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Workshop Report

Functional Consequences of Gene Expression in Health and Disease

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Workshop Organized and Report Compiled
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Summary

The workshop on *Functional Consequences of Gene Expression in Health and Disease* was held in response to a report by the Health and Environmental Research Advisory Committee (HERAC) of the Department of Energy (DOE). HERAC presented its report entitled "Genes, Molecules, and Human Biology" to the DOE in November 1995. The report emphasized the particular opportunity for the Office of Biological and Environmental Research (OBER) to enhance investigations of *homoeostatic controls as a part of gene expression in the human body*. OBER has, over the past few years, acquired the capability for managing such projects, that are clearly connected with the genome project.

The workshop aimed at identifying:

1) Functional units in terms of *biochemical circuits within complex adaptive systems*, such as the human body, that can be observed *in vivo* and coherently described as a consequence of interacting substrates in response to specific gene expression.
2) Useful, practical, and economical *tools for in vivo observations* of metabolic and functional circuits in response to specific gene expression, within individual phenotypes.
3) Promising *applications* of the above concepts and tools for medical research and practice.

The workshop had *22 experts* representing genetics, biochemistry, molecular and cellular biology, physiology and physiological modeling, oncology, radiology, and nuclear medicine. Nine participants, including the co-chairman of the workshop, were from DOE.

The participants were encouraged not to be parochial by discussing the importance of pressing scientific questions without identifying specific tasks for any agency, and to invite the wider scientific community to participate in creative ways in meeting the challenge.

All participants initially stated their *opinion on the aim and issues* of the workshop. This evolved into a broader discussion that clarified individual perceptions as to concepts, tools, and applications. The statements indicated the need for clarifying individual views from the various perspectives of expertise. *Experimental data* were presented on various aspects of signal transduction and pathways in cellular metabolism, on the technology of studying the relationship between genes and particular metabolic reactions and phenomena in specific organs, and on respective modeling and data interpretation. In this context, the linkage between genotype and phenotype as active in both directions was reemphasized. Immediate research opportunities are in studying various specific metabolic reaction circuits in recognized linkage to gene expression in neurology, cardiology, oncology, and gerontology.

The workshop *concluded with a plea* for exploiting the new opportunities created by the success of the genome program, for *integrating the diverse efforts and optimizing resources*. The meeting was considered to be an entree into the post-genome era in medicine; this demands appropriate choice, development, improvement and optimization of diverse measurement techniques and corresponding modeling of data, especially of nuclear medicine, which can be applied to *in vivo* and *in vitro* studies of specific phenotypic attributes of disease populations properly stratified.

In the text that follows, opening comments and research presentations are preceded by the names of the respective participants wherever appropriate.

Introduction

(Wagner): The *opening overview* stressed the advanced knowledge regarding *etiology and mechanisms of diseases* that has led to improved concepts and tools of therapy. Yet, therapy is still largely empirical. Of 5000 molecules developed for potential use in clinical treatment, about 250 reach preclinical trials, five will enter clinical trials and only one receives approval by the Food and Drug Administration (FDA). The *cost of developing a useful drug* has risen from about 54 million Dollars in 1976 to about 400 million Dollars in 1990. The demand for more efficient production of effective pharmaceuticals is obvious, and drug tailoring is one important avenue to reach that goal. For this, known genes and their immediate protein products are increasingly important and even crucial. In *testing the fate of pharmaceuticals in the human body* and drug specificity, tracer techniques have proven superior in terms of efficacy to other diagnostic procedures. The *nuclear medical imaging methods* are especially sensitive. Thus, positron-emission-tomography (PET) and single-photon-emission-computerized tomography (SPECT) have lower false positive results than most other diagnostic procedures in clinical medicine.

Indeed, the combination of *information on specific gene products, on their metabolic role in living systems, on the homoeostatic circuits to which they belong, and the relatively high specificity of nuclear medical imaging promises success* in attempting to study the bidirectional interactions of gene expression on functional responses, mainly in terms of biochemical pathways. Genetics will lead to an entirely new approach to many diseases. All diseases involve genes in that diseases may be caused by them or affect them. *Homoeostatic circuits in biochemical and functional pathways respond to gene expression and vice versa*. The response of these circuits to defined perturbations may be viewed as a trait of the individual phenotype. The tracer principle appears most effective to investigate such traits. Indeed, the application of appropriately labeled compounds and metabolic substrates for *functional imaging in relation to the genome* will allow a new and basic level of diagnostic observation.

Opening statements

The participants made opening statements—presented here in alphabetical order—as to their personal views regarding the task of the workshop:

(Anderson): The gap between the mass of new genetic sequence data and understanding the *meaning of gene expression in a complex system* is most difficult to bridge at present. A relatively narrow task in this context is the control of gradients of substrates and enzyme functions in regulating the cell cycle. In view of the minute success in answering related questions today, the challenge is getting involved in biological system analyses.

(Balaban): Mechanisms and effectiveness of *control of physiological and biochemical processes of a given phenotype* are largely unknown. The primary modifications essential for adaptive complex systems to operate are equally unknown. Basic research into these processes is needed and particular demands may evolve from the discussions at the workshop. Models that describe these processes are critical for testing hypotheses and develop new questions. Suitable technologies demand appropriate tools which still need to be designed. A case in support of the statement is the finding of myocardial hypertrophy in more than two thirds of a group of "knock-out mice" even if the knocked-out genes were expected to have nothing to do with myocardial structure and function.

(Barnhart): Prior to 1984, the *dogma on radiation effects* related high doses to genetic mutations. However, the survivors of relatively high doses of radiation in Hiroshima and Nagasaki did not show genetic mutations. At a conference in 1984, this discrepancy was highlighted and led in 1986 to a conference with 52 participants in Santa Fe, that was organized by the DOE. Here it was recommended that *DOE launch a multidisciplinary approach to sequence the human genome* in order to satisfy the need for such a resource for the health effects program. The national laboratories with their interdisciplinary experiences were tagged as ideal sites for the project. An initial $5.5 million were set aside. The scope and duration of the project was rationally projected to be eventually very demanding so that the initial aim was to focus and limit the project to get it started. The success of this seminal decision in Santa Fe is obvious today as the project is now a world wide effort.

(Budinger): The task of linking a genotype to its phenotype requires *suitable models* that are applicable to analyzing measured data. Hints come from knowing genes involved in causing cystic fibrosis, or might come in the near future from schizophrenia with erroneous DNA methylation and genetic abnormalities on chromosome 1 or 3; or manic depression with possible errors in the cellular sodium-potassium pump; or Alzheimer's disease with abnormal acetylcholine-transferase; or myocardial hypertrophy with defects in myosin expression, proline hydroxylation, or angiotensin-renin system. Another example is obesity with questionable depression of certain nerve cell receptors, or with obesity protein production and catabolism. Whatever it may be, non-invasive imaging is potentially invaluable in understanding the metabolic disturbances that are linked to specific gene expression. Indeed, the *non-invasive assessment of biochemical abnormality* can give powerfully effective clues to the search for genome abnormalities leading to disease.

(Cole): The workshop is seen as an extension of present DOE programs in Nuclear Medicine. The concept of *magic bullet* is expected to be broadened step-by-step into discovering the *function of homeostatic networks* to be eventually applied in clinical practice.

(Dawson): One area of research and clinical evaluation that will be important for taking advantage of the human genome is *in vivo cell biology*. Many of the tools available for study of *in vitro* cell biology and

biochemistry are not applicable to the study of these same cells in their *in vivo* environment. In addition, studies of *in vivo* cell biology generally need to account for the effects of local blood perfusion, *substrate diffusion though complex tissues*, and other complicating factors that are not generally controllable as they are *in vitro* studies. The development of *tools for in vivo phenotyping and analysis* of functional circuits involving cooperating genes at the cellular level within functioning tissues and organs requires *interdisciplinary cooperation* among physiologists, engineers, chemists, etc., which is not commonly available in conventional research laboratories. *Development of tracer probes, delivery, and detection methods* remains a key problem for advancement. The past history of the development of various imaging modalities and probes provides a model approach. However, additional development will be necessary, particularly in terms of an expanding *repertoire of indicator probes* for important cellular processes that are not now accessible. Also the time resolution for measuring dynamic processes need improvement, and appropriate *mathematical models* for estimating quantitative parameters should reflect the functional state.

(Drell): The completion of genome sequencing will unravel an *extreme complexity of causal effects in the evolution of phenotypes* and specific diseases. The cystic fibrosis gene alone has some 550 different polymorphisms of which many are capable of leading to the disease. A similar situation is known for various cancers. Also, 1/2–2/3 of newly sequenced genes of bacteria are totally new entities with as yet undiscovered biological roles, and not found so far in any other biosystem. More surprises will come. One thing one needs to approach is system-wide or "synthetic" biology, e.g., how genes and gene products work together in complex systems.

(Feinendegen): The workshop should address the selection of particularly useful metabolic circuits within the context of a complex adaptive system. Such *circuits may be analyzed in the functioning system by multiple tracer techniques.* One needs to relate the circuit's capacity of adaptation not only to momentary external interventions but also to the genes responsible for the component elements of the circuit. Such traits may become immensely helpful in the detection of individual risks of certain diseases, especially in individuals carrying specific oncogens. In this way, genetic risk is complemented by the actual risk that includes the phenotypic consequences of multiple gene expression.

(Guilarte): A major challenge today is the *genetic basis and control of learning and memory*; a hundred genes are likely involved. Both protein and gene expression may change in association to a given phenotypic mental expression. *In situ* hybridization may help in studying these phenomena.

