Nutrition and Genomics Workshop

Executive Summary

On June 18, 2001, the National Cancer Institute (NCI), U.S. Department of Agriculture (USDA), and American Society for Nutritional Sciences (ASNS) convened a group of expert scientists in a wide range of fields, including nutrition, genetics, cancer, and plant sciences. The meeting was organized by Drs. Richard G. Allison, Executive Officer, ASNS; Kathleen C. Ellwood, National Program Leader, Agricultural Research Service (ARS), USDA; and Young S. Kim, Special Expert, Nutritional Science Research Group, Division of Cancer Prevention, NCI.

Introduction and Welcome-Drs. Young Kim, Peter Greenwald, and Caird E. Rexroad, Jr.

Dr. Kim welcomed participants to the meeting and thanked them for attending. She said the goal of this meeting, a joint undertaking of the NCI, USDA, and ASNS, was to raise awareness of the linkages between genes and nutrients as determinants of growth, development, and disease risk. To achieve this goal, novel approaches are needed to promote the use of a new and innovative gene technology in the science of nutrition. Genetic technologies should assist in defining the role of diet in the operation of gene expression.

Dr. Greenwald, Director of the Division of Cancer Prevention, NCI, emphasized the crucial linkage between nutrition and genomics. There is convincing evidence that diet contributes to cancer risk, and recent advances have led to a much greater understanding of what this means in terms of molecular pathways and genomics. New technologies must be applied to nutritional science-utilizing these approaches is essential for convincing others that this field deserves a major allocation of resources. An interdisciplinary approach combining plant genomics and nutritional genomics is required to bridge the considerable gap between what is known about plants and what is known about mammals.

Dr. Rexroad, Associate Deputy Administrator, ARS, USDA, noted that determining the meaning of the human genome is a difficult process and that a multi-interdisciplinary approach will be required to meet the challenges presented by issues of nutrition and genomics. The USDA recognizes the importance of nutrients as a critical part of how foods interact with genes, particularly genes that are being expressed as they relate to cancer. New methods of using existing tools as well as new approaches to understanding these interactions are needed. Dr. Rexroad noted that USDA laboratories currently are conducting functional genomics, genotyping, and proteomics studies.

SESSION I-Moderator: Dr. Steven M. Kappes

The Impact of Nutrients on Health and Disease Risk: From Genomics to People-Dr. Vernon R. Young

Dr. Young, Professor in the Laboratory of Human Nutrition at the School of Science and Clinical Research Center, Massachusetts Institute of Technology, explained that the aim of his presentation was to help begin identifying an effective strategy for exploiting new biological knowledge to understand and define the role of diet and its constituents as determinants of human health and their contribution to disease risk. He outlined the three components of diet: toxicants, nonnutrient constituents, and nutrients. Dr. Young defined nutrients as chemically and fully characterized constituents of a diet, natural or designed, that serve as: (1) significant energy-yielding substrates; (2) precursors for the synthesis of macromolecules and/or compounds needed for normal cell differentiation and growth, renewal, repair, defense, and/or maintenance; and (3) required signaling molecules or cofactors and/or determinants of normal molecular structure or function and/or promoters of cell integrity. This definition is much broader than the traditional textbook definition of a nutrient.

Understanding the role that nutrients play in disease risk and health maintenance is a tremendous challenge, in part because nutrients have multiple functions. Dr. Young explained that nutrients may: (1) catalyze reactions and play cofactor roles; (2) act as substrates for macromolecules; (3) act as computers or signal molecules, executing a sequence of instructions; (4) alter macromolecular structure; and/or (5) function as molecules that determine the integrity of various structures within the cell. Researchers are beginning to learn more about the signaling function of nutrients-this is an important role in terms of the consequences of diet regarding chronic diseases, including cancer. Genomics and proteomics can be used to define the signaling function of nutrients in more detail. Also, researchers are beginning to understand the mechanisms by which nutrients induce transcriptional events and regulate translational events. Dr. Young explained that nutrients interact not only at the level of DNA, but also at the level of RNA.

Dr. Young stated that nutritional scientists should be asking themselves what the human genome sequence means to them and their research at their institution. If no attempt to answer this question is made, the field of nutritional science will die. He described the various levels of gene-nutrient analysis that need to be considered in attempting to determine how best to exploit modern knowledge and technology for advancing the contribution that nutritional science will play in understanding human health.

The DNA sequence provides the basic math of the amino acid sequence of the various proteins in the presence of cells. However, gene function is regulated at many steps, including DNA methylation and mRNA editing and stability-DNA sequence alone does not equal protein function or biology. Although information from the DNA sequence provides a good deal of information about proteins themselves, information from genomic data often does not rise to the level of conclusions that nutritionists, physiologists, or biologists prefer. Dr. Young commented that the nutritional challenge is to put proteomics into a real-time dynamic quantitated context with respect to the interaction between genes and nutrients.

