrigenomics specialists would provide services to medical doctors and other health care practitioners. For example, a physician might contract with a company that generates nutrigenomics reports for the physician's patients and that also provides the medical staff with training and guidance.

These three models have one thing in common: they each provide individualized nutrigenomics services. In the consumer model, the health care practitioner model, and the blended model, the focus is on the benefits and risks to the individual. They all involve individuals taking advantage of nutrigenomics technology one person at a time.

The fourth model is the public health model, in which nutrigenomics provides generalized nutrition guidance to the public. It gives up some of the power of personalized dietary advice to focus on nutritional advice that can benefit population groups. In the United States, much of the focus has been on models that deliver individualized nutrigenomics advice, but the question about which model is most appropriate for service delivery raises a number of ethical issues and raises political and societal questions about health care delivery as well.

To illustrate, Castle described debate taking place in the United Kingdom, where the Department of Health recently released a report called *Choosing Health: Making Healthy Choices Easier*.[1] The focus of the report is on the role of government provision of health information and the development of social structures that allow individuals to control their health decisions, with the hope that such an approach would lead to greater public interest in disease prevention. The concept is to put tools into the

[1]Available at: http://www.dh.gov.uk/en/Policyandguidance/Organisationpolicy/Modernisation/Choosinghealth/index.htm.

hands of individuals and move toward a system with little direct government involvement. This approach is similar to that used in models in the United States that provide nutrigenomics technologies to individuals and allow them to make better decisions about nutrition and health.

In response to *Choosing Health: Making Healthy Choices Easier*, the Food Ethics Council, a nongovernmental organization in the United Kingdom, released its own report. The report argues against the idea of personalization, claiming that personalization is "actually just one small cog in a broader neo-conservative agenda to save costs and reduce government involvement in people's lives." Rather than viewing personalization in terms of personal empowerment, the Food Ethics Council sees the issue as a way of separating individuals from their government and disengaging government from the provision of health care to its citizens. Instead of personalization, the report argues for a move in the opposite direction, toward a rights-based way of thinking about food and health, where people have a right to safe and healthy foods and, thus, government has an obligation to see that they are provided. "So what they are fundamentally disagreeing with," Castle said, "is the portrayal of personal choice as the key issue for improved public health."

In the course of making its arguments, the Food Ethics Council offers some points that have particular relevance for the future of nutrigenomics in the United States. First, there is a natural economic barrier to taking advantage of something like nutrigenomics that will skew its group of users. "The early adopters tend to be well educated," Castle noted. "They tend to be affluent, certainly more affluent and educated than average." Individuals with the money, time, and education to learn about nutrigenomics will tend to be those who use it, while others will lag behind. "This is a significant problem," Castle said. "The knowledge key that somebody uses to get into the nutrigenomics room comes with a pretty high socioeconomic barrier to it, and not just paying for the services."

Another point that the Food Ethics Council makes is that the personalization agenda can distract people from paying attention to the state's responsibility for public health initiatives—to the extent that the state does indeed have such a responsibility. However, whether one accepts the arguments of the Food Ethics Council or not, Castle said, they offer a clear statement of the potential problems with the personalized model for delivering nutrigenomics products. The greatest power of nutrigenomics may well lie in offering specialized advice to individuals, and a focus on public health can dilute that promise. Ultimately, the objective is that, through intervention strategies like nutrigenomics, individuals will live longer, happier, healthier lives. The best way to achieve this goal is to provide tailored advice and then to document its impact on the health and well-being among individuals in the population. If health advice from

nutrigenomics is diluted, possibly through the public health approach, it will be difficult to document success and thus launch the science more broadly.

Related to this issue is how best to communicate information about nutrigenomics so that the public can understand it and apply it. Too much information can create confusion, resulting in noncompliance with dietary guidelines. The problem, according to Castle, is that no one has done systematic public consultation and engagement work on nutrigenomics to determine the drivers for applied nutrigenomics research and to determine how individuals form perceptions and the role of the media in informing those perceptions.

In closing, Castle raised the issue of whether it is possible to use both a personalized approach and a public health approach to the application of nutrigenomics. This possibility is frequently offered as a compromise to obtain the best of both approaches. The answer is not known at this time; it is an issue that must be addressed, however, if nutrigenomics is to reach its potential in improving people's lives and health.

SCIENCE JOURNALISM AND THE NUTRIGENOMICS REVOLUTION

Presented by Sally Squires, Health and Nutrition Columnist, The Washington Post, and Susan Okie, Contributing Editor, New England Journal of Medicine

Although basic researchers may not spend much time thinking about the best ways to communicate their findings to the public, in the case of nutrigenomics, such communication will be essential. Not only will public attitudes have great influence on funding and regulation, but ultimately, it will be members of the public who are the consumers of nutrigenomics information.

One advantage that the field of nutrigenomics holds in terms of communicating results to the public is that it will not be necessary to work to get the public's attention. "The public is absolutely hungry for this information," Sally Squires told the workshop. "They couldn't be hungrier." That hunger, however, also holds a risk for the field. Since the public is so eager for results, the temptation will be to offer results—even when they are tentative—or to speculate about the future; and that is something that should be avoided. "It is going to be really, really important not to oversell this field and not to promise more than is there." Susan Okie echoed that advice: "I think that the worst thing that this field could do right now would be to try to sell it to journalists or to the public on the basis that

this field is soon going to come up with personalized dietary prescriptions for everybody based on their genes," she said.

The key to communicating effectively with the public will be to explain clearly what nutrigenomics is and what it has to offer. "It needs to be a very simple message," Squires said. "If you are looking to get your message across, I urge you to look at the way politicians get their messages across in terms of sound bites. You are going to have to have good spokespersons who can really give a clear message, and you will probably be dealing with a lot of reporters who are not necessarily educated in either science or medicine."

The task will be made much more difficult by the innate complexity of the field of nutrigenomics. Okie warned that this topic, that is, the whole idea that genes could determine an individual's response to dietary guidelines, is complicated and highly nuanced. As an example, Okie pointed to studies that have identified differences in individual responses to dietary changes, for example, how dietary modification may reduce blood pressure in some individuals and have no effect in others. This individual variability increases the difficulty of trying to craft guidelines for the public, and it raises the question of how one creates general advice for the public that everyone can follow. Furthermore, there is no guarantee that the public will heed the advice.

Referring to the two delivery models that Castle spoke about, the public health model and the individualized-advice model, Okie pointed out that it has been very difficult to get people to modify personal choices even in the face of overwhelming evidence that they can improve their chances of good health by following a particular path. "If you think about it," she said, "a lot of people have a sort of a genetic profile—it is known as their family history." Maybe they have family members who are obese or suffering from type II diabetes, or perhaps they have a father who had a heart attack at age 50. Is there any evidence, she asked, that having this kind of a genetic profile actually motivates people to be more faithful to following preventive dietary guidelines when they themselves do not actually have the disease? "Even with high public awareness and concern about the health risks of obesity, for example, and even with the stigma that exists now against obese people, most people are not very successful at avoiding weight gain and are very unsuccessful, for the most part, at keeping weight off, even if they manage to lose it." It therefore seems likely that one of the most difficult parts of conveying nutrigenomics information to the public may well be to do it in a way that makes it likely that people will actually apply that information and change their behavior.

NEEDS AND OPPORTUNITIES IN THE BIOMEDICAL SCIENCES: INTERACTING NETWORKS

Presented by Ralph Greenspan, Dorothy and Lewis B. Cullman Fellow in Experimental Neurobiology, The Neurosciences Institute, San Diego, California

Ralph Greenspan explained why basic research is essential to progress in any area of science and particularly in a developing area like nutrigenomics. "The dust bin of research history is starting to fill up with models of disease which were cured in the laboratory in an animal; those cures had absolutely no effect when they were taken to clinical trials." Such events occur because biological networks accomplish things in multiple ways. Although superficial similarities or even bona fide pathologic similarities between a disease model and a human disease may exist, the cure does not happen in the same way in the animal model and humans.

For that reason, a greater level of understanding things is absolutely essential. Whether or not model animals will acquire diabetes in exactly the same way that humans do is not the point. Of importance is understanding the principles of operation to identify the similarities between mechanisms in the human context. *Drosophila* (the fruit fly) is often used as a model because at least 50 percent of its genes are counterparts to human genes. Furthermore, among the human genes that are known to cause or increase susceptibility to disease, fully two-thirds are represented in the *Drosophila* genome, even though neither genome is particularly large, about 14,000 genes for *Drosophila* and 20,000 to 30,000 genes for humans.