(Hershman): A particular challenge is the *interplay of different genes* in a given gene constellation that is responsible for a given phenotype. In this way, the reductionist approach leads to answering the question as to the difference in gene expression that regulates a resting cell to divide, or a nerve cell to respond to neurotrophin by differentiating appropriately, and in analyzing what is involved in mental learning and remembering. Another particular challenge to investigation is the *genetic screening for high throughput functions*. The human genome project will deliver an enormous wealth of genetic data with often unknown biological roles. In collaboration with capable imaging devices, some of these questions may be answered by tracers in the living system. An initiating step is the use of a reporter gene that is bound to a gene of interest. When successfully transferred with the help of a proper vector to tissue cells, the transcription of both genes is locally observed by imaging of the defined product of the reporter gene. This approach with the thymidine-kinase gene as the reporter gene and labeled acyclovir as ligand for the enzyme has already been successfully used for *imaging of gene transcription in vivo*.

(Kingsbury): The human genome project presently targets *nucleotide sequencing and gene description* using a wide array of sequencing technologies and annotation tools. Building the infrastructure for an enormous database will bring a core tool for biomedical science in the future. This is a relatively new challenge to biologists, whereas physicists are more familiar with such tasks. Interpreting the information in the databases requires consideration of gene redundancies and genetic polymorphisms, a new challenge to informatics. Next, one requires information on *how a polymorphism of a specific gene is related to disease*. The problem lies in selecting a target of clinical relevance. Furthermore, the low structure resolution of the

majority of proteins involved hinders the immediate applicability for targeting. Nonetheless, targets must be defined first before special probes are designed.

(Kirkwood): A special challenge comes from *studying tumor cells* and the corresponding reaction of the host to the tumor. The number of interacting peptides in such interactions is enormous. The immune system activation and the answer to tumor cell derived antigens may fit into models that may augment the success of *clinical trials of immune-tailored therapy*. This also requires linking to the given genotype.

(Kung): For success in *applying genomics to pathophysiology and biochemistry* multiple tasks need coordination and constant interaction involving all natural sciences. Despite their different approaches and sources of funding, *radiopharmaceuticals play a key role in all*. The radiopharmaceutical tools need testing, and animal experiments are indispensable and expensive. Also, the FDA and local IRB's approval is crucial.

(Liu): *Oncology faces multiple scenes*. Cell biology, epidemiology, computational biology, gene libraries, protein data banks, etc., all are important in oncology. Regarding diagnosis, micro metastases and identification of people at risk, and not so much detection of advanced disease, are major challenges. A broad array of techniques to answer these challenges exists, which includes biopsies, as well as micro-imaging, *functional organ interrogation in various ways*, biochemical assays for testing the degree of gene expression and RNA translation into proteins, enzyme kinetics, and informatics for integrating systems at various levels of complexity. Acknowledging complexity illuminates the importance of *applied technologies with interdisciplinary interactions* for the purpose of functional screening. The present rate of advances in technology shows a doubling time of about five years.

(Murphy): From the medical point of view, etiology and pathogenicity of disease need to be understood in terms of *evolution of disease* that deviates from the coherent path of development of normal physiology. The conventional reductionist's approach suggests primarily the role of genes. Equally important is the question of how genes have evolved. Evolution operates, however, not primarily on genes but on the phenotypes derived from the genes. It is crucial to understand the interplay of genes in creating a given phenotype that is exposed for selection in a given environment. The feedback from phenotype to genotype is inescapable. The workshop must address *both directions of interaction between genotype and phenotype*.

(Palsson): Modeling may be of limited use, when it comes to real complexity and *simulation of detailed kinetic behavior*. However, models help in describing system matrices regarding the metabolically active genotypes and corresponding phenotype through structural analysis. Unknown is the degree of redundancy underlying the core metabolic pathways. Deletion studies, such as in knock-out mice, show the lethal consequences of some genotypic changes, but some 2/3 of the deletions do not change the phenotype at all. Thus, *buffering effects of phenotypic circuits* exist that may perhaps be interpretable by suitable modeling. Flexibility in the cell's genetic circuits is likely to become the key feature of the genotype-phenotype relationship.

(Phelps): Cross *fertilization between scientific disciplines* is mandatory for succeeding in linking genetics to clinical medicine. A case of such integrated biomedicine is functional imaging of gene expression by appropriate imaging techniques. Thus, reporter genes may help to assay transferred genes *in vivo*; also, *in situ* hybridization and the consequential use of antisense nucleotide chains *in vivo* promises *observation of gene function in the living body*. This development requires tailored labeled probes and preclinical work with animal imaging with specially designed micro-positron-emission-tomography (micro-PET). Also high-speed autoradiography may help in studying initial tracer feasibility in model tissue. This technical development in interdisciplinary settings will also benefit the many biotechnology companies, some 1400 in the U.S. alone and most of them with severe financial constraints.

(Piwnica-Worms): The potentials of *phenotypic system analysis* with reference to the genome appear clinically immediately relevant in *oncology*. Radiopharmaceuticals for diagnosis will lead to better therapy agents, and the reverse is also true. Several examples presently document this *bidirectional interplay in compound development for the diagnosis and treatment of cancer*. For

example, the time vs concentration behavior of 99m-technetium-Sestamibi in breast cancer has shown the potential for determining drug sensitivity of the tumor.

(Reba): From a clinical point of view, *inter- and intracellular communication* is revealing and also relies on the interplay of activation and inactivation of genes. For example, differentiating between simple dementia and Alzheimer's disease needs to consider the fact that perhaps 4–5 types of clinical Alzheimer's disease arise from different spatial and sequential expression of a given set of genes. Phosphorylation, acetylation and other transfer reactions are crucial in cell communication systems. In order to study such phenomena, allosteric properties determine specificity. An important question for *designing imaging tools and drugs* as well, thus, relates to the predictability of secondary and tertiary protein structures from knowing the respective amino acid sequences that are deduced from the particular genes. Defining specific ligand binding sites from such investigations promises to pay off well clinically.

(Rodbell): The questions aired at the workshop are relevant and indeed widely asked. The *organization of the tasks and coherence in execution* now is needed in the post genome era. One particular aspect is *signaling between and within cells in tissue*. As with the discovery of the role of G-proteins, data collection precedes modeling, and in both, individual creativity not only is exciting but essential for success. Signals operate in adaptive systems, and a single signal may elicit quite different phenotypic responses. These operate in part in *cellular compartments*, and their importance in cellular function is emerging now. Thus, proteins in the cell just do not move around freely but have their compartmentalized function that is linked to the cytoskeletal structures. A future task is to *view the phenotype as a set of compartmentalized functions*, the study of which needs new tools, interdisciplinary cooperation of biologists especially with physicists and physical chemists. One of the examples relates to the function of membrane embedded proteins under gene direction. Such large interdisciplinary tasks are well suited to being managed by appropriately experienced agencies such as the DOE.

(Srivastava): Modern nuclear medicine is based on a central premise that every given pathology has at least one associated *biochemically significant event*. Such events, as increased glucose metabolism in growing tumors; increased glucose consumption in hypoxic heart muscle; dopamine deficiency in Parkinson's disease; decreased acetylcholine synthesis in Alzheimer's disease; all can be identified by radionuclide tracer imaging for diagnostic accuracy and quantification of dysfunction. Similarly, such events may be exploited for targeting therapy. Individual events need to be identified in the context of *cellular signaling upstream and downstream* in the sense of linking ligand-receptor interaction to signal transduction, to gene expression, and to protein synthesis. A paradigm shift appears inevitable.

(Strauss): Functional imaging will have a leading role in characterizing gene function in the living system. This, however, relies on *assessing multiple genes in coordinated action* in order to understand phenotypic functional circuits. Also, the influence of the *environment likely influences gene expression* and needs to be seen in the context of life being determined by the maintenance of adaptive reserves. Genetic instability increases with accumulation of DNA damage upon aging which again predisposes to more damage, stress intolerance, and disease. Indeed, analyses of function may allow one to refer back to genetic mechanisms and stability. *Apoptosis*, as signal induced cell death, can now be identified in complex tissue by way of imaging a labeled ligand that exclusively binds to a specific receptor that is expressed in the membrane of cells undergoing apoptosis.

(Taegtmeyer): Complexities call for interdisciplinary interactions, as was recognized decades ago by de Hevesy, Schoenheimer and Krebs at the University of Freiburg. They called their little club: *panta rhei* (everything flows). An especially revealing *complex system is the heart*. Biochemical, electrochemical, and mechanical functions are interrelated and primarily based on *energy supply by ATP*, of which about one ton is produced and consumed per human heart per month. Finely tuned metabolic interactions are genetically determined and involve the essential substrate *glucose*. The quantification of its transport within the tissue cells, its phosphorylation and degradation in the cell uncovers the role of competitive use of *fatty acids*, another group of substrates

that are the major energy source in healthy heart muscle cells. Many questions arise as the biochemical interplay between substrates, *local blood supply* and cardiac function come to light. Examples relate to genetically controlled enzyme expressions, exercise conditioning, nutritional history, environmental and mental stress to cardiovascular control systems. *Linking an individual genotype to the phenotypic individual reactions with functional consequences* promises great returns in clinical research and practice.

(Varma): A special *niche for* DOE should be identified and then specific targeted research should be pursued. More specifically related to the workshop is the program of *computational structural biology*. This program will provide essential data for developing tailored compounds and tracers for serving the theme of the workshop.

(Wagner): One can approach the study of disease from genotype to phenotype, or "from the top down", begin by *characterizing disease* in terms of molecular abnormalities, and then use abnormalities of these *molecular processes as markers in searching for abnormal genes*. In patients with cancer, for example, the lesions are often characterized by over-expression of the gene coding for the enzyme hexokinase II, which results in avid accumulation of the radiotracer, 18-F-2-deoxy-glucose. Other molecular characteristics of cancer are the over-expression of plasma membrane receptors, such as somatostatin, vasoactive intestinal peptide, or dopamine receptors. The passage back and forth—*from phenotype to genotype and genotype to phenotype*—can often be studied by the use of radiotracers, both positron and single photon emitting tracers, that can characterize disease in terms of regional physiology and biochemistry. Today, *nuclear medicine* is underutilized, and has been called "*the best kept secret in medicine*".

