2002 Factbook

National Institute on Aging

Intramural Research Program

Gerontology Research Center
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Foreword

The mission of the NIA is the*"conduct and support of biomedical, social and behavioral research, training, health information dissemination, and other programs with respect to the aging process and the diseases and other special problems and needs of the aged."*

Research on Aging Act of 1974, as amended in 1990 by P.L. 101-557.

The Intramural Research Program (IRP) of the National Institute on Aging (NIA) comprises ten scientific laboratories and a research program that include the scientific disciplines of biochemistry, cell and molecular biology, structural biology, genetics, behavioral sciences, epidemiology, statistics, and clinical research and the medical disciplines of neurobiology, immunology, endocrinology, cardiology, rheumatology, hematology, oncology, and gerontology. Medical problems associated with aging are pursued in-depth using the tools of modern laboratory and clinical research. The central focus of research is understanding age-related changes in physiology and the ability to adapt to environmental stress. This understanding is then applied to developing insight about the pathophysiology of age-related diseases. The program seeks to understand the changes associated with healthy aging and to define the criteria for evaluating when any change becomes pathologic. Thus, not only are the common age-related diseases under study (e.g., Alzheimer's disease, atherosclerosis, osteoarthritis, diabetes, cancer), but the determinants of healthy aging are also being defined.

The bulk of the NIA intramural research program is based at the Gerontology Research Center at Johns Hopkins Bayview Medical Center in Baltimore, Maryland. The Laboratory of Neurogenetics and the Brain Physiology and Metabolism Section operate basic research programs at the Bethesda campus of the National Institutes of Health. The IRP provides a stimulating, academic setting for a comprehensive effort to understand aging through multidisciplinary investigator-initiated research. The program offers many excellent training opportunities in both laboratory and clinical medicine with a wealth of valuable resources. The NIA is committed to training researchers for lifetime careers in the biomedical and behavioral sciences.

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Laboratory of Cardiovascular Science

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The **Laboratory of Cardiovascular Science (LCS)** was established in 1985 as an outgrowth of the Cardiovascular Section of the Clinical Physiology Branch. LCS is presently organized into two sections: Cardiac Function and Behavioral Hypertension. The Cardiac Function Section, which comprised the entire LCS at its incipience, is organized into six functional units, each headed by a tenured or senior scientist. The Behavioral Hypertension Section was formerly part of the Laboratory of Behavioral Science and joined LCS in 1997.

The overall goals of the Laboratory of Cardiovascular Science are (1) to identify age-associated changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to study myocardial structure and function and to determine how age interacts with chronic disease states to alter function; (3) to study basic mechanisms in excitation-contraction coupling and how these are modulated by surface receptor signaling pathways in cardiac muscle; (4) to determine the chemical nature and sequence of intermediate reactions controlling the movement of ions through ionic channels and pumps present in myocardium, and how these are affected by aging and disease; (5) to determine mechanisms that govern neuro-hormonal behavioral aspects of hypertension; (6) to determine mechanisms of normal and abnormal function of vascular smooth muscle and endothelial cells; and (7) to establish the potentials and limitations of new therapeutic approaches such as gene transfer techniques. In meeting these objectives, studies are performed in human volunteers, intact animals, isolated heart and vascular tissues, isolated cardiac and vascular cells, and subcellular organelles.

Each section/unit/group independently conceptualizes and implements its research portfolio. Opportunities for collaboration among units/sections, however, are fostered and encouraged. In addition to independent work, substantial interaction occurs among scientists both within and between the sections/units. The stimuli for such interactions originate from individual scientists and from the Lab Chief, who commits substantial

energy to encourage (but not to demand) these research collaborations. Consequently, many of the LCS projects become multi-faceted, spanning a range from humans to molecules. Using this approach, scientists recognize that future research advances require the integration of discoveries within and among individual research areas. The networking among individuals within LCS also extends to individuals in other institutes within the NIH, academic institutions, and industry. We believe that such networking among individual facets of the biomedical research community is required for integration of discoveries that is tantamount to practical application of these research discoveries. The broad overall LCS mission permits tenured scientists, senior fellows, and new fellows appointed to the Lab to chose their specific research projects. In other words, individuals are most productive when working on projects on which they develop their own "passion." The resultant LCS environment has become somewhat unique: it is not strictly akin to a university department in which each individual dictates his/her mission and applies for individual funding in order to implement the proposed program; however, neither is the LCS environment strictly "mission oriented" in the sense that each individual is mandated to work on a given project in a "top down" design. The LCS environment is best described as a balance between the above approaches; and in the broad sense, the collective research output of the Lab can be considered to be a "bottom up" approach. Specifically, most projects originate at the investigator level but are coordinated by the Lab/Section/ Unit Chiefs to achieve a meaningful mosaic within the broad framework of the Lab mission.

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David Tweedie	Visiting Fellow
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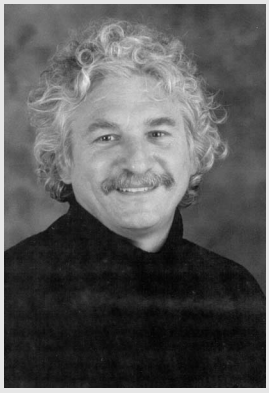

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Biography: Dr. Lakatta received his M.D., Magna cum laude, at Georgetown University School of Medicine. Following an internship and residency in Medicine at Strong Memorial Hospital, University of Rochester, Rochester, N.Y., he trained in basic research for two years at the NIH. Subsequently, he completed his cardiology fellowship at Georgetown and Johns Hopkins University Schools of Medicine. This was followed by a year of basic research training in the Department of Physiology, University College and the Cardiothoracic Institute, London England. Dr. Lakatta is the Chief of the Laboratory of Cardiovascular Science, National Institute on Aging. He also holds adjunct appointments as Professor, Department of Physiology, University of Maryland School of Medicine, and Professor, Cardiology Division, Johns Hopkins School of Medicine. Dr. Lakatta is recognized as both nationally and internationally as an expert in cardiovascular research. He has authored over 250 original publications in top peer reviewed cardiovascular journals, written over 150 invited reviews/book chapters and delivered over 300 invited lectures. He is a member of multiple scholarly societies and journal editorial boards. He has received several awards, among which has been election into the American Society for Clinical Research and the Association of American Physicians. He is the recipient of the Eli Lilly Award in Medical Science, the Paul Dudley White Award in Cardiology, the Allied Signal Achievement Award in Aging, the Novartis Prize in Gerontology, the Irving Wright Award of Distinction of the American Federation for Aging Research (AFAR), a Distinguished Service Medal, Public Health Service, National Institute of Health, National Institute on Aging and an Honorary Degree from the Universite D'Auvergne in Clermont, France. In 1994 he chaired the Gordon Conference on Cardiac Regulatory Mechanisms, and was the Chairman of the Scientific Program Committee of the International Society for Heart Research (ISHR) World Congress in 1998, a position he will hold again in 2004.

Keywords:

cardiovascular aging
G protein coupled cardiac receptors
cardiac apoptosis
vascular cell chemotaxis

Recent Publications:

Kass DA, et al. *Circulation* 2001; 104(13): 1464-1470.

Vaitkevicius PV, et al. *Proc Natl Acad Sci USA* 2001; 98(3): 1171-1175.

Wang SQ, et al. *Nature* 2001; 410(6828): 592-596.

Bogdanov KY, et al. *Circ Res* 2001; 88(12): 1254-1258.

Dr. Lakatta directs the **Cardiac Function Section (CFS)** which has a broad based research program ranging from studies in humans to molecules. The program is comprised of the following units:

Human Cardiovascular Studies Unit: This unit's studies deal with the interactions of age, lifestyle, and disease on cardiovascular structure/function in humans. The study panel for the bulk of the studies is the Baltimore Longitudinal Study of Aging (BLSA). Initially, age-associated changes in cardiovascular structure and function are defined in healthy individuals and subsequent studies define mechanisms for these changes and their prognostic significance. Additional populations that provide a diversity of lifestyle and disease have been added to the study panel for specific projects. Acute or chronic interventions in these individuals or in the BLSA are utilized to determine the responsiveness of age-associated

changes to pharmacological therapies or lifestyle changes, for example exercise habits. Several areas of related research in animal tissue and cells implemented in other units of the Section complement these studies in humans.

Molecular Cardiology Unit: The main focus of this unit is to define the molecular bases of aging in the heart. Many features of the age-associated changes in heart cells resemble those found during fetal development. For this reason, emphasis has been placed both on studies of development and on that of aging. The focus on early cardiac gene expression has relied greatly on the use of an embryonic stem (ES) cell differentiation model system. In these studies, potential early cardiac gene transcription factors will be identified and the proteins responsible for activating expression are being targeted using standard molecular biological techniques. For aging, a number of model systems are being developed so that specific genes can be targeted during senescence to examine their functional consequences. Each project area has multiple components, and it is hoped that through integration of developmental with aging studies, we will be able to obtain a global view of cardiac gene expression and how alterations in individual gene expression patterns lead to physiological and pathophysiological consequences.

Excitation-Contraction Coupling Unit: This unit's main research focus is on the control of cardiac cell calcium regulation. Substantial evidence indicates that the triggering of sarcoplasmic reticulum calcium release in cardiac muscle depends upon the interaction of the L-type sarcoplasmic calcium channel (dihydropyridine receptor) and the sarcoplasmic reticulum (SR) calcium release (ryanodine receptor) via local calcium gradients. This unit has developed quantitative mathematical models that embody this "local control" hypothesis. To test the predictions of these models, we require the ability to alter the behavior of these channels, while preserving their natural geometrical relationship in the cardiac myocyte. To achieve this, models are developed in which the relevant proteins (DHPR, RyR, FKBP-12.6) are mutated by homologous recombination in mouse embryonic stem cells. Genetically engineered myocytes produced are studied by biophysical techniques (patch-clamp and confocal microscopy). Additional projects deal with identifying how cardiac cell regulatory mechanisms become altered with aging and disease (anoxia, ischemia, hypertension, heart failure). The initial mechanistic focus of this unit has broadened from the study of biophysical mechanisms in cardiac cells to endothelial and vascular smooth muscle cells (VSMC) as well. These studies, which combine fluorescence and confocal imaging, link strongly to projects within the Vascular Studies Unit.

Cardiac Function Section: Further studies examine the functional effects of reactive oxygen and nitrogen species on cardiovascular function. There is considerable evidence that these play important roles in health and in disease states, including myocardial ischemia, congestive heart failure and atherosclerosis. These reactive species may frequently exert dramatically opposite biological effects, yet the spectrum of molecular targets overlaps to a considerable degree, particularly with respect to critical or regulatory thiol sites on proteins. Experiments are designed to examine how the dynamic competition between these species may be important in the evolution of various pathophysiological states, and how local control over nitric oxide and reactive oxygen species (ROS) production, and hence targeting, is responsible for some of the most important aspects of their physiologic and/or pathological roles. Specific areas of interest include, (1) the relationship between ROS, the redox state, and the function of mitochondria, and, (2) the role of NO in excitation-contraction coupling in heart.

Receptor Signaling Unit: The unit's focus is on elucidating signal transduction mechanisms for G protein-coupled- receptors, e.g., α and ß-adrenergic and opioid receptors and their subtypes in the heart. The interaction of signals emanating from stimulation of these with other receptor-mediated signaling pathways are also investigated. Studies are designed to integrate information gleaned from genetic manipulations, including gene transfer by adenoviruses, transgenic and gene targeted animal models, in conjunction with electrophysiologic, confocal imaging and cell biological techniques to probe novel intracellular regulatory mechanisms. In addition, considerable efforts have been put on cardiac aging and heart failure associated changes in G-protein coupled receptor signaling to understand the pathogenic mechanisms and develop new therapeutic strategies for the treatment of human heart failure.

Endogenous Sodium Pump Ligand in Blood Pressure Regulation: (A.Y. Bagrov, and O.V. Fedorova) Research in the group is primarily focused in pathophysiology of experimental hypertension. The main goal of the ongoing research is to better understand the cellular and molecular basis of salt sensitivity of hypertension. Primarily, research efforts concentrate on the regulation of the sodium pump, a major sodium transporting system in the kidney and cardiovascular tissues, by endogenous digitalis-like sodium pump ligands (SPL), such as ouabain and marinobufagenin. These studies utilize Dahl hypertensive rats, in which genetically determined sodium sensitivity due to mutation of α-1 subunit of the sodium pump underlies the development of hypertension. These studies are paralleled by investigations of SPL in patients with various types of hyperten-

sion. A major effort is made to define the sodium pump isoform profile of the SPL. In addition, the group is increasingly interested in co-regulation of the sodium pump in cardiovascular tissues by SPL and protein kinases, and in the involvement of SPL in tissue growth control and hypertrophic signaling.

Gene Therapy Unit: Investigators in the unit used constructs of endothelial growth factor (VEGF) with different vectors such as adenoviruses or plasmid/liposome complexes in experiments to deliver genes to promote angiogenesis. The major efforts are directed to characterize different experimental models of cardiac pathology in animals using the "cutting edge" *in vivo* technology such as pressure/volume analysis of cardiac function and Doppler echocardiography in mice. Great importance is assigned to the development of the optimal methods of delivery of appropriate genetic constructs to targeted tissue *in vivo* and to assess their therapeutic effectiveness. The Gene Therapy Unit interacts with other LCS units/sections, serves as a resource for other GRC labs, and collaborates with industry and academic institutions in animal trials that employ gene targeted therapy.

Vascular Studies Unit: Research areas of this unit include characterization of vascular smooth muscle cells (VSMC), VSMC properties (migration, secretion, invasion) *in vivo*, i.e., from neointimal lesions in restenosis injury, or from atherosclerotic plaque, and *in vitro*, i.e., in VSMC cells in tissue culture. A major focus is directed at discovering novel aspects of growth factor receptor-coupled signaling pathways that regulate cell migration and how these pathways change with age. Similar studies on signaling mechanisms of advanced glycation end-products (AGE) via their receptors (RAGE) on VSMC form an additional facet of the Unit's work. This Unit is also responsible for molecular biology studies on apoptosis in the cardiovascular system, focusing at this time on the regulation of cardiomyocytes death and survival. The Unit is highly interactive with other parts of a LCS-wide "vascular initiative" composed of Gene Therapy and Excitation-Contraction Coupling and Human Studies Units within the Cardiac Function Section of the Membrane Biology Section. The Vascular Unit also networks widely with academic institutions and industry.

Collaborators: Rui-Ping Xiao, M.D., Ph.D., Kenneth R. Boheler, Ph.D., Michael Crow, Ph.D., Heping (Peace) Cheng, Ph.D., Steven Sollott, M.D., LCS, NIA; Jerome L. Fleg, M.D., National Heart, Lung and Blood Institute, NIH; George Krause, Ph.D., Max Delbruck Centre for Molecular

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Biography: Dr. Bagrov received his M.D. and Ph.D. at Ivan Pavlov Medical University, Leningrad, USSR. He subsequently completed his cardiology training and held clinical and academic appointments in St. Petersburg, Russia. In 1992-1994 and 1998-2001, he worked at the NIA as a Visiting Associate and NRC Senior Associate.

Keywords:

Na, K-ATPase
endogenous inhibitors
hypertension
protein kinases

Recent Publications:

Fedorova OV, et al. *Hypertension* 2001; 37(2): 462-466.

Fedorova OV, et al. *Am J Physiol* 2001; 281(1): R-352-R358.

Fedorova OV, et al. *Circulation* 2000; 102(24): 3009-3014.

Bagrov AY, et al. *J Hypertens* 2000; 18(2): 209-215.

We are studying regulation of the activity of Na,K ATPase by endogenous digitalis glycoside-like ligands. The overall objective of our work is to clarify the role of endogenous digitalis-like ligands of the sodium pump (LSP) in the development of hypertension. We have shown that mammalian tissues contain a sodium pump inhibitor, similar to amphibian bufodienolide hormone, marinobufagenin (MBG) MBG and another endogenous inhibitor, ouabain-like compound (OLC) have different sites of origin and different effective stimuli, different kinetics in salt/stress induced hypertension, and interact with different subunits of the Na/K ATPase (NKA).

Our work has three major goals: (i) To define cause and effect relationships between LSP and hypertensive phenotype, (ii) To investigate, in Dahl hypertension, how LSP synergistically with the other vasoconstrictors contribute to cardiovascular remodeling, and whether this synergism involves protein kinase dependent mechanisms, and (iii) To study signaling pathways, which underlie the effects of LSP, and test the hypothesis that protein kinases potentiate the effects of LSP via isoform-specific phosphorylation of the sodium pump. Substantiation of these hypotheses may provide new approaches towards understanding the pathogenesis of NaCl sensitive hypertension and potentially provide new methods of early detection of the risk and prevention of pressor responses to high salt intake.

Goal 1. The studies of MBG and OLC in pathogenesis of Dahl hypertension include the experiments in which central and peripheral effects of MBG and OLC in rats with NaCl induced hypertension are blocked by MBG and ouabain antibodies which will permit assessment of whether each LSP contributes to hypertension. These experiments are paralleled by

a study investigating (i) whether doses of MBG/OLC administered to rats which are sufficient to promote hypertensinongenic effects are (a) comparable to *in vivo* plasma levels of CS, and (b) associated with the changes in activity of the NKA and expression of NKA isoforms in cardiovascular tissues.

Goal 2. In Dahl hypertension, the development of compensatory cardiac hypertrophy is followed by the development of heart failure. We investigate interactions of LSP with other vasoconstrictor systems (endothelins, angiotensins) during the development of left ventricular hypertrophy and congestive heart failure in Dahl rats. Plasma levels of LSP, endothelin, angiotensin II and atrial natriuretic peptide will be monitored in Dahl rats on high NaCl diet. In parallel, the combined action of LSP, endothelin, angiotensin II and atrial natriuretic peptide on Na/K ATPase activity from cardiovascular tissues is studied. We expect, that co-regulation of NKA by LSP and other cardiovascular hormones occurs via protein kinase C dependent mechanism. Studies of this mechanism may provide important data on both hypertensinogenic and growth promoting properties of LSP (**Goal 3**).

Goal 3. The studies of mechanisms of action of LSP focus on (i) NKA isoform specificity, and (ii) on the co-regulation of NKA by CS and protein kinases. These experiments will utilize NKA activity and receptor binding assays. The major questions to be answered are: (i) Do protein kinases unmask the effect of LSP via phosphorylation of the sodium pump?, (ii) Do protein kinases affect Na/K pumping and receptor properties of NKA?, and (iii) Is there an isoform specificity in the co-regulation of NKA by LSP and protein kinases? This Project is a continuation of ongoing studies testing the hypothesis that isoforms of the sodium pump represent receptor sites specific for different LSP, OLC and MBG in particular. The expression of a-1 and a-3 isoforms of the sodium pump in membrane fractions (sarcolemma and nerve ending plasmalemma) from rat heart and human mesenteric arteries will be studied. Concentration response curves of the inhibition of NKA in membrane fractions by LSP, including MBG and ouabain, will be determined in the absence and in the presence of activators of protein kinase C.

Collaborators: Peter A. Doris, Ph.D., Institute of Molecular Medicine, Texas University, Houston, TX; Joseph I. Shapiro, M.D., Medical College of Ohio, Toledo, OH; Atsuo Goto, M.D., School of Medicine, University of Tokyo, Tokyo, Japan; Denis Lopatin, M.D., Institute of Obstetrics and Gynecology, St. Petersburg, Russia; Nikolai Kolodkin, Ph.D., Institute of Highly Pure Biopreparations, St. Peterburg, Russia; Edwin E. Zvartau, M.D., Ph.D., Ivan Pavlov Medical University, St. Petersburg, Russia.

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Biography: Dr. Michael Crow received his Ph.D. in Physiology and Biophysics from Harvard University in 1981 and did postdoctoral studies in cellular and molecular biology of skeletal muscle development at Stanford University. In 1984, he joined the Faculty of the Department of Pharmacology at the University of Texas, Houston and moved to his current position in the NIA in 1991, shifting research interests from skeletal muscle to smooth muscle and cardiomyocyte cellular and molecular biology.

Keywords:

heart
vascular smooth muscle
cell migration
apoptosis
adrenergic receptors

Recent Publications:

Chesley A, et al. *Circ Res* 2000; 87(12): 1172-1179.

Neuss M, et al. *J Biol Chem* 2001; 276(36): 33915-33922.

Petrache I, et al. *Am J Physiol* 2001; 280(6): L1168-L1178.

Yeh C-H, et al. *Diabetes* 2001; 50(6): 1495-1504.

Cardiovascular Cell Biology: We study the behavior of vascular smooth muscle cells (VSMCs) and cardiomyocytes directed toward the goal of identifying the molecular mechanism through which alterations in these cells contribute to the pathogensis of cardiovascular disease.

Intracellular Signaling Pathways Regulating VSMC Migration: The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. Migration of resident VSMCs requires that the cells undergo a phenotypic switch from a contractile to synthetic/proliferative state. We previously showed that a key factor in this switch was the ability of VSMCs to activate the multifunctional protein kinase, calcium/calmodulin-dependent protein kinase II (CamKII). Our current work is focused on identifying the intracellular targets for CamKII, its upstream regulation, and its unique role in ß3 integrin-mediated signaling of ß1 integrin function. We have shown that engagement of ß3 integrins along with occupancy of the associated protein known as IAP (integrin-associated protein) are required for CamKII activation in response to chemoattractant recognition. Activated CamKII inhibits nonmuscle myosin light chain kinase (MLCK), altering VSMC myosin light chain (MLC) phosphorylation to attenuate stress fiber formation and promote migration. CamKII regulation of MLCK activity is also involved in ß3 integrin signaling, not only in VSMCs, but numerous other cell types, including macrophages, the erythroleukemic cell line K562, and HEK 293 cells. Interestingly, calcineurin, which is activated in the cell by the same signals that activate CamKII (i.e., calcium and calmodulin), can have the opposite effect of MLC phosphorylation resulting in inhibition of cell migration. These studies have what is likely to be general concept

regarding migration, i.e., that the relationship between migration and MLC phosphorylation is bell-shaped, with low or no migration occurring at very low or very high levels of phosphorylation. The practical consequence of this is that some cells may require increased phosphorylation for migration, while others decreased phosphorylation so that CamKII (and possibly calcineurin) may play different roles in promoting or inhibiting migration in different cell types.

Another potentially important target of CamKII regulation is TIAM, a protein first identified as a promoter of migration/invasion in T cell lymphomas. TIAM has subsequently been shown to be a guanine nucleotide exchange factor (GEF) for rac1 and to be expressed in many different cell types, including VSMCs. Phosphorylation of purified TIAM by CamKII leads to increased GEF and rac activity, promoting membrane ruffling and inhibiting stress fiber formation. Current studies are directed at developing dominant negative inhibitors of TIAM to test the signficance of this CamKII target in migrating cells. Our studies have identified a unique intracellular signaling network in VSMCs that is triggered by chemoattractant recognition and modulated by growth status, secretion of growth factors and extracellular matrix (ECM) components, and ECM-VSMC interactions with ramifications for other cell types in other settings.

Advanced Glycation Endproducts, Their Receptors, and Vascular Disease: Advanced glycation endproducts of proteins (AGE) accumulate in the plasma and in tissues with age and at an accelerated rate in diabetes. In isolated vascular cells, AGEs induce a proxidant stress, leading to activation of pro-inflammatory events such as increased activity of MAPK and NF-κB, increased monocyte chemoattractant protein-1 (MCP-1) production, and increased PDGF B chain activity, all of which have been implicated in vascular lesion development and the recruitment of inflammatory cells to atherosclerotic lesions. We have demonstrated that many of the effects of AGEs on gene expression are mediated through a unique immunoglobulin-type receptor called RAGE. We have constructed epitope-tagged wild type and mutant RAGE molecules and have shown that transfection of wild type receptor leads to increased MAPK activity and (MCP-1) RNA and protein levels in response to AGEs. Mutant receptors in which the cytosolic tail has been removed, however, do not result in increased MCP-1 production, but in fact block the ability of co-transfected wild type receptors to signal. These observations demonstrate that RAGE acts not merely as an AGE-binding protein but a bona fide transmembrane receptor, engaging intracellular signaling molecules to affect changes in

gene expression and protein production and secretion. Current studies are concentrated on exploiting the truncated receptor as a dominant negative to block the effects of RAGE-mediated signaling during vascular lesion development in transgenic mice. In addition, interaction cloning techniques are being used to identify intra-cellular proteins associated with the receptor.

Molecular Mechanisms of Cell Death in the Cardiovascular and Musculoskeletal Systems: Cardiac cell loss marks the transition from compensatory hypertrophy to heart failure and is a key event in the remodeling of the heart after ischemic insult. Cell loss is due predominantly to the death of cardiomyocytes and is mediated in part by apoptosis. Because adult cardiomyocytes are terminally differentiated cells and there is no identifiable cardiac stem cell present in the adult heart, their loss is currently permanent. We use various experimental models coupled with transgenic and extragenic approaches to study the process of cell death in the heart, with the goal to curtained and potentially fully protect cardiomyocytes from death-inducing stimuli. In a search for proteins that regulate apoptosis in the heart, we and others have identified a muscle-specific protein known as ARC (Apoptosis Repressor with CARD [Caspase Recruitment Domain]). Our studies show that ARC expression is downregulated by a number of death-inducing stimuli and that forced expression of a modified ARC fully protects isolated cardiomyocytes from these stimuli. We have also shown that ARC protects cells from death through multiple mechanisms, including direct inhibition of caspase activation, prevention of cytochrome c release and the mitochondrial permeability transition (MPT), and regulation of the NF-κB activity, an important regulator of apoptosis in a variety of cells. Transgenic mice carrying the ARC transgene under the control of a cardiac-specific promoter have been generated and are currently being used to assess whether forced expression of ARC will reduce infarct size and prevent cardiac remodeling following infarction. A conditional ARC knock-out is currently in development and additional studies are focused on how ARC expression is regulated and its pleiotropic effects achieved.

In addition to the heart, ARC is also expressed in injured blood vessels and skeletal muscles. Expression in injured blood vessels is confined to the neointima, an area of the blood vessel lumen into which vascular cells migrate and accumulate following vessel injury. Accumulation of a neointima is an important event in restenosis following balloon angioplasty and in cardiac transplant atherosclerosis. We have shown that vascular smooth muscle cells (VSMCs) isolated from the neointima (NI) express up to 5 times more ARC than cultured VSMCs from the medial

(M) cell layer. This difference in ARC expression correlates with the increased sensitivity of M-VSMCs vs. NI-VSMCs to apoptosis-inducing stimuli. Forced expression of ARC in M-VSMCs confers resistance to apoptosis comparable to that seen with NI-VSMCs. These results suggest that increased resistance to apoptosis caused by upregulation of ARC expression is an important factor that could prevent neointima growth and its associated pathogenic effects.

We are also currently examining the role of ARC in skeletal muscle. The initial focus of these studies was the potential role of ARC in the skeletal muscle atrophy arising from disuse, chachexia, sarcopenia, as well as various neuromuscular disorders. We discovered that ARC is necessary for the fundamental process of skeletal muscle formation. The differentiation of muscle precursors cells known as myoblasts into multinucleated myotubes, in fact, ARC is expressed at relatively low levels in proliferating myoblasts, but is markedly upregulated upon switching to media that promotes cellular and molecular differentiation. Differentiation also leads to increased nuclear accumulation of ARC. Muscle precursor cells in which the upregulation of ARC is inhibited or markedly delayed as a result of targeted RNA interference do not undergo morphological differentiation and show a marked reduction in the expression of markers of differentiation, such as the accumulation of sarcomeric-specific contractile proteins and creatine kinase activity. These ARC-deficient myogenic cells do undergo growth arrest in response to conditions that normally favor differentiation, but they fail to upregulate expression of myogenin, a critical mediator of the differentiation process. Coversely, enforced expression of ARC accelerates differentiation and leads to early upregulation of myogenin gene expression. Other cell death regulators, such as BCL-2 or the pan-caspase inhibitor, zVAD-fmk, or other CARD containing protein including the ARC-related protein NOP30, are all unable to substitute for ARC in stimulating differentiation, as are mutants of ARC in which the CARD domain is disabled. Together, these results identify a novel cellular function for the CARD protein interaction motif of ARC, suggesting that it may serve as a molecular link between cell differentiation and survival in myogenic cells.

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Biography: Dr. Boheler received his B.Sc. from Duke University and his Ph.D. in Physiology and Pharmacology from the University of California, San Diego. After completing a post-doctoral fellowship and working as a Researcher in Molecular Biology at Unit 127 of the National Institutes of Health and Medical Research (INSERM) in Paris, France, he was appointed Assistant Professor (Lecturer) at Imperial College School of Medicine in the Department of Cardiothoracic Surgery, London, United Kingdom. In October 1996, he joined the NIH in Baltimore to head the Molecular Cardiology Unit of the Cardiac Function Section of the Laboratory of Cardiovascular Science.

Keywords:

heart
development
calcium handling proteins
molecular biology

Recent Publications:

Boateng SY, et al. *Am J Physiol* 2001; 280(3): H1029-H1038.

Anisimov SV, et al. *Eur J Heart Fail* 2001; 3(3): 271-281.

Koban MU, et al. *Mech Dev* 2001; 109(2): 267-279.

Boheler KR, et al. *Cells Tissues Organs* 1999; 165(3-4): 237-245.

The focus of our research program over the past several years has involved examination of the expression and regulation of a number of proteins involved in regulating calcium movements in cardiac myocytes, including the sarcoplasmic reticulum calcium ATPase (SERCA), the Na/Ca exchanger (NCX1) and the sarcoplasmic reticulum calcium release channel (Ryanodine Receptor). The work has involved examination of the spatial and temporal expression of these mRNAs and proteins in the developing myocardium. Using simpler *in vitro* models, the regulation of presence of the mRNAs encoding some of these gene products has been studied through examination of signal transduction pathways.

Recent work is focused on use of an *in vitro* differentiation model of mouse embryonic stem cells and embryonic carcinoma cells in an attempt to further understand the consequences of development and of altered gene expression on function of these proteins. Additionally, research in the laboratory has been strongly directed towards the development of mouse models having temporal and spatial control of gene expression. This system is currently being tested and plans are underway to actively apply this system to mouse transgenic models and to differentiating ES cells.

Spatial and Temporal Analyses: Previous studies were performed in collaboration with the laboratory of Dr. Antoon Moorman, Amsterdam. With the development of molecular cell markers specific for contraction and relaxation, functional aspects of myocardial differentiation had been addressed through the use of *in situ* hybridization. We reported how expression of SERCA2 and PLB in the rat may partly explain why the

embyronic atrium and ventricle function essentially as they do in the adult. SERCA2 is expressed in a craniocaudal gradient; whereas that of PLB is expressed in a gradient essentially opposite to that of SERCA2. Accumulation of the NCX and RyR transcripts also occurs very early, similar to that for SERCA2, but do not show gradients of expression. With development, SERCA2 and PLB expression increase during late fetal and perinatal development; whereas that for NCX1 decreases at or around birth in a compartment dependent manner. NCX1 expression is, however, increased with aging. We have currently prepared a number of transgenic mice containing the promoters for rat NCX upstream of the b-galactosidase gene. The aim of this work is to identify sequences important for regulating cardiac restriction of this gene's products. Secondly, SERCA2 promoter constructs are being used similarly to understand how this promoter can regulate gradients of expression throughout the developing and adult myocardium.

Signal Transduction Pathways Mediating SERCA2 Expression: Using a model of neonatal rat cardiomyocytes, we have been able to determine that adrenergic agonists can play a critical role in regulation SERCA2 mRNA accumulations. Activation by alpha adrenergic agonists and protein kinase C isoforms reduces both SERCA2 mRNA expressions in a time and dose dependent mechanism probably through activation of the MAP kinase system. Beta adrenergic activation only results in decreased SERCA2 mRNA expression through a pathway that requires extracellular calcium and entry via the voltage dependent sarcolemmal calcium channel. The regulation however does not appear to be primarily transcriptional. Transfection into neonatal rat cardiomyoctyes of the 2.8 kb human SERCA2 promoter constructs linked to reporter sequences indicate a lack of response with any of the adrenergic agonists. Recent studies with Nuclear run-on assays have also indicated that transcriptional control of SERCA2 gene expression is not the primary mechanism responsible for increased mRNA, protein and function of SERCA2 seen perinatally. Studies are underway, to elucidate the mechanisms responsible for the post-transcriptional regulation, one possibility of which may relate to an alternatively spliced isoform of SERCA2 seen in the fetal myocardium, whose expression is greatly reduced late in gestation.

Expressional Analysis of Cardiac NCX in Development and Senescence: We have examined the mRNA expression of the Na/Ca exchanger (NCX) in rat heart during perinatal development and with aging. NCX is highly expressed in late fetal and neonatal rat hearts, decreasing to adult levels by 20 days after birth. The lowest level of accumulation is seen in 6

and 18 month old animals. In the 24 month old senescent rat, NCX expression is increased by almost 50% above that seen at 6 and 18 months ($p<0.05$), but is not different from that at 15 neonatal days. Results from nuclear run-on assays indicate that NCX expression during the perinatal period is regulated at least partially through transcriptional mechanisms. Relatively high transcriptional activity is seen at birth but by 20 post-natal days, no transcriptional activity from NCX can be detected. During development, there are no major changes seen in the use of the five identified transcription start sites, nor is there any major difference in the splicing patterns seen in the 5' untranslated regions. We have identified the presence of five different splicing variants in the cytosolic loop of the coding region, three of which have not been previously described in heart. We have also recently cloned a 2.8 kb fragment containing the putative cardiac NCX1 promoter and a consensus thyroid hormone responsive element which we are now examining. The work is now focused on the *in vitro* examination of this promoter. A number of putative GATA binding sites and Nkx binding sites have been identified. In transfection studies, GATA 4, 5, and 6 isoforms have been shown to be sufficient to transactivate this sequence. Constructs lacking these cis-binding elements or mutants of these sequences have been prepared and are being examined both *in vitro* and in the transgenic models described above.