Dr. Young noted that metabolism and genomics are equally important, and that metabolism and proteomics need to be integrated. This is a challenge that the NCI and USDA can meet if advances in biology and new techniques are appropriately exploited. He said that for the first time in a century, reductionists have yielded ground to those

trying to gain a holistic view of cells and tissues. Researchers must look beyond cells and tissues and gain a complete understanding of the role of organs, interorgans, and whole bodies. There are key life-cycle points at which researchers should think about how genomics and nutrition are used for a better understanding of how diet affects human health. The times at which nutrients and the genome interact at one level or another are particularly important to fully understand. Dr. Young briefly outlined a new technique of RNA interference described in an editorial in the journal *Nature*, and likened the impact of this technique to that of polymerase chain reaction. Nutritionists should capitalize on this technique as soon as they are able. Dr. Young said that problem-driven interdisciplinary research needs to be promoted, and among the challenges inherent in advancing the study of gene-nutrient interactions are creating coherent systems of research planning, developing appropriate approaches to operational monitoring and assessment, and obtaining long-term financial support.

Nutrition and Gene Regulation-Dr. Alfred H. Merrill, Jr.

The complexity of the fields of nutrition, genetics and cancer has historically slowed progress toward elucidating nutrition-gene interactions and the ability of researchers to give definitive, mechanism-based dietary advice to the public. Dr. Merrill, Professor in the Department of Biochemistry at Emory University School of Medicine (now in the School of Biology at Georgia Institute of Technology) gave examples of what is known about gene regulation by different categories of nutrients--such as fats, minerals, energy deprivation, and "functional" components--to illustrate how numerous facets of cell regulation must be considered in linking diet and cancer. Dr. Merrill pointed out that the effects of nutrition on gene expression are only the beginning of the story because dietary components impact every step from gene to protein to metabolic product(s), including signal transduction and post-translational modifications of proteins (and other structures, such as membranes). Technologies developed over the past few years are allowing these processes to be analyzed comprehensively (e.g., by "genomic," "proteomic," and "metabolomic" methodologies). Another benefit of the so-called "new biology" is its ability to cut across disciplinary boundaries. Dr. Merrill stressed that the lines between nutrition, pharmacology and toxicology are disappearing because all examine the ways organisms interact with molecules brought to them from external environments whether they be foods, synthetic drugs, toxins or toxicants. He reminded participants that what one individual may avoid as a toxin may be useful to another individual as a drug or antineoplastic agent.

Nonetheless, decisions concerning "optimum" nutrition will remain complex because the issues are not merely how to balance the components of the metabolic pathways to achieve the correct flux (while avoiding overloads), but also, how to chose among sometimes competing outcomes. Because humans and other animals are not evolutionarily "perfect" organisms (nor are their dietary requirements), diets that may be optimized to minimize the risk of one disease may increase the risk of another. It will be a challenge to choose among the trade-offs, but certainly the first step is to learn more about genetic variability, environmental factors, etc. that will help match diets to individual needs.

Dr. Merrill pointed out that many compounds that have previously not been considered to be important components of food are surfacing as highly relevant to diet and health. He briefly illustrated this with recent studies with a group of compounds known as sphingolipids. Sphingolipids are used by cells for structure as well as intra- and inter-cellular signaling, and their functions include regulation of cell growth and death (apoptosis). There is growing evidence that some of the enzymes of sphingolipid

metabolism and signaling may be defective in diseases ranging from neoplasia to diabetes.

Sphingolipids are also present in food in significant amounts: on a daily basis, humans consume approximately the same amount of sphingolipids as they do cholesterol. Dr. Merrill presented work recently conducted in his laboratory showing that intestinal neoplasia can be modulated by dietary sphingolipids using both chemically induced colon tumors and Min mice, which have genetic defects that resemble mutations found in human colon cancer. Mice fed sphingolipids in amounts close to those naturally found in food suppressed the development of colon tumors; furthermore, immunohistochemical studies of intestinal cells of the Min mice as well as human cancer cells in culture have found that the sphingolipids are normalizing the behavior of $?-catenin. This finding is significant because it demonstrates that this dietary intervention normalizes the phenotype that is defective as a result of this genetic mutation. Thus, dietary sphingolipids exemplify mechanism-based ("functional") food components.