Discussion of Issues

Medicine usually directs itself to phenotypic phenomena, and only more recently is addressing the *impact of the genotype and its role in disease development*. Although the mouse genome is less rigorously mapped than the human genome, the Oak Ridge National Laboratory targets *mouse mutations* and has a large number of "knock-out" mice available for studying gene function in phenotypic development. *Genetic counseling* on individual risks of cancer will increase. On the one hand, morphological and histochemical studies on tissue specimens obtained by biopsy, for example from the colon or the breast, may be decisive for molecular profiling of the individual at risk. On the other hand, *nuclear medicine may non-invasively contribute to risk assessment by using multiple markers properly labeled for imaging*. Both approaches also improve preventive diagnosis of different non-malignant diseases such as hypertension. The future hopes of better understanding disease evolution demands this type of *"molecular pathology"* with histopathology being superior to imaging technics in correlating altered function to microscopic structure; yet, imaging techniques permit the *description of function within the controlling network of the intact organism*, however with a lower degree of spatial resolution. For example, 30% of patients with breast cancer have an increase in certain serum factors, but imaging may elucidate even remote actions of these factors and, thus, can emphasize function over an individual diagnostic signal, be it histopathologically localized or expressed by factors circulating in the blood.

Diagnostic *procedures should have minimum risk and preferentially not disturb the tissue system* under observation. For many diseases, including age dependent diseases such as Alzheimer's disease, and known gene related diseases such as acetylcholinesterase deficiency—both affecting brain function—imaging of gene linked reactions is a demanding goal. However, the number of possible reactions to be considered for imaging gene related reactions is an enormous task and opportunity as well. In this context, *imaging of molecular reactions* within a given metabolic circuit may be more revealing *when the observed circuit is perturbed* on purpose, so as to evaluate a system tolerance. For this to be done, different types of perturbations and tracers may be useful, including physical, chemical and "natural" tracers and perturbations.

For stratification and classification, several tracers or markers need be selected according to their specificity and sensitivity. For example, an excessive density of breast tissue is associated with an increased incidence of breast cancer, but is it a heritable marker? Also, *types of markers* need to change with the level of organization and, of course, with the purpose of a study. Clinical test results may be general markers, as flowers of pea plants were markers for Mendel's discovery of genetic inheritance. Other such markers are the degree of sunburn of exposed skin or the neurological symptoms of Parkinson's disease. Such markers are considered peripheral to the *central question of functional evolution of disease in relation to gene expression*. Indeed, one has to determine the primary aim of the investigation, be it the etiology of the disease, its degree or severity, or its type in relation to gene expression. Genetic counseling will be needed that demands ethical considerations and accordingly restraint.

In order to fully exploit all potential approaches to *creating a molecular profile of a disease*, *interdisciplinary work is essential*. This applies to the development of models, radiopharmaceuticals, instrumentation for optimally studying function in relation to structure, and of data analysis that is based on a chosen model. Neural network application to data analysis is powerful for imaging as it is for structural biology, where the success rate is about 60–70%. In fact, new insights arise from this approach to a better understanding of the complex workings within a "black box."

Another approach is the determination of *defined fluxes of substrates through a system*, and not so much the elucidation of a rate limiting single step of a substrate interaction within a reaction chain.

Adaptations and deadaptations may be described rather efficiently by measuring fluxes, as in the case of the development of myocardial hypertrophy or heart failure as examples of adaptation and deadaptation to an increased workload from essential hypertension.

The participants discussed the difficulties in progressing from understanding basic biochemistry to appreciating a clinical syndrome of a disease. Both *genetic information and metabolic reactions including signal transfers from the extracellular milieu into the cells are needed for describing genetically determined homeostatic circuits* that direct a given phenotype at various levels of organization.

The *infrastructure needed* for such comprehensive tasks must provide for the exploitation of interdisciplinary opportunities, different useful methods, data bases, radiopharmaceutical developments, and animal models. Envisaged are complementary uses of microimaging and *in-situ* imaging. The former analyzes tissue sections with regard to microstructural function, for example, using light sources and specific histochemically active dyes, and converts 2-dimensional information into a *3-dimensional display of tissue function*. *In-situ* imaging uses labeled compounds that allow insight into metabolic circuits without disturbing the tissue under observation whether at steady state and under specified perturbations. The linking of the data from such efforts with genomic information promises to be productive in functionalizing the genome.

The *DOE has initiated the human genome project*; now it has an additional challenge in understanding the *functional consequences of gene expression*. This knowledge will be applicable to clinical medicine and will require innovative uses of the tracer techniques of nuclear medicine. *The DOE has particular institutional expertise in answering the challenge of focussing development of tracer techniques towards functional genomics.*

Presentations of Examples at Issue

After the round table discussion on the scope of the challenge and possible solutions and answers, 14 participants presented concrete scientific data on:

A. Signal Transfer and Action

(Rodbell): Signal transfer from the extracellular space into the cell is complex, fast, and efficient. Various types of receptors are localized within the outer lipid bilayer membrane of the cell. Many different signals that may activate receptors include photons, odors, hormones, neurotransmitters, growth factors, peptides and proteins. There are tyrosine kinase activating receptors and those that activate G-proteins. These proteins are composed of different subunits which in part regulate their activity; as a consequence, a cascade of intracellular phosphorylations begins with proteins and peptides and results in subsequent structural changes of target molecules. A key protein in the transduction of signals is the RAS protein, one of the G-proteins. This leads to the eventual signal transfer for initiation of DNA transcription, cytoskeletal conformational changes, and many other well identified metabolic responses in a complex interplay.

All signal transfer processes and appropriate responses within the cell must be seen in the context of the cell's structural compartments built mainly by lipid membranes and the fibrillar cytoskeleton. Information may be transferred throughout the cell along the fibers of the cytoskeleton.

G-proteins are also involved in shaping the structures of the cellular compartments. For example, small cell surface indentations, the so-called caviola, may be shaped by way of G-protein function also involving actin. These caviola may prepare the cell for being recognized by specific antibodies. The living cell physically pulsates in compartments through the action of the cytoskeleton.

The relationship between these cell functions and gene activations is not understood. Nevertheless, during the transition from the DNA-synthesis-phase of the cell cycle into the premitotic rest-phase many dif-

ferent genes are activated to different degrees and the pattern of gene activation per cell responds to growth factors. The response is very fast and leads to increased protein synthesis within a few minutes. The review listed the major players in the network of generation and transfer of information within the cell so it may adapt to its environment within the given physiological limits of tolerance.

B. Approaches in Oncology

(Liu): The major challenge in treating cancer is the prevention and identification of metastases. For example, breast cancer susceptibility genes have been identified and this has led to molecular profiling of patients aiming at identifying individuals at risk. Other genes are involved such as those for P53 and HER-2. The effect of chemotherapy in part may be predicted from the genetic constitution. Hence, multiplex analysis of genes is needed, as on a microscopic slide or tissue specimen. This way, the genetic profile may be directly linked to pathology.

The HER-2 gene amplification occurs in 21% of lymph node positive breast cancer, whereas it is amplified in 48% of breast cancer *in situ*; the P53 gene is mutated in about 30% of these latter cases. Interestingly, in one prospective study of breast tissue regarding the P53 mutation, with a 7% incidence in benign tissue, only 3% of the affected individuals will develop somatic mutations; with 48% incidence in benign tissue, only 8% will show somatic mutations; with 29% incidence in tumor tissue, 15% will have lymph node metastasis. A similar pattern of discrepancies between initial profile of P53 mutations and clinical outcome regarding somatic mutations has been seen in colon cancer. This appears to indicate scavenging mechanisms that remove cells at risk; these mechanisms are little understood. Dynamic imaging with tracers *in situ* may be helpful to meet this challenge.

The National Cancer Institute has limited techniques but uses effectively the laser-capture microdissection technique. This again feeds into cDNA libraries. Now, on web-sites, all DNA rearrangements obtained will be displayed for many different cancers and be available to check against respective control DNA. This promises enormous benefits to clinical research and medical practice.

Regarding therapy, information on individual gene profiles of cancer with identification of the respective gene products may help to develop monoclonal antibodies against such products for the purpose of carrying alpha-particle emitters to tumor cells. The NIH may do all this on site.

Paramount to success, hopefully with the help of the DOE, appears to be the availability of data bases, computer rendering, system interactions, and eventual creation of virtual cells with at least some of their vital biochemical reaction circuits.

C. Imaging of Cell Metabolism, Apoptosis

(Strauss): Radiotracers may allow higher sensitivity than any morphological imaging technique in detecting very small tissue masses provided their metabolism differs significantly from the surrounding tissue in the body. A case in point are small lymph nodes with metastasis in breast cancer. These nodes have a much higher rate of glucose metabolism than their neighboring tissue and can be detected by 18-F labeled 2-deoxyglucose (FdG). This metabolic precursor traces glucose uptake into the node, where it accumulates without being metabolized like glucose. The rate of accumulation is imaged usually by positron-emission-tomography (PET) and is proportional to glucose metabolism. The "hot spot" in the image, then, shows the site of the node with metastasis in the body despite its very small mass that evades detection by normal imaging of structures, such as by computer assisted tomography (CT) or by magnetic resonance imaging (MRI).