Given that there are relatively few genes, the combinations of how they work is absolutely critical, including understanding the ways that different versions of different genes combine to give particular outcomes. In short, relatively subtle differences between networks can result in big differences in their behaviors, and if there is to be a good understanding of how biological actions arise from the interactions of different genes, it is essential to perform the fundamental research necessary to create a detailed picture of how biological systems work.

Gene Networks

As an example of the complexity of such interactions, the Greenspan laboratory studied a mutation in the gene for syntaxin 1A, which causes *Drosophila* to pass out quickly when it is exposed to temperatures of about 39°C, or about 102°F (normal flies without the mutation are also affected by these temperatures, and they eventually become uncoordinated and fall to the bottom of a glass tube holding them, but it takes far longer for

the heat to takes its toll). Sixteen other mutations that suppress this behavior were also identified, increasing the time that the flies stayed active at the high temperature. Thus, a fly with a mutation in the gene for syntaxin 1A and one of the other mutations would be less affected by the heat than a fly with the syntaxin 1A gene mutation alone.

To determine how the 16 mutations interacted with each other, investigators bred flies that each had two of the mutations, although not the syntaxin 1A mutation, and then observed the flies for their ability to endure heat. Comparison of the results for the various pairings led to a mathematical prediction of what effect each of the pairings should have on a fly's ability to withstand the heat if there were no interaction between the different pairs of genes, that is, if all the effects were additive. When the actual results were compared with the predicted results to discover which pairs of genes did have synergistic interactions, some pairs of genes had a positive interaction, that is, their combined effect on the fly's ability to withstand heat was greater than that which would be expected if their effects were just added together, whereas other pairs had a negative interaction. Still other pairs had no synergistic interaction at all. With these findings indicating that the genes are interacting in a network, a further experiment was performed to determine how this network changes when the flies differ in genotype at one locus: the syntaxin 1A gene itself. The result is that the synergistic interactions among genes are almost entirely different, depending on the genotype at the syntaxin 1A locus.

The implication of this finding is that a gene network of this kind is not stable; rather, it is dependent upon the particular variants of the genes within it. If one gene changes, the relationships between many other genes in the network will change in response—and those changes can be radical. Changes in a network have wide ramifications. In the example of syntaxin 1A, the genes isolated were not the ones that would be predicted to be intimately involved with the process of synaptic vesicles in neurons (which is where syntaxin has a role). A wide range of genes were affected, for example, genes affecting metabolism and genes affecting the cytoskeleton. In other words, it is a highly interconnected network.

Gene and Metabolic Networks

Gene networks also interact with metabolic networks in a number of different ways: they can alter the expression levels of genes, they can alter the protein structure (which can change the functionality of a gene), or they can alter signal transduction and metabolic pathways (Figure 4-1).

The manner in which genes can affect a metabolic network can be illustrated by two types of flies, rovers and sitters. A fly larva placed on a plate with food will exhibit two types of exploring behavior: it will either

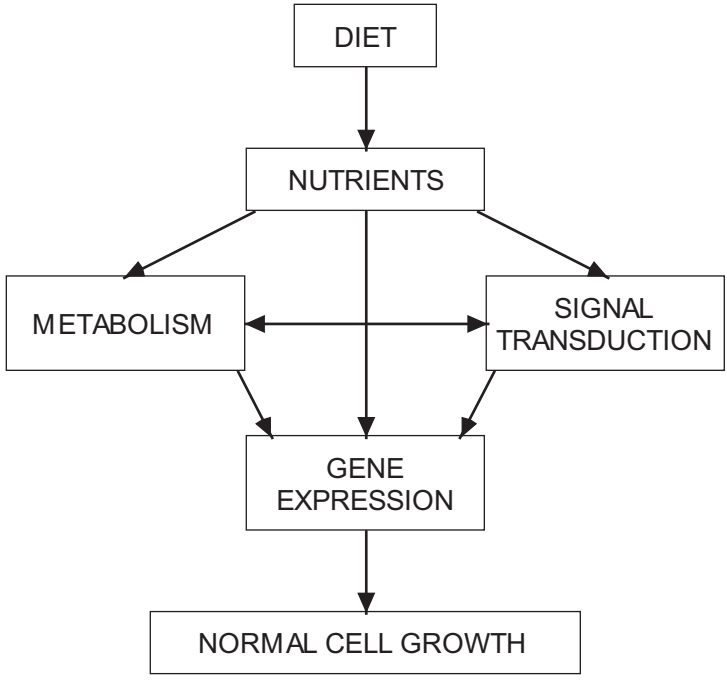

FIGURE 4-1 Gene network interactions with metabolic networks.

move around a lot or it will mostly remain in one place. This difference is apparent throughout the life span of the fly. The difference between the two types of behavior is due to a subtle difference in the level of expression of the gene for a cyclic guanosine monophosphate-dependent protein kinase. Rovers express slightly higher levels of the enzyme than sitters, and this is enough to produce the marked behavioral difference.

Because the difference is food related, the gene was expected to interact with the fly's metabolism. Experiments to determine whether gene variants affected metabolism revealed that the gene affects many of the enzymes involved in the citric acid cycle. Testing of the flies' responses to acetylcarnitine, a metabolite whose level is affected by the citric acid cycle, showed that one of the variants responds and the other one does not. Thus, the gene affecting food-related behavior also alters the response to an intermediate in nutrient metabolism. Figure 4-2 shows that there are a number of interacting networks through which nutrients modify gene expression, metabolism, and other physiologic events.

Because small differences in a network can have large effects on

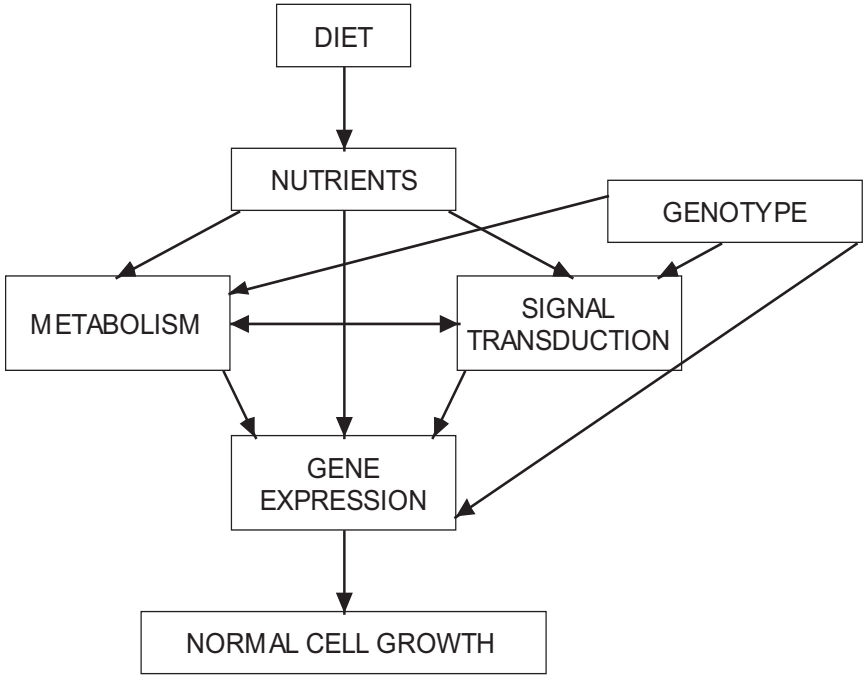

FIGURE 4-2 Genotype interactions with metabolic networks.

behavior, individual genetic variations can cause large differences in response to environmental stimuli. The interactions between the nutrient environment and the genome are extremely important, and there are many intervening steps. Evaluation of these interactions and the intervening steps offers a dynamic approach to addressing the interactions between nutrition and the genome.

NUTRIGENOMICS: INDUSTRY'S PERSPECTIVE

Presented by Peter Gillies, Adjunct Professor, Department of Nutrition Science, Pennsylvania State University; Adjunct Professor, Department of Nutrition, University of Toronto; and Senior Research Fellow in Central Research and Development, E. I. du Pont de Nemours & Company

The Business Challenge

Peter Gillies believes that businesses interested in nutrigenomics are in a bit of a dilemma: "This is clearly an area where the consumer is ahead

of all of us," he said. People are familiar with the idea of a relationship between diet and genes, they know that it may be possible to tailor diets to individuals, and they are already interested in products. "People are looking for things that we may not yet be able to deliver." Not only does industry not have many nutrigenomics-based products to offer at this time, but most companies are still trying to determine if they really need or even want to incorporate nutrigenomics strategies into their businesses, how much they should invest in it, and what the business model should be. At present, there is still a great deal of confusion and uncertainty surrounding the field and its practical impact on human health and nutrition.