Nutritionists, more than scientists in most other basic and applied fields, have been trained to participate in the "new biology" because they must think of many aspects of physiology and pathophysiology, from genetics to international health. As an example of how this type of thinking has not been incorporated sufficiently into many modern experiments, only a few of the laboratories that develop transgenic animals adequately define the diets that they are fed. One participant noted that a major problem facing nutrition researchers is that commercial suppliers of mice and other animals for research studies generally will not disclose what diets their animals are fed prior to shipment, and even if the investigators have a general idea of what the diet is, it may be changed without their knowledge. Genetically compromised mice can be bought from suppliers, but nutritionally compromised mice cannot. Dr. Merrill agreed that this issue is a major problem, and one that the participants in this meeting should try to resolve. Another participant noted that a new paradigm is needed and that researchers need to move away from looking at single genes or single gene-nutrient interactions only.

SESSION II-Moderator: Dr. Harold E. Seifried

How Can Population Genetics/Medical Genetics Assist in Identifying Critical Molecular Targets for Examination?-Dr. Martin Kreitman

Dr. Kreitman, Professor in the Department of Ecology and Evolution at the University of Chicago, said that the level of complexity in metabolic pathways and nutrient pathways is dwarfed by the complexity of the genetic variations between individuals that influence the individual phenotypic responses to nutrients. This represents a major challenge to understanding human variation, and further advances in the fields of genomics and proteomics are needed to assist in developing the science more as hypothesis-driven rather than driven by biochemistry and genetics. There are few good animal models for human variation, although there may be variations in mice that can be used to map genes that are involved in nutrient pathways. However, those variants will be different and probably not applicable to the genetic variations that might exist in humans. There are two approaches to identifying human genetic variants affecting any kind of trait, especially regarding nutrition. The first is the blind approach (e.g., quantitative trait loci mapping). The second approach is to use candidate genes. Dr. Kreitman discussed various approaches to using different candidate genes and molecular population genetic analyses to illustrate that polymorphisms in candidate genes can provide valuable information.

Dr. Kreitman discussed protein variation, noting that many of the most interesting protein polymorphisms are expression-level variants. The interesting variants are those that are acted on through selection. In these cases, the variation has risen to the level of phenotype, and the differences between individuals are meaningful-some aspect of those individual variants cause a difference in people's health, longevity, survivorship, fertility, and so on. The major theories explaining variation are: (1) genetic drift involving selectively neutral mutations; (2) mutation selection balance, in which most of the protein variants are going to be deleterious or slightly deleterious and are governed by the balance of the forward mutation rate; and (3) positive selection, in which a variant enters the population and under a set of environmental pressures has some selective advantage. Dr. Kreitman discussed techniques for identifying positive selection events, presented examples of adaptive polymorphism, and described recent work conducted in his laboratory on hereditary hemachromatosis in humans.

Neutral or effectively neutral mutations are small, between zero (completely selective mutation) and approximately 10^{-6} (thought to be the reciprocal of the evolutionary population). Dr. Kreitman said it is almost certain that amino acid polymorphisms cannot be modeled as completely neutral changes. One of the most relevant observations supporting this claim is that humans have approximately one-half as many protein polymorphisms as *Drosophila melanogaster*. Under strictly neutral models of genetic variation, the standing crop of polymorphism at a locus should be proportional to population size. The population size of *Drosophila* is orders of magnitude larger than that of humans, so there should be orders of magnitude differences in the levels of standing crop polymorphism for protein variance between the two, but there are not. He also noted that at the DNA level, there is only approximately one-twentieth the amount of silent polymorphisms in humans compared with *Drosophila*. Under neutrality, these ratios are expected to be the same.

The overwhelming majority of protein variants are still too common to be compatible with the model of strong mutation selection balance. There are two alternative hypotheses to explain why protein polymorphisms are common in humans: (1) these are effectively

neutral mutations, and they may have a relatively weak selective effect so that genetic drift can still influence their fate; and (2) many of these are under positive selection like the alleles that have higher frequencies and are common (i.e., the common variants are the ones that contribute most to phenotypic variation).

Evidence from both *Drosophila* and human studies suggest that protein variants can be subjected to positive selection, and a surprisingly large number of the best-documented cases of positive selection involve genes and metabolism. Dr. Kreitman concluded his talk by presenting some data from his laboratory suggesting that hereditary hemachromatosis, a relatively young mutation with a high frequency, is a positively selected mutation. He hypothesized that the rise of this mutation has been too fast to be compatible with genetic drift.