The high sensitivity of imaging of radionuclide-labeled metabolites that have a particularly high meta-

bolic specificity for certain tissue in the living body is also being used to study phenomena associated with cell death in tissues that would hardly be recognized in structural images. For example, programmed cell death, also called apoptosis, is essential for the development of biological structures such as during organ development. Also in malignant tumors, apoptosis occurs, as it does in certain diseases with an increased incidence of damage to cellular DNA. It can hardly be made out by CT or MRI. The induction of apoptosis in a cell destined to die depends on the proper functioning of a set of genes in that cell. A specific gene product in cells undergoing apoptosis moves to the outer cell membrane where it can be recognized in the human by annexin, a 30 kD large protein. It binds in humans only to the receptor that is displayed by apoptotic cells. With radionuclide-labeled annexin drug-induced and spontaneous apoptosis can be imaged either by PET or by conventional gamma camera either in the planar mode or three dimensionally by single-photon-emission-tomography (SPECT). The genetic control of apoptosis may allow the linking of the genotype to the specific phenotypic response, and its study in the attempt at pharmacological interventions.

Another example of linking gene function to its specifically controlled metabolic reaction is ion transport, such as of potassium, through the cell membrane. Ion transport is crucial, for example, for the proper response of heart function to different work loads. It depends on optimal oxygen supply. Between 0.5 and 1 mL of oxygen is consumed per gram of heart muscle each minute. In all, about 20% of the oxygen is consumed by basal metabolism, 15% by work determined by the volume of blood to be transported, and about 65% is used to work against the pressure within the circulating system. Changes in the physiological distribution of oxygen consumption is accompanied by functional changes in potassium transport that depends on oxygen metabolism. Functional changes are associated with alterations in the shapes of the constituent proteins of the ion channels. It will soon be possible to specifically recognize the function of protein components of the potassium channels of the heart muscle cells. Metabolic substrates are being engineered to bind to these proteins irreversibly if present in a particular shape. If appropriately labeled, tracer amounts of such engineered substrates allow the imaging of channel function under the direction of gene expression in normal and diseased states of the heart muscle. Eventually, the genetic origin of certain heart muscle diseases that are associated with insufficient oxygen supply may be recognized so that preventive therapy may begin early.

Other examples may serve to illustrate the power of radionuclide imaging of functional responses under the control of gene expression. Even simple measurements of blood flow through the brain may be linked to certain genetically influenced diseases such as attention-deficit-hyperactive disorders, ADHD. Pharmacological intervention, for example with ritalin, which has a mild stimulatory effect on the brain, may increase brain blood flow especially in the ADHD patients and others who might have this predisposition.

The bidirectional interaction between gene expression and phenotypic responses in the intricate net of metabolic reactions needs to be fully appreciated. Concepts, technologies and agents must be devised accordingly. Moreover, family histories of genetically determined phenotypic abnormalities will add to the genetic significance of *in vivo* metabolic studies. Such studies have proved their eminent importance in many diseases and will also help in recognizing metabolic circuits for example, in the formation of new blood vessels, i.e., angiogenesis, and apoptosis in cancer with its metastases.

D. Bioinformatics

(Palsson): Large data bases are already available now on DNA sequences, gene maps, protein structures, and individual cells. Bioinformatics will increasingly use such data and integrate their function into models. It is unlikely that even a few genes truly act alone. Genes collaborate, and for a given cellular function a minimal set of genes is required. Thus, in certain parasitic bacteria, about 20 different genes are required, on average, for a single cellular function. In humans, this ratio of genes per function is likely close to 50 or even more; 70,000 to 100,000 genes may be responsible for some 1000 different functions. Be it as it may, ge-

netic information feeds into enzymes that operate in circuits making the whole cell function. Cells are elements of complex biological structures and rely on energy metabolism for maintaining structural integrity on which rests cellular and tissue function, ultimately the function of the organism. The information transfer by signal compounds within and between cells, on the one hand, and by the sequence of transcription to translation and post-translational modifications, on the other, assures bi-directional interaction between the genome and its phenotype.

Individual genes may be likened to the periodic table of elements. As elements make molecules, genes construct metabolic circuits, as Eric Lander has said. Through computer aided design, existing biological data bases may eventually and effectively feed into models linking gene derived metabolic circuits to specific cellular functions such as energy metabolism, structural maintenance, substrate transport, information exchange and usage, whole cellular fates and at last tissue engineering. To be effective, such models for system analysis based on autonomous gene derived metabolic circuits must be robust, must include redundancies, have creative functions including oscillations produced by bifurcational interactions. The models must conserve components yet adapt to internal and external interventions. Models have been formulated that allow, for example, in red blood cells the description of metabolic oscillations of glucose metabolism in a series of sequentially occurring biochemical steps of phosphorylation processes. Feedback controls in the system are linked to structural changes, allosteric changes, of participating molecules. Such isolated metabolic circuits need to be seen within the context of the whole system where different pools of circuits cooperate.

In the discussion, the need for reducing complexity to simplicity in primary assumptions was stated to be paramount for approaching the new paradigm of system analysis and description over characterizing and defining the role of elemental reactions within the system. The DOE may play a key role in providing for the basic data and tools for analyzing gene controlled metabolic circuits. Methods, experiments on dynamics and kinetics of biochemical and physicochemical phenomena, mathematics, and infrastructures could evolve into a megaproject of potentially enormous consequences. Understanding whole cell descriptions and, what may be called, *ex vivo* tissue models will benefit broadly biomedical research and clinical applications in diagnosis and therapy not only of genetic diseases.

E. PET Imaging

(Phelps): Amongst the imaging modes for measuring radionuclide-labeled metabolic substrates in living tissues, PET provides the advantage of allowing any, even small molecular substrates, to be used. They can be labeled with gamma-radiation producing radioisotopes (positron-emitters) of the normal constituent elements of metabolites such as 11-C, 13-N, and 15-O; 18-F is used for labeling molecular hydrogen positions. Radioactive iodine may replace a methyl group; both are comparably large with similar diameters. Another advantage of PET is the relatively high spatial image resolution with a three dimensional display. The time resolution of PET is sufficient for sequential imaging for eventually constructing time-activity curves from selected regions of interest. These curves serve to describe the kinetic behavior of a labeled compound in the selected region. Appropriate models, then, allow the description of specific reaction rates and rate constants in which the labeled compound is metabolically involved. The rates of enzyme kinetics in addition to metabolic pools can, thus, be studied non-invasively even in normal individuals at very low radiation risks.

A breakthrough in 1975 was the labeling of 2-deoxyglucose with 18-F, FdG, for the purpose of observing *in situ* the site and the rate of glucose consumption. Imaging of the whole body following intravenous injection of FdG showed tracer accumulation mainly in the brain and heart muscle, and, if present, in malignant tumors as well. In Huntington's disease, a genetically determined and inherited malfunction of certain brain regions, FdG accumulation is significantly reduced in the caudate nucleus and the putamen

of the brain even before clinical symptoms have developed. Similarly, in early stages of Alzheimer's disease FdG uptake is already significantly reduced in parietal brain cortex regions. In early stages of Parkinson's disease, the neurotransmitter dopamine is significantly reduced or, depending on the severity of the disease, even absent. Other receptors of neurotransmitters are affected in a variety of genetically influenced diseases including schizophrenia. The power of PET imaging in establishing a diagnosis of an inherited disease is developing rapidly. Similarly, metabolic circuits under gene control may be assessed provided the needed technology of multiple tracer analysis by PET and appropriate models for data analysis are available.

In order to increase the spatial resolution of PET a micro-PET is being developed. Its resolution will allow the observation of metabolism within less than 10 mm^3 of living tissue. PET resolutions in the order of 1 mm are possible yet less cost-effective. With autoradiography of tissue sections and new digital methods, a resolution of 50 μm will complement metabolic studies *in vivo*.

The discussion of this topic on imaging devices with optimal resolutions emphasized the need of considering potential radiation doses to the observed tissue and whole body. Also, statistical errors tend to increase with improved resolution and consequentially increased noise to signal ratios. Small PETs with high resolutions, nevertheless, are desired for animal work that can efficiently help developing diagnostic applications to human subjects. Moreover, the coupling of different metabolic events to be observed may, at times, override the requirement of optimal spatial resolution. The same may be said regarding the coupling of metabolic imaging with electrophysiological studies using, for example, magneto-encephalography or -cardiography. The latter techniques have a superb time resolution and may serve to untangle images with a lesser time resolution but better spatial resolution, if the two modes of investigation are geared to the same phenomenon.

F. Imaging of Gene Function

(Hershman): Antibodies to specific gene products have immensely helped to identify gene function, as in embryogenesis. Fluorescing proteins are used for such studies. This principal technique is now widely used in basic cell biology, toxicology and, particularly, in teratology. Such approaches led to the improvement of non-invasive imaging of genes in selected regions of the living body. Labeled substrates are made to bind to gene products within the cell, or to bind to a specific receptor on the outside of the cell membrane. In short, targeted substrates will be used increasingly to define a given gene product.

In cancer therapy, the herpes simplex gene for the enzyme thymidine kinase is transferred to tumor cells with the help of an adenovirus as carrier of the gene. The intracellular expression of the thymidine kinase gene makes the enzyme available in the cells as the target of the toxin acyclovir, a metabolically inactive analogue of thymidine for phosphorylation; in fact, it binds to, and simultaneously inactivates, the enzyme. Acyclovir may be labeled with 18-F for PET. Indeed, experiments in mice have shown the labeled acyclovir to be a potent indicator of the activity of the transferred gene, as its transcription increases within a few days in the infected cells and eventually ceases functioning.

Another example is the transfer of the gene for the receptor of the neurotransmitter dopamine (D2 receptor) into cancer cells. The dopamine analogue spiperone is labeled with 18-F for PET and allows the imaging of the site of the transfected cells in the body. Simple imaging with FdG, the glucose analogue, would not differentiate between normal and tumor cells, if both use glucose at similar rates.

Both genes, for thymidine kinase and dopamine receptor, are useful reporter genes indicating a successful gene transfer into the target cell. Such reporter genes may also be linked stably to other genes. The successful transfer and transcription of a given gene in target cells is then recognized by the appropriately labeled specific substrates binding to the product of the chosen reporter gene.