Some companies have drawn an analogy between nutrigenomics and pharmacogenomics, thereby linking the science of food and pharmacology. Traditionally, the food and pharmaceutical industries have been worlds apart. The pharmaceutical industry operates in the world of unmet medical needs, rational drug design, clinical trials, and physicians who oversee and manage the interactions between drugs and patients. The food industry, by contrast, operates in the world of taste and convenience, food-related clinical trials are limited in number and scope, and products are promoted directly to consumers. The two worlds, however, have started to move a bit closer together. There are now foods for which there are valid health claims that are grounded in science and approved by the Food and Drug Administration (FDA). In this regard, the food industry has begun moving into the health arena through the manufacture of functional foods and nutraceuticals. Notably, scientists who have experience in the pharmaceutical industry have been moving into the food industry. The movement of such scientists may help bring the rigor of pharmacology into nutrition science that Gillies believes that it really needs. "These scientists bring with them a grounding in pharmacology [and] an understanding of biomarkers, and, more importantly, they bring with them their '-omic' tool kits."

However, it is one thing to migrate pharmacology scientists and their technologies; it is quite another thing to migrate pharmacology-like expectations. "I think what we tend to have done is to migrate some of the business models that we associate with the pharmaceutical industry and some of the consumer expectations that go along with it, and this is when we start to get into trouble." It is important to remember that foods are subtle bioactive entities, not potent drugs. Foods are not biologically targeted designer molecules; they are complex mixtures with pleiotropic nutritive and pharmacological activities. Collectively, the integrated effects of foods can have significant benefits based on changes in disease biomarkers, such as serum lipid levels; however, data on their direct

effects on underlying disease processes and associated clinical sequelae are much harder to come by.

Business models in the food industry are very different from those in the pharmaceutical industry. The food industry does not have a return on investment that is equivalent to that of the pharmaceutical industry. Whereas the pharmaceutical industry can readily protect its investments with patents, the food industry is more likely to do so with trade secrets. Furthermore, although the pharmaceutical industry operates in a "risk-benefit" paradigm, there is nothing like that in the food industry; the food industry uses a "safe and beneficial" paradigm. Ultimately, the food industry will have to find its own way in the development and marketing of nutrigenomics products, and for this to happen a new paradigm is needed.

The food industry can glean a number of lessons from the experience of the pharmaceutical industry. For example, the successes in pharmacogenomics were catalyzed by the availability of a major funding system. "The NIH [National Institutes of Health] allowed highly significant amounts of money to go to laboratories to enable the work to get done. The work got done. Research networks were formed." Unfortunately, there is little evidence thus far that this will happen in nutrigenomics. "We need to provide funding for this field to move ahead." Collaborations between industry and academia have been important to pharmacogenomics; something similar is needed for nutrigenomics.

A problem that has slowed the advancement and broader adoption of pharmacogenomics has been the limited ability to generate and share data from large clinical trials. Issues such as privacy, consent, and intellectual property intervened; and the field was not able to develop the databases that it had hoped it would be able to develop. Gillies emphasized the need for publicly accessible nutrigenomic databases.

As the field of nutrigenomics unfolds in the United States, one can look to Europe for some guidance. In 2004, for example, the European Commission formed the European Nutrigenomics Organization (NuGO) to foster the development of nutrigenomics in Europe (see Box 4-1). The organization's activities include training nutrigenomic researchers, validating and standardizing technologies, and bringing researchers together from across the continent to communicate and collaborate. "I think that what we need over here in the United States is a NuGO," Gillies said.

One area in which Europe excels is forming partnerships with a purpose. A good example of that is the LIPGENE consortium, an organization that comprises 25 research centers around Europe with the purpose of understanding how diet and genetics interact in the development of chronic diseases, such as metabolic syndrome. "In the LIPGENE project," Gillies said, "you have the agricultural industry, the dairy industry, and

BOX 4-1
The European Nutrigenomics Organization

The European Nutrigenomics Organization (NuGO) comprises 20 partner organizations in 10 countries. NuGO was formed in 2004 and is funded by the European Commission's Research Directorate General under the Food Quality and Safety Priority of the Sixth Framework Programme for Research and Technological Development.

NuGO aims to provide a forum for research integration and to enhance future research efforts in nutrigenomics. NuGO cites on its website six specific aims:

- Strengthen the European scientific and technological excellence in nutrigenomics by bringing together the critical mass of resources and expertise needed to offer leadership in the rapidly developing field of nutrigenomics.
- Define individual response to nutrients and refine the requirements for population subgroups on the basis of genetic variations, sex, and life stages.
- Determine the relative health benefits and risks of food compounds for different population subgroups and improve public health.
- Spread excellence in nutrigenomics beyond the partnership through training, sharing of methods and facilities, dissemination, and exploitation and enter into dialogue with stakeholder groups.
- Support the competitive aim of the European food industry, facilitating its growth for knowledge-based healthier food production.
- Promote understanding in the ethical, social, legal, economic, and scientific issues of concern in nutrigenomics.

SOURCE: The European Nutrigenomics Organization. Available at http://www.nugo.org/everyone/24017, accessed January 9, 2007.

the food industry coming together to develop novel products that have nutritional benefits; and they are doing so in a way that engages both society and members of the business community in a structured partnership." The goal is to modify the diet with products enriched with omega-3 fatty acids and then to evaluate their impact on metabolic syndrome.

Gillies closed with comments about the future of nutrigenomics in the United States. First, he said, it is important to recognize that at the moment nutrigenomics is "a very fragile paradigm." There are very few success stories. He suggested that they may well be coming in the future, but until then it is important to remain grounded and to not get too far out in front of the data. At the same time, it will not pay to be too timid. "Industry is in a catch-22. If we engage too early, we will most assuredly fall prey to the problems of hope and hype. If we engage too late, we will have lost the opportunity to shape our future." Perhaps, he said, the cor-

rect approach is to put together some sort of national agenda in this area. Finally, one of the keys to enabling the success of nutrigenomics will be having the proper bioinformatics infrastructure in place. "These are not the types of technical platforms that we have in the food industry," he said. "Somehow we need to make these available to the food industry in order for them to move ahead."

NEEDS AND OPPORTUNITIES IN THE FOOD AND AGRICULTURAL SCIENCES

Presented by Joseph Spence, Director, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture

No matter how great the potential of nutrigenomics to deepen the understanding of nutrition and to point the way to healthier eating is, that potential will not be realized without corresponding advances in other areas. One of those areas is agriculture and the food industry. Joseph Spence addressed the role that agriculture and the food industry can play in helping nutrigenomics meet its potential.

Spence pointed out that that role will develop in a number of different areas; one of the most important will be modification of the nation's food supply to reflect new understandings about nutritional requirements, something that is already being done on a limited scale. For example, the Agricultural Research Service (ARS) developed the heart-healthy NuSun sunflower as a variety high in oleic acid, a monounsaturated fatty acid. That variety now accounts for about 77 percent of the sunflowers produced for oil seeds in the United States.

The NuSun sunflower was developed by traditional breeding methods, but researchers are also using genetic engineering methods to create varieties with desired characteristics. Scientists at ARS have also produced transgenic tomatoes that contain four to eight times as much lycopene, a carotenoid known for its strong antioxidant properties, as nontransgenic tomatoes.

As nutrigenomics research reveals more details about the roles of various nutrients, the agriculture industry can modify food to take these findings into account for future research and development. Of particular importance will be demonstration of the clear nutritional benefits of these various nutrients for individuals, as illustrated in Figure 4-3. "We cannot fall into the trap of saying this probably will have a beneficial effect," Spence said. "We have to clearly identify the health benefits and get people to understand that these are long-term benefits."

Another role traditionally played by the U.S. Department of Agri-

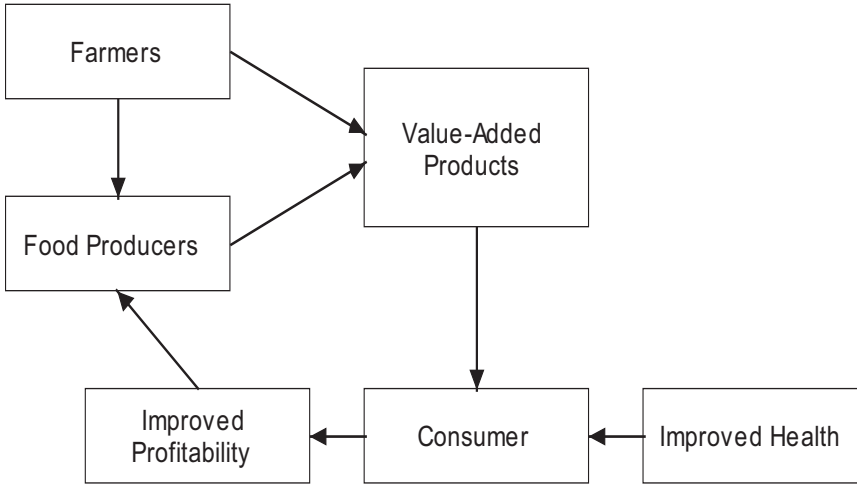

FIGURE 4-3 The role of value-added products, e.g., from nutrigenomics, in improved outcomes, particularly improved health, to consumers.

culture has been to make recommendations for a healthy diet with such tools as the food pyramid, originally released in 1992 and modified in 2005. The original food pyramid was a population-based, one-size-fits-all model with one set of guidelines intended for everyone, but the revised version (MyPyramid) consists of a number of pyramids that are matched to individuals on the basis of age, sex, and level of physical activity.