Are Animal Models Appropriate for Predicting Genetic Variation in Human Disease?-Dr. Joseph H. Nadeau

Dr. Nadeau, Professor in the Department of Genetics at Case Western Reserve University School of Medicine, outlined a series of questions for validating animal models: (1) Do animal models predict similar disease outcome? (2) Do they predict genetic variations relevant to the disease in humans? and (3) Do they predict pathways that modulate disease with diet or drugs? Humans and mice have about the same numbers of genes-approximately 30,000-and the list of genes in the two species is virtually identical. The average percent identity across those genes is approximately 85 percent, and the number of conserved segments in the genome is about 200. These similarities are important, but so are the differences, which can be exploited to gain a better understanding of genetic variation. Mice may react to a disease outcome in a way that humans cannot, but if researchers can gain a better understanding of how that disease process is modulated in mice, researchers may be able to use it therapeutically in humans.

With respect to disease outcomes, there are many examples in which a mutated gene in an animal, particularly in a mouse, provides a very good model for the human condition. Modifiers of colon cancer risk in mice do not predict genetic variation in humans, but they do predict the pathway that can be used to modulate colon cancer risk. There are some exceptions, however. the retinoblastoma gene in humans causes retinoblastoma in children. When it is knocked out in mice, the mice develop pituitary tumors. Even though the proteins in the two species are doing very similar things at the molecular level, the cancer outcome is quite different.

Dr. Nadeau noted that the identification of genetic modifiers has emerged as a very powerful approach for examining genetic variation between the two species, in part because it is based on the observation that, in many organisms, a variable phenotype can be found among individuals with the same genotype. It is hypothesized that by examining these modifiers and those that have a genetic basis, one can understand how the organism modulates the disease outcome of an individual with a different genotype. Different disease outcomes may be the result of another genetic modifier that suppresses the disease outcome. Dr. Nadeau characterized this as a naturally occurring way of dealing with the disease state. Modifiers are common, yet formal evidence for modifiers in humans is relatively sparse. The strongest evidence for these modifiers involves Mendelian traits. Modifiers can affect penetrance, dominance, expressivity, and pleiotropy. Dr. Nadeau discussed examples and presented data on predicting the correct pathways and of predicting the correct chromosomal locations for genetic variations.

To illustrate some of the opportunities and challenges associated with studying the link between nutrition and genomics, Dr. Nadeau described the homocysteine folate metabolism pathway using data from mouse genetics, genomics, expression, and profiling studies. He noted that the genes that adversely affect homocysteine folate all are in the Winn-Hedgehog BMP disheveled signal transduction factor. One of the most prominent success stories with respect to nutrition and diet is the effect of folate metabolism on neural tube defects. Neural tube defects are common, occurring in approximately 1 in 1,000 births. Research in this field has found that 70 percent of neural tube defects in mice and humans can be prevented by folate supplementation, although the mechanisms are not well understood. Dr. Nadeau described the problem of expression profiles and gene regulation among individuals who have different homocysteine folate levels, noting that expression profiles can be altered with diet.

The approaches to the problems discussed by Dr. Nadeau are multidimensional-they require interdisciplinary solutions in fields such as genetics, genomics, expression arrays, computational science, and biochemistry. He said one of the challenges facing researchers and participants at this meeting is bringing together the appropriate expertise and technologies to solve these problems. Mouse and rat models have predicted the chromosome location of polygenic disease traits, and mouse models have predicted pathways to modulate disease outcome. However, they are different species with similar but not identical genetics and biology, and researchers must be careful not to expect too much.

Can Dietary-Mediated Changes in Redox Status Influence Viral Genomics?-Dr. Melinda A. Beck

Dr. Beck, an Associate Professor in the Department of Pediatrics at the University of North Carolina, Chapel Hill, explained why malnourished individuals are more susceptible to a wide variety of diseases, including measles, tuberculosis, cholera, rotovirus, leprosy, and so on. All of these diseases can be affected by the nutritional status of the host, and it generally is thought that malnourishment affects the immune response, leading to a higher susceptibility to infectious disease. Malnourishment affects the immune system by reducing T cell activity, lowering antibody titers, reducing natural killer cell activity, and inhibiting macrophage function. Individuals with an infectious disease often have a decreased appetite, leading to a loss of nutrients, malabsorption, and altered metabolism-a cycle that enhances the disease. Dr. Beck stated that inadequate dietary intake can affect the disease without having to go through this cycle.

Dr. Beck described her research on Keshan disease, found in people living in certain parts of China in which the soil is selenium-deficient. Many individuals in this population also are deficient for vitamin E. The disease leads to cardiomyopathy, affecting mainly women and children, and can be prevented by selenium supplementation. Scientists in China found a seasonal and annual incidence associated with Keshan disease, even though the population is selenium-deficient throughout the year. This suggested that an infectious cofactor coupled with selenium deficiency was associated with contracting the disease. Coxsackieviruses, which are known to infect the heart, are thought to be the cofactor of Keshan disease. Scientists in China isolated coxsackieviruses from the blood and tissue of individuals infected with Keshan disease, and recently, using reverse transcriptase polymerase chain reaction, scientists were able to identify coxsackievirus fragments from archived tissue from Keshan disease victims. She explained that coxsackieviruses are members of the Picornavirus virus family-they have a high mutation frequency and can rapidly adapt to a change in host conditions, which is thought to be a survival mechanism.