Embryonic Stem Cells and Myocardial Development: This research area involves a model of *in vitro* differentiation of cardiomyocytes originating from embryonic stem cells (R1) and embryonic carcinoma cells (P19). The research is aimed at understanding the developmental processes involved in cardiac myocyte differentiation and development. To identify, atrial versus ventricular like cells, expression vector constructs have been made that link atrial and ventricular markers to the green fluorescence protein (GFP) and other selection markers. These constructs have been introduced into ES cells and positive transformants identified through neomycin resistance selection. From this work, we hope to use various molecular techniques to identify and analyze various transcription factors and growth factors that promote cardiac cell division and differentiation and importantly, the sequence of their activation and inhibition. Specifically, we are examining the expressed sequences of differentiating P19 cells through a technique called serial analysis of gene expression (SAGE). This technique takes advantage of PCR and type II restriction enzymes to isolate short sequences sufficient to identify RNA products expressed at any time point. Currently, SAGE analyses have been performed on adult mouse myocardium, 3+3 day *in vitro* differentiating P19

cells and a comparative analysis is underway with 3+0.5 day *in vitro* differentiating P19 cells. Through this technique, we hope to use the information gained about the expressed sequence pattern to target and specifically identify gene products that are important to cardiac differentiation.

Temporal/Spatial Regulation: The aim of this program is to develop conditional and inducible gene targeting models, limited to specific cardiac lineages (e.g. ventricular myocytes) and inducible at a desired developmental stage. The tools chosen to accomplish this program are the *Cre Recombinase-Lox P* recombination system and the tetracycline transactivator system. A number of mice have been prepared that carry the *Cre* recombinase transgene under control of a tetracycline-sensitive promoter. Secondly, a targeting construct containing *LoxP* sites has been prepared such that induction of *Cre Recombinase* expression by withdrawal of tetracycline should cause excision of a critical exon in a targeted gene. This system has been placed under control of a lineage-specific promoter so that a tissue-specific knockout can be made to occur at a specified time. Currently a tetop-Cre Recombinase and MLC2V-tTA construct has been injected into pronuclei of C57BL/6 oocytes and a number of founder lines positive for these transgenes have been identified. These lines are currently being studied for appropriate expression using another reporter mice. To inducibly knockout RyR2 expression, a 15 kb mouse 129/SvJ genomic DNA fragment has been cloned, sequenced and the genomic structure determined. This sequence has been appropriately modified and lox P sites and neomycin resistance cassettes placed appropriately within the sequence. This mutant mouse RyR2 targeting vector has also been successfully introduced into embryonic stem cells, injected into blastocysts, and positive chimeras have been identified. This work is on-going.

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Biography: Dr. Stern studied theoretical physics at Princeton and received an M.D. degree from University of Pennsylvania. Following internship, he was a Staff Fellow in the Laboratory of Technical Development of the NHLBI, where he invented a method to measure tissue microvascular blood flow using laser light scattering. Following an Internal Medicine residency at University of Michigan and Cardiology fellowship at Johns Hopkins, Dr. Stern joined the faculty in Cardiology at Johns Hopkins in 1981. His research on laser light scattering fluctuations in cardiac muscle, in collaboration with the Laboratory of Cardiovascular Science at GRC, led to the discovery that apparently resting heart muscle produces continuous, random asynchronous subcellular waves of contraction, which proved to be due to propagated calcium release from the sarcoplasmic reticulum. This led directly to his present interest in the basic mechanism of cardiac excitation-contraction coupling. His studies on the physiology of excitation-contraction coupling in single cardiac myocytes during extreme hypoxia led to the finding that reoxygenation injury is due to calcium shifts brought about by ionic conditions created during a vulnerable period of complete energy depletion. In parallel with this work, Dr. Stern carried out mathematic modeling of the basic mechanisms of sarcoplasmic reticulum calcium release. Based on this work he proposed, in 1992, the *local control* hypothesis of excitation contraction coupling, has become the leading theory of this process. In 1996, Dr. Stern joined LCS full time as a member of the Senior Biomedical Research Service.

Keywords:

calcium signals
excitation-contraction coupling
ryanodine receptors
mathematical modeling

Recent Publications:

Guia A, et al. *Biophys J* 2001; 80(6): 2742-2750.

Janczewski AM, et al. *Am J Physiol* 2000; 279(4): H2024-H2031.

Stern MD, et al. *Proc Natl Acad Sci USA* 1999; 96: 10756-10751.

Calcium Microdomain Signaling in Intracellular Communication: The heartbeat is initiated by the release of calcium from stores in the sarcoplasmic reticulum (SR). It is now well established that the trigger for this release is the entry of a much smaller amount of calcium through voltage-controlled L-type calcium channels in the cell membrane. This is the mechanism of *calcium-induced calcium release* (CICR), which is known to be mediated by ryanodine receptors, which are calcium sensitive calcium channels located in the membrane of the SR. Similar ryanodine receptors are located on intracellular calcium stores in a wide variety of cell types, where their function is not yet understood.

The release of SR calcium is a tightly controlled and smoothly graded function of the trigger calcium; this is paradoxical since CICR is an intrinsically self-reinforcing process which might be expected to lead to an all-or-none response. A possible resolution of the paradox is based on the fact that the L-type trigger channels and the SR release channels are known to be localized to opposite sides of the 15 nm dyad junctions between the cell membrane and the SR membrane. This means that the

trigger for CICR is not whole cell calcium, but rather the local calcium microdomain generated in the neighborhood of the triggering channel. We have shown mathematically that the interaction between the stochastic gating of individual channels and the fluctuating calcium microdomains which they generate can give rise to smoothly graded and controlled calcium release in the whole cell aggregate, even though individual release events may be nearly all-or-none. This is the *local control* hypothesis, which implies that whole cell calcium release depends critically on the details of the gating and ion permeation of the trigger and release channels, and on the local geometrical relationship between them. Over the past several years, considerable evidence has accumulated showing that this is the case. We have recently constructed a similar local-control model of the role of CICR in skeletal muscle excitation-contraction coupling. This model successfully explains many paradoxical observations, and leads to the insight that collective behavior of mesoscopic arrays of calcium-coupled release channels, which we term couplons, may be the basic functional unit of EC coupling.

In order to test the *local control* hypothesis more definitively, we hope to develop a model in which the full machinery of excitation-contraction coupling (junctions, ryanodine receptors, L-type calcium channel, auxiliary junctional proteins) is expressed and in which the components and the signals that control their localization can be manipulated genetically. This is the major project of our laboratory at the present time. Since cardiac myocytes are terminally differentiated and non-dividing, they cannot be used directly. In general, cultured cell lines do not form SL/SR junctions even when expressing the channel proteins. Our present approach to the problem is to make use of the well developed technique of gene targeting in mouse embryonic stem (ES) cells, together with *in vitro* differentiation of ES cells into embryoid bodies which contain beating cardiac myocytes. We have successfully established culture techniques which promote cardiac differentiation in a high percentage of embryoid bodies, and have demonstrated calcium sparks and waves, which are produced by RyR-mediated intracellular calcium release, in cells as early as 7 days of differentiation. These studies will be continued to characterize the biochemistry, ultrastructure and EC coupling physiology of these cells. These baseline studies will define that model and lead to increased understanding of the development of cardiac-type EC coupling. We will then use homologous recombination methods to obtain cardiac myocytes in which the key protein domains responsible for calcium sensing and release, and others (such as the enormous 2 megadalton “foot process” of the ryanodine receptor) whose function is unknown, have been altered. More impor-

tantly, we hope to discover the signals which give rise to the organized geometrical structure of the dyad junction, and to alter it in order to test the sensitivity of coupling to geometry which is predicted by the local control theory. A combined approach utilizing ES cell techniques, confocal calcium measurement, patch clamp and mathematical modeling will be used.

Since ryanodine receptors are ubiquitous, it is likely that the insights gained from this program will be important for understanding the way in which spatial and temporal localization of intracellular calcium signals leads to their diversity of function in many cell types.

Collaborators: Heping Cheng, Kenneth Boheler, Edward G. Lakatta, M.D., LCS, NIA; Eduardo Rios, Department of Physiology and Molecular Biophysics, Rush University; Phillip Palade, University of Texas, Galveston; Michal Horowitz, Hebrew University, Jerusalem.

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Biography: Dr. Cheng studied fluid dynamics, physiology and bioengineering, and then served as a faculty member in Peking University, China. To advance his career in biomedical sciences, he came to the United States in 1989, received his Ph.D. (physiology) from the University of Maryland and joined the Laboratory of Cardiovascular Science in 1995. During Ph.D. research, he discovered "Ca^{2+} sparks", now known as the elementary events of Ca^{2+} signaling in many types of cells. His current research interest focuses on local Ca^{2+} and cyclic AMP (cAMP) signaling in the context of excitation-contraction coupling and receptor-mediated signal transduction in normal and diseased hearts. These studies enlist an array of state-of-the-art techniques (e.g., confocal microscopy, electrophysiology and laser flash photolysis), gene-targeted animal models as well as mathematical modeling.

Keywords:

Ca^{2+} sparks
optical single-channel recording
Ca^{2+} sparklets
excitation-contraction coupling

Recent Publications:

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Ca^{2+} Sparks: Ca^{2+} sparks, extremely limited in space (~2 mm) and time (10-100 ms), are the elementary sarcoplasmic reticulum (SR) Ca^{2+} release packets. The detection of sparks was made possible with the advent of confocal microscopy and indicators that fluoresce negligibly when free of Ca^{2+} and have fast reaction kinetics. In heart muscle, the exquisiteness of excitation-contraction coupling is reflected by the ability of a single L-type Ca^{2+} channel to activate a Ca^{2+} spark, due to the large increase in local Ca^{2+} concentration ($[Ca^{2+}]_i$) in the vicinity of RyRs that are in close apposition of the L-type channel. Summation of Ca^{2+} sparks gives rise to global intracellular $[Ca^{2+}]_i$ transients; brillions ($>10^{12}$) of Ca^{2+} sparks are expected to ignite synchronously to drive each heart beat. Surprisingly, Ca^{2+} sparks relax, rather than constrict, vascular smooth muscle cells. The reason for this spark-induced relaxant effect is because local $[Ca^{2+}]_i$ gradients established by subsarcolemmal sparks activates Ca^{2+}-sensitive K^+ channels, and thereby hyperpolarizes surface membrane and shuts off Ca^{2+} influx. This is a classic case that a given signaling molecule may exert opposing physiological effects due to spatial compartmentalization.

Despite extensive studies over the last five years, the origin and the exact nature of Ca^{2+} sparks remain elusive: whether Ca^{2+} sparks are single-channel events or a collective phenomenon of clusters of RyRs? What makes the spark size twice that predicted by theory? How big is the Ca^{2+}release flux underlying a spark? What mechanism terminates Ca^{2+}sparks (see below)? To address these fundamental questions, we embark on novel imaging techniques, digital imaging processing algorithms and models of spark generation.

Nanoscopic Ca^{2+} Signaling Between Single L-type Ca^{2+} Channels and Ryanodine Receptors: Recently we have, for the first time, visualized single L-type channel Ca^{2+} transients, termed "Ca^{2+} sparkles," in intact heart cells. The novel optical recording of voltage-gated single-channels breaks the limits set by electrophysiological single-channel measurement, and makes it possible to track single-channel activity in circumstances when patch-clamp recording is precluded.

Ca^{2+} sparklets represent the smallest units of channel-mediated Ca^{2+} entry into the cell. In contrast, Ca^{2+} sparks reported previously by us involve many Ca^{2+} release channels/ryanodine receptors (RyRs), and constitute elemental events of intracellular Ca^{2+} release. Importantly, here we demonstrated that Ca^{2+} sparklet due to a single L-type channel opening is capable of triggering an elemental Ca^{2+} spark. The sparklet-spark coupling represents cardiac excitation-contraction (E-C) coupling at the molecular level. Our data provide direct evidence that intracellular Ca^{2+} release is under exquisite local control by individual Ca^{2+} channels in the plasma membrane, conferring high efficiency and specificity of Ca^{2+} signaling.

Employing a new generation of confocal microscope with improved time resolution and signal/noise ratio, we have demonstrated that the triggered Ca^{2+} spark rises on top of an ongoing sparklet, which is manifested as the foot of the triggered spark. This provides unequivocal evidence that sparklets directly trigger sparks. Furthermore, we found that the sparklet-spark coupling latency obeys a single-exponential distribution, with a time constant of 6.7 ms, and that the coupling fidelity is less than unity (δ=0.70) and varies in a use-dependent manner. These provide the first quantitative characterization of the kinetics, fidelity, and stoichiometry of the coupling between two signaling molecules, L-type Ca^{2+} channels and ryanodine receptors.

A fundamental question of E-C coupling has been whether a spark reflects a single RyR acting solo or a cluster of RyRs acting in concert. Here we determined that a single L-type channel couples to 4~6 RyRs to ignite a spark, resolving the long-sought molecular nature of Ca^{2+} sparks.

Since voltage-gated channels are essential to many vital cellular processes, and since Ca^{2+} is a ubiquitous second messenger, these novel approaches and exciting findings should be of great interest to a broad spectrum of investigators in the fields of ion channels, Ca^{2+} signaling and intermolecular signal transduction in general.

Termination of Ca^{2+}-Induced Ca^{2+} Release: In cardiac myocytes, Ca^{2+} release from RyR in the SR is activated by the Ca^{2+}-induced-Ca^{2+} release (CICR) mechanism. CICR, with its inherent positive feedback, is expected to operate in an "all-or-none" fashion. In order to generate Ca^{2+} transients of graded amplitude and robust stability, a regulatory mechanism must exist to counteract the regenerative CICR. Several mechanisms, including inactivation, adaptation, and stochastic closing of RyRs have been proposed, but no conclusive evidence has yet been documented. Our recent study has shown that FK506-binding protein (FKBP), an immunophilin and accessory protein of RyR, constitutes a prominent regulator of CICR via shortening the duration of the elementary release events (Ca^{2+} sparks) and accelerating the desensitization of RyR to Ca^{2+}. To elucidate the primary termination mechanism of CICR, we first developed a novel fluorescent technique. By combination of a fast, linear Ca^{2+} indicator, Oregon Green BAPTA 5N, and a high concentration of Ca^{2+} chelator, EGTA, Ca^{2+} release was visualized as discrete "Ca^{2+} spikes" restricted to T tubule-SR junctions, each consisting of single or a few Ca^{2+} sparks. Increasing the open duration and promoting the reopens of Ca^{2+} channels with the Ca^{2+} channel agonists, FPL64176, did not prolong or trigger secondary Ca^{2+} spikes, even though 2/3 of the SR Ca^{2+} remained available for release by caffeine. Latency analysis revealed that Ca^{2+} spikes coincided with the first openings, but not with the reopens, of L-type Ca^{2+} channels. Furthermore, after an initial maximal release (e.g., at 0 mV), even a multi-fold increase in unitary Ca^{2+} current produced by a hyper polarization step to -120 mV, failed to trigger additional release, indicating an absolute refractoriness of RyRs. When the release was submaximal (e.g., at +30 mV), tail currents upon hyper polarization did activate additional Ca^{2+} spikes; confocal images revealed that the tail release originated from those unfired during depolarization. These results indicate that Ca^{2+} release is terminated primarily by a highly localized, use-dependent inactivation of RyRs, but not by stochastic closing and adaptation of RyRs, or depletion of SR Ca^{2+} in intact ventricular myocytes.

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Biography: Dr. Rui-Ping Xiao has been working in the Laboratory of Cardiovascular Science since February 1990. She was trained as a physiologist and molecular pharmacologist at Tong-Ji Medical University, China, and at the University of Maryland, where she received her M.D. and Ph.D., respectively. Her main scientific focus has been related to receptor-mediated transmembrane signal transduction in the cardiovascular system. In addition, considerable efforts have been put on cardiac aging and heart failure associated changes in G-protein coupled receptor signaling. The breadth of our work covers four different areas: (1) signal transduction mechanisms which underlie the distinct functional roles of ß-adrenergic receptor (AR) subtype stimulation in cardiac myocytes; (2) age-and heart failure-related alterations in cardiac responses to ß-AR subtype stimulation; (3) interaction of the ß-adrenergic signaling pathway with other cardiac sarcolemmal receptor mediated signaling pathways (e.g., opioid, adenosine, and acetylcholine receptors); and (4) the role of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) in cardiac functional regulation. Most studies are designed to integrate information gleaned from genetic manipulations, including gene transfer by adenoviruses, transgenic and gene targeted animal models, in conjunction with electrophysiologic, confocal imaging and cell biological techniques. The mechanistic and interdisciplinary nature of our research has made the past few years particularly fruitful.

Keywords:

ß2-adrenergic receptor
G proteins
cAMP compartmentation
cardiac excitation-contraction coupling
cell survival
apoptotic cell death

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Zheng M, et al. *J Biol Chem* 2000; 275(51): 40635-40640.

Dual Coupling of Cardiac ß$_2$-Adrenergic Receptor to G$_s$ and G$_i$ Proteins: G protein-coupled receptors (GPCRs) constitute the largest class of cell surface signaling molecules in eukaryotes and in some prokaryotes. By activating their cognate heterotrimeric guanosine triphosphate (GTP) binding proteins (G proteins), GPCRs transduce stimulatory or inhibitory signals for a wide array of endogenous hormones and neurotransmitters, and ambient physical and chemical stimuli, as well as exogenous therapeutic reagents. ß-adrenergic receptors (ßARs) are archetypical members of the GPCR superfamily. There are, at least, both ß$_1$AR and ß$_2$AR present in heart muscle cells. Whereas both ßAR subtypes stimulate the classic G$_s$-adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade, ß$_2$AR can activate bifurcated signaling pathways through G$_s$ and G$_i$ proteins. Because of their distinct G protein coupling, these ßAR subtypes fulfill distinct, sometimes even opposite, physiological and pathological roles. Specifically, in the heart, whereas ß$_1$AR-generated cAMP signal can broadcast throughout the cell, the ß$_2$AR-stimulated cAMP signal is spatially and functionally compartmentalized to subsurface membrane microdomains by the concurrent G$_i$ activation, thus selectively affecting plasma membrane effectors (such as L-type Ca^{2+} channels) and bypassing cytoplasmic regulatory proteins (such as phospholamban and myofilaments). Of potentially greater importance, the ß$_2$AR-to-G$_i$ pathway also

delivers a powerful cardiac protective signal. As a consequence, ß$_1$AR and ß$_2$AR exhibit opposing effects on heart cell survival: ß$_1$AR activation can promote programmed heart cell death (apoptosis); in sharp contrast, ß$_2$AR activation can protect heart cells from a wide range of assaulting factors, including enhanced ß$_1$AR stimulation, hypoxia, and reactive oxygen species. The ß$_2$AR survival pathway sequentially involves G_i, Gß, phosphoinositide 3-kinase (PI3K), and Akt. Furthermore, *in vivo* overexpression of ß$_1$AR, but not ß$_2$AR, induces heart muscle cell hypertrophy and heart failure in transgenic mouse models. These findings indicate that the differential G protein coupling, to a large extent, accounts for the distinctly different physiological and pathological roles in the heart for ß$_2$AR versus those of ß$_1$AR. The delicate balance of G_s and G_i signaling in space and time might be crucial to normal cellular functions, whereas an imbalance may have important pathophysiological relevance and clinical implications. For instance, selective activation of cardiac ß$_2$AR may provide catecholamine-dependent inotropic support without cardiotoxic consequences, which might have beneficial effects in the failing heart.

In chronically failing heart, the ß$_2$AR/G_i coupling is exaggerated. The enhanced G_i signaling underlies the heart failure-associated dysfunction of ß$_2$AR. Based on the dual G coupling of ß$_2$AR, we conceptualize that receptor ligands may selectively activate a subset(s) of the post-receptor signaling pathways. By screening a variety of ß$_2$AR ligands, we have identified one ligand (fenoterol) that selectively activates G_S, bypassing the G_i signaling. Strikingly, fenoterol is able to restore the markedly depressed ß$_2$AR contractile response in two experimental chronic heart failure models. Our most recent studies provide compelling evidence that stimulation of ß$_1$AR, but not ß$_2$AR, induces cardiac apoptosis. The anti-apoptotic effect of ß$_2$AR stimulation in cardiac myocytes is mediated by G_i-Gγ subunits-PI3 kinase-Akt signaling pathway. These studies not only reveal the diversity and specificity of ß-AR subtype and G protein interactions, but also provide new insights for understanding the co-existence and different functional roles of ß$_1$AR and ß$_2$AR in healthy and failing hearts.

Roles of Ca^{2+}/Calmodulin-Dependent Protein Kinase II (CaMKII) in Regulating Cardiac Pacemaker Activity: The human heart faithfully supplies blood to the body by beating more than 3 billion times in a lifetime. The sinoatrial (SA) node possesses automaticity and serves as the primary physiological pacemaker of the heart. Our recent studies have shown that SA node pacemaker activity is critically dependent on Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)-mediated positive feedback regulation of the L-type Ca^{2+} current ($I_{Ca,L}$). In freshly dissociated rabbit single SA node cells, specific CaMKII inhibitors, a peptide CaMKII inhibitor or KN-93 (0.1 - 3.0 μM), but not its inactive analog KN-

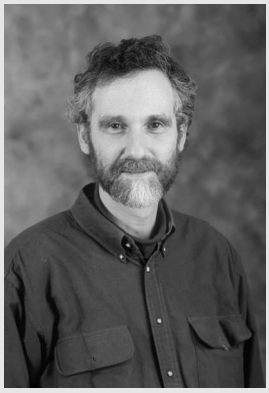
92, depressed the rate and amplitude of spontaneous action potentials (APs) in a dose-dependent manner. Strikingly, 3 μM KN-93 or 10 μM CaMKII peptide inhibitor completely arrested SA node cells, which indicates that basal CaMKII activation is obligatory to the genesis of pacemaker AP.

To understand the ionic mechanisms of the CaMKII effects, we measured L-type Ca currents and found that inhibition of CaMKII markedly decreased the current amplitude and slowed its recovery from inactivation. Similar results were observed using the fast Ca^{2+} chelator BAPTA, whereas the slow Ca^{2+} chelator EGTA had no significant effect, which suggests that CaMKII activity is preferentially regulated by local Ca^{2+} transients. Indeed, confocal immunocytochemical imaging showed that active CaMKII is highly localized beneath the surface membrane in the vicinity of L-type channels. Thus, CaMKII plays a vital role in regulating cardiac pacemaker activity via modulating properties of $I_{Ca,L}$ inactivation and local Ca^{2+} is critically involved in this process.

In addition to the robust modulatory effects, CaMKII also plays an important permissive role in cardiac pacemaker activity. For example, the CaMKII inhibitor, KN-93 (1μM), completely abolished the positive chronotropic effect of ß-adrenergic stimulation in SA node cells. In contrast, the effect of PKA is mostly modulatory because inhibition of PKA activity by H-89 (2μM), which fully prevented isoproterenol-induced chronotropic response, failed to abolish SA node pacemaker basal activity. Thus, CaMKII may afford an important integrating mechanism for diverse signals to regulate heart rate.

In summary, although previous studies focused on the role of ß-adrenergic and muscarinic stimulation in modulating the heart rate, our recent studies demonstrate a pivotal role of CaMKII in cardiac pacemaker performance. CaMKII-mediated regulation is unique as compared to the well established hormonal or neuronal control of cardiac pacemaking function, because it is an intrinsic and constant regulatory mechanism of cardiac pacemaker cell.

Collaborators: Dr. Robert J. Lefkowitz, Howard Hughes Medical Institute; Dr. Walter J. Koch, Duke University Medical Center; Dr. Ruth Altschuld and Charlene Hohl, Department of Medical Biochemistry, Ohio State University; Dr. E-G. Krause, Max Delbrück Center of Molecular Medicine, Department of Cardiology, Berlin, Germany; Dr. Brian Kobilka, Howard Hughes Medical Institutes, Stanford University Medical Center; Dr. Edward G. Lakatta, LCS, NIA.



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Biography: Dr. Sollott received his M.D. from the University of Rochester and completed his residency in internal medicine at a Cornell University program. He subsequently completed his cardiology fellowship at Johns Hopkins University and an NIH medical staff fellowship at NIA's Laboratory of Cardiovascular Science. His research attempts to bridge interests spanning basic and clinical science to therapeutics.

Keywords:

excitation-contraction coupling
calcium
nitric oxide
mitochondria
ischemia/reperfusion preconditioning
chemotaxis

Recent Publications:

Heldman AW, et al. *Circulation* 2001; 103(18): 2289-2295.

Vila Petroff MG, et al. *Circ Res* 2001;89(5): 445-452.

Vila Petroff MG, et al. *Nat Cell Biol* 2001; 3(10): 867-873.

Zorov DB, et al. *J Exp Med* 2000; 192(7): 1001-1014.

We are studying structure and function of cells from the cardiovascular system along two principal and distinct lines: 1) mechanisms of cardiac contractility, and 2) cellular changes after vascular injury. An underlying theme in both of these areas involves the pursuit and development of single cell biophysical methods to overcome certain limitations and complexities inherent in *in vivo* and in multicellular *in vitro* experimental systems, to gain an understanding of basic cell biological processes that may have implications for the pathophysiology and treatment of human disease.

Mechanisms of Cardiac Contractility: Principal research efforts, often employing these newly-developed biophysical methods, have focused on the regulation of contractility in intact cardiac myocytes, with particular emphasis on modulation of myofilament contractile activation by novel signaling pathways, for example, via alterations in the balance of specific kinase/phosphatase pathways, via cross-talk between the cGMP- and cAMP-dependent pathways, and via endogenous nitric oxide-dependent mechanisms. Recent work has focused on novel mechanisms of recruitment of contractile activation underlying the Frank-Starling response.

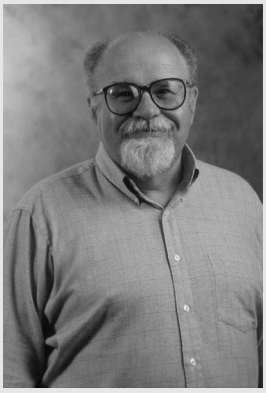
Mechanisms of Perturbed Mitochondrial Function in Cardiac Myocytes: Mitochondria play a central role in the regulation of apoptosis, and contribute to the pathogenesis of human degenerative diseases, aging, and cancer. Mitochondrial perturbations can have this result in a number of ways: by disrupting electron transport and energy metabolism, by releasing and/or activating proteins that mediate apoptosis, and by altering

cellular redox potential together with the generation of reactive oxygen species (ROS). Recent research is focusing on the relationship between the mitochondrial electrochemical gradient, ROS production, induction of the permeability transition pore, and functional sequella, including ischemia/reperfusion and myocardial preconditioning.

Cellular Response to Vascular Injury: The other major research direction involves the investigation of basic cellular responses of vascular smooth muscle cells during gradient-directed chemotaxis, in order to gain insight into fundamental events in the pathogenesis of vascular disease. These experiments with vascular smooth muscle cells have enabled an understanding of how focal receptor-tyrosine-kinase activation coordinates the cascade of signaling traffic and the reorganization of the cytoskeleton, leading to directed migration. Migration of vascular smooth muscle cells from the arterial media to the intima is a key event in the pathogenesis of occlusive vascular disorders, including atherosclerosis and post-angioplasty restenosis. We found that a unique intracellular Ca^{2+}-signaling profile is initiated via extracellular cues provided specifically by gradient exposure to PDGF, achieving an apparent threshold for activation of CaM kinase II (requisite during VSMC chemotaxis), and this phenomenon mediates VSMC chemotaxis. Differences in this specific Ca^{2+} signaling paradigm among individual cells underlies the asynchronous occurrence rate of chemotaxis seen in VSMC populations. Work is continuing to establish the mechanisms and coordination of subcellular Ca^{2+}-microdomains, compartmentalization of CaM kinase II activation and cytoskeletal rearrangements.

These ideas have been applied to the search for strategies to ameliorate the complications of vascular injury. We found that nanomolar levels of paclitaxel (taxol) blocked chemotaxis of VSMC in culture via specific interference with microtubule function, without killing cells. Subsequent *in vivo* experiments showed that paclitaxel, given systemically to rats at doses achieving blood levels some 2 orders of magnitude below that used in oncologic therapeutics (i.e., averaging well below peak levels of 50 nM), reduces the extent of neointimal proliferation following balloon injury by 70-80% without apparent toxicity. Currently, studies in larger mammals are under way to determine the feasibility of initiating a clinical trial in humans. Also, collaborative efforts are under way pursuing local paclitaxel delivery schemes, such as paclitaxel-coated stents, for this purpose. A microtubule-stabilizing-agent use-patent has been obtained for the applications of paclitaxel (etc.) in treatment of atherosclerosis and restenosis, and a CRADA has been established with private industry partners.

Collaborators: Salvatore Pepe, Ph.D., Baker Medical Research Institute, Melbourne, Australia; Kaikobad Irani, M.D., Johns Hopkins University; Pascal Goldschmidt-Clermont, M.D., Duke University; Jay L. Zweier, M.D., Johns Hopkins University; Ajay M, Shah, M.D., University of Cardiff, Wales, UK; Eduardo Marban, M.D., Ph.D., Johns Hopkins University; Robert S. Danziger, M.D., University of Illinois; Antoine Younes, Ph.D., Universite d'Auvergne Clermont, Aubiere, France; Edward G. Lakatta, M.D., LCS, NIA; Dmitry B. Zorov, Ph.D, Moscow State University; Jean-Luc Balligard, Ph.D., University of Louvain Medical School, Brussels, Belgium; Daria Mochly-Rosen, Ph.D., Stanford University School of Medicine; Suhn Hee Kim, M.D., Ph.D., Chonbuk National University Medical School, Chonjen, Korea; Kirsti Ytrehus, University of Tromso, Tromso, Norway.



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Biography: Dr. Talan was trained as a physician at the First Leningrad Medical School in Russia. He received his Ph.D. in Physiology at the Pavlov Institute of Physiology in Russia where he continued to work as a principal researcher before coming to the NIA in 1980. His studies at the NIA in the area of thermoregulation, regulation of hemodynamics, and operant conditioning of autonomic functions evolved into his present interests of development and assessment of genetic therapeutic interventions in cardiovascular pathology using different experimental models.

Keywords:

gene therapy
cardiac functions
hemodynamics
microcirculation
angiogenesis

Recent Publications:

Talan MI, et al. *J Thermal Biology* 2000; 25: 111-117.

Gowdak LH, et al. *J Vasc Surg* 2000; 32(2): 343-352.

Gowdak LH, et al. *Circulation* 2000; 102(5): 565-571.

Poliakova L, et al. *J Thorac Cardiovasc Surg* 1999; 118: 339-347.

Therapeutic Angiogenesis: The broad objective of this program is to perform preclinical experimentation on animal models of myocardial and hindlimb ischemia as well as on different experimental models of heart failure to evaluate the therapeutic potential of gene therapy with angiogenic growth factors. *In vivo* experiments are aimed at characterizing clinically relevant animal models and optimal conditions, vectors, and routes of delivery at which gene transfer of angiogenic growth factors induce therapeutic angiogenesis.

A) Adenovirus-mediated Gene Transfer of $VEGF_{121}$ Stimulates Angiogenesis in Normoperfused Skeletal Muscles: Administration of angiogenic factors has been shown to induce angiogenesis in the presence of tissue ischemia and to improve blood perfusion. However, there were no clear evidence that angiogenesis can be induced in normoperfused skeletal muscles. Furthermore, it is also unclear if once induced, the new-formed vessels can preserve blood perfusion upon induction of ischemia. Accordingly, we tested the hypothesis that adenovirus-mediated intramuscular (IM) gene therapy with vascular endothelial growth factor ($AdCMV.VEGF_{121}$) could augment collateral vessel development in nonischemic skeletal muscles and, subsequently, attenuate the hemodynamic deficits related to induced ischemia. Animals received IM injections of $AdCMV.VEGF_{121}$, AdCMV.Null, or saline in the thigh 4 weeks (rabbits) or 2 weeks (rats) before induction of ischemia in the injected limb. In rabbits, increased tissue perfusion (TP) to the ischemic limb was documented by a superior calf blood pressure ratio for $VEGF_{121}$ group versus controls, improved blood flow in the ischemic gastrocnemius ($P<.001$) and more angiographically recognizable collateral vessels (angioscore) ($P<.0001$), at day 1 after surgery. In rats, we found a 29% increase in

capillary density for $VEGF_{121}$ ($P<.03$ vs. saline) and an improvement of the bioenergetic profile of the gastrocnemius muscle obtained through ^{31}P NMR spectroscopy. We concluded that IM administration of $VEGF_{121}$ induces angiogenesis in normoperfused skeletal muscles and the newly formed vessels preserve blood perfusion once ischemia develops. This prophylactic approach could have therapeutic significance as part of an alternative treatment strategy for patients with peripheral vascular disease.

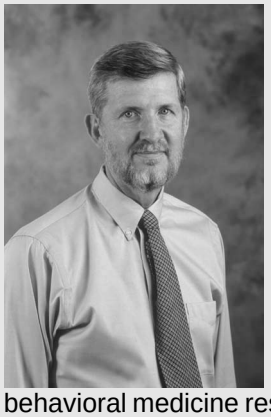
B) Treatment with $VEGF_{165}$ Encoded in Plasmid/liposome Complex Stimulates Angiogenesis in Rabbits Hindlimb Ischemia Model: Liposome-based vectors for gene therapy are considered to have lower transfection rate that adenovirus-based vectors. Nevertheless, comprehensive, *in vivo*, efficacy evaluation of liposome-based endothelial growth factors gene transfer for the treatment of tissue ischemia was not previously conducted. Two days after surgical removal of the femoral artery on one side, the ischemic tissue of different groups of rabbits was injected with different concentrations of plasmid/liposome construct encoded with $VEGF_{165}$, control substance (plasmid/liposome without expression cassette), or saline. Blood pressure distally to removed femoral artery, tissue blood flow, postmortem angiography and capillary density were assessed weekly, for four weeks. Accelerated development of new capillaries and larger vessels was confirmed by all assessment techniques during the first two weeks in $VEGF_{165}$ treated groups. *In vivo* angiogenic efficacy of plasmid/liposome vector encoded with $VEGF_{165}$ was not inferior to that of adenoviral vector.