Nutrigenomics will make it possible to extend this pattern and to offer dietary guidelines that are even more closely focused on individuals. "We have different genetic backgrounds, different life styles, and so on," Spence noted, "so it is very difficult, in the nutrigenomic age, to continue to make dietary advice that is based on population types of studies." In the future, he suggested, dietary guidelines will take into account an individual's genetic background and will vary the recommendations on the basis of that individual's ancestry. Although relatively little work has been done in this area so far, it seems likely that nutrigenomics studies will eventually pinpoint ways in which nutritional advice should differ between, for example, American Indian/Alaska Natives and descendants of northern Europeans, and nutritional guidelines should be written to reflect those insights.

THE ECONOMIC POTENTIAL OF NUTRIGENOMICS

Presented by Patricia Danzon, Celia Moh Professor, Wharton School of Management; Professor and Chair, Department of Health Care Systems; and Professor of Insurance and Risk Management, University of Pennsylvania

One of the driving forces behind the development of any technology is the expectation of financial reward. A technology that is expected to pay off handsomely will tend to attract more investment and develop more quickly than one whose financial prospects are less sanguine. Patricia Danzon spoke about the potential economic impact of nutrigenomics in managing health care costs.

Danzon said that because the science of nutrigenomics is still in its infancy, it would be foolhardy to try to estimate the economic impact at this point. She therefore devoted her presentation to offering ideas on the economic potential of nutrigenomics and on strategies to maximize that potential.

Figure 4-4 shows that expenditures on health care in the United States have been rising rapidly. About 15 percent of the gross domestic product is spent on medical care," she noted. "Interestingly," she commented, "we are now almost at the point where about half of that is coming out of public funds." In addition, the average cost of an insurance premium for the average family is about $12,000 a year. Furthermore, that 15 percent

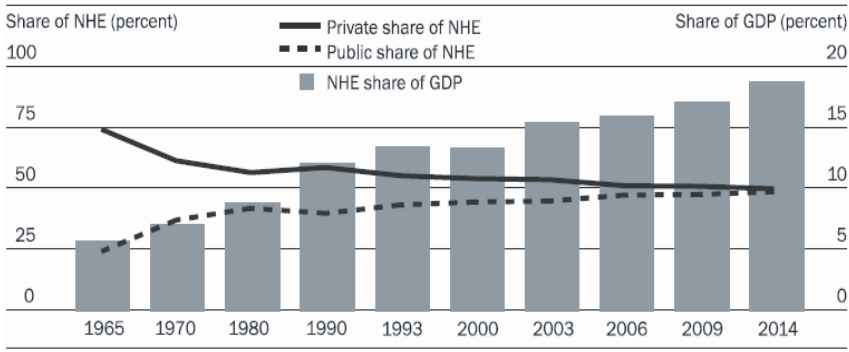

FIGURE 4-4 National health expenditures (NHEs) as a share of the gross domestic product (GDP) and private and public shares of NHE, selected years, 1965 to 2014.
SOURCE: Stephen Heffler et al. U.S. Health Spending Projections for 2004-2014. *Health Affairs*, web exclusive, W5-74. February 23, 2005. http://content.healthaffairs.org/cgi/content/full/hlthaff.w5.74/DC1.

of GDP spent on medical care is just the tip of the iceberg, she said. The entire economic burden of disease and poor health, including such things as lost productivity, is far greater.

Furthermore, most of the spending on health care goes toward treatment rather than prevention. Ninety-five percent of health care spending is on people who are sick, with only 5 percent spent on preventing people from getting sick, Danzon said, quoting Tommy Thompson, former secretary of the U.S. Department of Health and Human Services. If more money was spent on effective prevention, the economic benefit could be considerable. This is an area in which nutrigenomics would be expected to have a major economic effect.

Danzon offered two caveats. First, it is not clear how easy it will be to get individuals to change their behavior in response to nutrigenomics-based advice. Second, the apparent potential of a field is not always met. "I am struck by the fact that for the last 5 years so little has been delivered [in the pharmaceutical industry] compared to what was promised by the more optimistic people at the time of the mapping of the human genome." Nonetheless, nutrigenomics could yield a huge economic impact if the gains in scientific knowledge are significant.

The economic benefits of nutrigenomics are likely to be long term. The effects of dietary change, however, are likely to be cumulative and affect diseases that occur later in life. Thus, a marker of the benefit of nutrigenomics may be its impact on the severity of disease or disease progression.

In addition, efforts at disease prevention are the most cost-effective when they target high-risk diseases among the population subgroups most likely affected. This is because the cost of prevention is spread out among everyone who is a target of the prevention effort, whereas the savings come only when the disease that is the target of prevention is prevented in those who would have acquired the disease otherwise. Thus, disease prevention is more cost-effective when efforts to prevent diseases that are particularly costly are targeted to high-risk subpopulations.

Cost-effectiveness may also improve if the cost of prevention is decreased. According to Danzon, it makes sense to think in terms of modifying diets rather than adding supplements, which can add considerably to the cost of a nutritional regimen. Unfortunately, she noted, the current system is generally set up to favor the use of supplements rather than simply encouraging the consumption of a variety of foods. Insurance companies, for example, are likely to pay for medicines or dietary supplements that are prescribed for medical reasons, but they generally do not pay for healthier foods. Similarly, if the FDA approves supplements but says nothing about foods that may have a therapeutic impact, consumers' choices may be affected. "We are heading into an era where there are

going to be new types of regulations that need to be put in place and new ways of thinking about insurance coverage to make sure that we enhance this movement and don't distort it."

Finally, Danzon pointed out that it will be important to demonstrate conclusively the effectiveness of various nutrigenomics interventions. If the nutrigenomics data signal is obscured by the general "noise" that consumers are regularly bombarded with, not enough people will pay attention. Thus, it will be important to make the signal-to-noise ratio big enough to influence consumer behavior. If that can be done and if care can be taken to target high-risk diseases and subgroups and to rethink regulatory and insurance strategies, Danzon said, "I think that nutrigenomics research has the potential to have huge economic benefits."

NUTRIGENOMICS IN ACADEMIC AND PUBLIC HEALTH: HOW CAN WE MOVE THE FIELD FORWARD?

Presented by Harvey V. Fineberg, President, Institute of Medicine, the National Academies

To close the workshop, Harvey Fineberg summed up the proceedings of the previous 2 days and offered two new concepts to consider as nutrigenomics moves into the future.

First, Fineberg spoke of the challenge that nutrigenomics will pose to the existing public health paradigm. There are a variety of public health programs aimed at prevention, for example, programs that encourage the use of seat belts and smoking cessation and programs that issue dietary guidelines aimed at lowering the risk of cancer. For the most part, they are all applied uniformly and universally to the population. "Everyone is told, 'Wear a seat belt.' Everyone is told, 'You should stop smoking, and if you don't smoke, don't start.' Everyone is told what dietary guidelines are appropriate." The seat-belt advice is unlikely to change, since it is unlikely that it would be determined that some people are at less risk of bodily injury in an accident and thus do not need to wear them. It is at least conceivable, Fineberg said, that a small percentage of the population is not put at risk by smoking and that no matter how much tobacco smoke they inhale, they would still have a very small risk of developing lung cancer, but the smoking advice would likely remain the same.

Dietary advice is different, however. It is not just possible but likely that there are nutrients that affect some population groups differently than others, and public health guidelines will have to take such differences into account. That will put greater demands on the public, however. It will not simply be a matter of knowing that it is a good idea to wear seat belts whenever you go somewhere in a car. Instead, as Fineberg pointed

out, "a public health paradigm of universal education is going to have to be adapted to the scientific reality and scientific knowledge as it develops and it unfolds." This paradigm will demand a greater effort from both the people who develop the guidelines and those who follow them.