Another approach to measuring the rate of synthesis of a gene product is using the tracer dilution technique *in situ*. Thus, a defined amount of labeled substrate that competes with the naturally synthesized

substrate for a specific binding site in or on cells, is diluted in proportion to the rate of natural synthesis. In consequence, the rate of accumulation of the labeled substrates is inversely related to the rate of natural substrate synthesis in the tissue.

The simultaneous expression of several genes may also be studied using the respective labeled substrates for sequential imaging with the help of an image subtraction technique. Further techniques may involve the use of fluorescing probes that are recognized *in situ* by light, for example in light tomography. As with all protein probes, also with those permitting photochemical activity, cross reactions due to lack of specificity must be considered.

Another category of gene recognition *in situ* makes use of so-called antisense nucleotides that target specific nucleotide sequences in messenger RNA. This nucleotide targeting works for gene identification in isolated systems; however, it not yet known whether this also functions *in vivo*.

Radiopharmaceutical chemistry is paramount to success of the various approaches. Combinatorial chemistry will help tailoring of substrates for specific needs. Some substrates allow observing irreversible binding to functionally resting or activated targets of many different types, others are precursors for metabolic products. Such tracers will eventually open many more opportunities than exist today, as the human genome project progresses towards identification of all functionally relevant genes. Animal experiments are indispensable prior to clinical applications of *in vivo* imaging of gene expression.

G. Multidrug-Resistance (MDR)

(Piwnica-Worms): Chemotherapy of cancer fails if the targeted cells do not retain the drug but transport it out of the cells back into the extracellular space and the peripheral blood. Upon reaching the liver, a drug is usually detoxified and degraded. The mechanism by which cells neutralize one or more toxic agents can confer multidrug resistance (MDR) to the cells. MDR involves cell surface transporters made of phosphoglycoproteins. These are anchored in the cell membrane by 12 transmembraneous sections of the molecule. In all, some hundred different types of phosphoglycoproteins have been identified, and nearly all cells in the human body carry one or some of them. The function of the transporter proteins may be inhibited by substances like verapamil, quinidine, and cyclosporin-A. Also, newer compounds have been developed that bind irreversibly and specifically with high affinity to these proteins and thus block the transporter channels. Monoclonal antibodies against the specific proteins are also available.

For testing the nature and function of the MDR transporter proteins, metal complexes of isonitriles now play a major role. Such compounds are well known, can be easily labeled with 99m-technetium and are widely used for imaging of blood perfusion of heart muscle. This particular usefulness results from their easy solubility in lipids and their electrical, cationic, charge. Once inside the cells of a tissue they tend to concentrate in mitochondria. In cells with functioning MDR transporters, the 99m-technetium-labeled isonitrile derivative, called Sestamibi, is excreted and the rate of loss from the cells grades the functioning of the transporter. This has been well tested by introducing into the cellular genome the wild type gene for the transporter protein. A series of tests, also with specific blocking of the transporter, ascertained the reliability of the MDR transporter assay with Sestamibi. The function that is correlated with the levels of gene expression can be specifically blocked or altered by transferring an altered MDR protein gene; also, specific binding of compounds to these proteins can be competitively displaced. Now, new compounds are under investigation for testing the function of MDR transporter proteins. The screening uses single cells in culture. *In vivo* testing in genetically engineered mice will precede larger applications in clinical diagnosis in cancer patients. Indeed, monitoring prevention of the back transport of a chosen toxic agent out of the cell through the MDR transporter may improve the effectiveness of chemotherapy for cancer.

Initial clinical investigations with sequential imaging of Sestamibi in humans showed the liver to have two functional responses. The individual rates of tracer loss from the liver grouped with statistical sig-

nificance into two populations. This strongly indicates a genetic influence on states of function of the MDR transporter in healthy humans. The immediate challenge is the evaluation of the predictive value of the Sestamibi studies in cancer therapy and the appropriate correlation with biochemical and molecular biological findings obtained from dissected tissue specimen. The long term task is the further development of the approach to link specific gene expression to a well and easily observable function of membrane bound proteins on diverse cells in the intact body.

H. Brain Metabolism

(Fowler): Nuclear medical imaging, especially but not exclusively with PET, allows the targeted observation of interactions between neighboring cells in the brain, i. e., of the function of synapses between nerve cells. Here, neurotransmitters are the key molecules for transfer of excitation from one nerve cell to the other. They cause excitation in the recipient cell through binding to their specific receptors. Whereas the release of neurotransmitter by the nerve cells into the synaptic cleft is mainly a biophysical process, the back transport of these molecules into the transmitting cells is governed biochemically by specific transport channels that consist of particular proteins.

Synapses may be seen as metabolically fueled biological dynamos of brain function. Several thousands of such synapses are on each of the hundred billion nerve cells in the brain. Various neurotransmitters and their metabolic precursors, as well as their analogs have been labeled, mainly with either 11-C, 18-F or 123-I, for imaging. Also, compounds that bind irreversibly to transporter proteins and thus block the reuptake of neurotransmitter into the source nerve cell have been identified and appropriately labeled.

A large number of neurotransmitters and their analogs have been synthesized and used in clinical research and the diagnosis of various brain diseases. The imaging of receptors of neurotransmitters and of neurotransmitter release and reuptake, in addition to imaging of cerebral perfusion and glucose metabolism, has opened a new direction in the diagnosis of neurological and especially mental disorders. Into the latter category belongs the group of diseases caused by drug addiction.

For example, trace amounts of cocaine labeled with 11-C accumulated as rapidly in the brains of addicts as of normal individuals with a peak at about 10 minutes after injection. About 20–30 minutes later the labeled cocaine had nearly completely disappeared from the primary incorporation sites in the brain. Cocaine binds to the protein channels responsible for the back transfer of dopamine into the presynaptic nerve cells. The blocking of back transport of dopamine with the concomitant increase in the dopamine concentration for binding to the receptors causes the sensation of well being. Yet, the postsynaptic cells respond to the temporarily increased dopamine concentration by diminishing the number of receptor sites, and the blocking of the back transfer of dopamine into the presynaptic cells causes a temporarily diminished rate of dopamine release. The result of the cocaine induced adaptations in cellular metabolism causes a sensation of craving that is overcome again by blocking dopamine back transfer by renewed cocaine consumption; this results, again, in an increase in the dopamine concentration for binding to the available receptors. The cellular responses to surplus dopamine in the synaptic cleft with blockage of back transfer into the dopamine-synthesizing cells are under genetic influence; this will become amenable to study when the genes associated with the adaptive responses have been identified.

Various brain regions correspond in such a way that various types of neurotransmitters have far reaching consequences in the true sense of the word. Such synchrony of action has been observed for the neurotransmitters dopamine, acetylcholine, and gamma-amino-butyric acid (GABA). Blocking the action of GABA leads to a synchronous remote reduction of dopamine release. Also, cocaine addicts show a decrease in glucose metabolism especially in the prefrontal region of the brain, and such effects may last for several months after withdrawal from addiction. The functional circuits between various neurotransmitters and metabolism are, again, under genetic con-

trol and will be better understood when the corresponding genes have been identified.

In presynaptic nerve cells, the enzyme monamine-oxidase (MAO-B) degrades dopamine. Blocking this enzyme leads to an increase in the amount of dopamine to be available for release into the synaptic cleft. One of the compounds that binds to, and blocks, the MAO-B is L-deprenyl. It is used to increase dopamine availability in Parkinson's disease that is characterized by dopamine deficiency. In trace amounts and labeled, for example, with 11-C, L-deprenyl helps identify the amount of MAO-B at a given site in the brain. After destroying MAO-B with large quantities of non-labeled L-deprenyl in experimental animals, the resynthesis of the enzyme was titrated in images with trace amounts of labeled L-deprenyl. It took about 3 months for the reconstitution of the physiological level of MAO-B. This prolonged time for the brain cells to resynthesize the enzyme to its normal level is of relevance in the use of L-deprenyl as a drug. The *in vivo* description of the rate of synthesis of an enzyme is directly related to *in vivo* gene transcription, provided RNA translation and final protein synthesis are progressing normally.

The interaction of deprenyl with the enzyme was also studied. For this purpose, the L-deprenyl was labeled with deuterium in the various hydrogen positions and with 11-C in the molecular core. The rate of bonding of this double labeled L-deprenyl to MAO-B was measured. Only when the deuterated hydrogen position was involved in the bonding did the bonding rate slow down. In this fashion, the molecular site of L-deprenyl that binds to the enzyme could be identified *in situ*. This observation of the mechanism of substrate interaction with the help of the deuterium isotope effect on hydrogen bonding promises a wide application in the attempt to study metabolic circuits in the living system.

A new inhibitor of MAO-B is lazabemide (RO 19 6327). It binds reversibly on MAO-B in contrast to deprenyl, and is, thus, is easier to control in therapy. Using the 11-C-labeled L-deprenyl imaging technique for studying competitive inhibition of binding to MAO-B, the optimal dose of this new inhibitor could be determined in humans to be 50 mg twice a day for the treatment of Parkinson's disease.

Also cigarette smoking causes a temporary inhibition of MAO-B of about 40 %, without any effect on glucose metabolism in the specific region of interest. The substrate causing this inhibition is not known; yet, nicotine is not the inhibiting ingredient of tobacco smoke. The concomitant increase in dopamine availability to the synapse appears to be the reason for a lower incidence of Parkinson's disease in smokers. The sensation of well being caused by cigarette smoking may be related to the increased availability of dopamine for synaptic action. In this context it is noteworthy that about 88% of schizophrenic patients, about 75% of depressed patients, and about 80% of alcoholic patients smoke versus only 29% of seemingly healthy people.