C) Experimental Model of Post Myocardial Infarction Chronic Heart Failure: In keeping with a broad objective of the program, we mustered the techniques for *in vivo* assessment of cardiac function in rats and mice - the high resolution Doppler-Echocardiography and pressure/volume loop analysis with intracardiac pressure-conductance catheter. Using this “cutting edge” technology, we are conducting extensive functional and dynamic characterization of chronic heart failure which is developing subsequently to ligation of a coronary artery in mice and rats. This experimental model will be used for gene therapy experiments and for transgenic-based studies of the role of different receptors pathways in development of heart failure.

Using the experimental model of myocardial infarction in mice, we characterized the usually described “dilated” phenotype of chronic heart failure. This phenotype is associated with large, more than 40% of left ventricle, infarcts and characterized by ventricular dilatation. The reduction of LV elastance, which defines the decline of the cardiac pump function in this phenotype coincides with an increase of arterial elastance, so

that ventricular arterial coupling became mismatched. We also identified and characterized previously overlooked phenotype of chronic heart failure associated with small, non-transmural infarction. This phenotype was not accompanied by left ventricular enlargement and was characterized by increased ventricular elastance in end-systole and stiffness in end-diastole, i.e., it might represent a diastolic model of heart failure. Increased ventricular elastance in this phenotype matched the increased arterial elastance so that ventricular-arterial coupling remains preserved. In longitudinal study, we showed that development of this non-dilated phenotype was independent from usually described dilated phenotype characterized by reduced ventricular elastance.

Collaborators: Richard Spencer, M.D., Nuclear Magnetic Resonance Unit, LCI, NIA; Maurizio Capogrossi, M.D., Institute of Dermatology, Rome, Italy; Petro Anversa, M.D., Cardiovascular Research Institute, Valhalva, NY; Irni Kovesdi, Ph.D., GenVec Inc., Rockville, MD; Edward G. Lakatta, M.D., LCS, NIA.



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Biography: David E. Anderson was originally trained as a clinical psychologist, receiving his Ph.D. from the University of Oregon in 1966. His career interest in behavioral medicine research emerged while he was at the Johns Hopkins University School of Medicine (1968-1981), where he developed an animal behavior model of hypertension. He elaborated this model while at the University of South Florida (1981-1987), where he was a recipient of an NIH Research Career Development Award (1983-1987) and the Pavlovian Award for Biological Science in 1985. He joined the National Institute on Aging in 1987 as Chief of the Behavioral Medicine Section. He became a member of the Laboratory of Cardiovascular Science in 1997, where he continues his studies of interactions of stress, salt, and blood pressure in humans.

Keywords:

blood pressure
breathing
hypercapnia
hypertension
sodium pump inhibitors

Recent Publications:

Anderson DE, et al. *J Hypertens* 2001; 19(3): 459-463.

Anderson DE, et al. *Prim Psych* 2001; 8: 66-70.

Anderson DE, et al. *J Hypertens* 2001; 14: 761-767.

Scuteri A, et al. *Psychom Med* 2001; 63(3): 470-475.

Behavioral Medicine Research: It is generally agreed that many chronic diseases result from one or more environmental influences overlaid on a genetic predisposition. Genetic studies may facilitate new treatments of these diseases, while studies of the environmental components may result in an understanding of adaptive lifestyles that can delay, or even prevent, their onset. Cardiovascular diseases are perhaps the most prevalent example of those which may be amenable to lifestyle interventions. Within this group of disorders, the development of hypertension is thought by many to be potentiated by interactions of stress and dietary factors. However, the mediating mechanisms remain to be clarified. The goal of the research in the **Behavioral Hypertension Section** is to identify physiological and biochemical mechanisms by which behavioral interactions with the environment compromise the ability of the kidneys to regulate dietary sodium and thereby contribute to the development of chronic hypertension. From such studies may come interventions that can attenuate or prevent this important risk factor for heart disease, stroke, and kidney disease in older persons.

Stress, Salt and Blood Pressure: Behavioral stress can reduce renal excretion of dietary sodium, not only by increases in renal sympathetic nervous system activity or stimulation of adrenocortical sodium-retaining hormones, but also via inhibition of breathing. When respiratory removal of CO_2 is decreased relative to its metabolic production, plasma pH decreases. The kidneys respond by reabsorption of buffer compounds (e.g., bicarbonate ions), but also by decreasing sodium and water excretion, while increasing excretion of hydrogen ions. Experimental studies in our laboratory found that sustained voluntary slow frequency breathing at a

normal depth that increased pCO_2 was sufficient to decrease renal sodium excretion and stimulate endogenous sodium pump inhibitors that are sensitive to plasma volume expansion. In these experiments, the plasma volume remained increased even after plasma pH returned to normal.

Many studies have shown that psychological influences affect breathing frequency, with increases during excitement, and, of particular relevance to the present research, decreases during vigilant attention to the environment. Humans do not all breathe at the same frequency while at rest, but show large individual differences that tend to remain stable over time. In addition, substantial individual differences in resting pCO_2 are also observed, with individuals who tend to breathe slowly at rest maintaining higher resting end tidal CO_2 at rest than those who breathe more rapidly. Recent research in our laboratory found that high resting end tidal CO_2 ($PetCO_2$) is a risk factor for blood pressure sensitivity to high sodium intake. In addition, high resting $PetCO_2$ has been found to be an independent correlate of elevated resting systolic BP, especially in women who are low in trait anger. Thus, chronic hypoventilatory breathing might be a risk factor for some forms of high blood pressure. Recently, we have shown that high perceived stress is an independent predictor of the inhibited breathing pattern at rest, especially in women.

Ongoing Studies: A study is in progress to test the hypothesis that blood pressure sensitivity to high sodium intake in normotensive persons is a function of the inhibited breathing pattern and associated endogenous sodium pump inhibitors. Participants are placed on a low salt diet and a high salt diet for seven days each, during which ambulatory breathing pattern and blood pressure are monitored in the natural environment. In addition, blood and urine samples are collected systematically to determine the time course of changes in sodium balance and related hormones involved in blood pressure regulation. This study may provide a simple clinical test for sodium sensitivity and elucidate critical mechanisms mediating the role of behavior in the pathogenesis of chronic hypertension.

Collaborators: Alexei Y. Bagrov, M.D., Ph.D., Olga V. Fedorova, Ph.D., Edward G. Lakatta, M.D., Laboratory of Cardiovascular Science, NIA; Margaret A. Chesney, Ph.D., Office of Research on Women's Health, National Institutes of Health.

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The **Laboratory of Cellular and Molecular Biology (LCMB)** includes scientists formerly in the Laboratory of Biological Chemistry (LBC). The name was changed to better reflect the general interests of the group and the nature of ongoing studies. The LCMB is currently comprised of six independent research programs headed by either a tenure track scientist or a senior investigator. These programs include the Cell Stress and Aging Section, the T Lymphocyte Signaling Unit, the Stress Signaling Unit, the Cell Cycle Control Unit, the Cancer Molecular Genetics Unit and the Molecular Neurobiology Unit.

Major areas of emphasis common to the individual programs include: 1) the elucidation of signal transduction processes and gene regulatory mechanisms involved in mediating cellular responses to environmental signals such as growth factors, cytokines, and stress stimuli; 2) the determination of molecular mechanisms contributing to the maintenance of cellular homeostasis and cell cycle control; and 3) the contribution of dysregulated gene expression, or loss of critical gene functions to the development of cancer. As described below for the individual programs, a wide variety of *in vitro* and *in vivo* models are being employed to approach these issues. These processes have direct relevance to our understanding of critical events associated with various age-related deficits and/or development of age-related diseases including cancer and Alzheimer's disease. The ultimate goal of the programs is to uncover knowledge that can be applied to prevent or delay the onset of age-related disabilities and disease processes, and/or provide new strategies for their diagnosis or treatment.

While the individual research programs within the LCMB generally function as independent groups, they are highly interactive, conduct biweekly joint meetings, and engage in collaborative projects. Combined, the programs within the LCMB provide extensive and broad expertise in the areas of biochemistry, cellular and molecular biology and genetics. Specialized expertise in a variety of approaches used to analyze or manipulate gene expression is also available within the LCMB. The LCMB is equipped with state-of-the-art instrumentation and an extensive computer network.

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Biography: Dr. Gorospe received her Ph.D. from the State University of New York at Albany (New York) in 1993. She completed her post-doctoral training at the Section on Gene Expression and Aging (renamed Cell Stress and Aging, 2000), National Institute on Aging, and assumed the position of Investigator in the Spring of 1998. Her research program focuses on post-transcriptional mechanisms serving to modulate gene expression, particularly that of cell cycle regulatory genes.

Keywords:

mRNA turnover
cell cycle control
stress response
von Hippel-Lindau

Recent Publications:

Wang W, et al. *Mol Cell Biol* 2000; 20(3): 760-769.

Lin S, et al. *Mol Cell Biol* 2000; 20(21): 7903-7913.

Wang W, et al. *EMBO J* 2000; 19(10): 2340-2350.

Wang W, et al. *Mol Cell Biol* 2001; 21(17): 5889-5898.

Research Summary: Aging is characterized by a general decline in the ability of individuals to adequately respond to different stresses, either environmental or endogenously generated. Many stress-regulated genes have been identified and their expression is believed to play an important role in determining cell fate. While the transcriptional events serving to regulate the expression of these genes have been extensively studied, it is becoming increasingly clear that post-transcriptional regulatory mechanisms also play a critical role regulating gene expression during stress. These post-transcriptional processes, still poorly understood, include mRNA and protein turnover. Our long-term efforts are two-fold: 1) to search for RNA-binding proteins, target mRNA regions, and signaling pathways involved in regulating the stability of mRNAs encoding proliferative and cell cycle-regulatory genes; 2) to elucidate the tumor suppressive role of the von Hippel-Lindau gene product by investigating its influence on mRNA and protein stability.

Regulation of Stress-Response Genes Through Altered mRNA Turnover: We and others have shown that expression of the cyclin-dependent kinase inhibitor p21 is highly induced by various stresses, and this heightened expression often enhances cell survival. In response to short-wavelength ultraviolet light (UVC), we showed previously that this induction was due to the stabilization of the p21 mRNA and later demonstrated that this stabilization event requires the association of the RNA-binding protein HuR with the p21 3' untranslated region (UTR). We further implicated the increased presence of HuR in the cytoplasm with the enhanced binding of HuR to the p21 3'UTR and the p21 mRNA stabilization. Another stress agent, prostaglandin A_2 (PGA_2), potently repressed cyclin D1 expression by rendering the cyclin D1 mRNA unstable, while it increased the association of mRNA-destabilizing protein AUF1 with an instability element within the cyclin D1 3'UTR. Our results

suggested that AUF1 may participate in the degradation of cyclin D1 mRNA. Current efforts are aimed at identifying mRNA sequences and RNA-binding proteins that regulate the expression of additional stress-regulated genes encoding short-lived mRNAs.

Regulation of mRNA Turnover During the Cell Division Cycle: We recently reported that the increased cytoplasmic localization of HuR throughout the cell division cycle contributed to the cyclic stabilization of ARE-containing cyclin A and cyclin B1 mRNAs. HuR was almost exclusively nuclear during early G1 phase, increasing in the cytoplasm during the S and G2 phases. The expression and half-life of mRNAs encoding cyclins A and B1 likewise increased during S and G2, a time during which cytoplasmic HuR directly bound these mRNAs and modified their expression levels and half-life. Our results reveal that HuR plays critical role in cell proliferation, at least in part, by mediating cell cycle-dependent stabilization of mRNAs encoding cyclins A and cyclin B1. The expression of other cell cycle regulatory proteins encoded by short-lived mRNAs is the focus of intense ongoing investigation in the laboratory.

Regulation of mRNA Turnover During Cellular Senescence: Cellular aging is accompanied by alterations in gene expression patterns. Using two models of replicative senescence, we recently described the influence of HuR in coordinately regulating the expression of cyclin A, cyclin B1 and c-fos, whose expression decreases during senescence. We demonstrated that HuR levels, HuR binding to target mRNAs encoding cyclin A, cyclin B1 and c-fos, and the half-lives of such mRNAs, were lower in senescent cells. We further showed that HuR levels directly influenced the senescent phenotype and that mRNA turnover played a critical role during the process of replicative senescence. Although the link between *in vitro* cellular senescence and human aging remains controversial, a diminution in proliferative capacity is also a hallmark of *in vivo* aging. Therefore, knowledge of the mechanisms regulating gene expression during *in vitro* senescence is likely to aid in our understanding of *in vivo* aging, as well as contribute to our comprehension of age-related diseases such as cancer and hyperplasia, where control of proliferation is lost. Our findings further suggest that orchestrated gene expression during senescence may be regulated by proteins such as HuR that coordinately regulate the stability of critical proliferation- and senescence-associated mRNAs. The study of additional senescence-associated labile mRNAs and RNA-binding proteins has become an area of great interest within the Unit.

Signaling Events Regulating mRNA Turnover in Response to Stress: Several recent studies have provided increasing support for the notion that mRNA stability is regulated through mechanisms akin to those controlling

gene transcription, i.e., signal transduction pathways involving phosphorylation events. While transport of the RNA-binding protein HuR from the nucleus to the cytoplasm is emerging as a key regulatory step for HuR function, the mechanisms underlying this process remain poorly understood. We recently identified the AMP-activated kinase (AMPK), an enzyme involved in responding to metabolic stresses, as a potent regulator of the levels of cytoplasmic HuR. Inhibition of AMPK increased HuR presence in the cytoplasm, enhanced binding of HuR to p21, cyclin B1 and cyclin A mRNA transcripts, and elevated their expression and half-life. Conversely, AMPK activation resulted in reduced cytoplasmic HuR, decreased levels and half-life of mRNAs encoding p21, cyclin A and cyclin B1, and diminished HuR association with the corresponding transcripts. We thus propose a novel function for AMPK as a regulator of cytoplasmic HuR levels, which in turn influences the mRNA-stabilizing function of HuR and the expression of HuR target transcripts. Additional signal transduction pathways involved in regulating mRNA turnover are the subject of ongoing investigation.

Functional Analysis of the von Hippel-Lindau (VHL) Tumor Suppressor Gene: The precise mechanisms whereby the VHL gene product exerts its tumor suppressor function remain largely unknown. Using SAGE (serial analysis of gene expression), we have identified a number of genes whose expression is altered by VHL by using renal cell carcinoma (RCC) lines with different VHL status. The discovery of several genes involved in tumor necrosis factor (TNF)-α-mediated signaling events led us to find marked differences in the sensitivity that RCC cells with different VHL status exhibit towards TNF-α. We further demonstrated that VHL-deficient RCC cells expressed strikingly elevated levels of TNF-α mRNA and protein. This heightened TNF-α expression was due, at least in part, to the longer half-life and the enhanced translation of the TNF-α mRNA in VHL-deficient RCC cells. Current efforts aim to establish if the TNF-α mRNA stability is linked to its association with high molecular-weight polysomes, and if the VHL-mediated suppression of TNF-α expression contributes to the tumor suppressive function of VHL.

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DNA damage
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cancer
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Recent Publications:

Li J, et al. *Cancer Res* 2001; 61(4): 1493-1499.

Arrington E, et al. *Free Radic Biol Med* 2000; 29(11): 1166-1176,

Kumaravel TS, et al. *Neoplasma* 1999.

Research: DNA repair mechanisms are believed to play a vital role in the maintenance of genome integrity. Loss of fidelity in the replicative mechanism, accumulation of genetic lesions, and faulty DNA repair mechanisms facilitate tumorigenesis. Similarly, aging or cellular senescence is characterized by random accumulation of damage or mutation in DNA, RNA, or proteins and perhaps a diminished ability to repair DNA. The increased incidence of cancer as a function of age underscores the mechanistic relatedness of these two cellular processes. The diminished ability to repair DNA appears to be the crucial and convergent factor highlighting the important clinical manifestations associated with defects in DNA repair mechanisms. The overall thrust of our work has been to understand the role of DNA repair in cellular senescence and tumorigenesis in order to uncover ways to use measured DNA repair capacity as a clinical tool in the diagnosis and treatment of cancer and age-related disease and disability.

Breast Cancer and DNA Repair: Breast cancer is predominately a disease of older women. Pursuant to the hypothesis that DNA repair capacities decline over the lifespan in all tissues, it is logical to consider that this would result in the accumulation of both environmental and endogenous DNA damage in breast tissue. There is currently little available knowledge concerning the role of specific forms of DNA damage or the proficiency of specific repair pathways in breast cancer susceptibility and progression. We have begun to characterize the proficiency of DNA repair mechanisms required to remove mutagenic lesions from human breast tissue.

Several lines of evidence suggest that accumulation of DNA damage coupled with defects in DNA repair play an important role in breast cancer. Our own previous work has shown that nucleotide excision repair is defective in the Li-Fraumeni syndrome, a heritable cancer prone syndrome associated with increased susceptibility to breast cancer.

Several groups have shown that defects in the removal of UV- and X-irradiation induced DNA damage are present in newly diagnosed sporadic breast cancer patients and in their healthy first-degree female relatives. Other investigators have shown that levels of oxidative DNA damage (e.g. 8-oxo-7, 8-dihydroguanine) are increased in human breast tumors and in surrounding normal tissue. Finally, it is also known that the breast cancer susceptibility gene, BRCA1, is required for transcription-coupled repair of oxidative damage in mutant rodents. These data along with several other lines of evidence suggest that both the nucleotide excision and base excision repair pathways may be involved in breast cancer development. It remains to be established whether DNA repair mechanisms are defective in breast cancer cells and which specific repair pathways are of primary importance. We hypothesize that one of the critical steps in mammary carcinogenesis is the loss of the normal response to DNA damage and that specific defects in nucleotide excision repair (NER) or base excision repair (BER) may be critical in development and progression of the malignant phenotype.

To explore the possible role of DNA repair mechanisms in sporadically occurring breast cancer, nucleotide excision repair of UV-induced dimers has been studied in normal human mammary epithelial tissue and in hormone dependent and independent breast tumor cell lines. Studies of bulk DNA repair reveal a defect in the processing and repair of UV-induced cyclobutane pyrimidine dimers for both estrogen receptor positive and estrogen receptor negative breast cancer lines. Furthermore, results of gene-specific repair experiments performed on both tumor cell lines and normal human mammary epithelial cells, suggest that transcription-coupled repair in the two tumor cell lines is defective. Taken together, these results suggest that defective DNA repair may be important in both heritable and sporadic breast cancer; however, the mechanism and genetic changes that account for this repair phenotype are unclear. We examined mRNA and protein expression levels of nucleotide excision repair related genes (ERCC1, XPA, XPB, XPF, XPC and RPA) before and after UV irradiation and found that there is an alteration in the expression of the ERCC1 gene at the protein level possibly pinpointing a specific portion of

the nucleotide excision repair pathway. Ongoing work is examining mechanistic explanations is focused on alterations in repair related gene function by transfecting a vector containing the ERCC1 gene back into the breast cancer cell lines and evaluating whether this complements the defects in post UV and Mitomycin-C cellular survival and repair defects found.

Increased sensitivity to endogenous DNA damage and/or defective DNA repair of other lesions may also be important susceptibility factors in the development of sporadically occurring breast neoplasms as well. Increased levels of oxidative DNA lesions have been observed in human breast tumors, suggesting that oxidative DNA damage may play a crucial role in mammary carcinogenesis. Accumulation of oxidative damage may be a result of increased susceptibility to reactive oxygen species. Alternatively, impaired DNA repair mechanisms may fail to eliminate the oxidative lesions thereby resulting in accumulation of DNA damage and subsequent development of mutations and genetic instability. Due to the high levels of oxidative DNA lesions of breast cancer tissue, it is speculated that DNA repair capacity in these cells may be diminished. We have begun to examine the possible role of BER in breast cancer development by analyzing the capacity of nuclear extracts from these cell lines to recognize and remove the oxidative DNA adducts 8-oxo-7, 8-dihydroguanine (8oxodG) and thymine glycol (TG). Preliminary data suggest that nuclear extracts from MCF-7 and MDA-MB-468 cells are proficient in the incision of TG and 8-oxo-dG. This does not eliminate defective BER as a contributing factor in breast tumorigenesis. Recently it has been shown that mitochondrial DNA mutations in colorectal cell lines likely result from unrepaired oxidative damage. This work indirectly suggests that DNA repair in mitochondria of these cancer cells may be compromised. It is possible that this could also be the case for breast cancer cells. Therefore, our future plans for this part of the project will include examination of mitochondrial DNA repair of oxidative lesions.

The clinical relevance of nucleotide excision and base excision repair defects in tumor cells may lie in potential use of this DNA repair profiling as a tool in assessing metastatic potential of a specific tumor or in deciding upon appropriate cytotoxic chemotherapy.

DNA Repair as a Mechanism of the Adaptive Response: The adaptive response (AR) is a phenomenon whereby the harmful effects of high dose ionizing radiation or other genotoxic agents can be mitigated by prior exposure to a low dose of the same or similar genotoxic stress. The adapted cells show an increased survival, less chromosomal aberration and

decreased mutagenesis termed the adaptive response. It is not clear which biologic pathways are involved in the AR, speculation centers on cell cycle controls, signal transduction, and DNA repair mechanism. DNA repair mechanisms, once thought to be constitutive, have now been proven to be inducible. Wilson, Mitra, and others have shown that genes and gene products involved in base excision repair are induced after low doses of certain forms of DNA damaging agents. We are working on the hypothesis that DNA repair, particularly base excision repair, is an important underlying mechanism of AR. The components of the proximal limb of the p53 DNA damage response pathway are posited to be critical in the initiation and maintenance of the adaptive response. We hypothesize that DNA repair induced by low doses of ionizing radiation occurs through induction or activation of p53 related genes. There is evidence that PARP and ATM are required for AR. However, the role of DNA-PK in concert with these two components and possibly c-ABL is unclear.

Our work to date has focused on evaluating the role of DNA-PK in AR. Using SCID mouse models with different mutations in DNA-PK, we are evaluating AR in terms of biological endpoints that include apoptosis, cell survival, chromosomal aberrations, and persistence of DNA damage measured by the Single Cell Gel Electrophoresis (COMET Assay) after low doses of gamma irradiation. We chose to examine the role of DNA-PKcs in AR using a SCID mouse model, because DNA-PK is one of the initial molecules to recognize DNA damage and because it has been implicated in aspects of DNA repair, a mechanism believed to play a role in AR. Our data indicate that DNA-PKcs is not essential for the induction of AR in the SCID mouse model when evaluated in terms of apoptosis, chromosomal aberrations and comet assay as end points. It is interesting to note that even though, DNA-PK is an important component of the DNA damage and repair mechanisms, it is not involved in the augmentation of DNA repair that occurs following low doses of radiation. It is known that these SCID cells have defects in double strand break and possibly in nucleotide excision repair. However, there are no reports on altered base excision repair pathway (BER) in these cell lines. Since we believe that enhanced DNA repair is a mechanism of the adaptive response, we hypothesize that base excision repair is competent in these SCID cells. If these SCID cells show AR, then the DNA repair in these cells is accentuated via the BER pathway.

As part of our plan to document enhanced BER in SCID cells, we examined apurinic endonuclease 1 (APE-1) protein expression in the adapted and non-adapted cells because of this enzymes pivotal role in the base excision repair pathway. Ramana et al., have shown an up regulation

of APE-1 gene following low levels of oxidative stress and has suggested that APE-1 levels may be an indicator of BER induction. While the low dose priming irradiation did not induce APE-1 proteins, the adapted cells showed an exaggerated and more sustained expression of APE-1 than the non-adapted ones. The induction of APE-1 most probably occurred at the level of transcription, because the increased APE-1 transcripts were also seen in adapted cells on northern blot analysis.This suggests that BER is upregulated in the process of AR, in SCID cells. The control cells also showed similar response of APE-1 expression indicating that BER may be equally accentuated in normal cells as well. Therefore, it appears that AR induced by ionizing radiation may result from enhanced DNA repair, with contribution from enhanced BER.

Future plans include evaluation of other mutant cell lines with defects in p53-related genes and DNA repair related genes to dissect the pathways in the adaptive response. To date it is not clear how other p53 related genes such as interferon regulatory factors 1 and 2 (IRF1, 2), GADD45, and p21 are involved. GADD45 known to be induced by low-moderate doses of ionizing radiation and IRF1 a tumor suppressor protein and transcription factor known to regulate responses to DNA damage by interacting with p53 and p21 have yet to be examined in the AR pathway. We speculate that these genes are critical elements in the AR because they are essential in the DNA damage recognition mechanisms, and cell cycle check points and may activate or induce nucleotide excision and/or base excision repair mechanisms. This work may also allow us to dissect the potential interrelationships between the stress induced signal transduction pathways and DNA repair pathways as they relate to the adaptive response.

Understanding the mechanism of the adaptive response is clinically applicable because it may play a role in modulating the cellular response to chemotherapy and radiation therapy used for malignant disease. In addition, defects in the adaptive response may be correlated with the diminished capacity for senescent cells and elderly individuals to respond appropriately to exogenous environmental stress and genotoxic agents.

Oxidative DNA Damage and Age-related Ocular Disease: The prevalence rate of senile cataracts among Americans 65-74 years old is 122.1/1000 and 229.0/1000 among those 75 and older. Studies from numerous laboratories particularly from the laboratory of Abraham Spector have established that oxidative stress is one of the likely initiating events in the development of the senile or maturity onset cataract. Lens proteins, cellular membranes and perhaps most importantly the DNA of the lens epithelium are targets for reactive oxygen species and hydrogen

peroxide present in aqueous humor. There is evidence to suggest that compromise in the cellular function of lens epithelial cells may be directly related to cataract development particularly for cortical cataracts. Not only is cataract development a part of normal human aging it is also seen as a clinical manifestation of several heritable human progeroid syndromes including Werner, Cockayne, and Rothmund-Thomson syndromes. These entities are also characterized by DNA damage hypersensitivity and defects in DNA repair. We are examining whether defects in the processing of oxidative DNA damage in lens epithelial cell lines plays a role in the multi-factorial etiology of cataracts.

To assess the possible role of oxidative DNA damage and repair in premature cataractogenesis, we examined the cellular response of the premature cataract prone Nakano mouse lens epithelial cells (NKR11) to hydrogen peroxide-induced oxidative stress. NKR11 cells are more sensitive to H_2O_2 than normal mouse epithelial cells on the MTT-based cellular proliferation and viability assay. NKR11 cells also showed more apoptosis at 24 hrs after H_2O_2 treatment than the control cells. To examine whether this hypersensitivity is due to defective removal of DNA lesions induced by H_2O_2, we investigated the rate of removal of H_2O_2 induced DNA lesions in NKR11 and control cells, using the single cell gel electrophoresis (comet assay). NKR11 cells removed DNA lesions induced by 50-100uM H_2O_2, as efficiently as the control cell lines; however, they were deficient in the removal of lesions induced by 150uM H_2O_2. Comet Assay used with DNA repair enzymes as damage specific probes showed that NKR11 cells were defective in removal of both Endo III and fpg sensitive sites, suggesting defective base excision repair (BER). Western Analysis of BER related enzymes showed that NKR11 cells were deficient in flap endonuclease (FEN1) a protein that plays a role in BER and the maintenance of genomic stability. Other BER proteins including DNA Polymerase Beta, Proliferating cell nuclear antigen (PCNA) and AP endonuclease (APE1) were normal. Examination of chromosomal aberrations by premature chromatid condensation revealed that NKR11 cells had a higher level of breaks, gaps, fragments, and different modal number after treatment with 100uM H_2O_2 when compared with control cells, suggesting that these cells are indeed genetically unstable. Lens epithelial cells from the cataract prone Nakano mouse appear to have defect in processing DNA lesions induced by H_2O_2, suggesting that defective DNA repair could be one of the mechanisms of cataractogenesis. Further work on this project will involve assessing FEN-

1 activity in Nakano cell extracts as well as expansion of the project to investigate the base excision repair capacity in the Emory Mouse known to develop maturity onset cortical cataracts at a chronological age similar to humans.

Age-related cataract is not the only cause of visual decline in the elderly. Age-related macular degeneration is also an important entity about which even less in known in terms of the role of DNA damage and repair. Our future work will include a small project examining the role of oxidative damage and repair in age-related macular degeneration.

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Keywords:

neurodegeneration
Alzheimer's disease
amyloid
glutamate

Recent Publications:

Kusiak JW, et al. *Brain Res* 2001; 896(1-2): 146-152.

Liu A, et al. *J Biol Chem* 2001; 276(48): 45372-45379.

Luo JJ, et al. *J Neurosci Res* 2001; 63(5): 410-420.

Neurodegenerative Mechanisms in Aging and Alzheimer's Disease: Neurodegenerative diseases of aging including Alzheimer's and Parkinson's Diseases have distinct pathologies exhibiting severe neuronal cell loss. The etiology of these diseases is obscure although excessive oxidative stress, environmental factors, and genetic factors have been proposed as initiating elements. Recent clinical studies of Alzheimer's disease (AD) patients treated with anti-inflammatory or anti-oxidant drugs suggest a potential ability of these drugs to slow the progression of the disease. One of the hallmarks of AD brains is the presence of extracellular senile plaques. A major constituent of senile plaques is the Aß peptide derived from a larger precursor protein, the Amyloid Precursor Protein (APP). Clues to the disease process come from recent discoveries of mutations in the APP gene and in two genes, unrelated to APP, termed Presenilins 1 and 2 (PS - 1, PS - 2). Mutations in these genes are found in early-onset familial forms of AD and in each case lead to an increase in the production of longer forms (1-42) of the Aß peptide which have a greater tendency to aggregate and form senile plaques than 1-40. *In vitro* studies showed that the Aß peptide is toxic to neuronal cells and that cell death induced by Aß may be apoptotic in nature.

NMDA receptors are one type of glutamate receptor and play a pivotal role in several brain functions. However, over-activity of these receptors can lead to excitotoxic neuronal cell death. The type of cell death may be either necrotic or apoptotic depending upon the receptor subtypes involved

and the degree of receptor stimulation. Interestingly, the distribution of these receptors correlates with the areas of cell loss found in AD. The receptors are important in learning and memory, processes severely impacted in AD, and over-activation of these receptors is thought to initiate a common final pathway of neuronal cell death in both acute and chronic brain insults.

Work in this group focuses on two areas of research: (1) the role of APP and PS genes in the pathology of Alzheimer's disease and (2) the transcriptional regulation of expression of the NMDAR1 gene, a key subunit of all NMDA receptors.

Amyloid Precursor Protein and Apoptosis in Alzheimer's Disease: A major focus of this project is to discover the roles of APP and PS in the etiology and pathology of AD and to define the mechanisms involved in the neuronal cell death induced by mutant forms of these proteins. One of the aims of our laboratory is to discover how APP or PS mutations lead to specific neuronal cell loss in AD. Previously we showed that over-expression of mutated forms of APP in stably transfected PC12 cells leads to the increased production of intracellular, amyloidogenic C-terminal fragments of APP. This is accompanied by increased apoptotic cell death over several days. Recently, we showed that transient expression of mutated forms of PS-2 also increased the amount of apoptosis in growth factor-dependent PC12 cells.

Taken together, the above results suggest that in AD, the selective neuronal cell loss may be, in part, due to an apoptotic mechanism. This provides a rationale for targeting particular elements of an apoptotic pathway for therapeutic intervention in AD. We have generated adenoviral vectors for injection into rat brains in order to examine the *in vivo* effects of over-expression of APP mutations. We will examine the possible differential sensitivity of older animals to an increased Aß load.

Transcriptional Regulation of NMDA Receptor Subunit Genes: A major focus of this project is to discover the pathological roles that excitatory amino acid (glutamate) receptors play in neuronal cell loss in aging and AD and the mechanisms by which this cell loss occurs. One of our objectives is to determine how NMDAR1 and other family member genes are regulated at the transcriptional level. Since neurons expressing NMDA receptors are lost in AD, it may be important to determine which

factors are involved in regulating expression and consequent activities of NMDA receptors during development and in aging and disease. Another objective of this project is to determine the mechanism by which glutamate causes cell death and the role that activation of glutamate receptors plays in initiating a genetic cascade of programmed cell death.

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Keywords:

signal transduction
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aging

Recent Publications:

Hutter D, et al. *Biochem J* 2000; 352: 155-163.

Chen P, et al. *J Biol Chem* 2001; 276: 29440-29449.

Li J, et al. *Mol Cell Biol* 2001; 21: 8213-8222.

Regulation of MAP Kinase Phosphatase-1 and 2: Recent studies have provided convincing evidence to support a central role of the MAP kinase (MAPK) cascades in orchestrating the cellular changes that occur following exposure to a diverse set of extracellular stimuli including growth factors and cellular stress. Upon activation, MAPKs can undergo translocation from the cytoplasm to the nucleus, where they phosphorylate and activate a multitude of transcription factors including c-Myc, c-Jun, c-Fos, Elk-1 and ATF-2, ultimately resulting in enhanced gene transcription. The fact that a broad variety of extracellular signals conscript the MAPK cascades to convey their specific messages suggests that MAPK cascades serve a myriad of purposes and need to be tightly controlled. While activation of MAPK is achieved through phosphorylation by MAP kinases, attenuation of the MAPK activities is accomplished through dephosphorylation by a group of MAP kinase phosphatases (MKPs). Thus, in order to understand the molecular basis for the diversity in gene expression as well as cellular outcomes provoked by stress, it is critical to understand the regulation of the MKPs.