Furthermore, like efforts in physics to develop a grand unified theory that would unite a number of areas of physics into one, biology and health also have a challenge to develop an analogous grand unification theory. That is, many different factors are known to play a role in the development of disease. These range from socioeconomic and demographic factors, behavioral choices, and environmental exposures such as nutrients or chemicals all the way to the genetic makeup of an individual and how that interacts with the specific constituents of foods.

"Is it possible," Fineberg asked, "that over time we can identify what ultimately must be the biological common pathways through which all determinants of disease or health must ultimately exercise their effect?" Such an understanding would certainly require new theoretical developments and ways of expressing and calculating biological mechanisms, but there is no reason to believe that the development of such a grand unification theory is not possible.

Fineberg concluded with a question meant to be a provocation and a challenge to the audience and to those everywhere interested in nutrigenomics: "Is it not possible that nutrition science—bridging, as it does, everything from human behavior, cultural values, all the way through to nutrigenomics and metabolomics and so on—might not be the crossroads for such a grand unification theory for health and disease?"

Appendix A

Glossary

ABBREVIATIONS/ACRONYMS

CVD	Cardiovascular disease
Dnmt	DNA methyltransferase gene
FLP	Fibrinogen-like proteins
FRT	FLP recognition target
HDAC	Histone deacetlyase
IAP	Intracisternal A-particle
ICF	Immunedeficiency Centromeric region instability and Facial abnormalities
IPE	IAP proximal enhancer element
MeCP2	Methylene-CpG-binding protein
MTHFR	Methylene-tetrahydrofolate reductase
SHMT	Serine hydroxymethyltransferase
SNP	Single nucleotide polymorphism

DEFINITIONS

APC tumor suppressor

The APC tumor suppressor gene is mutated in familial adenomatous polyposis and in approximately 80 percent of sporadic colon cancers.

Chimera

An individual or organism whose cells and tissues have non-uniform or"mosaic" genetic elements.

Chromatin
: A complex of DNA and proteins that makes up chromosomes. Chromatin functions to package DNA into a small enough volume to fit in a cell, facilitate mitosis and meiosis, and help control gene expression

CRE-lox
: A system for rapid execution of homologous recombination-mediated insertion or deletion in the mouse genome. The bacteriophage P1 encodes the recombination system consisting of loxP, a short asymmetric DNA sequence, and the CRE recombinase enzyme. CRE mediates site-specific recombination between loxP sites.

Enhancer and silencer elements
: Specific genomic regions with genetic sequences that influence transcriptional activation.

Epigenetic
: Mechanisms, processes, and/or biological compounds that affect a cell, organ, or individual without changing or perturbing DNA.

FLP/FRT
: A technique of induced somatic recombination using the FLP site-specific recombinase and FRT (FLP recombinase target). When expression of FLP is induced by heat shock it catalyzes crossing-over and recombination of maternal and paternal chromosomes at the FRT site.

Knockout mutation
: A technique for targeted disruption of specific gene(s). A null mutation is introduced into a gene by designed alteration in a cloned DNA sequence; the sequence is then introduced into the genome by homologous recombination and replacement of the normal allele in embryonic stem cells, which, when injected into mouse embryos, gives rise to chimeric animals that no longer carry a gene they would normally have carried.

Nutraceutical
: Any substance that is a food or part of a food that provides medical or health benefits, including preventing or treating disease.

Phenotype
: The physical, biochemical, and physiologic makeup of an individual; determined by genetic and environmental factors. “Phenotype” is often used to refer to the overt, observable features that an individual has.

PI 3-kinase
: Phosphotidalinositol-3-kinase; consists of a p85 regulatory subunit and a p110 catalytic subunit. This kinase phosphorylates inositol phospholipids and can be activated by receptor tyrosine kinases as well as other cell-surface receptors, including G-protein-linked receptors.

Pleiotropy
: The ability of a single gene to produce multiple phenotypic effects.

Ras MAP kinase
: A signal transduction pathway that mediates the growth-promoting effects of the tyrosine kinase receptor family of hormones.

RNAi
: RNA interference; a tool to analyze the loss of function of individual genes, identify complex regulatory pathways, and silence genes through short-interfering RNAs (siRNAs). The process uses double-stranded RNA to suppress expression of a target gene by triggering specific degradation of the complementary messenger RNA sequence.

shRNA
: Short hairpin RNA; RNA structure that is processed intracellularly into short duplex RNAs that have gene-silencing properties similar to siRNAs (see RNAi).

Appendix B

Workshop Agenda

Nutrigenomics and Beyond: Informing the Future
June 1–2, 2006
National Academy of Sciences Auditorium
2100 C Street, NW
Washington, DC 20037

Day 1

8:30 a.m. **Welcome**
Ann Yaktine, Senior Program Officer, Food and Nutrition Board
Robert M. Russell, Chair, Food and Nutrition Board
Harvey V. Fineberg, President of the Institute of Medicine
Nicholas Schork, Food and Nutrition Board Member and Workshop Moderator

8:45 **Keynote Address**
Why Nutrition and Genomics Are Important
- Bernadine Healy, *U.S. News & World Report*

9:30 *Introductory Comments on Scientific Sessions*
- John Milner, National Cancer Institute, National Institutes of Health

9:45 **Scientific Session I: Human Genetic Variation and Nutrition**
Moderator: Nicholas Schork, University of California, San Diego

Human Genetic Variation: New Tools for Understanding Its Role in Health and Disease
- Francis Collins, National Human Genome Research Institute, National Institutes of Health

10:15 Break

10:45 *Implementation of the Human HapMap Initiative and Large-Scale Polymorphism Studies*
- David Cox, Perlegen Sciences, Inc.

Contemporary Nutrigenetics Studies
- Jose Ordovas, Tufts University

Discussion

11:45 **Economic Impact**
Estimating the Economic Impact of Nutrigenomics in Managing Health Costs
- Patricia Danzon, University of Pennsylvania

12:15 p.m. Lunch

1:15 **Scientific Session II: Epigenetics**
Moderator: Rowena Matthews, Life Sciences Institute

Critical Events; Genomic Programming and Reprogramming
- Rudolph Jaenisch, Massachusetts Institute of Technology

Nutrition Modulation of Epigenetic Programming and Reprogramming
- Patrick Stover, Cornell University

Role of Maternal and Infant Nutrition in Genetic Programming and Epigenetics
- Cutberto Garza, Boston College

Discussion

3:15 Break

3:30	**Scientific Session III: Systems Biology** Moderator: Robert Cousins, University of Florida
	Genetic Networks and Applied Systems Biology • C. Ronald Kahn, Joslin Diabetes Center, Harvard University
	Genome-Scale Reconstruction of the Human Metabolic Network: Basic Concepts and Practical Uses • Bernhard Palsson, University of California, San Diego
	Emerging Technologies Nanotechnology • Martin Philbert, University of Michigan
	Discussion
5:30	Adjourn for the Day

Day 2

8:45 a.m.	**Session IV: Implications for the Future** Introduction and Moderator: John Milner
9:00	*Issues in Ethics* • David Castle, University of Guelph
9:30	*Science Journalism and the Nutrigenomics Revolution* • Sally Squires, *The Washington Post* • Susan Okie, *New England Journal of Medicine*
10:00	*Genes, Metabolism, and Behavior: Interacting Networks* • Ralph Greenspan, Neuroscience Institute
	Nutrigenomics: Industry's Perspective • Peter Gillies, DuPont
11:00	Break
11:15	*Needs and Opportunities in the Food and Agricultural Sciences* • Joseph Spence, Agricultural Research Service, U.S. Department of Agriculture

Nutrigenomics in Public Health: Moving the Field Forward
- Harvey V. Fineberg, President, Institute of Medicine, The National Academies

12:15 p.m. Discussion and Next Steps

1:00 Adjourn

Appendix C

Speaker Biographies

David Castle, Ph.D., is Canada Research Chair in Science and Society, Department of Philosophy, University of Ottawa. Dr. Castle's research and teaching interests lie in the philosophy of the life sciences, with particular emphasis on evolutionary biology and ecology, environmental philosophy, and the ethical implications posed by biotechnology. He is a principal investigator of the Genome Canada-funded Canadian Program on Genomics and Global Health and the Social Sciences and Humanities Research Council of Canada-supported Legal Models of Biotechnology Intellectual Property Protection: A Transdisciplinary Approach. He is coeditor of *Genetically Modified Foods: Debating Biotechnology*, published by Prometheus Press, and *Aquaculture, Innovation and Social Transformation*, which is forthcoming from Springer. He has also published in *Postgraduate Medical Journal*, *Biology and Philosophy*, *Dialectica*, *Ethics and the Environment*, and *Trends in Biotechnology and Public Affairs Quarterly*.