Interestingly, MAO-B is also in the supporting cells of the brain, the glia cells. As the fraction of glia cells in the brain increases with age, so does the MAO-B concentration in brain tissue. This is demonstrated in brain images after deuterium-substituted 11-C-L-deprenyl administration. The use of deuterium-substituted 11-C-L-deprenyl was important in this study because the reduced rate of trapping of this tracer increased its sensitivity in regions of high MAO-B concentrations and low blood flow which is seen in aging.

The various consequences of interruption of the dopamine release—back transfer—MAO-B circuit as they are analyzed in different regions of the living brain, are examples of the power of nuclear medical imaging techniques for observing mechanisms of disease at the molecular level under the direction of genes.

I. Gender Differences in Brain Metabolism

(Wagner): Amongst the many neurotransmitting molecules produced by the human brain, opiates bind to specific receptors in various brain regions. Studies on the activity of opiate receptors in various brain regions in men and women indicate that significantly higher amounts of an analog of opium, carfentanil labeled with 11-C, bind in normal female brains than in normal male brains. This finding indicates a sex linked

genetic influence on opiate receptor density or function in specific nerve cells. These differences may be associated with the mediation of emotional and cognitive differences in men and women.

When activated opiate receptors in different brain regions may generate the sensation of well being. This is derived from a study involving patients with depression. When these patients received effective antidepressant therapy, 11-C-carfentanil binding was significantly increased in different cortical brain regions and in some basal ganglia. Again, female patients had a significantly higher receptor density in terms of tracer binding specifically in the temporal cortex and the putamen of the brain than did male patients.

The relation between opiate receptor activity and sensation of well being also emerged in studies involving cocaine addicts. Cocaine binds to the channel proteins that are responsible for reuptake of dopamine into presynaptic nerve cells. When cocaine addicts were in early withdrawal 1 to 4 days after the last cocaine use, significantly higher amounts of 11-C-carfentanil were bound to the opiate receptors in the basal ganglia and thalamus than seen in control subjects. After 4 weeks of abstinence, the amount of 11-C-carfentanil bound to these receptors had declined but had not reached the average level of controls. The degree of increased 11-C-carfentanil binding in the addicts was significantly associated with the degree of craving for cocaine. The prolonged response of opiate receptors over at least 4 weeks after cocaine use indicates a prolonged upregulation of genes responsible for the opiate receptors.

J. Brain Glia Cells

(Guilarte): Molecular markers of cellular reactions to brain damage are becoming available for clinical use. Such markers may also be applied to investigate whether the product of a known gene may be clinically useful. The peripheral benzodiazepin receptor (PBR), located in glia cells that support nerve cells in the brain, may be such a marker.

Upon physical or pharmacological damage to the brain, glia cells react by changing their metabolism. This leads to the accumulation, for example, of fibrillary acidic protein in the cells and also to an increased availability of the PBR. The gene for the PBR has been cloned from human cells. The upregulation of this gene in the cells that react to damage would allow the observation by imaging with PET or SPECT using an appropriately 18-F- or 123-I-labeled benzodiazepin. Indeed, experiments in mice with 3-H-labeled benzodiazepin and autoradiography of brain sections showed a significant correlation of tracer uptake and gene transcription in glia cells in response to chemically induced injury. The challenge for future work is to determine to what degree, over what spatial distance and during what time period signals from nerve cells may activate gene expression of PBR in glia cells in the living brain of humans.

K. Heart Muscle Metabolism

(Taegtmeyer): Normal heart muscle cells derive their energy by metabolizing mainly fatty acids; in a state of oxygen deficiency, as it occurs in coronary artery disease, glucose metabolism is preferred over that of fatty acids. Yet, glucose is the essential reserve for the cells to stay alive. Moreover, glucose is essential for storage as glycogen in the cells.

18-F-labeled 2-deoxyglucose, FdG, is transported, as in brains, into heart muscle cells very similarly to glucose, and it is also similarly accepted by the first enzyme in the chain of glucose metabolism, that phosphorylates glucose to glucose-monophosphate. Thereafter, the phosphorylated FdG accumulates in the tissue. The imaging of the rate of tracer accumulation in the tissue region of interest is proportional to the rate of glucose consumption by the cells in that region. In contrast to the brain, glucose uptake by the heart muscle is modulated by insulin and competing substrates. Both also strongly affect the tracer.

Glucose metabolism is essential to cellular metabolism for the purpose of balancing the energy supply by way of providing adenosine-triphosphate, ATP. The

major catabolite of glucose, pyruvate, is converted to acetyl-coenzyme-A that feeds into the citric acid cycle in preparation for the synthesis of ATP using oxygen. Acetyl-coenzyme-A is also the final catabolite of fatty acids. Incomplete glucose metabolism occurs in a type of short circuit for anaerobic energy supply; it yields lactate by reduction of pyruvate without conversion to acetyl-coenzyme-A for the citric acid cycle.

If the isolated rat heart in a closed perfusion setting is stressed to work harder, by epinephrine administration, fatty acid utilization stays nearly constant, whereas glucose utilization increases with a subsequent depletion of glycogen in the cells. If oxygen supply is reduced, in ischemia, the glucose consumption remains nearly constant; yet lactate production increases concomitantly with a decrease in glucose oxidation. At the same time fatty acid metabolism is reduced. The balance between the various metabolic pathways in the normal heart at rest and under workload, and in the state of acute oxygen deficiency serves as a model for studying adaptive processes under genetic control. To do this right, local blood flow, oxygen supply and hormonal actions on the heart muscle need careful control.

For the use of FdG in imaging studies of the human heart, the data need validation with glucose, for example, with 11-C labeled glucose; glucose labeled with beta-emitting radionuclides such as tritium or 14-C is used for observing metabolism in tissue sections with the help of autoradiography. The quantitation of glucose uptake along with FdG is paramount for assessing the mechanisms of altered glucose transport and phosphorylation.

Comparing the rates of phosphorylation, of the hexokinase reaction, the rate constant of FdG was lower than that of glucose. Addition of a relatively high dose of insulin to the rat heart in the perfusion system caused no effect on the phosphorylation of FdG, but it caused an increased rate of degradation of glucose, that was measured by the release of tritiated water after adding tritiated glucose to the system. Also, insulin decreased the affinity of FdG on hexokinase to a greater extent on the outer mitochondrial membrane than in the cytosol of the muscle cells; but with glucose being the substrate, the activation of the enzyme by insulin was the same in the two subcellular fractions. It appears that insulin may increase, on the one hand, the transport of glucose into the cells and, on the other, facilitate a transfer of hexokinase onto the mitochondria.

The different rate constants observed with glucose and deoxyglucose indicate the need for caution when FdG is used for imaging glucose metabolism in general. The ratio of rate constants varies with the type of tissue and is likely quite different in tumors than in the heart muscle. Some tumors have a reduced glucose metabolism not seen with FdG. This may be the consequence of derepression of the gene for an isoenzyme of hexokinase that phosphorylates FdG but not so readily glucose. The dual tracer analysis with FdG and labeled glucose may uncover certain gene influenced metabolic changes *in vivo* provided the base line ratio of rate constants for the two tracers is established. Quantitation is essential, but has thus far not yet been achieved.

L. Mitochondrial Function

(Bergmann): Nuclear medical imaging of metabolic reactions in the heart muscle can uncover genetic defects that cause severe disturbances in fatty acid degradation and thus in energy supply. The ensuing heart disease, cardiomyopathy, ususally appears symptomatically as heart failure only as individuals grow older. In some instances, the disease becomes manifest already in childhood. The underlying cause may be a defect in the transport of fatty acids into mitochondria through an inefficient so-called carnitine shuttle in the case of congenital lack of carnitine synthesis or of deficiency of the carnitine transporter in the body. Another genetically influenced illness comes from the inability of the heart muscle cell to degrade fatty acids of certain chain lengths. In the latter case, affected children may die suddenly, others may hardly develop symptoms; the reason for the different severities of symptoms is not known.

Common to this group of diseases is a deficiency in one or more enzymes, the acyl-coenzyme-A-dehy-

drogenases, that specifically degrade long-, medium- and short-chain fatty acids in the mitochondria. The diagnosis of such a disease is difficult and, thus far, relies on the biochemical analysis of small tissue specimens obtained by biopsies, and eventually on the analysis of the genes of the diseased individual and his or her family.

The degradation of fatty acids in the heart muscle cells may be measured using an appropriate radionuclide for labeling. PET or SPECT imaging in the sequential mode allows the construction of time activity curves that give the rates of release of the label from the observed tissue sites. The rate of tracer release describes the fatty acid transport from the cytosolic compartment of the cells into the mitochondria where degradation is comparatively very rapid with subsequent loss of the label, usually attached to a metabolic end-product, from the degradation site. A reduced rate of release of label alone does not indicate a reduced rate of degradation of the labeled substrate; a prolonged release may come from altered local blood flow, influences by substrates and hormones in the circulating blood, or from individual work loads on the heart. To overcome the difficulty of selectively measuring the capacity for fatty acid degradation in the mitochondria, a second tracer, 11-C-labeled acetate, is used that enters the degradation pathway directly without the need of any of the acyl-coenzyme-A-dehydrogenases. In this case, release of label from the tissue directly signals the rate of the final step in mitochondrial fatty acid degradation. If the rate of release of label from a given fatty acid coincides with that from labeled acetate, there is no enzyme deficiency. Yet, a relatively prolonged release rate from the labeled fatty acid indicates impairment in fatty acid metabolism. The severity of the enzyme deficiency shows by the degree by which the release of label from a fatty acid of a given chain length deviates from the control release of label from acetate.

Abnormal fatty acid degradation is common to different forms of congenital and acquired cardiomyopathies. Also, drugs may affect fatty acid metabolism. The differential diagnosis in such situations will profit from the more sophisticated nuclear medical imaging modes for unraveling malfunctions in lipid metabolism of the heart muscle. The particular challenge is the linking of metabolic findings to the individual genotype of the patient.