Dr. Beck and colleagues created an animal model to examine the relationship between the nutritional status of the host and coxsackievirus infection as a model for Keshan disease in C3H/HeJ male mice. Two strains of coxsackievirus were used: (1) a moderate-extreme strain, CVB 3/20, that replicates in the heart and causes inflammatory heart disease in infected mice; and (2) an amyocarditic strain, CVB 3/0, which replicates in the heart but does not cause disease. Mice were fed diets for 4-weeks that were either adequate or deficient for selenium, and either adequate or deficient for vitamin E. It was found that mice fed diets that were adequate in selenium and vitamin E and infected with the CVB 3/0 strain did not develop myocarditis. However, CVB 3/0-infected mice fed selenium- and vitamin E-deficient diets did develop myocarditis, when normally they would not. Dr. Beck presented data illustrating an increased viral replication in the hearts of CVB 3/0-infected mice fed the deficient diets compared with those fed adequate diets. Also, she summarized data indicating that, based on studies of T cell response and mRNA levels of chemokines and cytokines, a greatly decreased immune response was found among mice fed the deficient diets. Dr. Beck further summarized data that suggest increased oxidative stress is the mechanism for the increased pathology seen in these animals.

A viral passage experiment was conducted to determine whether the change in viral pathogenesis was due to the virus changing or due to the immune response. It was found that the virus itself was changing as a result of replicating in selenium- and vitamin E-deficient animals. She discussed specific changes in the nucleotide makeup of the virus that were found. These studies showed, for the first time, that a specific nutritional deficiency could drive changes in the viral genome, changing an avirulent virus into a virulent one.

There is evidence of other viral diseases that may be caused by nutritional deficiency. One possibility is an epidemic of optic and peripheral neuropathy that occurred in Cuba during the early 1990s that affected more than 50,000 individuals, mostly young adult males. The illness was associated with dietary limitations and increased physical activity. The epidemiological associations were a diet low in animal proteins, fats, B group vitamins, vitamin E, selenium, lycopene, and α- and ß-carotenes. Smoking, a pro-oxidant, was also a risk factor, suggesting an impairment in antioxidant pathways and increased oxidative stress in these individuals. Virologists in Cuba isolated a virus antigenically related to coxsackievirus A9 from cerebrospinal fluid samples of affected individuals. Serological evidence suggests that there was a high circulation of A9 just prior to the epidemic. It is hypothesized that coxsackievirus A9 replicated in nutritionally deficient hosts, which resulted in viral mutations, and that the mutated A9 virus has pathogenic properties that led to the optic and peripheral neuropathy. Dr. Beck also presented evidence suggesting that certain strains of influenza virus can be influenced by the nutritional status of the host.

SESSION III-Moderator: Dr. Richard G. Allison

The Cancer Genome Anatomy Project: A Model of the Application of Bioinformatics to Genetic Studies-Dr. Lynette H. Grouse

The overall goal of the Cancer Genome Anatomy Project (CGAP) is to achieve a comprehensive molecular characterization of normal, precancerous, and cancerous cells. CGAP is intended to facilitate the interface of genomics and cancer research. The CGAP Web Site provides public access to all CGAP data and resources. The site is located at http://cgap.nci.nih.gov. Dr. Grouse, Scientific Projects Manager in the Office of Cancer Genomics, NCI, described the contents of the CGAP Web Site, which include: (1) genomic data for both humans and mice, including expressed sequence tags (ESTs), gene expression patterns, single nucleotide polymorphisms (SNPs), cluster assemblies, and cytogenetic information; (2) informatics tools to query and analyze the data; and (3) information on methods and resources for reagents developed by CGAP. The Web site is organized with links to the following categories of information: genes, chromosomes, tissues, pathways, tools, methods, reagents, and a catalog of resources. Dr. Grouse noted that CGAP offers a wide range of tools for researchers. For example, virtual Northern blot assays can be viewed to see the expression of a gene, both EST and serial analysis of gene expression (SAGE) can be examined, whether a certain gene has been implicated in any cancer cases can be determined, and information about protein similarities can be found.