Francis S. Collins, M.D., Ph.D., is a physician-geneticist noted for his landmark discoveries of disease genes and his leadership of the Human Genome Project and is director of the National Human Genome Research Institute (NHGRI) at the National Institutes of Health (NIH). With Dr. Collins at the helm, the Human Genome Project consistently met projected milestones ahead of schedule and under budget. This remarkable international project culminated in April 2003 with the completion of a finished sequence of the human genetic blueprint. Building on the foundation laid by the Human Genome Project, Dr. Collins is now leading

NHGRI's effort to ensure that this new trove of sequence data is translated into powerful tools and thoughtful strategies to advance biological knowledge and improve human health. Dr. Collins is also known for his consistent emphasis on the importance of ethical and legal issues in genetics. In addition to his achievements as the NHGRI director, Dr. Collins's laboratory has discovered a number of important genes, including those responsible for cystic fibrosis, neurofibromatosis, Huntington's disease, and most recently, the gene that causes Hutchinson-Gilford progeria syndrome, a dramatic form of premature aging. Dr. Collins received a B.S. from the University of Virginia, a Ph.D. in physical chemistry from Yale University, and an M.D. from the University of North Carolina. He is a member of the Institute of Medicine and the National Academy of Sciences.

David R. Cox, M.D., Ph.D., is chief scientific officer of Perelegen Sciences, Inc. Dr. Cox is internationally recognized for his research on the molecular basis of human genetic disease. After receiving B.A. and M.S. degrees from Brown University in Rhode Island, Dr. Cox obtained M.D. and Ph.D. degrees from the University of Washington, Seattle. He then completed his pediatric residency at the Yale-New Haven Hospital in New Haven, Connecticut, and was a fellow in both genetics and pediatrics at the University of California, San Francisco. From 1980 to 1993, Dr. Cox held faculty positions in the Departments of Pediatrics, Biochemistry, and Psychiatry at the University of California, San Francisco. In 1993, he accepted a position as a professor of genetics and pediatrics at the Stanford University School of Medicine as well as the position of codirector of the Stanford Genome Center. In October 2000, Dr. Cox left his position at Stanford University to become the chief scientific officer of Perelegen Sciences, Inc. Dr. Cox is certified by both the American Board of Pediatrics and the American Board of Medical Genetics. He has served on several international and national councils and commissions, including the Council of the Human Genome Organization and the National Bioethics Advisory Commission. He currently serves as a member of the Health Sciences Policy Board of the Institute of Medicine. Dr. Cox's honors include election to the Institute of Medicine of the National Academy of Sciences.

Patricia Danzon, Ph.D., is the Celia Moh Professor at the Wharton School of Management, University of Pennsylvania, where she is also professor and chair of the Health Care Systems Department and professor of insurance and risk management. Professor Danzon received a B.A. from Oxford University, Oxford, England, and a Ph.D. in economics from the University of Chicago. Professor Danzon is an internationally recognized expert in the fields of health care, pharmaceuticals, insurance, and liability

systems. She is a member of the Institute of Medicine and the National Academy of Social Insurance. She has served as a consultant to the World Bank, the European Commission, the New Zealand Treasury, the Asian Development Bank, the U.S. Agency for International Development, the Institute for Civil Justice, the Alliance of American Insurers, and others. Professor Danzon is an associate editor of the *Journal of Health Economics* and the *International Journal of Health Care Finance and Economics*. She has published widely in scholarly journals on a broad range of subjects related to medical care, pharmaceuticals, insurance, and the economics of law. Selected publications include: Cross National Price Differences for Pharmaceuticals: How Large and Why? (with L. W. Chao, *Journal of Health Economics*, 2000); *Pharmaceutical Price Regulation: Global vs. National Interests* (AEI Press, 1997); *Medical Malpractice: Theory, Evidence and Public Policy* (Harvard University Press, 1985); The Impact of Price Regulation on the Launch Delay of New Drugs (with Y. Richard Wang and Liang Wang, *Health Economics*, 2005); Productivity in Pharmaceutical-Biotechnology R&D: The Role of Experience and Alliances (with Sean Nicholson and Nuno Pereira, *Journal of Health Economics*, 2005); and Biotech-Pharma Alliances as a Signal of Asset and Firm Quality (with Sean Nicholson and Jeff McCulloch, *Journal of Business*, 2005).

Harvey V. Fineberg, M.D., Ph.D., is president of the Institute of Medicine. He served as provost of Harvard University from 1997 to 2001, following 13 years as dean of the Harvard School of Public Health. He has devoted most of his academic career to the fields of health policy and medical decision making. Dr. Fineberg helped found and served as president of the Society for Medical Decision Making and also served as adviser and consultant to the U.S. Centers for Disease Control and Prevention and the World Health Organization. At the Institute of Medicine, he has chaired and served on a number of panels dealing with health policy issues, ranging from AIDS to vaccine safety. He is the author, coauthor, and coeditor of numerous books and articles on such diverse topics as AIDS prevention, tuberculosis control, assessment of new medical technology, clinical and public health decision making, and understanding risk in society.

Cutberto Garza, M.D., Ph.D., is academic vice president and dean of faculties of Boston College. He is an internationally recognized expert on infant and maternal nutrition. He has published extensively in this field, with more than 200 contributions to the peer-reviewed literature, monographs, and technical reports. Before joining Boston College, he served as director of the Division of Nutritional Sciences at Cornell University from 1988 to 1998, was reappointed to this position from 2003 to 2005, and served as vice provost of Cornell University from 1998 to 2000. He

served as director of the United Nations University's Food and Nutrition Program from 1998 to 2006. Before moving to Cornell, he held the rank of professor of pediatrics at Baylor College of Medicine and served as associate director for the U.S. Department of Agriculture/Baylor Children's Nutrition Research Center. He has served on numerous advisory groups for the U.S. government, the National Academies, the World Health Organization, the World Food Program, and other local and international agencies. Among these appointments are chair of the 1999 U.S. Dietary Guidelines Advisory Committee appointed by the secretaries of the U.S. Departments of Agriculture and Health and Human Services and cochair of the US-EU Biotechnology Forum, appointed by Presidents Bill Clinton and Romano Prodi. He served as chair of the Food and Nutrition Board of the Institute of Medicine from 1996 to 2002. He currently chairs the World Health Organization's Multicenter Growth Reference Study, a six-country effort designed to develop new international growth standards for infants and young children, and on the Food and Drug Administration's Food Advisory Committee. Dr. Garza received an M.D. from the Baylor College of Medicine in 1973 and a Ph.D. from the Massachusetts Institute of Technology in 1976. He was the recipient of the 1996 Feinstein World Hunger Prize for Research and Education, awarded by Brown University. He is a member of the Institute of Medicine and was named to the inaugural class of National Associates of the National Academy of Sciences in 2006 in recognition of his contributions to the work of the Academies.

Peter J. Gillies, Ph.D., F.A.H.A., is an adjunct professor in the Department of Nutrition Science at The Pennsylvania State University, University Park; an adjunct professor in the Department of Nutrition at the University of Toronto, Toronto, Ontario, Canada; and a senior research fellow in Central Research and Development at the DuPont Company in Wilmington Delaware. Dr. Gillies received a Ph.D. in medical science from McMaster University in 1978. His research interests are in molecular nutrition, nutritional genomics, and cardioinflammatory diseases; his technical expertise resides in the areas of lipoprotein metabolism, pharmacology, and toxicology.

Ralph Greenspan, Ph.D., has worked on the genetic foundations of behavior in the fruit fly (*Drosophila*) almost since the inception of the field, studying with one of its founders, Jeffrey Hall, at Brandeis University, where Dr. Greenspan received a Ph.D. in biology in 1979. He subsequently conducted research at Princeton University, the Roche Institute of Molecular Biology, and New York University before joining The Neurosciences Institute in San Diego in 1997, where he is the Dorothy and Lewis B. Cullman Fellow in Experimental Neurobiology. Dr. Greenspan's research

activities have included the demonstration that the fruit fly has sleep-like and attention-like behaviors similar to those of humans, the molecular identification of genes underlying natural variations in food-dependent behavior, and studies of the principles governing gene networks underlying behavior. Dr. Greenspan has been awarded fellowships by the Helen Hay Whitney Foundation, the Searle Scholars Program, the McKnight Foundation, the Sloan Foundation, and the Klingenstein Foundation. In addition to numerous research papers, he has also authored an article for *Scientific American* and several books, including *Genetic Neurobiology* (with Jeffrey Hall and William Harris) and *Fly Pushing: The Theory and Practice of* Drosophila *Genetics*, which has become a standard work in the field.