So far, ten MKP family members have been cloned from mammalian cells. Four of them, MKP-1, MKP-2, PAC-1, and B23, are predominantly localized in the cell nucleus and are encoded by immediate early genes. Since the expression of these nuclear MKPs is induced by conditions that also activate MAPKs, they are believed to serve an important function as feedback attenuators of MAPK-mediated gene transcription. Despite their critical role in regulating MAPK signaling, relative little is known about how the expression and function of these nuclear MKPs are regulated. With respect to function, recent studies have shown that the catalytic activities of several, but not all, cytosolic MKPs can be stimulated through

binding to their substrate MAPKs. Whether the activities of nuclear MKPs are altered as a consequence of substrate binding is not known. MKP activities can also be modulated through transcriptional induction. Although various nuclear MKPs have been shown to be highly inducible by various proliferative and stress stimuli, the mechanisms underlying their transcriptional regulation are very poorly understood.

To address the regulation of MKPs at the biochemical levels, we have focused on two nuclear MKPs, MKP-1 and MKP-2, and studied their biochemical regulations. We have found that MKP-1 can form complexes with p38 both *in vivo* and *in vitro* via a carboxyl-terminal domain of p38, and that this interaction enhances the catalytic activity of MKP-1. Point mutation of Asp316→Asn in the carboxyl-terminal of p38 dramatically decreases its binding to MKP-1 and substantially compromises its stimulatory effect on the catalytic activity of this phosphatase. Consistent with its defect in interaction with MKP-1, this p38 mutant also displays greater resistance to dephosphorylation by the phosphatase. Our studies provide the first example of catalytic activation of a nuclear MKP through direct binding to a MAPK.

It has been shown that MKP-2 preferentially inactivates ERK and JNK MAPK subfamilies, the mechanisms underlying its own regulation remain unclear. We have examined MKP-2's interaction with and catalytic activation by distinct MAPK subfamilies. We found that MKP-2's catalytic activity was dramatically enhanced by ERK and JNK but was only minimally affected by p38. By contrast, p38 and ERK bound MKP-2 with comparably strong affinities, while JNK and MKP-2 interacted very weakly. Through site-directed mutagenesis, we defined the ERK/p38-binding site as a cluster of arginine residues in the N-terminal domain of MKP-2. Mutation of the basic motif abrogated its interaction with both ERK and p38 and severely compromised MKP-2's catalytic activation by these kinases. Unexpectedly, such mutations had little effect on JNK-triggered catalytic activation. Both *in vitro* and *in vivo*, wild type MKP-2 effectively inactivated ERK2 while MKP-2 mutants incapable of binding to ERK/p38 did not. Our studies provided a mechanistic explanation for MKP-2's substrate preference. Taken together, our studies indicate that catalytic activation of nuclear MKPs upon binding to their substrate MAPKs may play a crucial role in the feedback control of the MAPK signaling in the nuclear compartment.

To address the mechanisms that regulate the expression of nuclear MKPs, we have investigated the relationship between histone H3 phosphorylation/acetylation and MKP-1 induction by various stress agents. We found that MKP-1 induction is associated with histone H3

phosphorylation/acetylation. Pre-treatment of cells with histone deacetylase inhibitor significantly increases the basal level of MKP-1 expression and further augments MKP-1 induction by arsenite, supporting the notion that chromatin remodeling plays a role in MKP-1 induction by stress. Chromatin immunoprecipitation assays indicate that histone H3 phosphorylation/acetylation is enhanced at the MKP-1 chromatin in response to a number of cellular stresses. Our results strongly suggest that chromatin remodeling after stress contributes to the transcriptional induction of MKP-1.

Age-associated Alterations in Signal Transduction Pathways: Using a number of biological model systems, aging has been shown to be associated with a decline in proliferative capacity. In primary cultured rat hepatocytes, treatment of cells from young adult animals (6 months old) with EGF results in a marked increase in DNA synthesis. This response is significantly attenuated in cells of aged (24 months old) animals, but the molecular mechanisms underlying the age-associated defect(s) are poorly understood. In recent studies we have demonstrated that aging is associated with a decline in the activities of both ERK and p70 S6 kinase. Both of these pathways are essential for G1 to S phase transition of cells. As these two pathways are for the most part distinct, a decline in the activity of both kinases in response to EGF stimulation suggests that aged cells may possess an alteration in an early upstream event common to both pathways, possibly at the level of growth factor receptor. To investigate this possibility, we examined tyrosine phosphorylation of EGFR, Shc, and the formation of EGFR-Shc complexes in young and aged hepatocytes treated with EGF. We have found that both EGFR and Shc become tyrosine-phosphorylated to a similar degree in both young and aged cells. However, EGFR-Shc complexes appear to be less stable in aged cells compared with those in young cells. The reduced stability of the EGFR-Shc complexes will likely impact the later events leading to activation of the ERK pathway. Consistent with this hypothesis, Ras activity in the EGF-stimulated old cells was found to be lower and sustained for a shorter time. Current efforts are focused on determining the causes of the aging-associated instability of the EGFR-Shc complexes.

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Keywords:

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Lin H, et al. *Cancer Res* 1999; 59(4): 807-810.

Morin PJ. *Bioessays* 1999; 21(12): 1021-1030.

Research Summary: Our laboratory's interest is twofold: molecular genetics of ovarian cancer and the role of the APC/ß-catenin pathway in human cancer.

SAGE Analysis of Normal Ovary and Ovarian Cancer: It is well documented that, in the process of going from normal to malignant, cells reprogram their gene expression. However, consistent changes that could be useful for diagnosis and/or therapy have remained elusive for most tumor types, including ovarian cancer. SAGE, one of the more powerful techniques currently available for the quantitative study of gene expression, is being used in our laboratory to analyze normal ovarian tissue, primary ovarian tumors and ovarian cancer cell lines. We have identified hundreds of transcripts differentially expressed during ovarian tumorigenesis. Interestingly, several of these genes represent novel genes. We are currently characterizing many of the differentially expressed transcripts using a variety of techniques including immunohistochemistry, quantitative (real-time) RT-PCR and functional assays. Some of these genes may become targets of novel strategies for early detection and/or mechanism-based therapy of ovarian cancer.

Search for Genetic Alterations in Ovarian Cancer: Surprisingly little is known about the molecular alterations in ovarian cancer. We have established a panel of matched normal tissue and primary ovarian cancer specimens and are using this panel, in conjunction with ovarian cancer cell lines, to identify genes important in ovarian tumorigenesis. Techniques used include representational difference analysis (RDA) and LOH studies. Of particular interest are chromosomal regions on Xq, 11p and 6q which are frequently lost in ovarian cancers, suggesting the presence of tumor

suppressor genes important in ovarian tumorigenesis. We have recently suggested that the *GPC3*, a gene located at Xq26 and previously implicated in an overgrowth syndrome, may be silenced during ovarian tumorigenesis.

The APC/ß-catenin Pathway in Human Cancers: The APC/ß-catenin pathway has recently been shown to be involved in human cancer. APC, a gene mutated in 80% of all colon cancers, is crucial for downregulation of ß-catenin and TCF-mediated transcription. Moreover, colon tumors containing wild-type APC, frequently contain activating mutations in ß-catenin, emphasizing the importance of this pathway for colon cancer progression. In addition, ß-catenin has now been found to be mutated in many tumors types such as ovarian, prostate and skin cancers. We are studying the regulation of this pathway in normal and in cancer cells using a number of approaches, including the construction of stable cell lines expressing inducible versions of the ß-catenin protein. One such line exhibits a highly inducible ß-catenin protein and has been used to generate SAGE libraries. These experiments should help us identify genes that are transcriptionally induced by the ß-catenin/TCF transcription complex and that may be relevant to a wide variety of human cancers.

Collaborators: Ellen Pizer, M.D, Ph.D., Johns Hopkins University; Myriam Gorospe, Ph.D., Laboratory of Cellular and Molecular Biology, NIA; Kathleen R. Cho, M.D., The University of Michigan Medical School.

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Biography: Dr. Wange received his Ph.D. from the Department of Pharmacology at Vanderbilt University in 1991. He received his postdoctoral training at the Cell Biology and Metabolism Branch of the National Institute of Child Health and Human Development (NICHD) before becoming an Investigator in the Laboratory of Biological Chemistry in 1997 (renamed Laboratory of Cellular and Molecular Biology, 2000). His research focuses on the signaling pathways involved in T lymphocyte activation.

Keywords:

T lymphocyte activation
signal transduction
protein kinases
lipid phosphatases

Recent Publications:

Shan X, et al. *Mol Cell Biol* 2001; 21(21): 7134-7149.

Veri MC, et al. *Mol Cell Biol* 2001; 21(20): 6939-6950.

Herndon TM, et al. *J Immunol* 2001; 166(9): 5654-5664.

Shan X, et al. *Mol Cell Biol* 2000; 20(18): 6945-6957.

Aging and T Lymphocyte Activation: The long term goal of our lab is to gain a better understanding of the mechanisms whereby immunosenescence arises in aging animals. Immunosenescence is characterized by a deterioration of both cellular and humoral immunity, and has been proposed to have its roots in declining T-cell function as a consequence of changes in the ability of the T cells in aged animals to respond to mitogenic stimuli. Studies have found no difference between young and old animals with respect to the expression level of the T-cell antigen receptor (TCR) or other cell surface receptors involved in responding to mitogenic stimuli. Therefore, we hypothesize that the decline in responsiveness to mitogenic stimuli may reflect changes in intracellular signaling pathways. In fact, many differences have been observed in some of the early TCR-initiated signaling events in T-cells isolated from young animals compared to old. However, none of these changes seem to account for the age-associated decline in T-cell function. Effective investigation of the signaling defects that give rise to declining T-cell function with age is hampered by the lack of a complete understanding of the signaling pathways involved in normal (i.e. young) T-cell activation. This being so, we are currently attempting to uncover new portions of the signaling pathway that are downstream of engagement of the T-cell antigen receptor.

Tyrosine Kinases in T-Cell Receptor Signaling: In order to understand the nature of the signaling defects in T lymphocytes from aged animals, one must first understand the signal transduction pathways used by normal T cells. Therefore, the laboratory is involved in identifying and studying the molecules involved in TCR signaling pathways. Certain tyrosine kinases have been found to be required for effective TCR signaling. Two of these kinases, ZAP-70 (zeta-chain associated protein) and Itk (Inducible

T cell kinase), are currently under investigation in the lab. ZAP-70 is required for all distal TCR signaling events, while Itk apparently plays a more limited role in modulating the activity of phospholipase C. Our studies focus on understanding the mechanisms regulating the activity of these kinases, as well as identifying the precise signaling partners that these molecules interact with. Recently we found that ZAP-70 regulates Itk activation by controlling the ability of Itk to interact with other signaling molecules. We are currently investigating the role of lipid kinases and phosphatases in regulating Itk localization and activation, and have found that the expression level of the lipid phosphatase PTEN plays an important role in regulating Itk activation, and in the general control of TCR signaling. If additional investigation confirms the importance of lipid phoshorylation in regulating TCR signaling, this may prove to be a fruitful area of inquiry with regard to T-cell immunosenescence, since the enzymes that regulate lipid phosphorylation show decreased activity with age.

Conjoint Re-engineering of ATP and Kinase ATP-binding Sites: A major frustration in studying signaling cascades that include protein kinases is the general inability to determine what the true *in vivo* substrates of a given kinase are. This stems largely from the very general nature of the catalyzed reaction, which precludes the generation of truly specific inhibitors. Even when available, selective inhibitors or the expression of dominant-negative kinase mutants can only indicate that a particular protein is downstream of a given kinase, not that it is a direct substrate. To overcome this difficulty the lab has initiated a project to make complimentary changes to the structure of ATP and to the structure of the ATP-binding site of protein kinases important in TCR signaling. The approach requires the synthesis of a radiolabeled ATP ortholog that cannot be used as a substrate by any natural kinase, but which can be used by the re-engineered kinase. Expression of the mutant kinase in cultured cells or in the whole-animal then allows the determination of which proteins are being phosphorylated by the kinase in response to any given stimulus. Using this approach in combination with knock-in transgenic techniques it will also be possible to measure how the substrate repertoire and sites of phosphorylation change with development and with age. This then should provide a new and potent tool for discovering differences in signal transduction pathways that occur with age. The initial kinases under investigation are ZAP-70, Itk and Lck, but in principle could be extended to any protein kinase.

Collaborators: Ezio Bonvini, M.D., Food and Drug Administration; Donna Farber, Ph.D., University of Maryland at Baltimore; Aideen Long, Ph.D., Royal College of Surgeons in Ireland; Joaquin Madrenas, M.D., Ph.D., University of Western Ontario; Dan McVicar, Ph.D., National Cancer Institute; Pamela Schwartzberg, M.D., Ph.D., National Human Genome Research Institute; Kevan Shokat, Ph.D., University of California, San Francisco; Tse-Hua Tan, Ph.D., Baylor College of Medicine; Dennis Taub, Ph.D., Laboratory of Immunology, National Institute on Aging.

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The **Laboratory of Clinical Investigation (LCI)** chiefly focuses on clinical research issues of importance in gerontology. Clinical work includes the activity with volunteers on the Baltimore Longitudinal Study of Aging (BLSA), and cross-sectional studies in a variety of age-related disease areas including diabetes, metabolism, cardiovascular disease, neurologic disease, and cancer.

The **Bioanalytical Chemistry Section (BCS)** has the overall goal of the development and application of novel bioanalytical methods to the etiology and diagnosis of diseases in the aging population and the optimization of treatment protocols for these diseases. The objectives of the Bioanalytical Section are to participate in the improvement of existing therapies and to lay the basis for the development of new approaches to clinical treatment. These objectives will be accomplished through the use of modern analytical techniques such as high performance liquid chromatography and capillary electrophoresis coupled with mass spectrometric detectors.

The **Diabetes Section (DS)** focuses on improving present methods for treating type 2 diabetic patients. Diabetes mellitus is one of the most prevalent diseases among the elderly. Approximately 40% of all adults over the age of 65 have diabetes or elevated fasting glucose. Diabetes is also a comorbid condition in other conditions of the elderly, especially cardiovascular disease. By definition, diabetes mellitus is a group of metabolic diseases characterized by high blood sugar resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes is characterized by both defects. It is generally accepted that it is the elevated sugar which leads to the complications of diabetes. Therefore, we in the Diabetes Section feel that our endeavors should be directed towards improving insulin secretion or restoring insulin action. Despite the fact that 3 new agents have become available in the past eighteen months to treat type 2 diabetes, they have proven less than adequate at normalizing blood sugars.

The **Endocrinology Section (ES)** conducts and facilitates (by collaboration with other intramural and extramural entities) research aimed at understanding the particulars of changes in regulation of hormones during the normal aging process. It explores the relationships of hormone secretion to states of nutrition and health and the interrelationships among various hormone axes during aging. ES elucidates the influence of alterations of endogenous hormone activity on risk factors for susceptibility to chronic diseases associated with aging. Current efforts focus on changes in the growth hormone and reproductive hormone (sex steroids) axes. Finally, the ES conducts research investigating the clinical utility and risk/benefit ratios of rationally selected hormone replacement interventions, designed to reverse documented age-related alterations of hormone balance.

The overall goal of the **Hematology/Oncology Section** is conducting both basic and clinical studies in cancer. These studies include basic tumor cell biology and tumor immunology as well as development of novel anti-tumor therapies and evaluation of these and conventional therapies in an aging population with ever increasing risk of developing cancer. As with many LCI sections, this will be accomplished both by direct Section efforts and by facilitating collaborations with other intra- and extra-mural groups. Critical basic studies include examination of molecular events controlling hematopoietic and breast epithelial proliferation/ differentiation, characterization of a new novel tumor suppressor gene and studies in basic tumor immunology and the effects of drugs such as bryostatin in modulating immune function in therapeutically useful ways. Critical clinical questions to be addressed in the aging population include: i) Can potential toxicity prior to treatment be predicted based on measures of DNA repair and other endpoints when conventional approaches to treatment typically utilized in younger populations are applied to common tumors such as those of lymphoid and breast origin; ii) Are there unique age related effects physical performance and cognition with commonly utilized cancer treatment and iii) Can novel synergistic and/or less toxic therapies be developed based on laboratory efforts that are then translated to the clinic. Efforts have been initiated or are planned to explore DNA repair and other potential predictive factors in treatment of lymphoma and breast cancer and to develop early phase I trials examining therapies such as combinations of IL-2 and bryostatin in treatment. Other work will bring together activities from other NIA laboratories to examine issues such as immune function during treatment and whether effects are different as a function of life span and in the future to examine whether by using “mini-

transplant" approaches, immune reconstitution can be accelerated in diseases such as chronic lymphocytic leukemia or offer therapeutic potential. With this work we hope to both improve the application of currently available anti-cancer therapy in the aging population as well provide new avenues and approaches to treatment of the more common cancers seen in this group.

The **Longitudinal Studies Section (LSS)** has a twofold mission. The first is to manage the operations of the Baltimore Longitudinal Study of Aging (BLSA), a multidisciplinary longitudinal study of human aging. Research on aging using this open panel of research volunteers is performed by scientists based in several NIA intramural research laboratories and numerous outside collaborators. The second is to perform research with the BLSA using both existing data and data from newly initiated projects.

BLSA Operations: LSS staff schedules and manages the activities of the men and women research volunteers during their biannual two and half-day visits during which time the volunteers participate in numerous research studies. LSS staff conducts the clinical evaluations that establish health status of all active participants on every visit. The results are used in many investigations and also are used to determine the safety of research procedures for various participants. The results of the clinical evaluations are given to participants and to their physicians if requested by the participant. Between visits, LSS staff maintain communication with participants, provide information about the findings of the study to participants both individually and by means of a periodic participant newsletter. They also maintain periodic contact with those who either are unable or unwilling to come in for regularly scheduled visits. LSS staff manages the recruitment of new research volunteers from a large group of applicants on a waiting list. LSS staff employs numerous mechanisms to learn about deaths in the study sample, obtain information about deceased BLSA participants and manage the autopsy program.

BLSA Research: LSS was given the responsibility to analyze, report and recommend continuing, changing or stopping a number of existing research projects without active investigators. Most had been started in the 1960s or 1970s and had either been recently discontinued or were ongoing. Project areas for which longitudinal analyses and reports were completed included: pulmonary function; hearing and vision, reaction time, reciprocal movement speed, nerve conduction velocity, power and strength measurements, self-reported participation in physical activities, blood pressure, and a variety of studies using clinical data.

New studies were initiated in the areas of prostate aging and disease, neuromuscular changes with age, hearing, physical functioning and disability and age differences in the dynamics of cerebral blood flow. All were designed to take advantage of the unique BLSA longitudinal database and all required the development of research teams from other laboratories and outside collaborators.

LSS staff developed a number of statistical approaches that facilitated the analysis of longitudinal data and have applied these approaches to a number of historical data sets in the BLSA.

The **Metabolism Section (MS)** has played a critical role in evaluating diagnostic standards and in determining whether an adjustment for age is appropriate. In two areas, diabetes and obesity, the standards in general use to define these diseases have not been age-adjusted during the adult years of life. The primary technique used to establish standards has been the relationship between levels (fasting glucose and glucose tolerance for diabetes, the Body Mass Index for obesity, and the waist circumference for the pattern of fat distribution) and the subsequent development of complications that are strongly related to the diseases. The BLSA has provided an unparalleled data source for this effort. In both areas, the analyses suggest that adjustment of standards for age is required. In further studies in collaboration with other intramural and extramural scientists, factors influencing glucose/insulin homeostatic mechanisms and quantification of the obese state are under study.

The **Molecular and Clinical Pharmacology Section (MCPS)** studies the role of age- and disease-related changes in calcium signaling in vascular smooth muscle on vascular responses in aging, hypertension, and atherosclerosis and seeks to understand how such changes affect drug responses. The high prevalence of hypertension and atherosclerotic disease in the elderly and their contribution to morbidity and mortality make understanding therapeutic responses and development of new therapies a priority.

In the laboratory, study of calcium channel variants is allowing improved understanding of how changes in cellular calcium homeostasis change cellular function. In addition such studies give insight into mechanisms of drug action and provide possible new targets for drug action. Clinical studies of forearm vascular responsiveness allow testing of the cellular and molecular findings as well as evaluation of proposed new therapeutic

targets. The applied goal of these studies is the development of new approaches to reverse impairments in vascular response in hypertension and atherosclerosis. In addition, these studies often provide insight into mechanism of effect of currently used therapies.

The **Nuclear Magnetic Resonance Unit (NMR)** performs biophysical and physiological studies on human subjects, experimental animals, and tissue and cellular preparations.

Current research includes imaging studies of engineered cartilage tissue, with particular emphasis on correlates between NMR-derived parameters such as matrix fixed charge, magnetization transfer, and local diffusion coefficient, tissue biomechanics, and tissue biochemistry. The response of engineered cartilage to a variety of growth conditions and pharmacologic interventions may be assessed in detail using our methods. We have also initiated studies of cartilage defects in small animals with the goal of investigating biological interventions.

Further work concerns spectroscopic studies of muscle metabolism under a variety of pharmacologic and physiologic conditions. Recent work has emphasized the bioenergetics of peripheral artery disease, including the effects of gene therapy with adenoviruses expressing vascular endothelial growth factor on acute hindlimb ischemia in the rat. We are also looking at adrenergic stimulation of the isolated perfused rat heart, with the goal of defining the bioenergetic correlates and patterns of substrate utilization of ß-1 and ß-2 agonists.

In addition, we continue to actively develop and apply novel noninvasive NMR methods for measuring enzymatic fluxes related to energy provision in the peripheral muscle of animals, as well as in normatively aging humans.

NMR Unit instrumentation consists of a double-resonance Bruker ABX 1.9T/31 cm Biospec with shielded gradients, and a triple-resonance wide-bore Bruker DMX 400 with microimaging and solids capability.

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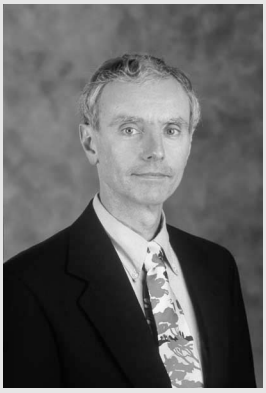

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Biography: Dr. Darrell Abernethy received his M.D. and Ph.D. (Pharmacology) degrees from the University of Kansas School of Medicine in 1976. Training in Internal Medicine through Board Certification was at the University of Miami/Jackson Memorial Hospital, and postdoctoral training in Clinical Pharmacology at Massachusetts General Hospital followed this. He joined the faculty at Tufts-New England Medical Center as an Assistant Professor. Following this he was at Baylor College of Medicine where he became Associate Professor of Medicine. Dr. Abernethy then moved to Brown University School of Medicine as Chief of the Division of Clinical Pharmacology and became Professor of Medicine at that institution. He then moved to Georgetown University School of Medicine as Francis Cabell Brown Professor of Medicine and Pharmacology and Director of the Division of Clinical Pharmacology. Dr. Abernethy joined NIA in April, 1999. Early in his career Dr. Abernethy made fundamental contributions to understanding of drug tissue distribution and the factors that regulate drug distribution. He then worked in the area of cardiovascular drug responses and their changes in aging and hypertension. This led to his current focus on understanding mechanisms of calcium homeostasis, its changes with age and disease, the effects of calcium antagonist drugs in these systems, and identifying new targets for therapy for hypertension, atherosclerosis, and other diseases of altered calcium homeostasis.

Keywords:

calcium
calcium antagonists
hypertension
pharmacodynamics

Recent Publications:

Abernethy DR, et al, *Clin Pharmacol Ther* 2001; 69(3): 96-103.

Romanin C, et al, *FEBS Lett* 2000; 487(2): 301-306.

Abernethy DR, et al, *Circulation* 2000; 101: 1749-1753.

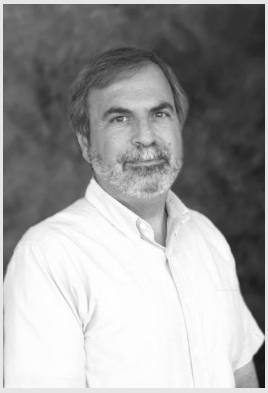
Abernethy DR, et al, *N Engl J Med* 1999; 341: 1447-1457.

Calcium Channel Variants in Aging and Disease: Alternative splicing generates diversity of the calcium channel alpha subunit, but does not significantly change the overall topology of the protein, which is highly conserved in the regions of calcium antagonist drug binding. Instead regions of diversity appear to regulate function of the calcium channel, in particular with regard to the rate of its inactivation following stimulation. The alternatively spliced variants of the calcium channel have been identified in different tissues, and appear to be expressed differently as a function of age. We are exploring the molecular correlates of calcium gating in this channel and how gating differs in the various naturally expressed channel variants. In addition we are studying the heterogeneity, distribution patterns and regulation of the splice variants in human cardiac and vascular tissues in relationship to age, hormonal, and pathological stimuli. L-type calcium antagonist drugs have become very important in cardiovascular therapeutics for the treatment of angina pectoris and hypertension. For further improvement of calcium channel targeting drugs, these studies will provide understanding of the molecular bases of regulation of the calcium channel.

Mechanisms of Calcium Antagonist Drug Action: Mechanism of calcium antagonist drug induced arterial vasodilatation is generally assumed to be due to L-type calcium channel blockade on vascular smooth muscle. Interference with other systems has not been well appreciated. We demonstrated in clinical study that calcium antagonist drugs block angiotensin II and endothelin mediated vasoconstriction. It was unclear if this was a specific effect; however, we have recently shown that calcium antagonist drugs alter angiotensin II signaling at the molecular level, suggesting that there is specificity to the clinical finding and that this is a further explanation of the mechanism of these drugs. We currently are studying this effect in calcium channel variants and extending these studies to understand the role of the vascular endothelium in calcium antagonist drug effect.

Role of Genetic Variants in Vascular Responses: Recently a number of genetic polymorphisms in systems that have important roles in vascular contraction have been identified. For example 5-10% of the population appear to have an altered endothelial nitric oxide synthase enzyme which has been suggested to be associated with myocardial infarction. The role of such a variant in altered responsiveness to drugs is not well appreciated. We very recently showed that the individuals with the altered nitric oxide synthase gene have markedly increased platelet aggregation that may be associated with cardiovascular disease. A large number of these kinds of genetic variants are being discovered; however, many do not have disease and/or drug-associated consequences. We are developing strategies to select those variants which we believe will have pathophysiological and pharmacological importance in aging and disease and in clinical studies determining if our strategies are effective. In the longer term we believe these studies will be critical for the development of patient-specific therapeutics and in the individualization of drug therapy in a way to minimize drug toxicity.

Collaborators: Nikolai Soldatov, Ph.D., NIA; Irving Wainer, Ph.D., NIA; Richard Spencer, M.D., Ph.D., NIA; Jane Freedman, M.D., Boston University; Deanna Kroetz, Ph.D., University of California, San Francisco; Michel Eichelbaum, M.D., Bosh Research Institute, Stuttgart, Germany; Mary Ann Mascelli, Ph.D., Centocor, Inc.



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Biography: Dr. Irving W. Wainer graduated from Wayne State University in 1965 with a B.S. in chemistry and then received his Ph.D. degree in chemistry from Cornell University in 1970. He did postdoctoral doctoral studies in molecular biology at the University of Oregon and clinical pharmacology at Thomas Jefferson Medical School. From 1978 to 1986 he worked for the Food and Drug Administration (FDA) as a Research Chemist. His duties included the development of the FDA's program on the stereoisomeric purity of drugs. In 1986, he left the FDA to become Director of Analytical Chemistry, Clinical Pharmacokinetics Lab, and Associate Member, Pharmaceutical Division, St. Jude Children's Research Hospital in Memphis. He stayed in Memphis until 1990 when he moved to Montreal where he assumed the position of Professor and Head of the Pharmacokinetics Laboratory, Department of Oncology, McGill University. He is still an Adjunct Professor at McGill. In 1997, he moved to Georgetown University Washington, D.C. as a Professor of Pharmacology. In 2001 he moved to NIA to head the new Bioanalytical Chemistry Section in the Laboratory of Clinical Investigation.

He has published over 250 scientific papers and eight books. He was founding editor of the journal *Chirality* and is currently Senior Editor of the *Journal of Chromatography B: Biomedical Sciences and Applications*. His awards include: co-recipient with Dr. John E. Stambaugh of the "*Harry Gold Award*" from the American College of Clinical Pharmacologists; "*Sigma Xi Science Award*", FDA Sigma Xi Club; "*A.J.P. Martin Medal*" presented by the Chromatographic Society for contributions to the development of chromatographic science; Elected Fellow of the American Academy of Pharmaceutical Sciences; Elected Member United States Pharmacopeial Convention Committee of Revision for 1995-2000. His research interests include clinical pharmacology, bioanalytical chemistry, proteomics and the development of on-line high throughput screens for new drug discovery.

Keywords:

cancer cachexia
drug metabolism
immobilized receptors
high throughput screens

Recent Publications:

Williams ML, et al. *Br J Clin Pharmacol* 2000; 49: 485-488.

Lu L, et al. *Mol Pharmacol* 2001; 59(1): 62-68.

Cabal-Manzano R, et al. *Br J Cancer* 2001; 84: 1599-2001.

The Effect of Disease State on Drug Metabolism: We have identified a number of discordances between metabolic genotype and expressed phenotype in patients with advanced cancer and AIDS. For example, patients with extensive or fast genotypes for cytochrome P450 (CYP) 2C19 and N-acetyltransferase-2 (NAT-2) have displayed poor and slow phenotypes, respectively. In the case of CYP 2C19, this discordance was associated with metastatic disease. With AIDS patients, the discordance between NAT-2 genotype and expressed phenotype was observed during acute disease events. Treatment of the acute illness resulted in a reversion to concordance between genotype and expressed phenotype.

Since these observations were associated with advanced disease, we have initiated studies in patients suffering from terminal syndromes such as cancer cachexia. In particular, we have developed a direct measure of a "proteolysis inducing factor" (PIF) associated with cachexia. The PIF is measured in spot urines using capillary electrophoresis (CE). The presence

of PIF in urine has been correlated with clinical status and with the identification of PIF in tumor biopsies. We have also correlated the presence of PIF in urine with treatment response and clinical relapse. A longitudinal study of the use of PIF as a disease marker has been designed and will be initiated this fall.

Based on these results, we have initiated a study using CE coupled with mass spectrometry (CE-MS/MS) and MALDI-TOF spectrometry to quantify PIF in tissues and to examine the effect of cachexia on pre- and post-translational expression of hepatic enzymes and transporters. We will also use laser capture microdissection and CE with mass spectrometry or laser induced fluorescence to study these effects in single cells.

Immobilized Receptors, Transporters and Enzymes: We have developed liquid chromatographic stationary phases containing immobilized receptors, enzymes and transporters as an on-line, flow system for use in new drug discovery and in the characterization of lead drug candidates. These columns can range in size from standard lc columns to micro-columns, can be used to screen complex chemical mixtures, to characterize single compounds and to screen phage libraries. The columns can be used with characterized targets - e.g. nicotinic, GABA, NMDA, estrogen receptors, P-glycoprotein and other ABC transporters, cytochrome P450 and other enzymes including phenylethanolamine N-methyltransferase and dopamine b-hydroxylase - as well as orphan receptors and other expressed proteins. In addition, the columns can be placed on-line with mass spectrometers or any other structure or activity detectors and provide real-time data. We also have data that demonstrate that this technique can give you information that cannot be obtained using standard micro-titer plate approaches. For example, the immobilized nicotinic receptor column can be used to rapidly identify non-competitive inhibitors of this receptor. At the current time, non-competitive inhibitors can only be identified through functional ion-flux studies. Current research involves the development of other ABC transporter columns and the creation of an opioid receptor column.

Bioanalytical Chemistry: We have developed a wide variety of new and unique bioanalytical methods for the quantification of drugs in biological matrices. These methods have been applied to pharmacokinetic and clinical studies. In addition, we have begun studies in the area of proteomics for the identification of proteins in cellular matrices. These techniques will involve MALDI and ms/ms mass spectrometry.

Collaborators: Darrell Abernethy, NIA; Gerry Price, McGill University; Neil McDonald, McGill University; Robert Clarke, Georgetown University; Francois Gimenez, Hopital Necker, Paris, France; Carlo Burtucci, University of Bologna; Terumichi Nakagawa, University of Kyoto; Beverly Barton, Medical and Dental University of New Jersey; Celeste Lindlye, University of North Carolina; Joanne Lampe, University of Washington.

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Biography: Dr. Josephine Egan is a board certified endocrinologist who received her endocrine training at the University of Virginia, Charlottesville. She has been with the NIA since July, 1990. Her early work related to investigating and quantitating insulin release from individual beta cells in the islets of Langerhans. Using this methodology, she outlined the abnormalities that occur in the aging beta cells of rats. More recently she has been working on ways to reverse these abnormalities, on ways to increase insulin secretion in Type 2 diabetes mellitus and on outlining the growth factors involved in beta cell replication.

Keywords:

GLP-1
Exendin-4
insulin and diabetes
islets of Langerhans

Recent Publications:

Doyle ME, et al. *Endocrinology* 2001; 142(10): 4462-4468.

Wang X, et al. *Endocrinology* 2001; 142(5): 1820-1827.

Perfetti R, et al. *Endocrinology* 2000; 141(12): 4600-4605.

Clocquet AR, et al. *Diabetes* 2000; 49(11): 1856-1864.

Aging and Type 2 Diabetes: The goal is to design new drugs to restore glucose sensitivity to the beta cells in type 2 diabetes and to prevent deterioration of the beta cells which seems an inevitable occurrence in aging. The general strategy is to outline the abnormalities that occur in aging and type 2 diabetes in beta cells and search for agents that can alter these processes. New agents are first tested in beta cell lines. Next acute and chronic experiments are carried out in animal models of aging and diabetes. With the information gained from the animal models, we plan to go as quickly as possible directly into trials in type 2 diabetic humans.