Dr. Grouse described the various tools and resources available on the CGAP Web Site:

- Tools To Find Genes: Gene Finder, Gene Ontology Browser, and Nucleotide BLAST Tool
- Tools To Find cDNA Libraries: Library Finder
- Tools To Examine Gene Expression: Gene Library Sorter, cDNA xProfiler, Digital Gene Expression Displayer, SAGEmap xProflier, and SAGEmap vNorthern
- Tools To Examine Chromosomes: Mitelman Database of Chromosome Aberrations in Cancer, Database of Recurrent Chromosome Aberrations in Cancer, FISH-Mapped BACs, Expression-Based SNP Imagemaps, Genetic and Physical SNP Maps, and GenMap99
- Tools To Find SNPs: Expression-Based SNP Imagemaps, and Genetic and Physical SNP Maps.

Dr. Grouse provided examples of how to use some of these tools/resources.

Dr. Grouse described CGAP's efforts in supporting the development of technology called "laser capture by microdissection," which isolates specific cell populations from a tumor sample and uses them to create cDNA libraries. Typically, 1,000 clones are sequenced from each library. She also briefly described the mammalian gene collection, an initiative of the NCI that will result in a full-length clone for every human and mouse gene.

Dr. Grouse discussed the current movement to standardize EST nomenclature. This effort will result in a database containing all of the aliases of a particular gene. CGAP has identified approximately 1,600 SNPs that are in cancer-related genes, and its SNP Index is available for researchers to use for determining whether an SNP is in the gene being studied. CGAP is building ESTs through the following steps: (1) producing cDNA libraries from normal and cancerous tissue; (2) sequencing the 3' ends of each clone to evaluate library diversity; (3) sequencing the 5' ends of clones; and (4) sequencing

additional 5' ends to complete the clone insert. EST sequences are deposited into a database, dbEST, and clones are available from the Integrated Molecular Analysis of Genomes and their Expression (IMAGE) Consortium.

In discussion, one participant noted that it is important that CGAP include as much data as possible, not only for studies currently underway, but also for future studies. Dr. Grouse noted that she would be willing to work with meeting participants in discussions for possibly including nutrition links on the CGAP Web Site.

What Is the Future of Genomics and Proteomics in Nutrition Research?-Dr. Leonard H. Augenlicht

Dr. Augenlicht, Director of Molecular Oncology at Albert Einstein Cancer Center, described a method using array data to evaluate the potential efficacy and toxicity of certain compounds. These studies are useful for determining how similar certain compounds are and to what degree they mimic physiological inducers. The measurements resulting from these studies can be used as baseline measurements to compare how cells respond. The nonsteroidal anti-inflammatory agent sulindac is highly efficacious in inhibiting colon tumor formation. However, it has significant toxic side effects that generally prohibit its use in the clinic. There is a large investigative effort underway to try to understand these negative effects and define sulindac's beneficial effects so that it can be used therapeutically to inhibit colon cancer formation in humans. Dr. Augenlicht and colleagues studied gene changes in colonic epithelial cells in a series of three patients treated with sulindac. Two interesting points arose from this analysis: (1) the genetic changes resulting from sulindac treatment are highly complex, and (2) the genetic effect of sulindac treatment is highly heterogeneous, with very few sequences and the total effect showing commonality of change. In comparing *in vivo* sulindac data with *in vitro* data, it was noted that there was a population of genes that generally were downregulated by sulindac in patients and were not expressed or showed no change in the cells of tissue culture. These were all sequences that are commonly expressed in lymphocytes that are therefore not expressed in the colonic epithelial cells *in vitro*.

Dr. Augenlicht and colleagues also examined those genes that changed similarly *in vivo* and *in vitro* as a result of sulindac treatment. Only 0.1 percent of the entire data set demonstrated similar changes *in vivo* and *in vitro*. One gene in particular, p21, a cell cycle-dependant kinase inhibitor, is induced by sulindac and these studies found that sulindac induced p21 *in vivo*. To determine whether p21 was a marker of response to sulindac or was functionally important in the response, the investigators engineered a mouse that had an Apc mutation to initiate development of tumors in the small intestine. These mice were either wild-type for p21, heterozygous for p21, or homozygous for p21. Loss of p21 was associated with an increase in both tumor incidence, frequency, and size that appeared to be gene-dose dependent. This phenomenon was found to act additively for the formation of intestinal tumors when Apc knockout mice were fed a high-risk western diet that was high in fat and phosphate, and low in calcium and vitamin D.