Bernadine Healy, M.D., is a health editor for *U.S. News & World Report* and writes the On Health column for the magazine. A physician trained at Harvard University and the Johns Hopkins University, Healy is former president of the American Red Cross and is a past director of the NIH, where she started the Women's Health Initiative. Dr. Healy served as deputy science advisor to President Ronald Reagan from 1984 to 1985. She has also served as president of the American Heart Association and was awarded the American Heart Association Special Award for Service and the 1992 Dana Foundation's Distinguished Achievement Award for her work on promoting research on the health problems of women. She is currently a member of the President's Council of Advisors on Science and Technology and is a leader in patient care research and education. Dr. Healy has been a member of the Institute of Medicine since 1987.

Rudolf Jaenisch, M.D., is a founding member of the Whitehead Institute for Biomedical Research and a professor of biology at the Massachusetts Institute of Technology. He is a pioneer in making transgenic mice, some of which have produced important advances in understanding cancer, neurological and connective tissue diseases, and developmental abnormalities. These methods have been used to explore basic questions, such as the role of DNA modification, genomic imprinting, X-chromosome inactivation, nuclear cloning, and, most recently, the nature of stem cells. His laboratory is renowned for its expertise in cloning mice and in studying the myriad factors that contribute to the success and failure of this process. Dr. Jaenisch and colleagues have more recently gained insights into therapeutic cloning and, indeed, have rescued mice with a genetic defect through therapeutic cloning and gene therapy. Dr. Jaenisch also directs the new human stem cell facility at the Whitehead Institute. Dr. Jaenisch received an M.D. from the University of Munich. He is an elected fellow of the American Academy of Arts and Sciences and is a member of

the National Academy of Sciences and the International Society for Stem Cell Research.

C. Ronald Kahn, M.D., received undergraduate and medical degrees from the University of Louisville. After training in internal medicine at Washington University, he moved to the NIH, where he rose to become the head of the Section on Cellular and Molecular Physiology of the National Institute of Diabetes and Digestive and Kidney Diseases. In 1981, he moved to Boston to become research director of the Joslin Diabetes Center. In 1984 he became professor of medicine at Harvard Medical School and in 1986 the Mary K. Iacocca Professor. Under his leadership, the amount of research performed at the Joslin Diabetes Center grew more than 10-fold. In 2000, he was named president and director of the Joslin Diabetes Center. Dr. Kahn is the preeminent investigator of insulin signal transduction and mechanisms of altered signaling in disease. His laboratory has produced multiple seminal observations regarding the insulin receptor kinase, its substrates, the molecular components of the insulin-signaling network, and their alterations in disease; these finding have revolutionized the field. Dr. Kahn has received numerous honors and awards, including the highest scientific awards of the American Diabetes Association, the Juvenile Diabetes Research Foundation, the European Association for the Study of Diabetes, the British Diabetes and British Endocrine Societies, the International Diabetes Federation, the American Federation of Clinical Research, and the Endocrine Society, as well as the Hamdan Award for Medical Research, the Rolf Luft Award of the Karolinska University, the Lawson Wilkins Award of the Pediatric Endocrine Society, the Beering Award of Indiana University, and the Allyn Taylor International Prize in Medicine. Dr. Kahn has served on many national commissions and advisory boards, including chairman of the congressionally established Diabetes Research Working Group. Dr. Kahn has also served as president of the American Society of Clinical Investigation and on the editorial boards of multiple journals. In 1999, Dr. Kahn was elected to membership in the National Academy of Sciences and the Institute of Medicine for his distinguished achievements in original research. Dr. Kahn also holds honorary doctor of science degrees from the University of Paris, the University of Louisville, and the University of Geneva and is an honorary professor at the Peking University School of Medicine.

Susan Okie, M.D., is a physician and medical writer for the Health section of *The Washington Post*. She attended Swarthmore and Radcliffe Colleges and Harvard Medical School. Dr. Okie came to *The Washington Post* as a summer intern while in medical school, and after graduating, she joined the paper's metropolitan staff in 1979 as a medical reporter.

Her 1980 series on D.C. General Hospital won the Washington-Baltimore Newspaper Guild's Public Service Award. In 1981, she left *The Washington Post* to complete a residency in family practice at the University of Connecticut. She then taught part-time in the university's family medicine department and worked at a clinic in Hartford. From 1985 to 1991, Dr. Okie was a reporter on *The Washington Post's* national staff, covering medicine and biomedical research. She spent 3 years in Kenya as a special correspondent reporting on AIDS, wildlife biology, family planning, anthropology, and other scientific and social issues. Upon her return to the United States in 1994, she became *The Washington Post's* national science editor, and in 1996, she returned to reporting as a staff writer for the weekly Health section.

Jose M. Ordovas, Ph.D., is senior scientist and director of the Nutrition and Genomics Laboratory, Jean Mayer Human Nutrition Research Center on Aging, at Tufts University. Dr. Ordovas's major research interests focus on the genetic factors that predispose individuals to cardiovascular disease and their interaction with the environment and behavioral factors, with a special emphasis on diet, particularly omega-3 and -6 fatty acids. He has participated in the Framingham Heart Study for nearly 20 years and is carrying out multiple cross-cultural studies to determine cardiovascular risk in different populations around the world, including Asian Pacific and Mediterranean populations. He has published about 400 original research articles and has written numerous reviews and edited several books on diet and coronary heart disease, diet and genetics, and the role of omega-3 fatty acids on lipoproteins and atherosclerosis. Dr. Ordovas serves on numerous editorial boards and is active with several American Heart Association and NIH committees, including the National Heart, Lung, and Blood Institute Program Projects Parent Committee. Throughout his career, Dr. Ordovas has contributed his expertise to various global organizations. He has served as a nutrition expert for the American Soybean Association, consulting for Mexico and Central America; was named expert consultant to the Singapore Ministry of Health; and is the recipient of the Francisco Grande Memorial Lecture for Excellence in Nutrition.

Bernhard Ø. Palsson, Ph.D., is professor of bioengineering and adjunct professor of medicine at the University of California, San Diego (UCSD). Professor Palsson is the author of more than 200 peer-reviewed scientific articles. His current research at UCSD focuses on (1) the reconstruction of genome-scale biochemical reaction networks, (2) the development of mathematical analysis procedures for genome-scale models, and (3) the experimental verification of genome-scale models, with a current emphasis on cellular metabolism and transcriptional regulation in *Escherichia*

coli and yeast. He holds a Ph.D. from the University of Wisconsin that he earned in 1984. Professor Palsson held a faculty position at the University of Michigan for 11 years from 1984 to 1995. He received an Institute of International Education Fellowship in 1977, a Rotary Fellowship in 1979, and a North Atlantic Treaty Organization fellowship in 1984; was named the G. G. Brown Associate Professor at Michigan in 1989, a Fulbright Fellow in 1995, and an Ib Henriksen Fellow in 1996; and received the Olaf Hougen Professorship at the University of Wisconsin in 1999 and the Lindbergh Tissue Engineering award in 2001. Dr. Palsson sits on the editorial boards of several bioengineering and biotechnology journals. He holds more than 20 U.S. patents, many of which are in the area of hematopoietic stem cell transplantation, cell culture technology, bioreactor design, gene transfer, cell separations, high-throughput single-cell manipulation, network reconstruction, in silico model building, and metabolic engineering. He cofounded a biotechnology company, AASTROM BIOSCIENCES, in 1988, where he served as the vice president of developmental research for 2 years. Dr. Palsson is also the founder and cofounder of ONCOSIS, a company that is focused on the purging of occult tumor cells in autologous bone marrow transplants; CYNTELLECT, a company that is focused on instrumentation for high-throughput screening and in situ cell sorting and processing; GENOMATICA, a company that is focused on in silico biology; and the Iceland Genomics Corporation, a company that is focused on tracing the genetic basis for common human diseases in the Icelandic population.