Type 2 diabetes develops, for the most part, because with increasing age, adiposity and changing lifestyle, insulin becomes less effective at its target tissues. This puts increased demand on the beta cells of the pancreas which then must supply more insulin. When supply cannot keep up with demand, blood sugars rise which then lead to complications such as blindness, nephropathy and neuropathy as a direct result of the elevated blood sugars. With increasing age, beta cells respond less to glucose stimulus. They also do not replicate at the same rate as beta cells in younger animals. Thus, in principle, we need to find agents that would restore glucose responsivity to the beta cells and that would prevent the decrease in replication that occurs in beta cells of aging mammals.

Design of Drugs of Potential Use in Type 2 Diabetes: We have been concentrating on a group of peptides known as incretins. They are released from the gut in response to food and they augment the insulin response to glucose. One of these peptides, GLP-1, is effective at increasing insulin release when given systemically even in long-standing type 2 diabetes. It also appears to be a trophic agent to the pancreas in pharmacological doses. This is a major difference from other agents that are presently used to treat diabetes as studies show that even with good control of blood sugars there is an inexorable decline in beta cell function. GLP-1 has a short half-life and consequently has to be given at least three times a day subcutaneously to maintain high insulin levels in the blood if used in outpatients. We have shown that when given continuously, intravenously to type 2 diabetic patients admitted because of stroke, blood glucose can be controlled. We are presently working with a peptide called Exendin-4 that is secreted in the saliva of the Gila monster (a lizard) and that is 53% homologous to human GLP-1. It also is very effective at inducing insulin release and, of great significance, when given subcutaneously or intraperitoneally, it has a much longer biological action than GLP-1. We have completed animal testing of this compound and have begun human testing. When given intravenously to normal and type 2 diabetic subjects, its biological action lasts about twelve hours and it is extremely potent at inducing insulin release. We are involved in a 31-day study in type 2 diabetic subjects using Exendin-4 once or twice daily. We are also testing Exendin-4 that has been “humanized” i.e. we are replacing the amino acids of Exendin-4 with those of GLP-1 and hope to find out where the crucial amino acids that are responsible for the prolonged biological activity of Exendin-4 lie. Current efforts show that GLP-1 is a true growth factor for beta cells in the pancreas and perhaps is involved in cell differentiation in other organs besides pancreas.

Collaborators: Dr. Doris Stoffers, University of Pennsylvania; Drs. Dariush Elahi and Joel Habener, Massachusetts General Hospital; Dr. Seamus Sreenan, University of Chicago Medical School; Dr. Nigel Greig, Laboratory of Neurosciences, NIA; Dr. Andrew Young, Amylin Pharmaceuticals, San Diego; Dr. Grady Maneilly, University of British Columbia.

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Biography: Dr. Bernier received his Ph.D. from the University of Montreal, Canada, in 1983, and completed two postdoctoral fellowships. The first one was held at INSERM U.162 in Lyon, France, and the second one at the Johns Hopkins University School of Medicine in Baltimore. He was an assistant professor of Biochemistry at McGill University in Canada before joining the NIA in 1990. He became a Tenure-Track Investigator in July 1994. His current projects include investigation of the molecular aspects of insulin receptor signal transduction. He is a member of the American Diabetes Association and the Endocrine Society.

Keywords:

insulin
receptors
signal transduction
transcription factor NF-κB

Recent Publications:

Pandey SK, et al. *Endocrinology* 2002; 143(2): 375-385.

Garant MJ, et al. *Biochemistry* 2000; 39(24): 7178-7187.

Park D, et al. *Biochemistry* 2000; 39(41): 12513-12521.

Garant MJ, et al. *Biochemistry* 1999; 38(18): 5896-5904.

Protein-protein Interactions in Insulin Signaling: The insulin receptor is a central element in the transmission of insulin pleiotropic signals within the cells by interacting with a growing number of regulators and effector proteins. Indeed, functional interaction between the insulin receptor and caveolin, a major component of caveolae plasma membrane domains, has been recently established. Caveolin is tyrosine phosphorylated *in vivo* by Src-related kinases upon insulin stimulation, which could trigger the increase or the suppression of its capacity to interact with signaling proteins and, possibly, their redistribution into other areas of the cell. We have recently shown that the insulin receptor forms a trimeric complex with caveolin and a thiol-reactive membrane-associated protein (TRAP) in response to insulin and that oxidative stress markedly attenuates the formation of this multiprotein complex. We are currently designing strategies to purify TRAP and examine its role as a proximal downstream element in the signal transduction cascades activated by insulin. Because interaction of caveolin and TRAP with the insulin receptor may differentially modulate insulin signaling, it will be important to assess their intracellular compartmentalization in insulin-responsive cells subjected to a number of manipulations known to produce insulin resistance (e.g., hyperglycemia, cytokine or glucocorticoid stimulation).

Role of Short-lived Farnesylated Proteins in Insulin Action: Apoptosis, also termed programmed cell death, is an active, genetically controlled process that has been identified as a key phenomenon in the pathogenesis of a wide array of diseases, including diabetes. We have shown that isoprenylation of short-lived proteins may play a role in mediating the antiapoptotic properties of insulin through activation of the

phosphatidylinositol 3-kinase/Akt pathway. Of interest, a relationship between activation of Akt and the transcription factor NF-κB has been found, suggesting that the survival function of insulin may depend on NF-κB signaling. We have recently established that insulin-induced NF-κB activation involves a protein phosphorylation cascade that is distinct from that of the proinflammatory cytokine TNFα, a prototypical activator of NF-κB. Work is underway to assess the role of manumycin A, a potent and selective inhibitor of farnesyltransferases, on the ability of insulin and TNFα to stimulate NF-κB function. Obesity-linked insulin resistance and changes associated with aging have been linked to cytokine signaling and dysregulation of upstream kinases involved in NF-κB-dependent gene expression. We are also exploring the possibility that subcellular distribution of farnesylated proteins may differentially modulate NF-κB signaling to enhance insulin action but inhibit TNFα's effects in insulin responsive cells.

This is a wide-ranging set of investigations spanning modern techniques of protein biochemistry, molecular biology, and *in vitro* manipulations in cultured cells. Progress in our understanding of factors regulating insulin action shall allow the development of effective interventions for obesity-related insulin resistance and diabetes, two major contributors to morbidity and mortality in the U.S. and other Western societies.

Collaborators: Michael T. Crow, Ph.D., LCS, NIA; Magdalena Juhaszova, Ph.D., RRB, NIA; Irving Wainer, Ph.D., LCI, NIA.

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Biography: Dr. Eric Westin received his M.D. from Albany Medical College in 1976 with board certification in Medicine and Oncology, having received his Oncology training at the Medicine Branch of the National Cancer Institute. He has been on the faculty of the Medical College of Virginia/ Virginia Commonwealth University in Richmond Virginia from 1984 to 1997 and subsequently Professor of Medicine and Chief of the Section of Hematology/ Oncology and Medical Director of the Mary Babb Randolph Cancer Center at West Virginia University prior to joining the GRC in May 2000. His research interests from fellowship training have been focused on the role of proto-oncogenes in control of hematopoietic cell differentiation with evolution to examining the role and regulation of the c-*myb* transcription factor in this process. Current laboratory work examines the role of this and other genes in control of proliferation and differentiation using both hematopoietic and breast tumor epithelial models coupled with cDNA micro-array analysis of gene expression and how these processes may be modulated by chemotherapeutic and other signaling agonists or antagonists. In addition, significant new studies have been initiated characterizing a novel potential suppressor gene on chromosome 6q, discovered due to its proximity to the c-*myb*. This and other work in this section will lead to translational studies within the clinical research unit to directly test the clinical diagnostic of therapeutic potential of basic findings.

Keywords:

c-*myb*
hematopoiesis
breast cancer
proliferation
differentiation
tumor suppressor gene

Recent Publications:

Jeng MH, et al. *Endocrinology* 1998; 139: 4164-4174.

Qian Y, et al. *Oncogene* 1998; 16: 2185-2195.

Control of Hematopoietic and Epithelial Differentiation:

Hematopoiesis and breast epithelial proliferation and differentiation represent processes of terminal differentiation leading to cell death in the case of hematopoiesis or reversible differentiation and proliferation in the case of the breast epithelium. When viewed in the context of aging, each mimics aspects of cellular aging where other factors such as number of cell divisions and oxidant stress and damage are thought to limit cellular life span but nonetheless are likely to have effects through many of the same cell signaling processes. When viewed in the context of the treatment of malignant diseases, pharmacologic manipulation of signaling pathways responsible for controlling the balance between differentiation and proliferation in conjunction with chemotherapeutic agents may well provide methods to increase the specificity of conventional agents thus increasing both efficacy and reducing toxicity. Both are critical components needed to improve treatment in patients with co-morbidities most frequently associated with aging where the balance between benefit versus risk of intervention becomes increasingly problematic with age.

Hematopoietic and Epithelial Proliferation/ Differentiation: The cellular *myb* gene is a member of the transcription factor class of proto-oncogenes originally transduced in the avian myeloblastosis (AMV) and E26 acute transforming retroviruses of chickens. It is capable of either transactivation (target genes such as *mim*-1) or transrepression (target genes such as c-erbB-2) depending on the context of binding to the promoter. Based on our and other studies, down regulation of human c-*myb* expression occurs during hematopoietic differentiation through use of a transcription attenuator within the first intron of the gene. This down regulation is required for differentiation to occur. Introduction of a constitutively expressed c-*myb* gene will block both withdrawal from cell cycle as well as acquisition of differentiated features in a variety of differentiation models including Friend murine erythroleukemia (FMEL) cells.

Though progress has been made in understanding the role, regulation and function of *myb* in hematopoiesis, a number of critical questions remain. These include such basic issues as: i) what are the relevant target genes activated or repressed by c-*myb*; ii) what are the functions of products of c-*myb* produced by extensive alternative mRNA processing and found in a variety of cell types and; iii) is c-*myb* mechanistically involved in leukemogenesis in humans as it is in the mouse and chicken.

This laboratory program previously has focused on the human c-*myb* gene and its function in hematopoiesis using models such as HL60 and more recently in breast tumor cell proliferation and differentiation. We have elucidated both a number of mechanisms regulating c-*myb* expression during the process of hematopoietic differentiation as well as defined diversity in c-*myb* expression based on extensive alternative mRNA processing which has significant effects on c-*myb* protein function. Important future directions from this work will include the use of dominant/negative forms of *myb* to further define the role of this gene in hematopoiesis and in response of cells to chemotherapeutic agents and to elucidate potential targets of c-*myb* action and assess whether these are lineage and/or tissue specific.

Interest in *myb* in breast epithelium and tumors derives from the finding that c-*myb* is expressed in more than 60% of clinical breast tumor specimens and that while expression is positively correlated with estrogen receptor (ER) and progesterone receptor (PR) status, significant numbers (approx. 30%) of ER-/PR- tumors also express *myb*. Recent studies indicate that: i) c-*myb* is expressed in all ER+ breast tumor cell lines examined to date and is also expressed in some ER-/PR- cell lines, providing models in which c-*myb* regulation and function can be studied;

ii) *myb* is regulated in response to estrogen in the ER+ cell line MCF-7 following withdrawal and restimulation though a direct effect of the estrogen receptor; iii) c-*myb* expression is regulated in response to breast tumor cell differentiating agents such as retinoic acid and dexamethasone as in hematopoietic models of retinoic acid differentiation; iv) the mechanism of regulation of *myb* expression is radically different from hematopoietic models where we have shown that most if not all regulation is at the level of the transcription attenuator within intron 1. In the case of both *myb* expressing and non-expressing breast tumors, the promoter remains active with no evidence of attenuator function based on run-on assays.

Beyond an interest in *myb* regulation, an equally important question from a biologic perspective is what effect *myb* expression has on breast tumor cell behavior. In the case of *myb* expression in ER+ cell lines, transfectants are currently being developed. The hypothesis to be tested is fairly clear in this case. It has been known for many years that one mechanism by which estrogen stimulates growth of estrogen dependent tumors is by induction of a “second” wave of growth factors and their receptors including IGF-1. It is now known that a specific function of c-*myb* in some cell types is stimulation of IGF-1 and IGF-1R expression. Thus, constitutive expression of c-*myb* would be expected to make cells such as MCF-7 estrogen independent and resistant to antiestrogens such as Tamoxifen. If true, then Tamoxifen resistance could occur through any mechanism that would uncouple estrogen regulation from *myb* expression. Development of this breast tumor model will also provide a complementary cell system for elucidation of transcription targets of c-*myb* action and whether these are lineage and/or tissue specific when compared with those of the hematopoietic system and provide an important added dimension to work examining chemotherapeutic agent effects coupled with modification of signal transduction pathways in proliferation, differentiation and cell death.

In addition to studies specifically directed to evaluation of *myb* in these processes, cDNA microarray technology has been applied to examine sequential changes in gene expression in HL-60 during terminal differentiation induced by DMSO and begun in estrogen induced breast epithelial proliferation. In HL60, expression patterns have been identified that are both early (prior to withdrawal from cell cycle) and late (during the development of differentiated features and cell cycle withdrawal) as well as unique to the commitment phase at 48-72 hours. These findings validate the use of this technique as a general approach for comparison of different differentiation pathways to identify changes specific to each inducing agent. They have also highlighted specific changes at the

commitment stage that can be examined mechanistically using pharmacologic manipulation. Integration of this work with work on c-*myb* will be used to identify potential target genes regulated by c-*myb*. These results also indicate that cDNA microarray approaches will provide a powerful approach to increasing our understanding of both hematopoietic differentiation as well as the critical events surrounding the commitment step at which cells within the hematopoietic system are no longer able to divide, mimicking some features of cellular aging.

Isolation of a Candidate Tumor Suppressor Gene from Chromosome 6q: As the population continues to age, the age dependent increase in cancers including non-Hodgkin's lymphomas and breast cancer become an increasingly important source of morbidity. Many of these cancers are associated with acquired genetic abnormalities that are linked to their pathogenesis. One common area of abnormality that has yet to lead to identification of one or more specific genes is the long arm of chromosome 6. Abnormalities including deletion, rearrangement and loss of heterozygosity in the chromosome 6q region surrounding the c-*myb* gene occur frequently in a variety of tumors including the non-Hodgkin's lymphomas, acute lymphocytic leukemias, breast cancer, sarcomas, melanoma and non-small cell lung cancer.

Based on earlier work, a rearrangement was identified in the T-cell leukemia cell line CCRF-CEM in which the promoter region of c-*myb* was found to be rearranged. Cloning of the rearrangement (termed *myb* rearranged region 1 or mrr1) provided junction fragments and sequence information that identified the rearrangement as a sub-microscopic deletion of 6q. Subsequent work using a P1 clone isolated from the deleted region in FISH studies indicated that this region was also deleted in a T-cell leukemia cell line carrying the common 6q deletion seen by cytogenetics in T-cell leukemia. While a candidate gene was not initially isolated, considerable sequence data from this region was generated and intermittently searched against the genomic sequence databases.

With the near completion of the draft human genome sequence earlier this year, previous sequence data has led to isolation of a candidate tumor suppressor gene termed mrr1. Using ests for analysis, a partial candidate cDNA has been isolated and based on sequence analysis of this and other available sequence, the mrr1 protein analyzed. This protein is predicted to be a nuclear protein containing a WD-repeat structure and SH3 domain indicating that it may interact with a variety of other nuclear proteins in a complex macromolecular structure. Northern blotting studies indicate expression in a variety of tissues including thymus, fetal liver, muscle and

kidney. To date, alterations including a frame shift mutation in a cDNA from a uterine sarcoma and an aberrant mRNA species in a non-small cell lung cancer harboring a t1:6 translocation have been identified in addition to the rearrangement in CCRF-CEM.

These results indicate that an important and novel tumor suppressor gene has been isolated that resides in a region of chromosome 6q that is the site of frequent alteration in a wide range of tumor types. Better understanding of the function of mrr1 may provide both important tools for cancer screening as well as models in which to evaluate both novel anti-tumor therapeutic and prevention strategies.

Collaborators: Drs. D. Flynn and K. Landreth, West Virginia University, Morgantown, West Virginia; Dr. T. Bender, University of Virginia, Charlottesville, Virginia.

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Biography: Dr. Espinoza-Delgado received his M.D. degree and internal medicine training from the Central University of Venezuela. He then joined the National Cancer Institute and obtained laboratory and medical oncology training at the Biological Response Modifiers Program and Medicine Branch respectively. In 1996, Dr. Espinoza-Delgado joined the Department of Medicine of Louisiana State University Health Sciences Center, New Orleans as Assistant Professor and Associate Director of the Cancer Immunotherapy Program. In early 2001, he moved to the Section of Hematology-Oncology, Laboratory of Clinical Investigation, at the National Institute on Aging.

Keywords:

monocytes
antigen presentation
cancer vaccine
immunotherapy
IFN-gamma
Bryostatin-1

Recent Publications:

Curiel RE, et al. *J Immunol* 2001; 167(9): 4828-4837.

Bosco MC, et al. *J Immunol* 2000; 164(9): 4575-4585.

Curiel RE, et al. *Blood* 1999; 94(5): 1782-1789.

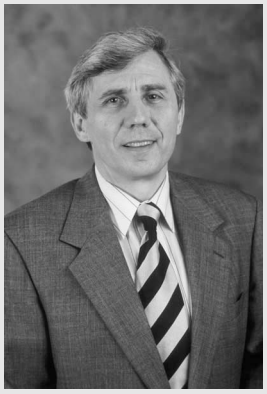
Monocytes, Antigen Presentation, and Cancer Immunotherapy: Pre-clinical studies have clearly demonstrated that antigen-pulsed DCs can generate protective immunity against tumors. More recently, pilot studies performed in humans suggest that monocyte-derived DCs are capable of eliciting antigen-specific immune responses some of which were associated with clinical response. The results of these experimental models and early clinical trials provide a compelling rationale for arduous exploration of antigen-based cancer vaccines using APCs as initiators of the immune response. One problem with this approach has been the DCs themselves. Although DCs have been characterized as the most immunologically powerful APCs, they have several drawbacks including the lack of a well defined phenotype, the expensive long-term ex-vivo culture conditions ranging from two days to more than one week, the limited availability of these cells and the variability, in terms of recovery, from patient to patient. Moreover, recent reports indicated that tumor bearing animals and cancer patients with advanced disease have a decreased number of DCs with a diminished APC function. Taken together, these factors may have a negative impact on the development of APCs-based cancer vaccine. Therefore, innovative approaches to circumvent the above mentioned problems are needed to further advance the cancer vaccine field. Our approach has been to explore the potential of human monocytes to act as professional APCs. In contrast to DCs, monocytes offer the advantage of being phenotypically well characterized, being available in large amounts from peripheral blood, and being effector cells. Studies examining human monocytes activated for short periods of time (18 hours) revealed that these cells express all the recognition, adherence and costimulatory molecules required for the induction of a

specific and efficient antitumor response. The studies demonstrated that IL-2 induces both B7-1 mRNA and surface protein expression in human monocytes. The studies also revealed that through processes requiring new protein synthesis, transcriptional activation of the B7-1 gene was responsible for the observed mRNA upregulation. Noteworthy, the expression of B7-1 in response to IL-2 was associated with an enhanced antigen presentation function by human monocytes. Finally, activated monocytes produced significant amounts of pro-inflammatory and inflammatory cytokines that might create the proper microenvironment required to induce a competent antitumor response. Studies are currently underway to examine the expression of chemokines and chemokine receptors to evaluate the ability of activated monocytes to migrate to regional lymph nodes and initiate the immune response. Studies in a xenogeneic model are planned to determine the *in vivo* relevance of this approach.

Th1 Response Induced by Bryostatin-1 and Low Dose IL-2 : Implications for Cancer Immunotherapy: Bryostatin-1 (Bryo-1), a potent ligand and modulator of PKC, has a broad antitumor activity. Our group has reported that Bryo in combination with vincristine resulted in an increased growth arrest and apoptosis of human B cell lymphoma compared to either agent alone. These effects were associated with changes in the expression of p53, bcl-2 and bax. In addition to its direct antitumor activity, bryostatin-1 may also inhibit tumor growth *in vivo* by indirect mechanisms related to its ability to stimulate host immune response. Studies to evaluate the effects of Bryo-1 on human monocytes and lymphocytes have revealed that Bryo-1 induces the production of IL-l, IL-6, IL-8 and TNF-a by human monocytes. Furthermore, our studies have demonstrated that bryostatin-1 selectively synergizes with IL-2 in activating lymphocytes or monocytes, and this effect seemed to be dependent on the ability of bryostatin-1 to induce the expression of IL-2Ra or IL-2Rg chains, respectively. More recently, our group has reported that a combination of Bryo-1 and low dose of IL-2 (LDIL-2) induces high levels of IFN-g expression. It was found that in primary human T cells through a process independent of PKC activation, a dual mechanism involving transcription and postranscriptional levels of regulation is responsible for the Bryo-1 plus LDIL-2 induction of IFN-g gene expression and protein secretion. The ability of Bryo-1 plus IL-2 to induce a Th1 phenotype might have clinical relevance. Studies in cancer patients and pre-clinical models have indicated that T cell responses to tumor cells are impaired. Furthermore, some reports have shown that in T cells from tumor bearing animals IFN-g production is deficient. Thus it has been hypothesized that the failure to protect against tumors is not due to a lack

of an immune response, but it is the result of the cytokine pattern deviation which impairs the proper development of an efficient antitumor response. Our studies suggest that Bryo-1 plus IL-2 may play a crucial role in controlling the polarization of the immune response in a clinical therapeutic setting by inducing IFN-g expression. Taking into account the well characterized antineoplastic and immunomodulatory activity of both Bryo-1 and IL-2, and having shown in a murine model with B16-F10 melanoma cells that a combination of Bryo-1 and LDIL-2 have antitumor activity without significant toxicity, we are currently conducting a phase I clinical trial to evaluate the immune effects and toxicity of this combination in patients with cancer.

Collaborators: Dr. Maria Carla Bosco, Giannina Gaslini Institute, Genova, Italy; Dr. William Murphy, NCI, NIH; Dr. Adrian Senderowicz, NIDCR, NIH.



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Biography: Dr. Nikolai Soldatov received his Ph.D. degree in bioorganic chemistry in 1981 from Shemyakin Institute of Bioorganic Chemistry of the USSR Academy of Sciences, Moscow. In 1983, while on postdoctoral training in Shemyakin Institute, he initiated research directed to the identification and isolation of skeletal muscle dihydropyridine-sensitive calcium channel. In 1986 he joined the Institute of Medical Biotechnology led by cosmonaut Prof. B. Egorov and studied the relationship between calcium channels and primary and secondary messengers of human fibroblasts proliferation and memory, learning and nootropic effects in the brain. In 1990 he joined the laboratory of Dr. G. Blobel at the Rockefeller University, New York. He cloned the human L-type calcium channel and investigated its genomic structure. In 1993 he worked at the Department of Pharmacology of the University of Bern, Switzerland. He constructed a representative panel of human calcium channel splice variants and investigated, in collaboration with Prof. H. Reuter, their pharmacological and electrophysiological properties. In 1996 he moved to Georgetown University Medical Center, Washington, D.C., where he worked as an Assistant Professor of the Department of Pharmacology. He studied mechanisms of calcium-induced inactivation, cross-talk between calcium channel and angiotensin receptor, and the role of C-terminal tail of the channel in calcium signaling in cardiac myocytes. In July 1999 Dr. Soldatov joined NIA as an Investigator. He is a member of the Editorial Advisory Board of the *Journal of Pharmacology and Experimental Therapeutics*.

Keywords:

calcium signaling
human L-type calcium channel
mechanisms of inactivation

Recent Publications:

Harms GS, et al. *Biophys J* 2001; 81(5): 2639-2646.

Romanin C, et al. *FEBS Lett* 2000; 487(2): 301-306.

Soldatov NM, et al. *J Membr Biol* 2000; 177(2): 129-135.

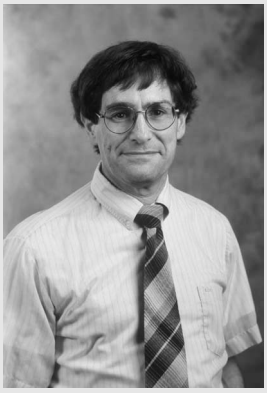
Calcium Sensitivity of Calcium Channel: The voltage-gated L-type Ca^{2+} channel is inhibited by Ca^{2+} but not Ba^{2+} ions on the cytoplasmic side of the pore. This Ca^{2+}-induced inactivation serves as an important feedback mechanism against Ca^{2+} overloading of the cell. We found that the 650-amino acid carboxyl-terminal tail of the channel is critically important for the feedback (Soldatov et al., *J Biol Chem*, 272: 3560, 1997). Our studies showed that Ca^{2+}-induced inactivation of the L-type Ca^{2+} channel and its modulation by calmodulin is differentially mediated by two short carboxyl-terminal motifs (Soldatov et al., *J Biol Chem*, 273: 957, 1998). One of these motifs is a Ca^{2+} sensor site that binds calmodulin at low resting free Ca^{2+} concentration. Increase in Ca^{2+} concentration causes release of calmodulin from this motif and in turn stimulates its binding to the IQ-region of the adjacent motif. These data imply that Ca^{2+}-dependent transfer of calmodulin between the two spatially close binding sites leads to Ca^{2+}-induced inactivation of the channel (Romanin et al., *FEBS Lett*, 487: 301, 2000). Further investigation of this region by NMR spectrometry and electron diffraction will allow us to compare involved structural determinants which may lead to the development of new drugs.

Molecular Determinant of the Voltage-dependent Ca^{2+} Channel Inactivation: Slow voltage-dependent inactivation is controlled by an annular determinant in the inner mouth of the pore. Previously we observed (Soldatov, *Proc Natl Acad Sci USA*, 89: 4628, 1992) the naturally occurring A752T mutation at the cytoplasmic end of transmembrane segment IIS6 of the α_{1C} channel. This mutation prevented a large (≈25%) fraction of the current from inactivation (Soldatov et al., *J Membr Biol*, 177: 129, 2000). Incorporation of similar mutations in the analogous positions of transmembrane segments S6 of each repeat of the α_{1C} subunit completely inhibited slow inactivation. The mechanisms of functional targeting of the outlined annular determinant of slow inactivation by C-terminal Ca^{2+} sensors of inactivation and regulatory β-subunit are the subjects of ongoing investigation using electrophysiology, immunochemistry, fluorescence resonance energy transfer (FRET), and α_{1C} transgenic animal models.

Functional Architecture and Regulation of Human L-type Ca^{2+} Channel: It is clear that inactivation is affected by multiple regions of the α_{1C} subunit, thus suggesting that substantial molecular rearrangements are involved. To study state-dependent molecular motions in the functionally expressed wild-type, non-inactivating, and Ca^{2+}-insensitive isoforms of the channel, we combined patch clamp technique with FRET between various Green Fluorescent Protein (GFP) variants fused to the termini of the expressed recombinant proteins. Confocal and fluorescence imaging techniques will be applied also to muscle and neuronal cells produced by GFP-α_{1C} transgenic mice expressing wild-type and mutated Ca^{2+} channels. To investigate the role of amino acids and motifs of the pore-forming α_{1C} subunit in regulation of the human Ca^{2+} channel using the most sophisticated techniques, we have initiated a multifaceted international collaboration between NIA (Dr. Soldatov), University of Linz, Austria (Dr. Romanin), University of Leiden, The Netherlands (Dr. Schmidt) and the University of Cologne, Germany (Dr. Hescheler). The method of fluorescent channel detection was successfully minimized to the level of single-molecule imaging in live cell (Harms et al., *Biophys J*, 81: 2639, 2001). The data obtained by single channel recordings have shown that membrane targeting, ion conductance, inactivation kinetics, Ca^{2+}-dependence and run-down of the human Ca^{2+} channel and two-site modulation by calmodulin all critically depend on amino acids in the motif 1572-1651 of the C-terminal tail (Kepplinger et al., *FEBS Lett*, 477: 161, 2000; Kepplinger et al., *J Physiol*, 529: 119, 2000). We plan to extend this

study to investigate the assembly of the channel from differentially labeled subunits. Our collaborative efforts with Prof. Meissner (University of North Carolina) on reconstituted ryanodine receptors, and with Prof. Morad (Georgetown University) on Ca^{2+} release sparks formation in cardiac myocytes have demonstrated the involvement of the Ca^{2+} channel carboxyl tail in cross-talk with Ca^{2+} release Ca^{2+} channel of SR. Further development of this collaboration is directed to the analysis of the architecture of Ca^{2+} signaling microdomains and identification of interacting motifs of both channels.

Collaborators: Darrell R. Abernethy, M.D., Ph.D., NIA; Evgeny Kobrinsky, Ph.D., NIA; Zuqin Nie, Ph.D., NIA; Elena Schwartz, Ph.D., NIA; Chengzhang Shi, NIA; Olga Carlson, Ph.D., NIA; Christoph Romanin, Ph.D., University of Linz, Austria; Thomas Schmidt, Ph.D., University of Leiden, The Netherlands; Jürgen Hesheler, M.D., University of Cologne, Germany; Gerhard Meissner, Ph.D., University of North Carolina, NC; Susan Hamilton, Ph.D., Baylor College of Medicine, Houston, TX.



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Biography: Dr. E. Jeffrey Metter received his M.D. from the University of California, Los Angeles in 1971. He completed a medical internship and neurology residency at the Mayo Graduate School of Medicine, Rochester, Minnesota in 1976. He returned to Los Angeles, where he became a staff neurologist and chief of the stroke rehabilitation ward at the Veterans Administration Medical Center, Sepulveda, California. He was also on the full time faculty in the Department of Neurology, UCLA School of Medicine. In 1987, he joined the National Institute on Aging as the physician for the Baltimore Longitudinal Study of Aging.

Keywords:

aging
longitudinal studies
neuromuscular
cerebrovascular
prostate

Recent Publications:

Brooks JD, et al. *J Urol* 2001; 166(6): 2034-2038.

Fang J, et al. *Urology* 2001; 58(3): 411-416.

Talbot LA, et al. *Med Sci Sports Exerc* 2000; 32: 417-425.

Carter HB, et al. *J Natl Cancer Inst* 1999; 91: 1733-1737.

Conwit RA, et al. *Clin Neurophysiol* 1999; 110: 1270-1275.

Prostate Aging and Disease: The Baltimore Longitudinal Study of Aging (BLSA) is characterizing normal aging in the prostate and identifying transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition, the research is using information about structure and function of the prostate to improve early detection of prostate disease. Clinical evaluations of prostate growth and function have been made in over 800 men with and without prostate disease and the availability of stored sera and genetic material. Prospectively, BLSA men aged from 30 to 79 have physiological, clinical and imaging of their prostate. To date, the major accomplishments have come from analyses of prostate specific antigen (PSA) which show that PSA increases more over a period of years in men who develop BPH than in those who do not. The rate of change in PSA is even greater in men who develop prostate cancer, and the increases goes up exponentially 5-7 years prior to diagnosis. Furthermore, the ratio of free to total PSA is able to distinguish men who develop prostate cancer from and those who do not about 10 years prior to diagnosis. Analyses of a subset of the men who developed prostate cancer show that the free to total PSA ratio is lower in men who have clinically defined aggressive tumors. Current work is showing that normal levels of PSA can be stratified to identify men at high risk of developing prostate cancer over a 20 to 30 year period. Alterations in prostate structure or function are studied in relation to the possible development of prostate disease, particularly BPH. Currently, magnetic resonance imaging of the prosate are performed at each visit. The data are being analyzed to estimate prostate volume as well as the percentage of epithelial and stromal tissue. Longitudinal evaluation of the change in prostate size was found to increase into the fifties and the rate of change declines in older age decades.

Neuromuscular Changes with Age: The purpose is to characterize and explain age associated losses of muscle strength. We seek to understand the time course of strength loss, factors that contribute to the loss, and to what degree the exercise response differs between old and young individuals. Our research has three main components:

1. Characterization of longitudinal strength changes in the BLSA: This consists of two parts. From 1960 to 1985, strength and power were measured in BLSA participants using an in house constructed equipment that measured isometric strength and power in the upper extremities. The purpose is to determine long term longitudinal changes (up to 25 years) in strength and power, and to relate these changes to changes in muscle mass, peripheral nerve function, daily and physical activity, and aerobic fitness. Starting in 1992, strength has been measured using a state of the art isokinetic dynamometer (Kin-Com). This equipment allows for the measurement of both concentric and eccentric strength at multiple velocities in both the upper and lower extremities. The specific purposes are to determine age-associated maximal force production of the upper and lower body musculature during the concentric and eccentric phases of exertion, at fast, slow and zero speed, and determine the angle of greatest force; determine relationships between changes in strength with age and changes in lean body mass, fat mass, bone mineral density, glucose homeostasis, functional abilities, physical activity and nutritional state. We are also interested in the contribution of muscle strength to functional performance and the development of disability, balance problems, and falls. In recent work, we have shown that the age associated declines are explained in part by change in muscle mass. However, other factors are also important as demonstrated by the fact that the amount of strength generated by muscle declines with increasing age, but is sensitive to the methods used to measure strength and muscle mass. In addition, age associated changes in nerve function are, independent of muscle mass and age, associated with changes in muscle strength across the adult lifespan. Similarly, free testosterone levels in men are associated with aging muscle strength.

2. Comparison of exercise response to resistive strength training in young and old subjects: This project was completed under contract with the University of Maryland, College Park, Dr. Ben Hurley, principal investigator. The specific purposes are: (1) Determine the relationship between changes in lean body mass or muscle mass and changes in glucose regulation with age and strength training. (2) To determine if changes in strength or muscle mass can predict changes in total or regional bone mineral density. (3) To determine what factors best explain strength

losses associated with aging and detraining and strength gains associated with strength training. We have found that young and elderly women and men respond relatively similar to resistive training. In all groups, strength increased 25-35%, with evidence of muscle hypertrophy. What was most striking was that the strength gains achieved over 9 weeks of training persisted for at least 6 months without further training.