The investigators also found that the presence of p21 was critical for the tumor-inhibiting effects of sulindac. This has implications for the subset of colon cancer patients who have a reduction in p21 expression. These patients have a poor prognosis, and one could predict that they would not respond to sulindac or to other agents that induce the same pathway. Dr. Augenlicht said the effects of sulindac on the intestinal mucosa probably are on reducing mitosis, not on inhibiting apoptosis. The investigators also found that loss of goblet cell phenotype was associated with loss of p21, suggesting that loss of p21 was associated with increased intestinal tumor formation initiated by Apc

mutation. It is not known what takes place in the intestinal mucosa in response to the Apc mutation that leads to the development of tumors. It is believed that differentiation, whether a direct or indirect effect of either initiators or preventive agents, may be the key step in development of these tumors. In support of this, Dr. Augenlicht presented data showing that targeted inactivation of the Muc2 gene in mice, which eliminates recognizable goblet cells, distorts crypt architecture, and alters intestinal cell maturation and migration, leads to intestinal, including colon and rectal, tumor formation.

Dr. Augenlicht reminded participants not to neglect the mitochondrial genome. He presented data showing coordinate downregulation of the mitochondrial genome in the mucosa at risk for tumor development and in colon tumors that develop. When differentiation of these cells is induced back towards normal, there is a complementary coordinate upregulation in the genome.

A variety of experiments have suggested that the metabolism of short-chain fatty acids is key to their ability to induce apoptosis and changes in maturation in the intestinal mucosa. Dr. Augenlicht and colleagues studied a mouse that had a homozygous deletion mutation in the gene called short-chain acyl hydrogenase, which encodes the first step in mitochondrial metabolism by ß-oxidation of short-chain fatty acids. They found that short-chain fatty acid metabolism was necessary for apoptosis in the mucosa, losing the ability to metabolize decreased apoptosis by approximately 90 percent in the proximal colon and the distal colon of these mice. He noted that a decrease in apoptosis was not observed in the duodenum, explaining that this pathway is not used in the duodenum. It is thought that the key to mitochondrial function in these pathways is the mitochondrial membrane potential, which can be measured using various mitochondrial-specific dyes. It is known that the membrane potential may influence the interaction of mitochondria with a large number of molecules that play key roles in cell maturation. Many pathways based on nutrient interactions impede on mitochondrial function and maintenance of a membrane potential, and the level of impedance is directly dependant on electron transport.

Dr. Augenlicht concluded his presentation by discussing two developing methodologies that will advance the science of nutrition-gene interactions. The first methodology is a fluorescent measurement using laser capture and novel software and hardware. It is hoped to link this technology to microarray analysis to examine hundreds of different messages simultaneously on a single-cell basis and identify cell populations that have unique signatures. The second methodology is a high-throughput approach for the structural determination of proteins. This technology uses an x-ray beam and will examine candidate genes from an array analysis, provide the structural determinations of those proteins, and allow investigators to see what the common features are of proteins that respond to various chemopreventive agents.

One participant discussed the differences between the duodenum, jejunum, and colon and asked whether the experiments in the acyl-hydrogenase mutated mice had been run with the colon interposed, with the duodenum down in the colon to determine whether the duodenum would express genes differently according to its position. Dr. Augenlicht replied that these experiments had not been done and that he would not expect genes to be expressed differently if the duodenum were interposed. In response to another question, he explained that changes in apoptosis in the flat mucosa are not as significant to tumor development; they play a greater role in later stages of tumor promotion and progression, but not in initiation. Dr. Augenlicht noted that: (1) databases are highly interactive, data from one database often can only be understood in light of data from other databases, so bioinformatics and the ability to continue to develop and expand

these databases interactively is extremely important; and (2) the systems are highly interactive, when tissue culture, mouse, and the human systems are studied interactively and confirm each other, investigators begin to feel that they are on the right track.

One participant asked how to make sense of the seemingly arbitrary variability associated with the expression of a large number of genes at one time. How many times does an experiment need to be run and what does the variability represent? Dr. Augenlicht said there are data to support that the variability in many experiments is real, and agreed that the array methodology is exquisitely sensitive. However, researchers can run control experiments, get an idea of what the confidence intervals are, and set the limits accordingly. Twice usually is enough to determine what the "noise" is in a given experiment. Dr. Augenlicht said experimentally proving how important the changes are is a challenge; it is not all noise, and it needs to be accounted for. Another participant said this research cannot stop at the gene level; it must go into the proteome.

SESSION IV-Moderators: Drs. John A. Milner and Kathleen C. Ellwood

Future Directions

Dr. Milner, Chief of the Nutritional Science Research Group in the Division of Cancer Prevention, NCI, said advancing the science of nutrition is critically important. Clearly understanding some of the variation in response requires a greater understanding of the genome and functional genomics from the perspective of proteomics and the functional proteome. Approximately $580 million is spent per year on nutrition research, and currently the NCI is spending about $120 million per year on nutrition research, but more funding is required.