Martin Philbert, Ph.D., received a Ph.D. in 1988 in neurochemistry and experimental neuropathology from the Royal Postgraduate Medical School of London University in England. There he received a Medical Research Council Scholarship in experimental neuropathology. In the spring of 1988, Dr. Philbert was recruited as a postdoctoral fellow in neurotoxicology at Rutgers University. While he was at Rutgers, Dr. Philbert investigated the mechanisms by which chemicals that gain access to the central nervous system produce specific neurotoxic effects. In 1995, he joined the Toxicology Faculty at the University of Michigan as an assistant professor. Dr. Philbert is a professor of toxicology and senior associate dean for research at the University of Michigan School of Public Health. He has provided service on a variety of committees at the university, including the President's Commission on Undergraduate Education, the University Taskforce on Multidisciplinary Teaching, and the University Committee on the Use and Care of Animals. Currently, Dr. Philbert provides consultation to the National Cancer Institute, the National Institute of Environmental Health Sciences, and the National Toxicology Program; is a scientific advisor to the International Life Sciences Institute in

Washington, D.C.; and is a member of the American College of Toxicology. He teaches courses in general pathology, toxicologic pathology, and mechanisms of neurotoxicity. Dr. Philbert's research interests include the development of nanotechnology for the intracellular measurement of biochemicals and ions and for the early detection and treatment of brain tumors. He is also actively engaged in the investigation of mechanisms of chemically induced energy deprivation syndromes in the central nervous system. He has published more than 100 scholarly manuscripts, book chapters, and abstracts and is the recipient of the 2001 Society of Toxicology Achievement Award. Dr. Philbert holds or has held grant awards from the National Cancer Institute, the National Institute of Environmental Health Sciences, the U.S. Department of Defense's Defense Advanced Research Projects Administration, the Environmental Protection Agency, and the W. M. Keck Foundation.

Robert M. Russell, M.D., is a professor of medicine and nutrition at Tufts University and director of the Jean Mayer U.S. Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University in Boston. He has served on national and international advisory boards, including the USDA Human Investigation Committee (chairman), boards of the Food and Drug Administration, the U.S. Pharmacopoeial Convention, the National Dairy Council Advisory Board, and boards of the American Gastroenterology Association and the American Board of Internal Medicine. He has worked on international nutrition programs in several countries, including Vietnam, Iran, Iraq, Guatemala, China, and the Philippines. Dr. Russell is a member of numerous professional societies, on the editorial boards of five professional journals, past president of the American Society for Clinical Nutrition, and editor of *Nutrition Reviews*. Dr. Russell coauthored the standards for parenteral and enteral nutrition to be used in U.S. long-term-care facilities. He is a staff gastroenterologist at the New England Medical Center Hospitals. Dr. Russell's primary work involves studying the effects of aging on gastrointestinal absorptive function. He is a noted expert in the area of human metabolism of retinoids and carotenoids. Dr. Russell served as a member of the Food and Nutrition Board's Panel on Folate, Other B Vitamins, and Choline and was chair of the Food and Nutrition Board's Panel on Micronutrients. Dr. Russell received a B.S. from Harvard University and an M.D. from Columbia University. He is currently chair of the Institute of Medicine Food and Nutrition Board.

Joseph T. Spence, Ph.D., joined the Agricultural Research Service (ARS), USDA, in 1993, when he was appointed director of the Beltsville Human Nutrition Research Center, Beltsville, Maryland. This is the oldest ARS-

funded human nutrition research center and, under his direction, has become the largest of the ARS-funded human nutrition research centers. The center is actively conducting research on nutrition and immunology, phytonutrients, food composition, nutrition monitoring, and the role of individual nutrients in maintaining health. He received a doctoral degree in nutritional biochemistry from Cornell University in 1977 and was an NIH postdoctoral fellow at the McArdle Laboratory for Cancer Research of the University of Wisconsin, Madison. He was a health scientist administrator at the National Heart, Lung, and Blood Institute of NIH. He was professor of biochemistry and associate dean for research and graduate studies at the School of Medicine of the State University of New York at Buffalo prior to his arrival at Beltsville. His research interest is in the regulation of gene expression in the liver in response to dietary and hormonal influences. In August 2003, he was appointed deputy administrator for nutrition, food safety and quality, where he oversees the ARS national programs related to food and nutrition as well as value-added products, product quality, and bio-based products.

Sally Squires is an award-winning, nationally syndicated columnist for the *Washington Post*. She holds a master's degree in nutrition from the Columbia University's Institute of Human Nutrition and a master's degree in journalism also from Columbia. In 2001, she began the Lean Plate Club, a weekly column about nutrition and physical activity that now has a national circulation of more than five million readers. She's also heard twice weekly on WTWP radio, hosts the popular Lean Plate Club (LPC) web chat at washingtonpost.com and writes an LPC e-mail newsletter that reaches more than a quarter million readers weekly. Her articles have appeared in numerous national magazines and she's the author of the newly published book *Secrets of the Lean Plate Clu*b (St. Martin's Press).

Patrick J. Stover, Ph.D., is professor and director of the Division of Nutritional Sciences at Cornell University. He is also director of the Cornell Institute for Nutritional Genomics. Dr. Stover's research interests are in the regulation of folate-mediated one-carbon metabolism, the development of mouse models to elucidate mechanisms of folate-related pathologies, and the translational control of gene expression by ferritin. In 1996 he received the Presidential Early Career Award for Scientists and Engineers, the highest honor bestowed by the U.S. government on outstanding scientists and engineers beginning their independent careers. He received the ERL Stokstad Award in Nutritional Biochemistry from the American Society for Nutritional Sciences in 1999 and has been selected as an Outstanding Educator four times by Cornell Merrill Presidential Scholars.

MODERATORS

Robert J. Cousins, Ph.D., holds the Boston Family Chair in Nutrition and is director of the Center for Nutritional Sciences and affiliate professor of biochemistry at the University of Florida. His research activities span the molecular and cell biology of zinc absorption, metabolism, and function; nutritional genomics; and technologies for nutritional status assessment. Dr. Cousins' laboratory has produced more than 65 graduate students and postdoctoral associates in nutritional sciences and biochemistry. In his area of research, Dr. Cousins ranks among the top one-half of 1 percent of the most highly cited authors, and he is an elected member of the National Academy of Sciences.

Rowena Matthews, Ph.D., is the G. Robert Greenburg Professor of Biological Chemistry, a research professor in the Life Sciences Institute, a research professor in the Biophysics Research Division, and a professor in the Department of Chemistry at the University of Michigan. She received a B.A. in biology (summa cum laude) at Radcliffe College in 1960 and a Ph.D. in biophysics from the University of Michigan in 1969. Dr. Matthews studies the mechanisms of folate-dependent enzymes and their regulation. Through skillful analytical analyses, she has gained important insights into the chemistry of a large number of transformations of central importance to cellular metabolism. Dr. Matthews is a senior fellow in the Michigan Society of Fellows, a fellow of the American Association for the Advancement of Science, and a fellow of American Society for Microbiology. In 2002 Dr. Matthews was elected to the National Academy of Sciences and became a member of the Institute of Medicine in 2004. In 2005 she was elected to the American Academy of Arts and Sciences.

John Milner, Ph.D., is chief of the Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute. In this position he promotes research that deals with the physiologic importance of dietary bioactive compounds as modifiers of cancer risk and tumor behavior. Previously, Dr. Milner was professor and head in the Department of Nutrition at The Pennsylvania State University, where he also served as director of the Graduate Program in Nutrition. Dr. Milner received a doctorate in nutrition, with a minor in biochemistry and physiology, from Cornell University in 1974. He is a member of the American Society for Nutritional Sciences, the American Association of Cancer Research, the American Society for Clinical Nutrition, the American Chemical Society's Food and Chemistry Division, and the Institute of Food Technology. Dr. Milner is a fellow in the American Association for the Advancement of Science and serves on the editorial boards of the *Journal of Medical Food*, *Journal of Nutritional Biochemistry*, *Nutrition and Cancer*, *Comprehensive*

Reviews of Food Science/Food Safety and Nutrition, *Nutrition and Foods*, and *Journal of Nutrition*.

Nicholas J. Schork, Ph.D., is Director of Research at Scripps Genomic Medicine, a division of Scripps Health, and a professor of Molecular and Experimental Medicine at The Scripps Research Institute. Previously, he was a professor of Psychiatry and Biostatistics at the University of California, San Diego. Dr. Schork's research focuses on human genetic and phenotypic variation, including sequence characterization of genetic variants and related bioinformatics analysis, the molecular physiologic impact of sequence variation, physiologic genomics, the clinical impact of polymorphism, pharmacogenetics, linkage and association analysis, and applied population genetics. His previous positions were as an associate professor of epidemiology and biostatistics at Case Western Reserve University, associate director of the Program for Population Genetics and adjunct associate professor of biostatistics at Harvard University, and an adjunct associate staff scientist at the Jackson Laboratory in Bar Harbor, Maine. Dr. Schork serves on a number of journal editorial boards, and is a frequent participant on NIH-related steering committees and review boards. Dr. Schork has published more than 200 scientific articles and book chapters on genomic analysis of complex traits and diseases. He previously served on the Institute of Medicine Committee on Twin Studies and is a member of the Food and Nutrition Board of the Institute of Medicine.