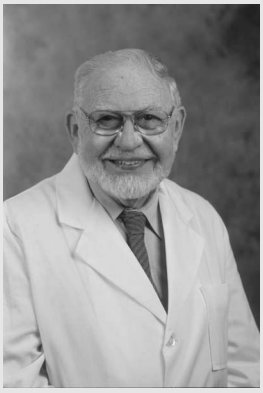
3. Examination of the motor unit and its relationship to muscle strength and exercise response: A clinical protocol has been developed that explores motor unit function at different levels of muscle exertion in the quadriceps. The goal of this project is to understand the changes that occur in motor units with aging, and the effects of these changes on muscle strength and how these changes affect the exercise response. Over the past 20 years *in vivo* techniques allow for the direct examination of the motor units in humans. Most studies that have examined age related changes in motor units have focused on old versus young rather than examining the entire adult life span. They do not allow for an assessment of where during the life span these changes begin, or the association between the motor units and strength. We have developed a clinical protocol that allows for the evaluation of motor units during the generation of fixed force levels. We have found a strong relationship between the size and firing rates of motor units and force generation. With resistive training, smaller units are able to generate fixed forces in the absence of improved strength to a nontraining task. We are now examining changes with age in the BLSA.

Age-Associated Race and Gender Differences in the Carotid and Intracerebral Arteries: This project is studying intracerebral blood flow velocity and resistance, carotid blood flow velocity, and carotid wall characteristics using doppler ultransonographic techniques in BLSA participants. The goal is to determine whether differences in either carotid or intracerebral parameters may explain racial and gender differences in stroke and coronary heart disease, and whether changes in arterial characteristics are associated with fitness and frailty. We have found that intimal-media thickness of the common carotid artery increases with age concommitant with dilitation. Greater carotid wall thickness is associated with increasing risk for the development of both overt and silent coronary heart disease after adjusting for age, and that the common carotid wall thickness is thicker in the presence of asymptomatic coronary disease. Carotid doppler ultrasonography is commonly used during evaluation of cerebrovascular disease. Our findings suggest that examining the carotid wall thickness can increase the suspicion for coronary artery disease. In a

related analysis, we found that women who use estrogen replacement postmenopausally show less arterial stiffness than women who are not on replacement. Improved arterial function may be another result of hormone replacement therapy that contributes to lower rates of heart disease. We have also observed that age change in flow velocities in the carotid artery is poorly correlated with the flow velocities in the middle cerebral arteries. We have compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease.

Body Composition and Bone Aging: This project was formerly in the Applied Physiology Section under the leadership of Dr. Jordan Tobin. With Dr. Tobin's retirement, the section was merged into the LSS. The focus of the work has been on the physiological and pathophysiological changes in bone and body composition that are associated with three of the most common problems of the elderly, osteoporosis, osteoarthritis, and sarcopenia. Most of the research at present is on the BLSA where we are examining longitudinal changes in bone mineral density and body composition. The recognition that bone loss occurs in males as well as in females is an important aspect of this work, and the potential for increased morbidity from hip fractures in males is becoming more important as more men live to an age at which hip fracture is common. The higher rate of loss of bone in women, with twice the incidence of hip fractures as compared to men, has led to the Perimenopausal Initiative that is examining the changes in the rate of bone loss in women as they traverse the menopause. In 1993, the BLSA initiated a study of the perimenopause by starting to recruit a cohort of 100 White and 100 African-American women 45-55 years old. In addition to the bi-annual BLSA visit, these women receive quarterly outpatient visits until menses have ceased for 2 years or hormone replacement is begun. These visits include a menopausal symptom questionnaire, endocrine profiles, anthropometry, dual energy x-ray absorptiometry, bone biochemistries, and psychosocial assessments. Analyses will proceed as more women complete the study.

Collaborators: Jerome Fleg, M.D., NIA; Michele Bellantoni, M.D., Robin Conwit, M.D., Christopher Earley, M.D., Ph.D., Johns Hopkins Bayview Medical Center; William Brown, M.D., Tufts University; Daniel Stashuk, Ph.D., University of Waterloo, Ontario, Canada; Benjamin Hurley, Ph.D., University of Maryland, College Park; Laura Talbot, RN, CS, Ed.D., Ph.D., Johns Hopkins University; William Palosky, Ph.D., NASA; S. Mitchell Harman, M.D., Ph.D., Kronos Research Foundation, Phoenix, Arizona.



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Biography: Dr. Andres received his medical degree and residency training at Southwestern Medical College in Dallas. His postdoctoral fellowship began at Johns Hopkins in 1950 and he has maintained his academic appointment there as Professor of Medicine. He came to the NIH in 1962 to be the Clinical Director and Assistant Chief of the Gerontology Unit in Baltimore, initially when it was in the National Heart Institute, then in the National Institute of Child Health and Human Development, and now in NIA. Dr. Andres is past president of the Gerontological Society, a member of the American Society of Clinical Investigation and the Association of American Physicians, and the recipient of the Kleemeier Award, the Allied-Signal Achievement Award in Aging, the Enrico Greppi Gerontology Prize (Italy), the Rank Prize in Nutrition, and the Albert Renold Award of the American Diabetes Association.

Keywords:

diabetes
body composition
insulin
nutrition

Recent Publications:

Iwao S, et al. *Obes Res* 2001; 9(11): 685-695.

Iwao N, et al. *Aging* (Milano) 2000; 12(6): 461-469.

Iwao S, et al. *J Am Geriatr Soc* 2000; 48(7): 788-794.

Andres R, *Obes Res* 1999; 7(4): 417-419.

Sorkin JD, et al. *Epidemiol Rev* 1999; 21(4): 247-260.

Sorkin JD, et al. *Am J Epidemiol* 1999; 150(9): 969-977.

Glucose/Insulin Homeostasis and Aging: Several diverse research approaches are in progress in order to understand the role of aging in the progressive changes occurring in this complex metabolic axis. (1) Factors influencing the age changes in fasting glucose and in glucose tolerance have been shown to be obesity and a central pattern of fat deposition, physical inactivity, dietary variables, physical inactivity, and a number of distinct diseases and medications associated with aging. (2) The glucose clamp technique (hyperglycemia and hyperinsulinemic/euglycemic) was devised in order to quantify, in intact humans, (a) beta cell responsiveness to glucose and to incretins (GIP and GLP) and (b) sensitivity of body tissues to insulin. (3) The implications of elevated fasting glucose and glucose tolerance values for the development of the characteristic complications of diabetes are being quantified in participants in the Baltimore Longitudinal Study of Aging. The development of coronary artery disease, the overt diabetic state, and all-cause mortality are under study. (4) The diagnostic cutpoints for the “impaired” state and for diabetes are being carefully examined with reference to the possibility that an adjustment might be required for older men and women. Data from the BLSA, the Rancho Bernardo Study, and the National Health and Nutrition Examination Survey III are being collated.

Collaborators: Dr. Dariush Elahi, Massachusetts General Hospital; Dr. Elizabeth Barrett-Connor, University of California, San Diego; Dr. Katherine Flegal, National Center for Health Statistics; Dr. John Sorkin, University of Maryland; Dr. Josephine Egan, Diabetes Section, LCI, NIA; Dr. Ballentine Carter, Johns Hopkins; Dr. Judith Hallfrisch, Beltsville Human Nutrition Research Center, USDA; Dr. Katherine Tucker, Human Nutrition Research Center, Tufts University.

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Biography: Richard Spencer obtained his Ph.D. in Medical Physics from the Massachusetts Institute of Technology (MIT) in 1987, working with Professor Joanne Ingwall at the NMR Laboratory for Physiological Chemistry of Harvard Medical School, and his M.D. from Harvard Medical School in 1988. He was a postdoctoral fellow with Professor Robert Griffin at the Francis Bitter National Magnet Laboratory of MIT before joining the NIH. Dr. Spencer joined the National Institute on Aging in 1991, as Chief of the Nuclear Magnetic Resonance Unit. He completed medical residency training at Johns Hopkins Bayview Medical Center in Baltimore. He is a Diplomate of the American Board of Internal Medicine and an Associate Professor of Medicine at Johns Hopkins Medical School in Baltimore, Maryland.

Keywords:

magnetic resonance
imaging and spectroscopy
heart
cartilage
muscle

Recent Publications:

Ellis SJ, et al. *Magn Reson Med* 2001; 46(4): 819-826.

Potter K, et al. *Arthritis Rheum* 2001, 44(4): 846-855.

Petersen EF, et al. *Magn Reson Med* 2000, 44(3): 367-372.

Horská A, et al. *Am J Physiol Endocrinol Metab* 2000; 279(2): E333-E339.

Nuclear Magnetic Resonance Unit: The interests of the Nuclear Magnetic Resonance (NMR) Unit are primarily in imaging (MRI) and metabolic studies of three-dimensional cartilage grown from chondrocytes in culture with particular emphasis on biological response modifiers, and spectroscopic studies of cardiac and muscle metabolism under a variety of pharmacologic and physiologic conditions. Methodology development in magnetic resonance imaging and spectroscopy is also ongoing.

A Bioreactor System for Magnetic Resonance Microimaging and Spectroscopy of Chondrocytes and Neocartilage: Osteoarthritis is the leading cause of joint pathology in the older population. One approach to control this disease is the use of chondrocyte transplantation. Accordingly, we have begun a detailed exploration of cartilage growth and development in a hollow fiber bioreactor specially designed for NMR studies. This system permits cells and the three-dimensional matrix which they elaborate to be studied longitudinally for several weeks in a non-invasive manner. Ultimately, we hope to define appropriate conditions for neocartilage development in osteoarthritic joints *in vivo*. In addition, our work may aid in the development of tissue engineering protocols for cartilage tissue suitable for transplantation.

In cartilage developing from whole chick sterna, we have investigated the correlation between histology and NMR microimages. NMRI revealed the development of stromal layers between growth units of neocartilage centered about each hollow fiber. Density images show decreased mobile

water content in these layers. Just outside the fiber walls, we find high proton density with relatively low mobility. Mobility increases with distance from the hollow fibers within the growth units, corresponding to differences in cell size and density. In magnetization transfer contrast images, we find that the lowest k_m values correspond to areas of high proteoglycan concentrations. These are prevalent in the mid-regions of the growth units. In contrast, the stromal layers and the regions around the fibers which are relatively proteoglycan-poor show the highest k_m values, potentially indicating greater collagen-water interactions.

We are also using ^{31}P NMR to gain insight into metabolic adaptations as chondrocytes mature. We have been able to establish the presence of phosphocreatine in this system, and have demonstrated a decrease in intracellular pH during early development of the tissue. This is consistent with the known tendency for developing chondrocyte cartilage systems to become increasingly dependent on anaerobic metabolism. We have also found indirect evidence for a premineralization state of the tissue, characterized by a decrease in phosphate mobility.

In addition, we are performing detailed studies of the correlations between biophysical properties of neocartilage as assessed by MRI, mechanical strength and tissue biochemistry in systems undergoing matrix degradation by enzymatic treatment. Current results indicate the ability of noninvasive MRI studies to predict the mechanical and biochemical properties of the tissue. Further studies are underway examining the effects of ibuprofen, acetaminophen and aspirin on biochemical and biophysical properties of developing tissue. These studies have particular relevance to the clinical scenario of cartilage regeneration *in situ*.

Collaborators: Maurizio Capogrossi, M.D., IDI-IRCCS, Rome; Mark Talan M.D., Ph.D., and Edward Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Walter Horton, Ph.D., Northeast Ohio University College of Medicine; Periannan Kuppusamy, Ph.D., Division of Cardiology, Johns Hopkins University School of Medicine.

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The **Laboratory of Epidemiology, Demography, and Biometry (LEDB)** conducts research on aging and age-associated diseases and conditions using population-based epidemiologic and biometric methods. Laboratory staff work collaboratively both within and among four groups: the **Epidemiology and Demography Section**, the **Neuroepidemiology Unit**, the **Geriatric Epidemiology Section**, and the **Biometry Section** and with other NIA and outside investigators. The mission of LEDB is to elucidate the etiology of diseases of old age by combining epidemiologic data with information from other disciplines; evaluate the consistency of epidemiologic data with etiologic hypotheses developed either clinically or experimentally; and to provide the basis for developing and evaluating preventive procedures and public health practices. These general principles have guided a research agenda that emphasizes three important and interrelated areas: Physical Function and Disability, Cognitive Function and Dementia, and Age-associated Diseases and Conditions – including successful or effective aging. In each area, studies are influenced by results of analytic efforts of current LEDB-sponsored studies and by opportunities created by advances in biology. The **Epidemiology and Demography Section** plans and conducts studies on chronic diseases, functional status and disability in the older population. The **Neuroepidemiology Unit** conducts interdisciplinary research on the association of genetic, molecular, and behavioral factors in relation to brain disease in old age. The **Geriatric Epidemiology Section** carries out interdisciplinary studies of the association of molecular and genetic risk factors with health outcomes in old age, including discrete diseases, disability and mortality. The **Biometry Section** conducts research in the mathematical, statistical and numerical aspects of aging and health. This Section provides statistical consulting, computing, graphics, and data

management services to the other units within LEDB. Senior LEDB staff consult with other components within the IRP, NIA, other NIH Institutes, other Government agencies, and the private sector. LEDB research interests use data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE), the Women's Health and Aging Study (WHAS), the Honolulu-Asia Aging Study (HAAS), Health and Body Composition (Health ABC) Study; Veterans' Study of Memory in Aging (VSMA); the MacArthur Studies of Successful Aging, and other epidemiologic studies.

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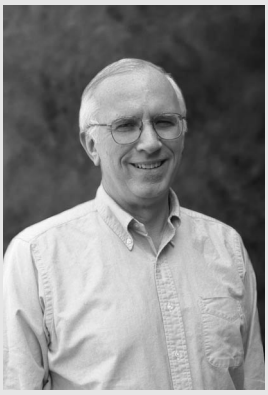

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Biography: Dr. Havlik received his master's degree in public health from The Johns Hopkins University, and his medical degree and training in internal medicine from Northwestern University Medical School. He first came to NIH as a research associate in the Epidemiology Branch, NHLBI. In 1980 he was appointed chief of NHLBI's clinical and genetic epidemiology section in the Epidemiology and Biometry Program. Prior to his NIA appointment in 1990, as NIA associate director, Dr. Havlik served as special assistant for biomedical applications at the National Center for Health Statistics, Centers for Disease Control. In the Laboratory of Epidemiology, Demography, and Biometry, he directs epidemiologic studies that look at aging processes and the onset of disease. In cooperation with other research groups, he develops projects to obtain data related to cancer, dementia, heart disease, and other major diseases of older persons.

Keywords:

epidemiology
chronic diseases
hypertension
dementia

Recent Publications:

Havlik R, et al. *Am J Cardiol* 2001; 87: 104-107.

Yancik R, et al. *JAMA* 2001; 285: 885-892.

Masaki KH, et al. *Neurology* 2000; 54: 1265-1272.

Havlik R, et al. *Neurology* 2000; 54: 1526-1529.

Cognitive Impairment and Dementia: The Veterans' Study of Memory in Aging (VSMA) is an investigation of early life head injury and its effect on late life dementia. The National Academy of Sciences Medical Follow-up Agency was identified as a source of WWII records on veterans and was established that a successful follow-up for dementia was possible. Noteworthy findings of this study are an almost 4-fold increased risk for Alzheimer's disease (AD) for men with a documented history of early life unconsciousness or amnesia for 24 hours or greater. Additional analyses of the possible effect on the findings of estimated intelligence from an entrance exam and early life head injury influencing late-life depression are underway. Veterans with head injury were more likely to report major depression in subsequent years and were more often currently depressed. The lifetime risk of depression increased with severity of the head injury. This research strategy fits with testing the more general hypothesis that the dementing process is likely a life-long affair. The Honolulu-Asia Aging Study (HAAS) is a study that provides a unique opportunity for investigating early life predictors for and protectors against late-life dementia. Building on the earlier published report of a relationship of mid-life blood pressure and poor cognitive function and the controversy over the exact role of cardiovascular risk factors in AD, an extension of these analyses of the relationship of mid-life blood pressure with neuritic plaques and tangles in brain autopsy specimens was suggested as another example of outcome predictors. The higher frequency of vascular

dementia in this population provided an opportunity to understand the interplay of various life-style factors in both major types of dementia. For example, the combination of vitamin E and C supplement use may act as a protective factor for vascular dementia.

Age-Associated Diseases and Conditions: Cardiovascular disease provides an example of the possibilities of collaborative research where individuals with similar interests but different types of expertise can come together to initiate and complete studies. As an example, the Laboratory of Cardiovascular Science has developed an indirect measure of vascular stiffness called Aortic Pulse Wave Velocity (APWV). This measurement had been used in the Baltimore Longitudinal Study of Aging and some correlates, such as systolic blood pressure and physical activity, were identified. The measurement of APWV was initiated in the Health ABC Study in order to test some of the previously identified correlates and to identify new ones. Active analyses of findings are underway. For example, an association between visceral fat and APWV has been identified. Another opportunity for population level assessment of this method occurred with the inception of the Activity Counseling Trial (ACT) by the National Heart, Lung, and Blood Institute. This was a randomized intervention trial of various educational strategies to boost physical activity over a 2-year period. The recommendation of adding the APWV measurement to ACT has provided an opportunity to investigate cross-sectional relationships as well as the effects of exercise on vascular stiffness. An inverse relationship between high density lipoprotein cholesterol and APWV has been reported. Another age-associated disease of great importance is cancer. Some years ago the valuable resource of the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Study was used to acquire additional information from hospital records on incident cancer cases at various sites in older persons. With case follow-up built into the NCI system, the accumulating mortality experience and any possible relationships with other co-morbidities can be assessed. For example, for colon cancer, the total co-morbidity or number of conditions was a significant predictor of mortality, even when age, gender and cancer stage were accounted for in the analysis. For breast cancer and prostate cancer comorbidity tends to minimize treatment options.

Genetic-Environmental Interactions: During the VSMA Study, there was a report about a possible interaction among APOE4, head injury, and AD. It was possible through DNA collection with buccal swabs to address this issue to a certain degree in the VSMA Study. There was only modest

evidence of an interaction, possibly because of small numbers. However, the lack of an interaction is more consistent with recent findings from other studies. In another study data from HAAS have shown that APOE4 predicts short-term incident AD. The HAAS is a valuable EDB resource to investigate interactions, such as with head injury and other environmental risk factors, although larger case numbers will be necessary. During the last few years, the potential of using unique populations to generate information on associations with candidate genes as well as with gene-searching activities has become quite evident. The AGES Family Diabetes Study, has completed the data collection phase in the HAAS. A number of siblings have been part of the HAAS cohort and a subset of the siblings manifested considerable evidence of glucose dysregulation. On this basis a supplement to HAAS was developed to investigate this aspect further. Analyses are being initiated. In the future there will be other studies that will have the potential to contribute toward the understanding of genetic influences on and genetic-environmental interaction with age-associated diseases and conditions. Besides the EDB-sponsored AGES Reykjavik (Iceland) Study, EDB will collaborate with laboratories at the GRC to implement a gene-searching study in Sardinia.

Collaborators: Dr. Edward Lakatta, NIA; Dr. Dennis Taub, NIA; Dr. David Schlessinger, NIA; Dr. Brenda Plassman, Duke University; Dr. Rosemary Yancik, NIA; Dr. Kamal Masaki, University of Hawaii; and Dr. Alexander Wilson, NHGRI.

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Biography: Dr. Guralnik received his M.D. from Jefferson Medical College in Philadelphia and his M.P.H. and Ph.D. from the School of Public Health, University of California, Berkeley. He practiced as a primary care and public health physician prior to his Ph.D. training. He is Board Certified in Public Health and General Preventive Medicine. Before coming to NIH he did research on predictors of healthy aging in the Human Population Laboratory Alameda County Study in Berkeley, California. He has been in the Laboratory of Epidemiology, Demography, and Biometry at the National Institute on Aging since 1985 and has been the Chief of the Epidemiology and Demography Section since 1991.

Keywords:

epidemiology
chronic diseases
disability
functional status

Recent Publications:

Guralnik JM, et al. *J Gerontol A Biol Sci Med Sci* 2000; 55: M221-M231.

Guralnik JM, et al. *Aging Clin Exp Res* 2000; 12: 65-76.

McDermott MM, et al. *Circulation* 2000; 101: 1007-1012.

Ferrucci L, et al. *J Am Geriatr Soc* 2000; 48: 1618-1625.

The **Epidemiology and Demography Section** plans and conducts epidemiologic studies of the risk factors for specific chronic diseases important in aging and pursues research on the consequences of disease, especially the effects of chronic disease on functional limitations, disability, and the ability to remain independent in the community. Assessing the roles of behavioral, psychosocial, and demographic risk factors in the development of disease and disability is also an important area of research. Particular attention has been focused on the development of mobility disability and how factors such as strength and balance, exercise, and measures of physical performance predict the loss of walking ability. Research interests have been pursued using data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE), the Women's Health and Aging Study (WHAS), and the Honolulu-Asia Aging Study.

Physical Activity and Exercise: A major research interest has been in examining the impact of physical activity and exercise on disability and other health outcomes in older people. Past work demonstrated the risk of incident disability related to sedentary lifestyle. Recent work using EPESE data has shown that active life expectancy, the number of years expected to be lived without disability, is strongly influenced by physical activity. We have also recently demonstrated that an active lifestyle is associated with both living to advanced old age and with dying with no major disability in the last year of life. Data from the WHAS have shown that many women with difficulty walking continue to walk for exercise while nearly half of the women without difficulty don't walk at all for exercise. The amount of walking for exercise done by older women is strongly

influenced by their level of disease and disability but many psychosocial variables also influence the amount of walking these women do. Recent findings indicate that even very modest amounts of walking are associated with lower rates of disability onset. A randomized clinical trial evaluating the impact of exercise in preventing disability in non-disabled but at-risk older persons is now being planned.

Assessment Methods: A number of research activities are directed at improving our ability to evaluate older persons in epidemiologic studies, including objective measures of physical performance, measures of exercise tolerance, and measures of muscle mass. Previous research that demonstrated that performance measures of functioning predict incident disability in previously non-disabled subjects has been replicated in several EPESE sites. Predictive equations developed from this work give risk estimates for disability onset so that sample size calculations for clinical trials of disability prevention may be made.

The Pathway from Disease to Disability: An important and ongoing area of research has been to develop an understanding of how the consequences of chronic diseases and the physiologic changes associated with aging cause important losses in functional status and affect the ability to remain independent in the community. A large amount of data collected in the WHAS and other studies provides the basis for empirical study of the steps in the causal chain of events in this pathway. A large research effort has gone into understanding muscle strength in older people and how it relates to functional limitations, disability and other outcomes. The impact on progression through the pathway of both specific conditions and co-occurring multiple conditions (co-morbidity) has been a long-standing area of emphasis in our research. A large effort has gone into identifying biochemical markers of subclinical diseases and frailty that are strongly prognostic of mortality and other adverse outcomes. Our previous work demonstrated increased risk of mortality associated with low serum albumin level and also a graded risk of mortality across the full spectrum of serum albumin values. Recent research assesses the impact of both low total cholesterol and HDL cholesterol on all-cause mortality and on cardiovascular and non-cardiovascular disease mortality.

Health Disparities: We have had a long-standing interest in the impact of social class on health and have demonstrated that educational status and income are powerful predictors of disability onset and mortality. We have also shown that active, or disability-free, life expectancy is considerably longer in persons with higher levels of education. Race also plays a role in the health of older persons although its influence, after adjustment for

education and income, has not been consistently demonstrated. Research has also been initiated on the impact of neighborhood characteristics on health outcomes.

Psychological Factors and Health: The influence of psychologic factors, particularly depression, in disease and disability outcomes continues to be a major topic of research. The 6-year longitudinal EPESE data permitted a classification of participants with depression into those who at follow-up 6 had new onset depression and those with chronic depression. Subsequent risk of cancer and heart disease was different for people with these categories of depression, with chronic depression being a risk factor for cancer and new onset depression in men being a risk factor for coronary heart disease. The importance of psychological factors and personality is also being explored in disabled women in the WHAS. We have identified a subset of the WHAS participants who have emotional vitality, defined on the basis of assessments of depression, happiness, personal mastery, and anxiety. A recent publication demonstrated that, even after adjustment for a number of indicators of disease and disability status, emotional vitality is protective of functional decline over a 3-year follow-up period.

Pain: Pain is a very common symptom in older persons and its impact on quality of life and disability has received insufficient study. Using extensive data in the WHAS on multiple pain sites we have begun to evaluate the extent of pain, the associations of pain with functioning, and the use of analgesics to treat pain.

Collaborators: Drs. Linda Fried, Judy Kasper, Karen Bandeen-Roche, Johns Hopkins Medical Institutions; Dr. Mary McDermott, Northwestern University School of Medicine; Drs. Marco Pahor, Brenda Penninx, Graziano Onder, Wake Forest University School of Medicine; Dr. Ann Shumway-Cook, University of Washington, Seattle; Drs. David Reuben and Teresa Seeman, UCLA; Dr. Stephanie Studenski, University of Kansas; Drs. David Curb and Kamal Masaki, University of Hawaii; Dr. Suzanne Leveille, Research and Training Institute of the Hebrew Rehabilitation Center for Aged and Harvard Medical School, Boston, Massachusetts; Dr. Jiska Cohen-Mansfield, Hebrew Home for the Aged, Rockville, MD and George Washington University School of Medicine; Drs. Kyriakos Markides and Siva Satish, University of Texas, Galveston; Dr. Dorit Carmelli, Stanford Research Institute; Dr. Luigi Ferrucci, Italian National Institute on Aging, Florence, Italy; Dr. Chiara Corti, University of Padua, Padua, Italy; Drs. Howard Bergman and Francois Beland, McGill University, Montreal, Canada; Dr. David Melzer, University of Cambridge, Cambridge, England; Dr. Sallie Lamb, Coventry University, England; Dr. Marja Jylhä, University of Tampere, Finland; Dr. Taina Rantanen, University of Jyväskylä, Finland.

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Biography: Dr. Harris received her M.D. degree from Albert Einstein College of Medicine, New York, New York in 1978. She trained in internal medicine at Montefiore Hospital, Bronx, New York and in geriatric medicine at Harvard University, Division on Aging, where she was a Kaiser Fellow in Geriatric Medicine. She obtained a M.S. in Epidemiology from Harvard School of Public Health and also has a M.S. in Human Nutrition from Columbia University College of Physician's and Surgeons. From Harvard, she joined the Office of Analysis and Epidemiology at the National Center for Health Statistics. Dr. Harris moved to the National Institute on Aging in 1991, where she is Chief of the Geriatric Epidemiology Section. Dr. Harris has developed the Geriatric Epidemiology Section to cover a broad range of topics ranging from molecular and genetic epidemiology and body composition to health disparities. The goal of this research is to identify new risk factors for disease and disability amenable to intervention.

Keywords:

molecular and genetic epidemiology
bioimaging
chronic disease
aging

Recent Publications:

Visser M, et al. *Pediatrics* 2001; 107: E13-E16.

Harris TB, et al. *Ann NY Acad Sci* 2000; 904: 462-473.

Taaffe D, et al. *J Gerontol A Biol Sci Med Sci* 2000; 55(12): M709-M715.

Resnick HE, et al.*Genet Epidemiol* 2000; 19(1): 52-63.

The role of the **Geriatric Epidemiology Section** is to integrate molecular and genetic epidemiology with interdisciplinary studies of functional outcomes, disease endpoints and mortality in older persons. This includes identification of novel risk factors and design of studies involving biomarkers, selected polymorphisms and exploration of gene/environment interactions. The Section has been particularly active in devising methods to integrate promising molecular or imaging techniques in ways that begin to explore the physiology underlying epidemiologic associations. The major areas of research include:

Health Studies in Relation to Weight and Body Composition: Despite the fact that overweight is well-accepted as a risk factor for disease, disability and death in younger populations, there remains controversy about the optimal level of weight in old age. This is further complicated by age-associated changes in body fat, bone and muscle and questions regarding the contribution of sarcopenia, or age-related muscle loss, to declines in aerobic capacity and function with age. The Geriatric Epidemiology Section initiated the Health, Aging and Body Composition Study (Health ABC) in 1996 to investigate these questions. The major study objective is to examine whether change in body composition, particularly loss of muscle, represents a common pathway by which multiple conditions contribute to disability. Since little was known about sarcopenia in an unselected population, the Health ABC population was selected as well-functioning and relatively health-stable, but at high risk of

health transitions secondary to age, race and gender characteristics. The Health ABC cohort consists of 3,075 black and white men and women aged 70-79 (46 percent of the women and 37 percent of the men enrolled are black) who initially reported no difficulty walking at least 1/4 mile and or up a flight of stairs. The major study outcome is report of new limitation in walking 1/4 mile or up stairs, complemented by assessment of performance on a 400-meter walk, quadriceps strength, and other objective functional tests. Morbidity and mortality are also assessed.

The study was designed around the hypothesis that factors affecting body composition and loss of muscle would be consistent across all four race/ sex groups and that factors in three key areas would modulate loss of muscle including: metabolic dysregulation, particularly inflammation or genetic factors; episodes of acute illness; and patterns of change in physical activity. A battery of detailed physiologic measurements and questionnaire material was developed to follow change over the 7-year period of examinations that is part of the study and that covers a period of rapid health transitions. All critical measures will be repeated during this time (see website: www.nih.gov/nia/research/intramural/edb/healthabc). We have established a large repository of specimens and continue to seek innovative ideas and collaborators for the use of these samples.

One important finding from this study is the characterization of the extent of fatty infiltration into muscle and the metabolic and functional correlates. The Geriatric Epidemiology Section has organized a series of studies to investigate this finding in more detail including collaborating with investigators who have a large library of full-body MRI scans to assess fatty infiltration by age, race and level of physical activity and molecular studies of muscle and fat tissue from several locations in the body.

Causes and Consequences of Inflammation in Diseases of Old Age: The focus of efforts in the Geriatric Epidemiology Section has been on the contribution of chronic low-level inflammation to health outcomes apart from cardiovascular disease, and to understanding what conditions and behaviors appear to be linked to low-level inflammation. A number of data sets have been used to explore the relationship of chronic low-level inflammation with health risks in old age.These efforts have involved studies of mortality, disability, cardiovascular disease, diabetes and glucose metabolism, smoking and pulmonary function, cognition, and weight and fat distribution. Visceral fat has been identified as the fat depot most consistently associated with higher levels of cytokines; however, fat infiltrating into muscle also appears to be associated with higher cytokines

as well. There is on-going analysis of these data to assess whether the poor health outcomes associated with elevated cytokines is due to direct effects of elevated cytokines or whether the elevated cytokines represent severity of the underlying condition and the condition ultimately is responsible for the increased health risk.

Assessing the Genetic Contribution to Diseases of Old Age: The Geriatric Epidemiology Section initiated and works collaboratively with the Neuroepidemiology Section on the Age, Gene/Environment Susceptibility (AGES) Study. This study, established collaboratively with the Icelandic Heart Association, consists of a follow-up examination of an established cohort of about 12,000 people in the birth cohorts of 1907-1935 previously examined in the Reykjavik Study. The AGES Study goals include: identification of genetic and other new risk factors for selected diseases and conditions including: atherosclerosis, cognitive impairment, dementia and subtypes (i.e. Alzheimer's disease), stroke, sarcopenia, obesity, osteoporosis, diabetes, and osteoarthritis; characterization of phenotypes for these diseases and conditions to study them in relation to genetic susceptibility, gene function and genetic/environmental contributions to disease; and identification of contributory molecular markers associated with these conditions including markers of cellular maintenance and repair, markers of oxidative stress, and immunologic and endocrine indicators.

The Geriatric Epidemiology Section has also carried out studies of selected polymorphisms pertinent to inflammation and body composition measures in nested case-control studies in the Health ABC Study and in other datasets developed for this purpose. Efforts have been made to broaden the application of emerging techniques for genomic and proteomic studies to populations by development of new methods in collaboration with laboratory-based investigators.

Collaborators: Eleanor Simonsick, Ph.D., Laboratory of Clinical Investigation, NIA; Lenore Launer, Ph.D., M.P.H, Neuroepidemiology Section, NIA; Dennis Taub, Ph.D., Laboratory of Immunology, NIA; Anne Newman, M.D., M.P.H., Lewis Kuller, M.D., Dr.P.H., Jane Cauley, Ph.D., Bret Goodpaster, Ph.D., University of Pittsburgh; Stephan Kritchevsky, Ph.D., Fran Tylavsky, Ph.D., Ron Shorr, M.D., University of Tennessee, Memphis; Steven Cummings, M.D., M.P.H., Michael Nevitt, Ph.D., Susan Rubin, M.S., Susan Averbach, M.S., Emily Kenyon, Ph.D., Thomas Lang,

Ph.D., Thomas Fuerst, Ph.D., Charles Peterfy, M.D., University of California, San Francisco; Russell Tracy, Ph.D., University of Vermont; Marjolein Visser, Ph.D., Free University, Amsterdam, Netherlands; Stefania Maggi, M.D., M.P.H., University of Padua, Padua, Italy; Mauro Zamboni, M.D., University of Verona, Verona, Italy; Dennis Taaffe, Ph.D., University of Brisbane, Australia; Luigi Ferrucci, M.D., Ph.D., IRCA, Florence, Italy; Dympna Gallagher, Ph.D., Columbia University College of Physicians and Surgeons, New York, New York; Helaine Resnick, Ph.D., Washington Hospital Center, Washington, D.C.; John Robbins, M.D., University of California, Davis; Teresa Seeman, Ph.D., David Reuben, M.D., University of California, Los Angeles; Harvey Cohen, M.D., Duke University; Vilmundur Gudnason, M.D., Ph.D., Palmi Jonsson, M.D., Gudmundur Thorgeirsson, M.D., Ph.D., Gunnar Sigurdsson, M.D., Ph.D., University of Iceland and Icelandic Heart Association.