Dr. Milner asked participants for recommendations/comments on what can be done to advance this field of research. The following recommendations/comments were made:

- The paradigm currently being used throughout nutrition sciences is a deficiency model, studies examining low levels of vitamin C, vitamin D, calcium, and so on. That is how nutrition scientists have been taught, and the public has been given "one size fits all" recommendations when it comes to nutritional intake. A new paradigm needs to be developed that focuses on the nutrient-gene interaction and where the field of plant genetics is in relation to human genetics. The definition of nutrients needs to be expanded beyond essential nutrients. To receive increased levels of funding, nutrition researchers will need to be able to articulate this new paradigm. Defining and articulating this new paradigm would be an appropriate agenda item for the Interagency Committee on Nutrition.
- Unless there is a change in the way NCI study sections view nutrition research, few of the potential advances in nutrition research discussed in this meeting will occur. One major problem is that reductionist thinking takes place in the study sections. Nutrition researchers should try to become members of study sections to change this way of thinking. The field of nutrition requires a holistic approach, examining the totality of the diet, this was characterized as a "reconstructionist" approach as opposed to a reductionist approach. The reductionist model has been the core of molecular biology, so changing this will require a paradigm shift.
- True interdisciplinary research activities across institutions, beyond departments, and beyond individual institutions are required. The USDA and National Institutes of Health (NIH) should seriously consider how this is going to come about; it will not be easy, and it will require changing attitudes. Nutrition researchers should try to learn from and imitate other branches of science in which there is much greater collaboration across disciplines. Interagency committees should be used to promote nutrition issues as well as multidisciplinary collaborative efforts. It would be helpful to build a network that funds interdisciplinary research activities.
- USDA's ARS is a good example of interdisciplinary collaboration. ARS laboratories have plant scientists, soil scientists, and human nutritionists working side-by-side on common issues. One of these laboratories is developing a program to use plant genomics approaches to examine metabolic biosynthetic transport pathways for the accumulation of potential nutritionally important compounds.
- Increased training opportunities for future generations of nutrition researchers are required. These training opportunities should emphasize multidisciplinary collaboration programs.

- Few individuals are applying for NIH predoctoral/postdoctoral fellowships in nutrition, and the award series is nominal in terms of nutrition researchers. Part of the problem is that the field of nutrition sciences is poorly defined. A study of reasons for not entering a Ph.D. or postdoctoral program in nutrition conducted at the University of California, Los Angeles, found that many individuals reported not knowing what "nutritional science" meant.
- A greater understanding of the roles and functions of nutrients is needed, particularly in terms of the impact of diet and lifestyle, as well as the interaction between diet and the genome, so that ultimately, individualized dietary recommendations can be made.
- It is important to develop a strategic plan for nutrition and genomics and define what deliverables the nutrition community wants to have 5, 10, and 15 years from now and define how they are going to be produced. If these deliverables are defined and funders are requested to work towards those deliverables, it may result in a different kind of community or different kind of research. It is easy to focus on the reductionism aspect of research because it is a good way to get some answers, but it does not always answer the big questions or move towards supplying deliverables. Examples of deliverables include understanding more about signaling factors and how they work, determining how many kinds of foods and nutrients impact individual genotypes, and predicting the consequences of changes in diet on various disease processes.
- Creating a database or information source similar to CGAP for nutrition researchers would be extremely useful, although funding such an effort would be difficult.
- Program project plans can be used to develop funding opportunities to build bridges across disciplines for nutrition-oriented research.
- Other model organisms beyond the mouse could be developed for nutritional research. Drosophila, yeast, and zebrafish were suggested as possible models.
- It would be extremely useful to include a dietary component to population-based longitudinal studies that currently are underway in the United States.
- Nutrition grants need to focus more on mechanisms, not just dietary recommendations or guidelines. Effective mechanistic definitions often are missing from grant applications in the field of nutritional sciences.
- Nutritionists and molecular biologists need to work side-by-side so that these scientists can appreciate the contributions made by the other field and use them to make advances in their own field.
- It was suggested that a nutrition-oriented center or institute be established at the NIH. Alternatively, it also was suggested that each NIH Institute have a nutrition-specific laboratory. New approaches to promoting the interaction between nutrition researchers at the NIH and those outside of the NIH are needed.

Dr. Milner concluded the meeting by thanking the organizers of the meeting and the participants. Dr. Kim briefly described the relationships between molecular targets for nutrients; genes involved with growth, development, and disease; and familial traits. She also thanked the attendees and noted that their insights into nutrition and genomics will contribute greatly to advancing the field of nutritional science