M. Lung Metabolism

(Dawson): The gas exchange function of the lungs is of obvious primary importance. However, the lungs play a role in controlling the composition of the arterial blood in other ways, such such as by removing and/or activating various vasoactive hormones and by changing the redox status of certain redox active compounds that reach the pulmonary circulation via the venous return. These "non-respiratory" functions of the lung are largely carried out by the pulmononary endothelial cells as the venous blood becomes arterialized on passage through the lungs. Endothelial functions of the lungs may severely respond to many inflammatory processes, may suffer injuries from chest irradiation with hyperthermia used in cancer therapy, or sometimes from chemotherapy; acute injury may result from lung storage and reperfusion or in the course of the adult respiratory distress syndrome. Measurements of some endothelial functions may relate to the metabolic status of the endothelial lining of the pulmonary vessels that are critical to maintaining the organ's gas exchange function.

Two approaches attempt the description of endothelial metabolic function. One is functional imaging using PET and a labeled ligand, such as 18-F-captopril; this compound is trapped by the angiotensin converting enzyme in the pulmonary endothelial cells. More such specific probes are needed for fully describing the metabolic endothelial function. The other approach is the multiple indicator dilution, MID, method. This allows the evaluation of pulmonary endothelial cell reactions with time constants on the order of the pulmonary capillary transit time, less than one second. Following the simultaneous intravenous injection of a vascular reference tracer, such as labeled albumin, and a specific metabolic tracer, such as a labeled ligand or metabolic substrate, both tracers are rapidly and frequently measured in the arterial blood. This demands either frequent blood sampling or on-

line detection in flow-through detectors. The separation in time and concentration between the reference tracer and the substrate tracer is the signal to be decoded using kinetic analysis. The kinetic analysis uses models of distribution in space and time; they take into account the longitudinal concentration gradients within the capillaries. These gradients come from the reactions involving the tracers. This approach principally differs from the conventional compartment analysis of PET data.

In the intact lung, the overall rate of substrate utilization depends not only on the metabolic status of the endothelial cells, but also on the number of cells exposed to flowing blood. This requires the estimation of separate parameters that reflect both the intensive and extensive properties of the cellular function under investigation. One approach uses the injection of a sufficient amount of unlabeled substrate to dilute the labeled substrate; this reveals the degree of saturation of the metabolic pathway of interest. Substrate and tracer are dispersed after intravenous injection resulting in a distribution of their concentrations with time at the entrance of the capillaries. This has been referred to as the "bolus sweep method"; it allows for the application of models that include non-linear kinetics, such as expressed in the Michaelis-Menton equation.

In this example, the maximal velocity, Vmax, is an extensive property reflecting the number of cells perfused, whereas the substrate concentration at half maximal velocity, Km, is an intensive property reflecting the cell function independent of perfusion.

Considerable infrastructure is now available for the analysis of MID data through the National Simulation Resource at the University of Washington that is directed by Dr. James Bassingthwaighte. The lung has some specific advantages for the application of the MID method. One of these is the relatively direct and non-invasive access to pulmonary blood flow and effluent.

However, the method is also applicable to other organs *in vivo*. Development of probes and rapid online detection systems will expand the applicability of the MID method and stimulate further theoretical development as well. This promises to eventually reveal the *in vivo* metabolic phenotypes of the cells within a functioning organ, and the role of specific genes in homeostatic metabolic circuits, so that appropriately targeted therapies may be developed.

N. Magnetic Resonance Imaging (MRI)

(Balaban): Magnetic resonance imaging, MRI, primarily observes structures as they are expressed by specific molecular configurations. With more recent advances of MRI technology changes in these configurations are observable as they occur over time spans of minutes. The time resolution depends on the structures observed and is constrained by the physics of signal generation from the target atoms and molecules and of signal measurement.

Hydrogen is especially sensitive to signal generation by the strong magnetic field and by the radiofrequency pulse of the apparatus. Perturbation of structures becomes visible through changes in signals mainly from hydrogen in the observed system. Among the natural elements in tissues, oxygen, phosphorous and the stable carbon isotope 13-C in the various molecular bonds are less sensitive but most useful signal generating elements. Thus, the MRI technique has advanced to include spectroscopy which allows measuring various rates of phosphorylation of different compounds. States of oxygenation and even the tissue pH are observable. Sequential MRI now routinely analyzes blood flow in a living tissue such as the brain, and is now preferred to nuclear medical tracer imaging with PET or SPECT. Crucial for this is the MRI signal change that correlates with the ratio of oxygenated versus non-oxygenated hemoglobin in the circulating blood.

Present advances also include the recognition of bonding of water molecules; here, proteins, lipids, and the 2-valent iron, for example in transferrin, influence the signals of closely located water molecules. This type of analysis even allows the measurement of the motion of water in the tissue.

The various advances in MRI technology now allow many studies on the metabolic state and reactions in tissues. Particularly interesting may be the progress made in analyzing mitochondrial function.

The generation of adenosine-triphosphate, ATP, as a consequence of oxygen consumption in mitochondria can be perturbed by various metabolic substrates and by changes in physical demands, for example, on heart muscle cells. Extra-mitochondrial metabolic reactions can be visualized by MRI as changes in the distribution of phosphorylated compounds. The pattern of distribution responds, for example, to the calcium concentration in the cells.

A relatively frequent heart disease, familial hypertrophic cardiomyopathy, CMP, stems from the mutated gene for β-myosin heavy chain, an essential component of heart muscle cells. This disorder is expressed as a derangement of muscle cells that interferes with the coordinated and unidirectional contraction that is necessary for the heart to act as a pump. The pattern of signals from substrates such as creatine, creatine-phosphate and ATP in skeletal muscle is quite different from that registered in the heart muscle from patients with CMP. The work of the heart may now be expressed *in situ* in terms of Joules per second in relation to ATP produced per second. The spectroscopic measurements for this kind of work limits the spatial resolution of conventional equipment with a 1 Tesla magnetic field to about 1 cm^3 for phosphorous and to about 2–3 cm^3 for the tracer 13-C. With a magnetic field of 4 Tesla, the resolution for phosphorous can be improved to about 0.5 cm^3, if 15 to 18 minutes are permitted for signal collection.

In attempting to evaluate metabolic circuits influenced by genes *in situ*, MRI methods, even if they are constrained by low sensitivity to detect chemical composition, will open many new avenues and complement tracer work using radionuclide-labeled compounds. Whereas tracer methods have much higher sensitivity than MRI or MR-spectroscopy, for example, in the evaluation of receptor concentrations, MR-spectroscopy can show chemical composition in larger tissue volumes with great accuracy.

Tools, Models and Approaches

The general discussion emphasized *the need for coordinated efforts*. This demands input from bioinformatics, physiological modeling, radiopharmacology, physiology and pathophysiology, biochemistry, molecular biology, cell biology, and imaging techniques, in col-laboration with clinical medicine. Only then will the growing possibilities from knowing the human genome be fully exploited. *Future studies* may focus on the function of memory, on mental diseases such as schizophrenia, on aging, obesity, cancer, and tissue adaptations to temporary injury. An example of the latter is the so-called hibernation of heart muscle cells after oxygen depletion in coronary artery disease.

(Budinger): Regarding *mathematical modeling* including the general area of biocomputation, *three categories* are presented: 1) modeling of connectivity or the relationships between genes and phenotypic expressions in terms of biochemical contents and reactions; 2) modeling and simulation to quantify traits; 3) modeling of parameters of population norms.

The *first* of the categories of modeling profits from renewed interest and developments in strategies such as *Hidden Markov Fields*. This theory may help in creating a *learning computational engine* for quantitatively establishing connections between traits and genetic mechanisms

The *second* of these categories includes *heuristic compartmental modeling*. It simulates known biology by using measured data sets in a modeling engine for allowing the explicit description of cause-effect relationships and influencing factors. Enzyme catalyzed reactions and ligand-receptor interactions are investigated by compartmental modeling.

(Feinendegen): The *special case* of analyzing *glucose transport across the blood brain barrier* is a simple example of this category of modeling. The transport was investigated with the help of a labeled glucose analog, 11-C-3-methyl-glucose, that behaves very similarly to glucose but does not enter metabolism. It is transported back into the circulating blood from the pool of free glucose in the extravascular tissue space. A two compartment analysis was applied. Two sets of measurements of tracer concentrations in the peripheral blood and brain tissue were made at various times after tracer injection, each at two different levels of blood glucose. The two compartment analysis gave the rate constants of inflow and outflow of tracer across the blood brain barrier. The rate constants at the two levels of blood glucose for each individual showed *the outflow but not the inflow to be regulated individually*. In other words, the data *indicate a genetic control of glucose utilization* in the human brain but not of the glucose transport into the brain tissue. The challenge is to link this finding to the genes that control glucose transport on the one hand, and glucose metabolism, primarily phosphorylation, on the other.

(Budinger): The *third* category of modeling analyses *relationships between structural composition and action*, or between growth and aging, or between nerve cell action and physiological responses, or between defined biochemical patterns such as reaction circuits, and environmental influences. In the latter case, control theory also helps describe adaptive responses in complex systems.

For all the modeling modes *various techniques* exist; they include compartmental simulation, Bayesian methods, neuronal networks, Hidden Markov strategies, homologies, threading models, fuzzy logic, and chaos theory strategies. For the analysis and data optimization of radionuclide imaging with PET or SPECT, many of these modes are being tried; they promise to improve especially the spatial and time resolution of metabolic parameters obtained from sequences of stochastically poor images.