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Biography: Dr. Brock received a B.S. in Mathematics in 1967 from Southern Methodist University. He received an M.S. in Statistics in 1969 and a Ph.D. in Statistics in 1971, also at Southern Methodist. He joined the Office of Statistical Methods at the National Center for Health Statistics as a Mathematical Statistician in 1971, conducting research in scientific sampling and survey methodology until 1974, at which time he joined the staff of the NCHS Division of Analysis as Mathematical Statistician, conducting research in analytic methodology for complex sample surveys. In 1976 Dr. Brock joined the staff of the NCHS Office of Statistical Research where he was named the Acting Chief of the Statistical Research Branch and directed a staff in research in variance estimation, analytic methods for complex survey data and small area estimation. In 1981 Dr. Brock was appointed Chief of the Biometry Section of the Laboratory of Epidemiology, Demography and Biometry at the National Institute on Aging, where he has directed a staff of statisticians and programmers in research on the mathematical, numerical and computing aspects of the epidemiology and demography of aging.

Keywords:

longitudinal studies
mathematical modeling
complex sample surveys

Recent Publications:

Brock DB, et al. *Encyclopedia of Epidemiologic Methods* 2000; 794-812.

Izmirlian G, et al. *Biometrics* 2000; 56: 244-248.

Izmirlian G, et al. *Stat Med* 2000; 19: 1577-1591.

Havlik R, et al. *Am J Cardiol* 2001; 87: 104-107.

Longitudinal Analysis and Modeling: Recent work in longitudinal data analysis involves development and application of models to study associations between changes over time in multiple outcomes. While most previous work has focused on a single outcome measure, chronic diseases often involve changes in more than one system, and thus multiple measures of disease severity may be used. If some common feature of the underlying pathology or the individual participant affects two different measures of disease severity, we would expect correlations between the individual trajectories. One approach to estimating these correlations is through the fitting of simultaneous random effects models. In one example this approach is being used to study simultaneous decline in physical and cognitive function in the HAAS. Multiple observations over time have been obtained using the Cognitive Abilities Screening Instrument (CASI) for measuring cognitive function and performance measures of physical function at Examinations IV, V, and VI. The hypothesis is that the association between decline in physical and cognitive function in this cohort is substantially stronger than would be concluded if models were fitted to separate outcomes. Additional work in longitudinal analysis is being done to study changes in arterial stiffness measures in response to educational exercise interventions in the NHLBI Activity Counseling Trial. Finally, work is being proposed to extend the use of order-restricted categorical data models to analyze change in physical function data from the EPESE in order to take into account floor and ceiling effects in the

functional measures and account for mortality among participants to eliminate possible survival paradoxes which can occur when survivor-only analyses are performed.

Estimation of Incidence and Prevalence of Dementia: The estimation of incidence and prevalence of dementia in the HAAS provides an appropriate example for the role of statistical methodology in the conduct of this important study. The nature of the HAAS as a community-based study of the prevalence and incidence of dementia and the substantial resources required to conduct clinical evaluations leading to a diagnosis of dementia led to the selection of subsamples of individuals from the cohort for neurologic and neuropsychological evaluation. Since not all cohort members were given clinical evaluations, cohort-level estimates of incidence and prevalence had to be based on up-weighting the data from the clinical evaluation subsamples to represent the entire cohort. This was accomplished jointly for prevalence and the first wave of incidence by the use of a model-based technique known as Mean Score Imputation to correct for missed cases, since only a relatively small group of men was evaluated at both waves of evaluation. As additional waves of clinical evaluation subsamples become available, additional development of the estimation technique will be required to account for design changes which were implemented in later stages of the HAAS follow-up evaluations. Similar considerations will apply to cognitive evaluation in the AGES study.

Analysis of Data from Complex Sample Surveys: Many of the data sets used in LEDB studies have been based on complex sample surveys. Examples include the NHANES and its epidemiologic follow-up, the New Haven and North Carolina EPESE sites, and the HAAS selection of subsamples for clinical evaluation. The complexities inherent in the designs of these studies, utilized primarily for the sake of efficiency and cost control in conducting the studies, introduced correlations among individual observations because of geographic clustering and complex post-sampling adjustments to survey estimators. This in turn has necessitated the use of adjustments in the estimators and especially in the variance estimators used in analyzing the resulting data. Recent efforts to analyze data on driving behaviors from the Asset and Health Dynamics in the Oldest Old (AHEAD) survey required the development of a jackknife-type estimator of variance for estimates of driving life expectancy based on a life table function constructed from responses to survey questions concerning risk factors for driving cessation among older adults. Other analyses involve the study of the lifetime risk of dementia from questions asked of the next of kin of a nationally representative sample of older decedents in the 1993 National Mortality Followback Survey.

Statistical Consultation: Recent examples of consultation activity include use of Poisson regression in the analysis of neuropathologic data on neuritic plaques and neurofibrillary tangles in the autopsy subsample from HAAS; power and sample size calculations for the neuropathologic substudy in the AGES; and design issues concerning a study on familial aspects of longevity in Trieste, Italy. Effort is underway to assist in the design of the Healthy Aging in Nationally Diverse Longitudinal Samples (HANDLS) study to extend it from a convenience sample to a population-based cohort study representative of the population living in West Baltimore.

Collaborators: Dr. Laurel A. Beckett, Biostatistics Division, University of California, Davis; Dr. Jon H. Lemke, Department of Biostatistics, University of Iowa; Dr. Richard J. Kryscio, Sanders-Brown Center on Aging, University of Kentucky; Dr. Douglas J. Lanska, Tomah VA Medical Center, Tomah, Wisconsin; Dr. Julia Bienias, Rush Institute for Healthy Aging, Chicago, Illinois; Dr. Michele Evans, Dr. Alan Zonderman, NIA.

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Biography: Dr. Launer received her Ph.D. in epidemiology and nutrition from Cornell University. From 1990 to 1999 she held academic appointments in the Netherlands (Erasmus University Medical School, Free University, National Institute for Public Health) where she collaborated in many epidemiologic studies of neurologic diseases including dementia and migraine headache. Dr. Launer joined NIA as Chief of the Neuroepidemiology Section in February 1999.

Keywords:

epidemiology
neurologic diseases
genetic and environmental risk factors

Recent Publications:

Geerlings MI, et al. *JAMA* 2001; 285(11): 1475-1481.

Kalmijn S, et al. *Arterioscler Thromb Vasc Biol* 2000; 20: 2255-2260.

Launer LJ, et al. *Neurology* 2000; 54(Suppl 5): S1-S8.

Launer LJ, et al.*Neurobiol Aging* 2000; 21: 49-55.

Studies in the **Neuroepidemiology Unit** focus on understanding the contribution of genetic, inflammatory, metabolic, vascular, and hormonal factors to sub-clinical and clinical outcomes in brain disease and investigating the links between brain disease and other common diseases of old age. Research is conducted using large epidemiologic studies, which allow us to test in the general population, hypotheses on risk/ protective factors and mechanisms identified at a more basic science level.

Vascular Factors and AD: Main research interests have focused on the role of vascular factors in brain disease. Genetic epidemiologic studies suggest the known mutations in amyloid processing, tau genes and alpha synucleins hypothesized to play a role in neurodegenerative processes, do not explain the great majority of dementia cases in the general population. These dementias are likely the result of an interaction between environmental factors and multiple genes that make small contributions to processes leading to neurodegeneration. The contribution of modifiable and genetic vascular factors to these dementias is not known. Vascular factors can contribute to neurodegeneration or lead to co-morbidity that increases the severity of dementia. Vascular factors may influence different stages of the dementing process. To this end, several studies have been conducted to examine the relation of vascular factors to different anatomical and functional markers of brain disease including: memory and executive domains of cognitive function; MRI measures of white matter lesions, (sub)-clinical stroke and regional (lobar and hippocampal) brain atrophy; clinical dementia and sub-types (AD and vascular dementia); and neuropathologic markers of AD. Studies are published or in progress to examine blood pressure, diabetes, smoking and lipids. The Honolulu Asia Aging Study (HAAS) has provided the basis for much of the research conducted on vascular factors and dementia. The HAAS is a prospective

population-based study of Japanese American men that was initiated in 1965 as a part of the Honolulu Heart Program (HHP). The original cohort consisted of 8,006 Japanese-American men living on Oahu and born 1900 through 1919. When the HAAS was initiated in 1991-1993 there were 4,426 survivors, and 3,734 (80 percent) completed the total examination.

Metabolic Risk Factors for Dementia: Steroidal hormones are hypothesized to modulate (improve) cognitive and affective behavior. There are few population-based studies of the association of steroidal hormones to these behaviors and they are mainly on women. We recently investigated the association of length of reproductive years (as a measure of exposure to endogeneous estrogen) and the risk for incident dementia in a large population-based cohort of women. We found, contrary to expectation, that a longer reproductive period was associated with an increased risk for incident dementia. This raises questions about the role of endogenous steroidal hormones. Investigations into the association of steroidal hormones and incident cognitive impairment and dementia are underway in the HAAS Japanese-American men. We are also investigating the role of thyroid hormones in cognitive function. Much is known about the effects of clinically low and high thyroid hormones, but there is little research on the effects on brain function of thyroid hormones in euthyroid women. This is being investigated in a series of studies on men and women.

Genetic Epidemiology of AD: Alzheimer's disease is a complex genetic disease meaning many genes contribute each with a small contribution. Studies are in progress to identify accurate phenotypes to let us better identify genes that regulate pathology in the pathways leading to dementia. We are also investigating the association of identified candidate genes and the risk for AD. This research is conducted in the context of the HAAS study and the newly initiated Age, Gene/Environment Susceptibility (AGES) study, which is conducted together with the Geriatric Epidemiology Section and in collaboration with the Icelandic Heart Association (IHA). The AGES examination is based on a well-defined cohort of 12,000 persons born between 1907-1935 that was established in 1967 by the IHA and followed by them as a part of the Reykjavik Study.

The Neuroepidemiology Unit has also carried out studies in the epidemiology of migraine headache, and is developing collaborations with laboratory scientists to bridge the gaps between our knowledge gained in epidemiologic studies with that gained through more basic research.

Collaborators: T. Harris, M.D., M.S., J. Guralnik, M.D., Ph.D., Laboratory of Epidemiology, Demography, and Biometry, NIA; K. Blennow, Ph.D., Gotenborg University, Sweden; M.M.B. Breteler, M.D., Ph.D., A. Hofman, M.D., Ph.D., Erasmus University Medical Centre, Netherlands; L. Farrer, Ph.D., Boston University, MA; M. Ferrari, M.D., Ph.D., M. van Buchem, M.D., Ph.D., Leiden University Medical Centre, Netherlands; R. Friedland, M.D., Case Western University, Ohio; S. Giampaoli, M.D., Institute of Health, Rome, Italy; V. Gudnason, P. Jonsson, M.D., G. Thorgeirsson, M.D., Ph.D., G. Sigurdsson, M.D., Ph.D., University of Iceland and Icelandic Heart Association, Iceland; M. Luster, Ph.D., NIOSH, W. Virginia; M. Mattson, Ph.D., P. Scheltens, M.D., Ph.D., Laboratory of Neurosciences, NIA.

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The **Laboratory of Genetics (LG)** includes a Human Genetics Unit, directed by David Schlessinger, a Human Genetics and Integrative Medicine Section, headed by Clair Francomano, a Transcription Remodeling and Regulation Unit directed by Weidong Wang, the Developmental Genomics Section under the direction of Minoru S.H. Ko, and a Gene Recovery and Analysis Unit headed by Ramaiah Nagaraja.

The interests of the Laboratory are based on the view that aging has genetic determinants as an integrated part of human development, with a profound dependence on the interplay of synthetic and degradative processes that are initiated in utero. Six major types of study are in progress:

1. Transitions between immortal and mortal cells, particularly at the level of large-scale regulatory phenomena at the level of chromatin. For example, the transition of immortal embryonic stem cells to mortal differentiating cells is a fundamental feature of the initiation of aging in metazoans. The genes specifically activated and repressed during such transitions are being studied in mice, by differential assays of gene expression in 3.5 days post coitum (dpc) mouse embryos and in developing embryonic stem cells (in the Developmental Genomics and Aging Section).

2. Cohorts of genes involved in the development of selected “nonrenewable” systems. For example, to understand and ultimately try to compensate for loss of cells and tissues during aging, skin appendage development is being studied. Studies start from human or mouse hereditary defects that have been attributed to single genes, such as the ectodysplasin-A involved in X-linked ectodermal dysplasia.

3. Mechanisms and treatment of heritable disorders of connective tissue. In addition to studies of lesions and their effects in skeletal dysplasias, systematic studies are determining gene cohorts involved in skeletal growth and development. The work is complemented by efforts to understand the potential roles of alternative and traditional medical practice in the care of persons with genetic conditions, with particular attention to the prevention or alleviation of chronic pain.

4. Nuclear organelles that determine large-scale chromatin remodeling events. Such events are involved in chromosome dynamics related to large-scale control of gene expression. The Transcription Remodeling and Regulation Unit is using a combination of approaches to isolate and characterize critical complexes, including the one that is modified to cause the Werner premature aging syndrome.

5. Genes involved in embryonic events that prefigure aging-related phenomena. For example, the Human Genetics Unit is involved in studies of overgrowth syndromes, in which the set point of size of tissues and organs is determined in fetal life; and in studies of premature ovarian failure, in which the aging phenomenon of early menopause is determined by an increased rate of follicular atresia during fetal life.

6. The genetics of aging-related complex conditions is being approached by interactive studies of the “founder” population in Sardinia. Initial phenotypes to be studied along with epidemiological factors include arterial stiffness, selected psychiatric/psychological traits. For this project investigators from Laboratory of Cardiovascular Science (Edward Lakatta and Angelo Scuteri), Laboratory of Personality and Cognition (Paul Costa, Antonio Terracciano, and Alan Zonderman), and Laboratory of Epidemiology, Demography, and Biometry (Tamara Harris and Richard Havlik) are working with Antonio Cao and Giuseppe Pilia, human geneticists at the University of Cagliari, Sardinia.

The laboratory is equipped with state-of-the-art resources for genomic approaches in the Gene Recovery and Analysis Unit, including large-insert clones and recovery methods, automated sequencing, and chromatin analysis techniques. Among the specific projects of the Unit is the detailed mapping and sequencing of the mouse t-complex, a region important for embryonic development and developmental genetics. In addition, the Laboratory houses the Mass Spectrometric Protein Analysis facility.

Among specific technological improvements that are being developed are techniques for the recovery of complete genes and YACs in circular, autonomously replicating clones (in the Gene Recovery Unit), and protocols to make and analyze high-quality cDNA libraries from very few cells from subregions of embryos (in the Developmental Genomics and Aging Section) and in collaborating with the Microarray Laboratory run by Kevin Becker (see Research Resources Branch) to develop gene expression profiling with microarrays of the cNDAs. The laboratory also benefits from joint efforts with other groups and resource providers both within NIA and at a number of extramural sites in the United States and abroad.

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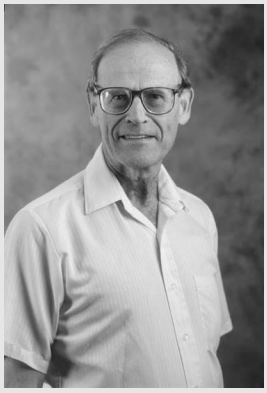

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Biography: Dr. Schlessinger received his Ph.D. from Harvard University in 1960. Following postdoctoral training at the Pasteur Institute in Paris, he joined Washington University in St. Louis, where he served as Professor of Molecular Microbiology, Genetics, and Microbiology in Medicine until his move to NIA in September, 1997. He has contributed both to microbial and human genome studies. He has served as President of the American Society for Microbiology in 1995, and as the Director of the Human Genome Center at Washington University from 1987-97. During his tenure as Center director, he oversaw the development of the X chromosome map and of much related technology, with the concomitant finding of a number of disease genes. He is currently a councillor of the Human Genome Organization (HUGO) International, and President, HUGO Americas.

Keywords:

X chromosome
gigantism/overgrowth syndromes
ectodermal dysplasia
premature ovarian failure

Recent Publications:

Crisponi L, et al. *Nat Genet* 2001; 27(2): 159-166.

Schlessinger D, et al. *Mech Ageing Dev* 2001; 122(14): 1537-1553.

Srivastava AK, et al. *Hum Mol Genet* 2001; 10(26): 2973-2981.

Cocchia M, et al. *Nucleic Acids Res* 2000; 28(17): E81.

Cocchia M, et al. *Genomics* 2000; 68(3): 305-312.

Human Genetics Unit: The program is designed to study embryonic and developmental events critical for the aging of specialized mammalian cells and concomitant aging-related phenomena.

1. Studies at the level of gene regulation in chromatin. Projects are designed to understand tissue- and developmentally-restricted expression of the genes in which mutation causes the inherited conditions Simpson-Golabi-Behmel Syndrome (SGBS) or Anhidrotic Ectodermal Dysplasia (EDA) (see below), or placental-specific expression (PLAC1). Promoter and enhancer element functions are being analyzed in those instances. The regulatory processes involve features of chromatin; analyses of open and closed chromatin are projected for the genes recovered in chromatin form in artificial chromosomes.

2. Cohorts of genes involved in selected processes, using a “genome approach” to developmental phenomena. The approach starts from human inherited conditions and relevant embryological studies in mouse models (where sets of genes from embryonic stages can be easily mapped in the genome and localized in sections, and knockout technologies are available). Examples include:

Premature ovarian failure. A set of translocation breakpoints in a “critical region of the X chromosome” are associated with POF. We are analyzing the breakpoints to look for genes or structural features in the chromosomal

DNA that can limit ovarian function. In related work on POF associated with eyelid dysplasia (BPES, the blepharophimosis-ptosis-epicanthus inversus syndrome), we have identified a “winged helix” transcription factor, FOXL2, that is mutated to cause both the eyelid and ovarian follicle defects. In correlated developmental work, systematic studies are beginning of gene cohorts specifically expressed during the development of ovarian follicles, and the target genes controlled by FOXL2.

Simpson-Golabi-Behmel syndrome (SGBS). Gigantism and overgrowth, particularly of mesoderm-derived tissues and organs, results from mutational lesions in a matrix glycoprotein, glypican 3. The speculative model for the etiology of the disease sees the determination of the set point for organ size as based on a system interacting with IGF2. A mouse model has strongly supported the existence of the putative growth-regulatory system, and studies are ongoing to reveal additional genes involved in gigantism.

X-linked anhidrotic ectodermal dysplasia (EDA). The gene provides an entree to an embyonic branch point that leads to teeth, hair follicles, and sweat glands. The Tabby mouse has been shown to be an experimental model for the human condition, and interacting genes can be found both by genomic approaches and by genetic studies of some of the other 170 inherited ectodermal dysplasias. Transgenic Tabby animals containing various isoforms of the EDA protein are revealing both the capacity of isoforms to initiate or maintain the restoration of skin appendages, and the other genes with which EDA interacts.

The projected work will depend on the Gene Recovery and Analysis Unit and collaborating groups, both for the developmental analysis of gene cohorts and for studies of physiology in aging populations with the aim of facilitating long-term patient benefit. The genetic potential provided from the Sardinia population provides an increasingly promising resource for genetic risk assessment and the determination of critical genes involved in aging-related conditions.

Collaborators: Professor J.M. Cantu, University of Guadalajara Medical School; Dr. Michele D’Urso, International Institute of Genetics and Biophysics, Naples; Professor Raj Thakker, M.D., Royal Postgraduate Medical School, London; Professor Antonino Forabosco, University of Modena; Dr. Giuseppe Pilia, Italian Research Council, Cagliari; Dr. Juha Kere, University of Helsinki; Dr. Anand Srivastava, Greenwood Genetics Center.

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Biography: Dr. Clair A. Francomano is a clinical and molecular geneticist whose research interests focus on applications of the Human Genome Project to molecular analysis of human disease. Her laboratory efforts are directed toward molecular aspects of the heritable disorders of connective tissue and skeletal dysplasias. Dr. Francomano received her M.D. degree from Johns Hopkins University in 1980. Her post-graduate training included a residency in Internal Medicine and Fellowship in Pediatric and Medical Genetics, both at Johns Hopkins. She joined the Johns Hopkins University School of Medicine faculty in 1984, in the Departments of Medicine and Pediatrics. In 1994 she was recruited to the National Human Genome Research Institute where she served as Chief of the Molecular Genetics Branch and Clinical Director until 2001. She has been with NIA since March 2001 as a Senior Investigator and Chief of the Human Genetics and Integrative Medicine Section in the Laboratory of Genetics. Dr. Francomano and her group will continue their clinical and molecular studies in genetic diseases of connective tissue and management of pain in those disorders.

Keywords:

skeleton
connective tissue
genomics
musculoskeletal pain

Recent Publications:

Iwata T, et al. *Hum Mol Genet* 2001; 10(12): 1255-1264.

King LM, et al. *Genomics* 2001; 71(2): 163-173.

Rose PS, et al. *Spine* 2001; 26(4): 403-409.

Ho NC, et al. *J Bone Miner Res* 2000; 15(11): 2095-2122.

The **Heritable Disorders of Connective Tissue** are a heterogeneous group of conditions affecting multiple organ systems, including skeleton, skin and vasculature. While rapid advances have been made in recent years toward understanding the genes underlying many of these disorders, genes responsible for many others remain to be found. In addition, much remains to be done if we are to understand the pathogenesis of those disorders for which genes are already known, the relationship between genotype and phenotype, and additional genes that act to modify the phenotype of known mutations. Moreover, the availability of molecular markers for many of these conditions facilitates prenatal or presymptomatic diagnosis, raising multiple ethical and social issues that merit exploration.

Specific **Skeletal Dysplasias** under investigation in the laboratory include the Ellis-van Creveld syndrome and Cartilage-Hair Hypoplasia, two recessive phenotypes common among the Old Order Amish. The relationships between specific mutations and phenotype are being explored in the type II collagenopathies and Marfan syndrome, a connective tissue disorder caused by mutations in the fibrillin gene on chromosome 15.

A major effort is underway to characterize mutations in the gene encoding **Fibroblast Growth Factor Receptor 3** (FGFR3) and to understand the biochemical pathways leading to specific FGFR3-related phenotypes, including achondroplasia, hypochondroplasia and thanatophoric dysplasia.

Recent studies have found highly specific FGFR3 mutations in a previously unrecognized phenotype of severe skeletal dysplasia with acanthosis nigricans and mental retardation.

We have embarked upon a major project designed to identify and map genes involved in skeletal growth and development. It is anticipated that genes and gene expression data derived through this effort will accelerate our understanding of skeletal differentiation and development, as well as the pathogenesis of rare Mendelian and more common complex disorders of skeletal growth and aging bones.

The section is also involved in several projects aimed at understanding the potential roles of alternative and complementary medical practices in the care of persons with genetic conditions. Many persons with **Hereditary Connective Tissue Disorders** (HDCT) suffer from chronic musculoskeletal pain. We are designing studies aimed at understanding whether there are fundamental differences in the neuro-biology of patients with HDCT that contribute to chronic pain. Interventions designed to ameliorate chronic pain in this population include mindfulness-based stress reduction in the Ehlers-Danlos population and “dry needling” of myofacial trigger points in patients with several different disorders of connective tissue. In collaboration with Dr. Helene Langevin of the University of Vermont, we are attempting to understand the role of connective tissue in the mechanism of acupuncture, and to understand whether variations of connective tissue seen in the HDCT influence the efficacy of acupuncture.

Collaborators: Dr. Michael Ain, Professor, Orthopedic Surgery, Johns Hopkins University School of Medicine; Dr. Benjamin Carson, Professor, Neurosurgery, Johns Hopkins University School of Medicine; Dr. Harry C. Dietz, Professor, Pediatrics and Genetics, Johns Hopkins University School of Medicine; Dr. Linda Fried, Professor, Medicine, Johns Hopkins University School of Medicine; Dr. Jacqeline Hecht, Professor, Pediatrics, University of Texas Houston; Dr. Robert Hotchkiss, Chair, Hand Surgery, Hospital for Special Surgery, New York; Dr. Mark Mattson, Chief, Laboratory of Neurosciences, National Institute on Aging; Dr. Daniele Rigamonti, Professor, Neurosurgery, Johns Hopkins University School of Medicine; Dr. Paul Sponsellar, Professor, Ortbopedic Surgery, Johns Hopkins University School of Medicine.

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Biography: Dr. Wang was trained as a biochemist and a molecular biologist at both UCLA, where he obtained his Ph.D., and Stanford University, where he worked as a postdoctoral fellow. His research has focused on the regulation of mammalian gene expression at the chromatin level. He has purified to homogeneity one of the first ATP-dependent chromatin-remodeling complexes in mammals, and has subsequently cloned all the subunits within one complex. His current projects include characterization of novel chromatin-remodeling complexes involved in human ATRX syndrome (X-linked mental retardation and α-thalassemia), DNA methyltransferase complexes involved in ICF syndrome (immunodeficiency, centromere instability and facial abnormally), as well as helicase complexes involved in the Werner premature aging syndrome, Bloom syndrome, Fanconi Anemia, and Rothmund-Thompson syndrome.

Keywords:

chromatin-remodeling
DNA methylation
SWI/SNF
helicase
genome instability
cancer

Recent Publications:

Bochar DA, et al. *Proc Natl Acad Sci USA* 2000; 97: 1038-1043.

Bochar DA, et al. *Cell* 2000; 102: 257-265.

Nie Z, et al. *Mol Cell Biol* 2000; 20(23): 8879-8888.

Xue Y, et al. *Proc Natl Acad Sci USA* 2000; 97: 13015-13020.

Research Description: Recently, multiprotein complexes have been implicated in the regulation or modulation of many cellular processes. Often, one protein can be discovered in several complexes, with each complex performing its unique function. Thus, the biological functions of a given protein can be understood only when the consequences of its association in complexes are defined. The Transcription Regulation and Remodeling Unit studies selected nuclear regulatory complexes.

In the eucaryotic nucleus, the chromatin structures that allow efficient storage of genetic information also tend to render the DNA inaccessible to metabolizing enzymes. The repressive chromatin structure must be remodeled to allow transcription and other metabolic reactions to occur. Chromatin-remodeling multiprotein complexes are critically involved in processes that include transcription, replication, chromatin assembly, and chromosome condensation. Furthermore, multiple human diseases, including several types of cancer, are caused by mutations in remodeling complexes; and aging in several lower species (and in several human disorders with features of premature aging) can be modulated by alterations in remodeling enzymes. Our Unit aims to discover novel chromatin-remodeling molecules and investigate their composition and mechanism of action. We have taken a biochemical approach to defining targeted complexes, starting with the development of a highly efficient immunopurification protocol to isolate the endogenous complexes from mammalian nuclear extracts in highly purified form. We have focused on studies of two families of multiprotein complexes involved in DNA expression and genome stability, in two corresponding projects:

Project I. Chromatin-remodeling Complexes that Participate in Gene Regulation

1. Mammalian SWI/SNF-Related Chromatin-Remodeling Complexes: The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. In mammals, the SWI/SNF-related complexes is involved not only in gene regulation, but also in targeting of HIV integration, cell cycle regulation, and in tumor suppression by interacting with Rb protein. Mutation of the hSNF5 subunit has been shown to be a cause for pediatric rhabdomyosarcoma. We have completely purified several distinct mammalian SWI/SNF-related complexes. By microsequencing, we have cloned all subunits from two major complexes of human KB cells. We have recently isolated a novel complex with tissue-restricted distribution and are in the process of characterizing it. Identification and subsequent characterization of these complexes is critical to understand their roles in gene regulation and other cellular processes.

2. NURD, a Novel Complex with both ATP-dependent Chromatin-remodeling and Histone Deacetylase Activities: ATP-dependent remodeling complexes are known to facilitate transcriptional activation by opening chromatin structures for activators. We recently identified a new human complex, named NURD, which contains not only ATP-dependent nucleosome disruption activity, but also histone deacetylase activity that is usually associated with transcriptional repression. Our results suggest that ATP-dependent chromatin-remodeling can participate in transcriptional repression by assisting repressors to gain access to chromatin. One subunit of NURD was identified as MTA1, a metastasis-associated protein with a region similar to the nuclear receptor corepressor, N-CoR; and antibodies against NURD partially relieve transcriptional repression by thyroid hormone receptor.

3. Purification of DNA Methyltransferase Complexes: DNA methylation plays critical roles in gene silencing, genomic imprinting and X-chromosome inactivation. The establishment and maintainance of methylation patterns are essential for mammalian development and differentiation. Inactivation of any of the known DNA methyltransferases causes lethality in mice. Mutation of a DNA methyltransferase in humans causes ICF syndrome (immunodeficiency, centromere instability and facial anomaly). A fundamental question regarding DNA methyltransferases is how do they find their target sites? One hypothesis is that proteins that associate with methyltransferases may recruit them to

their sites. We are in the process of purifying the complexes containing methyltransferases and identifying their components. The study may provide important clues to how these methyltransferases are targeted.

4. Chromatin Remodeling in Disease: ATR-X Complex and its Mechanism: ATRX syndrome represents a combination of α-thalassemia, mental retardation, and multiple associated developmental abnormalities. The gene defective in ATRX has been localized to the X chromosome and recently cloned. The ATRX gene encodes a gene product containing a SWI2/SNF2-type DNA-dependent ATPase domain. Thus, it has been hypothesized that ATRX could function in an ATP-dependent chromatin-remodeling complex and participate in regulation of gene expression. We have now isolated an ATRX-containing complex and identified most of its components. ATRX fractionated as a complex of 1.5 Mda by gel-filtration chromatography. Several ATRX-associated subunits have been previously identified as transcription factors. This study will provide important clues to how this protein works in transcriptional regulation, and how its malfunction causes disease conditions.

Project II. RecQ DNA Helicase Complexes Involved in Genome Instability Syndromes

1. Purification of a Complex Containing WRN, the Helicase Involved in Werner's Premature Aging Disease: Many human helicases discovered to date are related to diseases, including Werner Syndrome (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, Bloom's Syndrome and ATR-X (α-thalassemia with X-linked mental) Syndrome. Many of the gene products have only been identified recently and their mechanisms of action are not known. We recently found that the gene product encoded by WRN is present in a high molecular weight complex in HeLa cells. We have now purified this complex and identified all of its subunits by microsequencing. We are now studying the functions of the WRN complex. Hopefully, this will lead to better understanding of the human aging process.

2. Purification of a Complex Containing BLM, the Helicase Involved in Bloom Syndrome: This disease resembles Werner syndrome in genomic instability and cancer predisposition; but the patients do not display premature aging conditions. The gene defective in this disease belongs to the same family of RecQ helicase as WRN. We have now purified a complex containing BLM and identified all its components. Its components are completely different from those in the WRN complex. We are in the process of characterizing its components and setting up assays to study its biochemical activity.

3. Purification of a Complex Involved in Rothmund-Thompson Syndrome: The patients of this disease also exhibit genome instability and have higher risk of cancer. The gene mutated in the disease belongs to the same RecQ helicase family as WRN and BLM. We have now partially purified this complex.

4. Purification of Complexes Involved in Fanconi Anemia: This disease is also a genome instability disease and the patients have higher risks to develop cancer. Genetic studies have identified 8 complementation groups for the disease. Among them, six genes have been cloned. However, these gene products show no sequence homology to known proteins in the database and they have not been reported to have any biochemical activity. We have now purified a complex containing 5 gene products of this disease. The complex also has several other subunits that could provide crucial information on how the complex functions.

Proteomics: Protein identification and analysis by mass spectrometry. HPLC-coupled Mass Spectrometry has become the most powerful tool in protein identification and post-translational modification studies. It requires at least 10-fold less material than previous methods for protein identification. We have used this technique to identify the subunits of NURD and WRN protein complexes. In collaboration with the Research Resources Branch, we have assisted in creating the Mass Spectrometry Unit of the Central Laboratory Services Unit. We are using the facility to identify new proteins important in gene regulation and aging.

Collaborators: Dr. En Li, Massachusetts General Hospital; Dr. Richard Gibbons and Doug Higgs, Oxford University; Dr. Jacques Cote, Laval University Cancer Research Center; Dr. Jiemin Wong and Jun Qin, Baylor College of Medicine; Dr. Xiao-Long Zhang, Smith-Kline Pharmaceuticals; Maureen Hoatlin and Hua Lu, Oregon Health and Sciences University; Hans Joenje, Free University, the Netherland.

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Biography: Dr. Ko received his M.D. degree in 1986 and his Ph.D. in 1991 from Keio University School of Medicine in Tokyo. He held positions as Researcher from 1988 to 1991 and as Group Leader from 1991 to 1992 at the Furusawa MorphoGene Project, ERATO, JST, Japan. In 1992, he moved to the United States as Assistant Professor at the Center for Molecular Medicine and Genetics, Wayne State University in Detroit, Michigan, where he was promoted to Associate Professor and received tenure in 1997. He joined the NIA in Fall of 1998 to establish the Developmental Genomics and Aging Section within the Laboratory of Genetics. In one earlier study, using a steroid hormone inducible gene, he demonstrated a stochastic component in the regulation of expression of individual genes at a single cell level. He has also developed three methods that aid in profiling systematic gene expression in specific cell types. These are: 1) PCR-based amplification of a complex mixture of cDNAs, which allows the analyses of a cohort of genes expressed in the small number of cells; 2) a way to construct a normalized cDNA library in which the abundance of individual cDNA species is equalized; and 3) an efficient PCR-based method for localizing mouse cDNAs or ESTs on the genetic map. His group has recently established a 15,000 unique gene collection in mouse and used it to establish the NIA 15k mouse developmental cDNA microarray, facilitating some of the approaches in his research program.

Keywords:

cDNA library
EST project
mouse cDNA microarray
cellular immortality and pluripotency
pre- and peri-implantation mouse development
stem cells

Recent Publications:

Tanaka TS, et al. *Proc Natl Acad Sci USA* 2000; 97: 9127-9132.

Ko MSH, et al. *Development* 2000; 127: 1737-1749.

Hudson TJ, et al. *Nature Genet* 2001; 29: 201-205.

Sano Y, et al. *Genome Res* 2001; 11: 1833-1841.

Ko MSH, et al. *Trends Biotechnol* 2001; 19: 511-518.