The general discussion addressed the *applicability of modeling*. This is, of course, limited by the complexity of the system under study and by the demand for robustness. The latter may be an inherent curse. At times, only general solutions apply to

complex problems, and crude statements may be sufficient even if they are not fully satisfactory. Other demands pertain to the making of models of biological function in a se-lected system. Here, the biologist is challenged more than the statistician so that a decision may emerge re-garding, for example, the estimate of risk versus benefit in a bifurcational step derived from the biologist's data.

Linking phenotypes to their genotypes in the sense of linking proteins and their functions to their genome must consider the *bidirectional interdependence* of the two. Moreover, the phenotype reacts partly independently of its genotype. *Environmental influences* may modulate phenotypic reactions derived from genetic effects in such a way that adaptations to the environment result in a better chance of the system to survive. Also, many diseases often develop at certain body sites consequent to *multiple inherent metabolic alterations*; their causal relationship in the evolution of disease is difficult to describe qualitatively and quantitatively. A case in point is that *congestive heart failure* resulting from ischemic heart disease, hypertension, or cardio-myopathy is a major cause of death not just due to damaged myocytes but due to mechanisms not yet understood. Similar challenges are well known in link-ing genes to the development of the embryo.

Different strategies are needed regarding *modeling, measuring and data analysis* for untangling the mechanisms relating a genotype to its phenotype, and vice versa. Obviously, observing a single parameter in a complex adaptive system is not sufficient to solve these basic questions. Preferentially *more than two distinct targets of observation* need to be followed in their reactions and responses to interventions in the system. The *required tracers, instrumentation and data processing* must accordingly be geared to the particular problem. Only a few such attempts have been made thus far. As experience especially from animal studies accumulates, *data bases* will be created and should contribute to functionally linking phenotypes to their genotypes at the molecular level of observation.

Potential clinical applications

The question is how to clinically apply phenotype function based on biochemical reactions as a consequence of gene expression. This led to the *presentation of concrete suggestions*. These related to the brain and aging, heart, and cancer.

A. Neurology and Aging

(Fowler): The phenomenon of *aging* encompasses the entire body, and some tissues are more involved than others. A common denominator of aging is the decline of cellular and tissue function in its ability to adapt to internal and external metabolic demands and compensate for deficiencies. This decline of regulatory response of cells is potentially genetically determined, as well as caused by exogenous factors.

Especially regarding the brain, *aging needs to be differentiated from degenerative diseases* involving brain cells. Alzheimer's disease is a well known example. In the aging brain, on the other hand, the physiological function of neurotransmitters may decline progressively. This is seen in the *dopamine system*. Both dopamine related functions, of receptors on postsynaptic nerve cells and of transport back into the releasing presynaptic nerve cells, significantly decline progressively with age. Yet, the activity of the enzyme monamine-oxidase, MAO, that inactivates dopamine in the presynaptic cells, increases with age. In parallel, local blood flow in the brain decreases with age and to different degrees in various brain regions. On the other hand, *glucose consumption* may be unaffected in some brain regions but decline in others, especially in the frontal lobes. Evidence also suggests that the age related progression of deficiencies in brain function may be slowed by mental exercise. To what degree the *nerve cells or the supporting glia cells* in the brain are affected first is an open question. It will be answered with combined imaging of brain structure such as with MRI and of local function using radionuclide tracer methods preferably with PET. The *age related decay of homeostatic circuits* in the brain is determined to a large extent by genes, or, in other words, by structural changes in the DNA. The linking of the two changes will enhance the understanding of the bidirectional interaction between the two functions, of genes and circuits.

B. Cardiology

(Bergmann): Regarding the heart, available radiopharmaceuticals, imaging instruments and models for data analysis now allow the *observation of mitochondria*, the cellular site of energy provision for the heart's pump function.

Multiple tracer analysis, as discussed before, uses *labeled fatty acids* of different chain lengths together with labeled acetate as tracer for the final step of mitochondrial energy supply from fatty acid degradation. This permits analysis of various *genetic controls of mitochondrial activity* in the utilization of different fatty acids and glucose for providing the energy for heart function. When such observations are coupled with measuring local blood flow through the heart muscle, effects from local metabolic interventions due to altered oxygen supply may be analyzed and used diagnostically for guiding therapy decisions.

Such diagnostic observations may be quite distinct at various times after the beginning of local oxygen deficiency as it occurs as ischemia in coronary artery disease. On the other hand, studies on mitochondrial function may enable the accurate diagnosis within the group of heart diseases, called *non-ischemic cardiomyopathies*. These may have quite different causes but all express similar early symptoms. Both groups of diseases, *coronary artery disease* and non-ischemic cardiomyopathies, are linked to genetic dispositions as they appear as traits in families. A better understanding of this linkage promises improvement in treating the affected individuals and may bring great public health benefits.

C. Oncology

(Kirkwood): The incidence of *cutanoeus melanoma*, the example of cancer in this discussion, has risen rapidly in the last 40 years. This provides unique opportunities for the study of factors related to *disease progression*, and to response to new immunologic interventions with antibodies, effector T-cells, and cytokines/interferons. When *lymph nodes* are involved at the time of primary treatment, more than 50% of the patients relapse within 1 year and die on average 6–9 months later. Hence, pressing tasks are the *early diagnosis* before the melanoma metastasizes, and the definition of the role of progression factors, as well as immune response variables that now can be manipulated.

Early melanoma expresses a number of protein/-peptide antigens and gangliosides such as GM-2 on the surface of melanoma cells in the skin. These antigens may be recognized by T-lymphocytes and cause the production of cytotoxic cells or antibodies that can be used to identify the antigens and their coding sequences. The ensuing *immune interactions* between cancer cells and T-cells or antibodies may be similar to those in other cancers such as breast cancer. The importance of immune responses to melanoma appears to be crucial for the control of melanoma and the survival of the patient. Early diagnosis and treatment to alter cell proliferation as well as to enhance immune responses to the tumor, for example with interferon-α-2, has led in the last year to the first evidence of improved prognosis in patients with high risk, i.e., node positive melanoma.

Attempts at early diagnosis include examinations of specific metabolic markers and immune response components that can be evaluated not only in malignant tissues but also in potential precursor or *atypical moles* in the skin. *Different probes* are already available for tissue studies and include antibodies to adhesion molecules, CAM's, various cellular growth factors and receptors, and/or labeled antisense nucleotides for cellular messenger RNA. Some of the probes may be labeled radioactively or by appropriate dyes for *in vivo* analysis. Signals from genes associated with the tumor may be amplified and allow early diagnosis of the systemic spread of melanoma, as for other tumors marked by the particular products distinctive in the host.

Paramount to clinical success is the understanding of the molecular and immunologic features of *local and remote micrometastasis*. This will allow both diagnostic and therapeutic refinements, potentially including imaging ligands, selected metabolic substrates, and antibodies also with the help of combinatorial chemistry. The power of imaging in the search of micrometastasis is limited by the given spatial resolution; improvements to few mm resolution with PET are under way. This also applies to *follow-up investigations* in treated patients. Early recognition of micrometastasis may make the tumor curable by existing therapies be they surgical, radiological or pharmaceutical, alone or combined. Similar arguments apply to other tumors; and breast cancer was discussed in this context.

Much progress is under way in *nuclear medical diagnosis* of cancer. Whole body imaging with 18-F-labeled 2-deoxyglucose, FdG, and in the near future targeting more specific peptides and gangliosides are at hand. For chemotherapy, the prior recognition of the state of genetically determined multidrug-resistance of the tumor cells with 99m-technetium labeled Sestamibi, as was discussed above, promises to be decisive for therapeutic efficacy. Obviously, many interdisciplinary resources and cooperation will be essential, and the disease stages that may be most fruitful for intervention may be earlier ones than have hitherto been explored.

Concluding recommendations

The workshop concluded with a discussion of the *future role of the DOE* in studying functional consequences of gene expression in health and disease. This will be a major challenge in the post human genome era. The consensus of the participants was that some 95% of work and data that were presented at this workshop have their roots in DOE-funded research. DOE has had a *strong role in developing concepts and tools* for modern biomedical research and application; other funding agencies joined DOE later in several instances. *DOE should not abandon its engagement* in dedicated biomedical research as new tasks now arise out of what has been achieved. The scope of future work is expected to surpass the capabilities of DOE alone, so *partnerships are envisaged* for the benefit of all contributors. Such cooperation may be particularly fruitful for a *joint program of DOE with the NIH*, to which other agencies may well contribute as work progresses.

For this type of interinstitutional enterprise, *infrastructures need support* that bridge the genome project to the investigation of phenotypic expressions under genetic control. The demand for new techniques, investigational approaches, models, data bases, analyses and applications may require particular *hierarchical structures* with a broad involvement of clinical medicine. On the other hand, indispensable *freedom of interaction and integration* of various talents, and the fostering of creativity by the individuals within groups and as groups, all must be balanced against the requirement of *coherence as the program develops*. This was seen by the participants not to be the traditional issue of NIH funded research, yet that agency was viewed essential for the success of such a large cooperation at least in its early phase, for example, involving jointly the DOE and the NIH. Each partner in the cooperation has to *defend the program*; the DOE must do this to Congress. Reports need to justify the continued *funding of creativity*, as the future is only vaguely predictable on the basis of accomplishments. Research may appear like a seamless garment; yet incremental steps by creative individuals are often inspired by the interplay between funders and recipients.

The participants agreed:

1. to assess the *challenge* of understanding the relationship between genome and functions in organisms and of the significance of this understanding for biology and medicine;
2. to offer some *critical steps* to be taken over a period of approximately 10 years to meet the challenge identified in 1;
3. to explain how *success* in taking these steps will enable addressing major biomedical problems by the end of this period of time;
4. to ensure that all involved have the opportunity to suggest *examples*, biomedical and technological;
5. to convey to the agencies that fund and manage research, both public and private, the importance of *addressing the scientific questions* without identifying specific tasks for any agency;
6. to invite the *wider scientific community* to participate in solving the questions.

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