The long-term goal is to understand the fundamental mechanisms for the maintenance of self-renewal, immortality, and pluripotency of early mouse embryos and stem cells. Replicative senescence has been an important focus of aging research for many years, though studies have concentrated on the senescence of cells already committed to mortality; here we rather concentrate on the critical distinction between immortal early embryonic cells and mortal differentiating derivative cells. Studies utilize the potential of a systematic genomic approach - embryogenomics - to analyze global gene expression regulations. The approach includes the construction of cDNA libraries from a small number of cells followed by large-scale cDNA sequencing, in situ hybridization to mouse embryonic and fetal preparations, and simultaneous gene expression analyses by DNA chip/microarray technologies. We believe that such global studies will provide greater understanding of mechanisms that will aid in the adaptation of stem cells to replacement therapy for aging and dysfunctional cells and organs. We focus on three complementary research programs.

1. Mouse Embryonic cDNA Clones and Microarrays: A catalog of genes in the form of cDNA clones is the complement to the sequence of genomes, providing not only the confirmation of predicted gene structures, but also the materials for cDNA microarrays and for functional analyses or

proteomics. Primary means of achieving this goal have been expressed sequence tag (EST) projects, which essentially comprise single-pass sequencing of randomly picked cDNA clones. One major difficulty to construct a cDNA library from early embryonic materials is the scarcity of the starting materials. We have recently developed a novel design of linker-primer that allows one to amplify differentially long tracts (average 3.0 kb with size ranges of 1 - 7 kb) or short DNAs (average 1.5 kb with size ranges of 0.5 - 3 kb) from a complex mixture. The method allows one to generate cDNA libraries enriched for long transcripts without size selection of insert DNAs. All our recent cDNA libraries have been made by this new method, and thus, a significant fraction of these cDNA clones contain complete open reading frame ("full-length"). We have thus far generated ~140000 ESTs from early mouse embryos and mouse stem cells (http://lgsun.grc.nia.nih.gov/cDNA/cDNA.html).

To use these mouse cDNA clones for the cDNA microarray applications, we have selected ~15,000 unique mouse cDNA clones from ~52,000 cDNA clones and rearrayed them into 96-well microtiter plates. The clone set, called "NIA 15K Mouse cDNA Clone Set," consists of 2132 genes from E7.5 extraembryonic tissue cDNA library, 893 genes from E7.5 embryonic tissue cDNA library, 7692 genes from preimplantation cDNA libraries (unfertilized eggs, fertilized eggs, 2-cell, 4-cell, 8-cell, 16-cell embryos and blastocysts), and 4620 genes from E12.5 mesonephros and newborn ovary cDNA libraries. In May 2000, we distributed the clone set free-of-charge to 8 academic centers, all of which in return agreed to redistribute the clone set to at least 10 other research institutions at a nominal charge. The system has worked out well and more than 100 microarray facilities worldwide are using the NIA 15K Mouse cDNA Microarray (http://lgsun.grc.nia.nih.gov/cDNA/cDNA.html). We are currently working on the production of an additional 11,000 cDNA clones ("NIA 11K Mouse cDNA Clone Set").

2. Preimplantation Mouse Development: Preimplantation development is an important model system to study the pluripotency of mouse cells. Concerning the differentiation potential of cells, preimplantation development can be seen as a process in which totipotent stem cells (fertilized eggs) lose their totipotency. Preimplantation development also has many other interesting features as a biological system. First, it involves dynamic switching from a process governed by the activity of maternally stored RNA/proteins to a process governed by the genes of zygotic activation. Some oocyte mRNAs are translated, but fertilization triggers massive mRNA degradation. Transcription from the zygotic genome begins at the late one-cell to two-cell stage in mouse. Although it is

well established that this transition is regulated by a “zygotic clock,” it is not known what type(s) of genes is activated first or how genes are activated. Second, the first cell differentiation event in the mammalian development occurs in preimplantation embryos. The process, “compaction,” occurs at the 8- to 16-cell stage, when cells that were previously loosely associated begin to adhere in the tightly organized cell mass of the morula. This is the starting point for cell differentiation into Inner Cell Mass (ICM) (which eventually becomes the embryo) and Trophectoderm (which eventually becomes the placenta). Despite its importance, the molecular study of preimplantation development has been significantly delayed, mainly because of the scarcity of the materials for molecular biological/biochemical approaches).

In our previous work, we identified many genes that show stage-specific expression patterns during preimplantation mouse development. However, these genes have been identified by EST frequency, which is a relatively inaccurate and far from ideal way to do gene expression profiling. The cDNA microarray-based gene expression profiling will provide more reliable information. To this end, we have been working on a large-scale gene expression profiling of each stage of preimplantation mouse development using the NIA mouse 15K cDNA microarrays.

3. Embryonic and Somatic Stem Cells: Embryonic stem cells are derived from the inner cell mass (ICM) of the blastocyst and are pluripotent, i.e., give rise to all fetal tissues, including germ lines, *in vivo* and *in vitro*. The ES cells also have the capacity for “self-renewal,” i.e., undergoing an unlimited number of symmetrical divisions without differentiation. Thus, they are naturally immortalized cells with stable and normal karyotypes. Since the first establishment of mouse ES cell lines, these two features have been used to manipulate the mouse genome for the functional studies of genes. The embryonic germ (EG) cells that have similar characteristics have also been derived from mouse primordial germ cells. Recent establishment of human ES and EG cells increases excitement about the possibility of using these embryonic stem cells for therapeutic purposes. For such applications, it is paramount to understand how the ES cells maintain their pluripotency and self-renewal, and how the ES cells differentiate into specific cell lineages *in vitro*.

As a first step to delineate the pathways involved in pluripotency and self-renewal of mouse embryonic stem (ES) cells, we have been examining global changes of gene expression patterns in ES cells during differentiation, using the cDNA microarray containing ~15,000 distinct mouse genes.

In another approach, we have started from the question: Does any group of genes make stem cells stem cells? In another word, can we find a group of genes that makes committed cells become pluripotent and self-renewing embryonic stem cells? To gain some insight into this problem, we have obtained RNAs from a variety of stem cells in collaboration with experts in the field, and have done large-scale gene expression profiling with the NIA 15K mouse cDNA microarrays. We would like to emphasize that at present this microarray is well adapted to this experiment, because the array includes many genes that are expressed in early mouse embryos and stem cells. We have thus far conducted expression profiling of the following stem cells: Embryonic stem (ES) cells, Trophoblast stem (TS) cells, Mesenchymal stem (MS) cells, Neural stem (NS) cells, Hematopoietic stem (HS) cells and Embryonic germ (EG) cells. Initial goals are to identify genes that are commonly expressed in all the stem cells and genes that are uniquely expressed in individual stem cell systems.

Collaborators: Dr. Kuniya Abe, Kumamoto University, Japan; Dr. John Schimenti, Jackson Laboratories, Bar Harbor, ME; Dr. Janet Rossant, Mount Sinai Hospital, Toronto, Canada; Dr. Ryuzo Yanagimachi, University of Hawaii, HI., Dr. Roger Reeves, Johns Hopkins University, Baltimore, MD; Dr. Chen-Ming Fan, Carnegie Institution of Washington, Baltimore, MD; Dr. Hitoshi Niwa, Osaka University, Osaka, Japan; Dr. Keiko Ozato, NICHD, Bethesda, MD; Dr. Michael R. Kuehn, NCI, Bethesda, MD; Dr. Michael Q. Zhang, Cold Spring Harbor Laboratory, NY; Dr. Winston Hide, South African National Bioinformatics Institute, South Africa; Dr. S. K. Dey, University of Kansas Medical Center, KS; Dr. Takashi Yokota, The University of Tokyo, Japan; Dr. Akihiro Umezawa, Keio University, Japan; Dr. Gary Van Zant, University of Kentucky, KY; Dr. Angelo L. Vescovi, Institute For Stem Cell Research, Italy; Dr. Ken Boheler, Laboratory of Cardiovascular Science, NIA; Dr. Michael Seidman, Laboratory of Molecular Gerontology, NIA.

Laboratory of Immunology

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The interests of the **Laboratory of Immunology (LI)** cover a wide range of topics devoted to a greater understanding of the biological, biochemical, and molecular alterations in immune functions that occur within individuals during both normal and disease-associated aging processes. A common goal of these research programs is the elucidation of the age-related deficits in immune function that could be potentially targeted by various therapeutic strategies. Current research efforts include examining (1) a role for various cytokines, hormones, and chemokines in leukocyte trafficking, cellular activation, and apoptosis; (2) the biological and molecular mechanism of HIV-1 entry and propagation in Th/Tc subsets and mononuclear cells obtained from young and elderly individuals; (3) the preclinical and clinical development of immunologically-based protocols focusing on promoting cellular responses in elderly populations with the ultimate goal of improving the immune function of aged and cancer-bearing individuals; (4) the molecular examination of telomere length, telomerase activity, and the various factors and genes that appear to be differentially regulated during human lymphocyte development, differentiation, and activation; (5) identification and characterization of immunosuppressive factors associated with cancer-based immunosuppression; (6) defining various oncogenes and signaling/ cytoskeletal components involved in various signaling pathways within lymphocytes; (7) the development of protein-conjugate vaccines for *Streptococcus pneumoniae* for use in various immunoglobulin transgenic and knockout animal models as well as in the highly susceptible elderly populations; and (8) the process of generating the development of the B cell repertoire for antigen responses.

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Biography: Dr. Dennis D. Taub received his Ph.D. from the Department of Microbiology and Immunology at Temple University School of Medicine in Philadelphia in 1991. He subsequently entered the laboratory of Dr. Joost J. Oppenheim as a staff fellow at the National Cancer Institute in Frederick, Maryland. From 1994-1997, Dr. Taub headed the vaccine monitoring laboratory within the Clinical Services Program at the National Cancer Institute. In early 1997, he moved to the Laboratory of Immunology at the National Institute on Aging as the Chief of the Clinical Immunology Section and the Acting Chief of the Laboratory of Immunology.

Keywords:

chemokines
T cells
aging
HIV
Th1/Th2
immune senescence
inflammation
trafficking
G protein

Recent Publications:

Crow M, et al. *J Hematother Stem Cell Res* 2001; 10(1): 147-156.

Lillard JW Jr, et al. *J Immunol* 2001; 166(1): 162-169.

Kalehua AN, et al. *Gerontology* 2000; 46(3): 115-128.

Nilsson G, et al. *Immunology* 2000; 99(2): 314-319.

Alexandroff AB, et al. *Mol Immunol* 2000; 37(9): 515-526.

Chemokines, Aging, and Immune Responses: The recruitment of lymphocytes into inflammatory sites requires several activation events including endothelial cell activation by inflammatory cytokines, the expression of adhesion molecules, cellular adhesion, diapedesis, and migration via established chemotactic gradients. Over the past 10 years, members of the *chemokine* super family have been shown to induce adhesion, chemotaxis, activation, and degranulation of human and rodent leukocytes and lymphocytes both *in vitro* and *in vivo*. We are currently examining a role for chemokines in lymphocyte activation and as immunodjuvants in vaccine-based studies with hapten-carrier protein complexes. In addition, the laboratory is also examining the ability of various chemokines and other G-protein receptor ligands to modulate other T, B, and NK cell effector functions as well as antigen-presenting cell activities. Furthermore, studies examining the differential expression of various cytokines, chemokines and their cell surface receptors, post cellular activation via mitogens, hormones, lipids, and stress factors are also under investigation. As no cytokines or chemokines are ever alone within an inflammatory site, it is critical to determine how these various growth factors influence each other's signals and functions. We believe that a better understanding of the complexities of leukocyte extravasation and the mediators that induce cell trafficking and activation will greatly assist our ability to orchestrate, regulate, and control various pathological disease states associated with aging as well as our understanding of normal leukocyte trafficking.

A number of studies are currently underway examining leukocyte migration and signaling in response to various chemotactic stimuli including chemokine, complement components, bacterial-derived peptides, and several hormones. Using purified rodent, primate, and human immune cell subsets, a significant dampening of aged lymphocyte and mononuclear cell migration, adhesion, and chemokine receptor signaling was observed in response to ligand stimulation compared to younger control populations. The age-related changes that appear to play a role in this chemokine hyporesponsiveness include signaling defects through cell surface receptors, differences in cell surface receptor expression post cellular activation, and preferential expression or lack of expression of certain chemokine receptors on circulating immune subsets within an aged host. Studies using larger cohorts of elder donors are currently in progress examining chemokine signaling within young vs. aged immune populations as well as the influence of various growth factors at restoring chemokine receptor activity. As these age-related defects may play a significant role in the diminished capacity of elderly subjects to mediate vaccine and immune responses *in vivo*, we believe that the characterization of the chemokine response deficits within aged immune cells may provide some insight into possible interventional therapeutics which promote immune cell trafficking and boost immune and vaccine responses.

Differential HIV-Mediated Replication and Cell Death in Aged Immune Cells: The human immunodeficiency virus type 1 (HIV-1) is the etiological agent of the acquired immunodeficiency syndrome (AIDS) that develops in HIV-1-infected individuals of all ages after a long clinical latent period. HIV-1-infected elder individuals have a shorter AIDS-free period and shorter life expectancy than individuals aged 13-49 years.

Despite the extensive documentation on HIV-1 infectivity, replication within target cells, mechanism(s) of viral immunopathogenesis, and the development of AIDS in adults, no specific cellular- and/or molecular-based studies have been published to date examining any differential infectivity or propagation of HIV-1 within immune cells derived from elderly subjects or within HIV-1-infected elderly patients. Preliminary results from our laboratory have demonstrated significant differences in viral growth between young and aged mononuclear cells. Increased titers of virus were observed in HIV-1-infected aged mononuclear cells and lymphocytes compared to virally-infected cells from younger donors. We believe that aged lymphocytes may be less susceptible to HIV-1-mediated cell death and may serve as a reservoir promoting virion production. Given T cell phenotypic alterations that have been observed in various

chronic inflammatory disease states, we believe that a similar systemic phenotype change may occur in circulating T cells of elderly subjects making elder T cells more susceptible to HIV-1 disease. Based on these findings, we are examining various parameters of HIV-1-mediated signaling, replication, apoptosis, and immunopathogenesis using young and aged mononuclear cells, monocytes, and T lymphocytes. In addition, a clinical trial is being initiated using a cohort of age-, race- and gender-matched control and HIV-infected young and older subjects to assess the *in vivo* immune and physiological alterations associated with HIV infections in elderly patients. Such information should provide invaluable information on any age-related differences in AIDS pathogenesis.

Additional studies are underway examining the ability of various HIV-1 viral isolates, gp120 proteins, and chemokines to directly induce gene expression in young and old human lymphocytes and neuronal cells. We believe that active transcriptional signals through CD4 and/or chemokine receptor molecules are required for optimal HIV-1 infectivity and propagation as well as for normal lymphocyte adhesion and migration. Using differential display analysis and microarray gene filters and chips, we are examining the expression of known and unknown genes induced post chemokine receptor ligation or viral infection. We believe that the identification and examination of induced or suppressed genes will not only provide insight into HIV pathogenesis but may also elucidate the molecular mechanisms of inflammation and the various signaling defects observed in aged lymphocytes.

Role for Lipid Rafts and Cholesterol in the Maintenance of Chemokine Signaling: Chemokine receptors (CRs) have drawn much attention since their description as human immunodeficiency virus (HIV) co-receptors by several groups in 1996. Prior to that time, HIV tropism was defined as either macrophage (M)- or T cell (T)-tropic, which corresponded to non-syncytia- or syncytia-inducing viruses, respectively. Today, the classification of HIV tropism is defined by chemokine receptor usage of CCR5, CXCR4, or both receptors. Chemokine receptors are a family of seven transmembrane spanning G protein-coupled receptors (GPCRs) that are differentially expressed by a number of immune and non-immune cell populations. CCR5 has been shown to be palmitoylated and targeted to cholesterol-and sphingolipid-rich membrane microdomains termed lipid rafts. Lipid rafts is a broad term for the collection of membrane microdomains enriched in cholesterol, sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins, and acylated signaling molecules. Lipid rafts are believed to be important signaling platforms enriched in many signaling proteins, including but not limited to

src kinases, Gα subunit, H-Ras, LAT, and NOS. Signal transduction through the T and B cell receptors as well as the IgE receptor involves the recruitment of signaling assemblies to lipid rafts. CCR5 has been shown to be present in lipid rafts, colocalizing at the leading edge of migrating cells. This receptor has also recently been shown to be palmitoylated, which is one of the important modifications in lipid raft targeting of proteins. However, the role of cholesterol and these lipid rafts on T cell chemokine binding and signaling through CCR5 remains unknown. We found that cholesterol extraction by beta-cyclodextrin (BCD) significantly reduced the binding and signaling of MIP-1b using CCR5-expressing T cells. Reloading treated cells with cholesterol but not 4-cholesten-3-one, an oxidized form of cholesterol, restored MIP-1b binding to BCD-treated cells. Antibodies specific for distinct CCR5 epitopes lost their ability to bind to the cell surface after cholesterol extraction. Moreover, cells stained with fluorescently-labeled MIP-1b extensively co-localized with the GM1 lipid raft marker while using anti-CCR5 antibodies, the majority of CCR5 on these cells co-localized with CD59 and only partially with GM1 suggesting that active ligand binding facilitates receptor association with lipid rafts or that raft association promotes a higher affinity conformation of CCR5. Together, these data demonstrate that cholesterol and lipid rafts are important for the maintenance of the CCR5 conformation and are necessary for both the binding and function of this chemokine receptor. Similar studies were performed examining the role of cholesterol on CXCR4 function. We have determined that cholesterol extraction by beta-cyclodextrin (BCD) also inhibits the CXCR4 ligand, SDF-1a, binding to CXCR4 on human T cells. Intracellular calcium responses to SDF-1a, as well as receptor internalization, were similarly impaired in treated T cells. Loss in ligand binding appears to be due to conformational changes in CXCR4 and not increased sensitivity to internalization. Reloading cholesterol effectively restored SDF-1a binding. These data, along with microscopic evidence that chemokines only bind receptors present within lipid rafts, strongly suggest that cholesterol is essential for chemokine receptor function and conformational integrity within lipid rafts.

Clinical and Preclinical Vaccine Development: The Clinical Immunology Section is also continuing its involvement in the preclinical and clinical development of immunologically based protocols focusing on promoting T-cell responses in elderly patients. Peripheral blood leukocytes obtained from normal healthy volunteers and/or elderly patients treated with various human hormones such as growth hormone (GH), prolactin (PRL), and DHEA have been examined for alterations in innate immune function and leukocyte trafficking. In addition, some clinical trials

examining the *in vivo* immunoadjuvant effects of PRL and GM-CSF in elderly patients are currently being planned. Preclinical studies from this laboratory have already revealed that GH, PRL, DHEA, retinoids, or GM-CSF provide costimulatory signals during T cell activation both *in vitro* and *in vivo*. Additional studies examining the ability of various cytokines, receptor antagonists, and signal transduction inhibitors to facilitate immune tolerance have been performed with the hope of implementing such methodology in trials involving bone marrow transplantation. We believe that additional immunological research on cytokine- and hormone-immune cell interactions may provide insight into the various homeostatic mechanisms that control immunocompetence during aging and cancer.

Collaborators: Nicholas Lukacs, Ph.D., Steven Kunkel, Ph.D., University of Michigan; Richard Horuk, Ph.D., Berlex Pharmaceuticals; Milan Fialo, M.D., UCLA; Francis Ruscetti, Ph.D., William Murphy, Ph.D., National Cancer Institute, NIH; James Lillard, Ph.D., University of Alabama at Birmingham; Rachel Caspi and Robert Nussenblatt, M.D., National Eye Institute, NIH; Barbara Webb, Ph.D., University of Florida; Bruce Blazer, M.D., University of Minnesota; John Cambier, Ph.D., National Jewish Center; Gunner Nilsson, Ph.D., Uppsala University, Sweden; Hermann Schlvesener, Ph.D., University of Tuebingen, Germany; Nancy Belman, Ph.D. and Robert Klein, Ph.D., University of Kansas Medical Center.

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The Regulation of Growth Fraction in Tumor Cells: The vast majority of solid tumors have a very low growth fraction at the time they become clinically evident, usually in the range of 3-7%. When the tumor is treated, the growth fraction increases in an effort to maintain the tumor cell mass. This is reminiscent of the organization of most organ systems. Resting bone marrow stem cells are recruited into cycle under the influence of a myelotoxic stimulus. Surgical removal of a portion of the liver stimulates the recruitment of hepatocytes into the cell cycle to replace the removed tissue. Other examples could also be cited. What is of interest to us is how a tumor cell, with its many genetic abnormalities that tend to promote proliferation, is pulled out of the cell cycle in the first place. Some gene product that is working in the resting tumor cells has managed to antagonize all the oncogene mutations and missing or malfunctioning tumor suppressor gene products and stop the cell from dividing; and it does this reversibly. When the tumor perceives an attack that reduces its volume, cells can be recruited back into the cell cycle. We are separating fresh lymphoma specimens into dividing and nondividing populations, isolating cDNA, and using microarray techniques, characterizing genes that are expressed in resting cells but not in dividing cells. Such messages will be isolated, their genes identified, and then the message will be introduced into dividing cells to look for growth arrest.

Tumor-induced Immunosuppression: We initially observed, and it has been widely reproduced, that T cells from tumor-bearing hosts are defective in their signalling in response to antigen and in their function. A variety of defects are noted including defective nuclear translocation of the p65 NF-κB transcription factor, shortened half-lives for a number of cellular proteins such as TCR-ζ chain and signalling kinases of the src family, among others, and a deviation of the cytokine production profile toward Th2 cytokines (IL-4, IL-10) and away from Th1 cytokines (interferon-γ, TNF). Evidence of suppression of immune function in mice in whom tumor is growing in hollow fibers in the peritoneal cavity without any cell-cell contact in the host suggest that a soluble tumor factor is responsible for the defect in cellular immunity. We have devised a method of reproducing these tumor-induced changes in normal T cells *in vitro* and are in the process of isolating the tumor-derived factor(s) responsible for the changes. In agreement with this finding, we are able to demonstrate the immunosuppressive properties of the pleural fluid isolated from cancer patients. We are in the process of isolating and characterizing the tumor-derived factor(s) from the pleural fluids of cancer patients.

Role of Cadherins in Tumor Progression: Cadherins are a class of cell surface proteins that function in maintaining tissue organization, intercellular communication, and cytoskeletal integrity. The loss of cadherin in prostate cancer predicts for a poor prognosis and malignancy. A goal of our laboratory is to investigate the molecular role of cadherin in tumor progression and regulating cellular function. The expression of the cadherin gene in a prostate cancer line facilitated intercellular contact, redistribution of the actin cytoskeleton, and growth suppression. Alteration of the extracellular portion of the molecule eliminates cell contact but retains growth suppression. In contrast the truncation of the cytoplasmic portion cell contact is unaffected but the growth suppressive activity is lost. In addition, the reorganization of the actin cytoskeleton and the redistribution of β-catenin mediated by cadherin are closely associated with the growth suppressive activity. These findings suggest that the growth suppressive property of cadherin involves the alteration of the cytoskeleton and perhaps intracellular signaling. To address these possibilities, the laboratory focus is on the alteration of catenin proteins, which link the cadherin to the actin cytoskeleton. The disassociation of cadherin with the actin cytoskeleton should result in the loss of cadherin mediated growth suppression. Furthermore, it is predicted that the regulation of genes involved in growth suppression will be affected with cadherin expression and analysis of differential gene expression will be investigated.

Cyclosporin A-Resistant Costimulation of T Cells via CD28: The CD28-mediated co-stimulatory signal plays a pivotal role in many immune responses including T cell responses against tumors, virus-infected cells, and transplanted alloantigens. Depending on the nature of primary stimulation, CD28 can initiate multiple intracellular signaling pathways that can be broadly classified into two groups: one is calcium-dependent and sensitive to cyclosporin A (CsA), and the other one is calcium-independent and resistant to CsA. The CsA-resistant pathway has been thought to be responsible for the ineffectiveness of CsA in the treatment of *graft-versus-host disease* following allogeneic bone marrow transplantation. Our primary objectives are focused on three areas: first, characterization of the CsA-resistant co-stimulatory pathway; second, examination of the physiological significance of this pathway; and third, evaluation of the effect of aging on this pathway.

Role of Mutant p53 in TGF-β-mediated Growth Suppression in B Cell Lymphoma Cells: The tumor suppressor p53 is well known for its ability to inhibit the growth of cells that have suffered genetic damage or stress induced by their environment. It is able to inhibit cell growth by a number of mechanisms including apoptosis and senescence. The importance of p53 as a tumor suppressor is further documented by the facts that p53 null mice have dramatically higher rates of tumor formation than their wild type counterparts and p53 gene is mutated in approximately half of all human cancers. Although many of the biochemical functions of wild type p53 have been elucidated, the “gains-of-function” associated with p53 mutations are still, for the most part, poorly understood. However, evidence suggests that these functions may promote enhanced cell growth and tumor cell metastasis. While mutant p53 is a target for cancer drug development (mainly antisense strategies), little is known about the regulation of its expression. We have reported that TGF-β treatment resulted in growth suppression associated with a decrease in expression of mutant p53 of a B cell lymphoma cell line. The goal of this study is to understand the mechanism underlying TGF-β-mediated down regulation of mutant p53 and subsequent growth arrest, and analyze the gain-of-function properties for different mutant p53s.

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Keywords:

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immunological memory
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learning and memory
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Luo Y, et al. *J Mol Neurosci* 2001; 17: 127-134.

Weng N-P, et al. *J Leukoc Biol* 2001; 70: 861-867.

Research Interests: The research interests of this laboratory are focused on three areas: 1) the molecular and cellular mechanisms of lymphocyte differentiation and immunological memory; and 2) the molecular basis of learning and memory formation and aging influence on this process. The function of the immune system is dependent on the ability of lymphocyte division during development, differentiation and activation. It is unknown how naïve lymphocytes differentiate to become memory cells, what is the molecular basis of long-lived memory cells, and how aging influence the immune functions. A large-scale analysis of gene expression in naïve and memory lymphocytes allows us to identify genes that are involved in these processes. Further study of these differentially expressed genes and an understanding of their function will provide a rational basis for developing strategies of experimental and clinical intervention. Another area of interests is the mechanisms of learning and memory formation and aging effects on this process. We use rat stone maze as a model and analyze gene expression dynamics in hippocampus during maze learning. This analysis will allow us to identify and to characterize genes that are involved in the normal maze learning and the changes in aging.

Regulation of Telomerase Expression in Human Lymphocyte Development, Activation and Aging: Telomere, the terminal structure of chromosomes, has captivated considerable attention recently for its function involving the regulation of cellular replicative lifespan. Every telomere consists of an array of tandem hexamer repeats, $(TTAGGG)_n$ and the binding proteins, such as TRF1, TRF2, Pot1 etc. The inability of DNA polymerase to completely replicate the ends of chromosomes results in a loss of 50-200 basepair telomere repeats with cell division in normal human somatic cells. It has thus been proposed that a minimal length of telomeres is essential for cellular replication and telomere reduction is a mechanism for limiting replicative lifespan in normal somatic cells.

Telomerase is a unique reverse transcriptase consisting of two essential components, telomerase RNA template (hTER) and telomerase reverse transcriptase (hTERT), and functions to synthesize telomere repeats which serve to protect integrity of chromosomes and to prolong replicative lifespan of cells. The selective presence of telomerase in the germline and malignant cells but not in most normal human somatic cells has been hypothesized as a basis for the immortality of the germline and of malignant cells. Our previous studies demonstrated that the telomere length is longer in naïve than in memory T cells, reflecting their replicative history *in vivo* and paralleling replicative capacity *in vitro*; and that telomerase is also expressed in lymphocytes in a strictly regulated manner during lymphocyte development, differentiation, activation, and aging. Furthermore, in contrast to the recent findings that transcription of hTERT determines telomerase activity in normal somatic cells, human lymphocytes express hTERT independent of the presence, absence, or quantitative level of detectable telomerase activity. Instead, human T lymphocytes regulate telomerase function through novel events independent of hTERT protein levels; and hTERT phosphorylation and nuclear translocation may play a role in regulation of telomerase function in lymphocytes. Current ongoing studies are focused on the mechanisms of telomerase regulation, and age influence on the dynamics of telomere length and telomerase activity in subsets of lymphocytes.

Identification and Characterization of Differentially Expressed Genes in Memory and Naïve CD4$^+$ Lymphocytes: Immunological memory is one of the defining features of the immune function, yet its underlying mechanisms are not completely understood. Human memory and naïve CD4$^+$ T cells are distinct both phenotypically, memory cells express CD45R0 and naïve cells express CD45RA, and functionally, memory cells require only one signal and naïve cells require two-signals for activation. In an attempt to understand the molecular basis for the immunological memory response, we have utilized cDNA microarrays to measure gene expression of human memory and naïve CD4$^+$ T cells at rest and after activation. Our analysis of over 45,000 cDNA clones provides the first glimpse into gene expression patterns of memory and naïve CD4$^+$ T cells at the genome-scale and reveals several novel findings. First, memory and naïve CD4$^+$ T cells expressed similar numbers of genes at rest and after activation. Second, we have identified 14 cDNA clones that expressed higher levels of transcripts in memory cells than in naïve cells. Third, we have identified 135 (130 known genes and 5 ESTs) up-regulated and 68 (42 known genes and 26 ESTs) down-regulated cDNA clones in memory CD4$^+$ T after *in vitro* stimulation with anti-CD3 plus anti-CD28. Interestingly, the increase in mRNA levels of up-regulated genes was

greater in memory than in naïve $CD4^+$ T cells after *in vitro* stimulation, and was higher with anti-CD3 plus anti-CD28 than with anti-CD3 alone in both memory and naïve $CD4^+$ T cells. Finally, the changes in expression of actin and cytokine genes identified by cDNA microarrays were confirmed by Northern and protein analyses. Together, we have identified approximately 200 cDNA clones whose expression levels changed after activation, and suggest that the level of expression of up-regulated genes is a molecular mechanism that differentiates the response of memory from naïve $CD4^+$ T cells. Current ongoing studies focus on elucidating the functions of those differentially expressed genes in the generation and maintenance of memory T lymphocytes.

Analysis of Gene Expression in Rat Hippocampus in Maze Training and Aging: Learning and memory are complex neurological processes that involve acquisition, storage and/or retrieval of information. Long-term memory formation is involved in several areas of brain including hippocampus, requires de novo RNA and protein synthesis, and declines with increase of age. Although progress has been made in defining the anatomic areas and elucidating the importance of synaptic plasticity in learning and memory in the past three decades, the molecular mechanisms underlying learning and memory formation as well as the aging influences this process are essentially unknown. In an attempt to dissect the memory process at the molecular level, we used cDNA microarray to assess changes in gene expression associated with learning and memory processes (a 14-unit Stone T-maze, a task that is dependent on normal hippocampal function) in male Fischer-344 rats. Through a sequential analysis involving large-scale commercial filters (over 15,000 unique cDNA clones) and a custom-made filter (consisting of 1,124 cDNA selected clones), we have identified 28 unique cDNA clones (18 known genes and 10 ESTs) whose expression was enhanced in rat hippocampus following Stone maze learning. Some of those genes appear to be involved in calcium signaling, Ras activation, kinase cascades, and extracellular matrix function, suggesting that these genes may function in regulating neural transmission, synaptic plasticity, and neurogenesis. In addition, we have focused on one induced gene, neuroleukin, for further analysis and found: First, mRNA levels of NLK and gp78 were significantly increased in rat hippocampi following training in the Stone T-maze and the Morris water maze; second, a parallel increase was found in hippocampal NLK and gp78 proteins after maze learning; third, NLK and gp78 mRNA and protein expression in hippocampus was reduced in aged rats that showed impaired learning in the Stone maze, compared to young rats; finally, application of recombinant NLK to hippocampal neurons significantly enhanced glutamate-induced ion currents, functional changes that have

been correlated with learning *in vivo*. Taken together, our results identify a novel association of hippocampal expression of NLK and gp78 with rat maze learning. Interaction of NLK with gp78 and subsequent signaling may strengthen synaptic mechanisms underlying learning and memory formation. Current ongoing studies focus on extending the gene expression analysis during rat normal brain development and aging and neuroleukin function *in vivo*.

Collaborators: Richard J. Hodes, M.D., National Cancer Institute and National Institute on Aging, NIH; Carl H. June, M.D., University of Pennsylvania School of Medicine; Peter Lansdorp, M.D., Terry Fox Laboratory; Peter Munson, Ph.D., Center for Information Technology, NIH; Donald Ingram, Ph.D., Laboratory of Neurosciences, National Institute on Aging, NIH.

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The **Laboratory of Molecular Gerontology (LMG)** investigates DNA related mechanisms such as genomic instability, DNA repair, DNA replication, and transcription. Increased DNA damage accumulation in senescence is a major molecular change with aging, and this DNA damage may eventually inactivate individual genes and lead to a deterioration of the organism, which is characteristic of the senescent phenotype. DNA damage maybe a major cause of age-associated diseases, notably cancer. The goal of LMG is to understand the underlying mechanisms involved in DNA damage formation and its processing as well as the changes that take place with aging that render aging cells susceptible to cancer. DNA repair is likely to play a critical role, and we have a special interest in understanding the mechanisms involved in the major DNA repair pathways of nucleotide excision repair and base excision repair. We are investigating the molecular mechanisms involved in DNA repair and in genomic instability in normal, senescent and cancer cells. Studies are carried out *in vivo* and *in vitro*, in fractionated cell extracts, and in intact cells. We are also interested in the molecular processes that interact with DNA repair. They include transcription, replication, somatic mutation and mitochondrial functions.

The accumulation of DNA damage with age could be a result of a gradual decline in DNA repair capacity. Work from this and other laboratories suggests that this decline is not readily detectable in the overall genome, but may rather be a decline in the gene specific or transcription-coupled component of the DNA repair process.

The area of oxidative DNA damage and its processing is of particular interest to us. Repair of oxidative DNA base lesions is investigated in whole cells, in mitochondria and in cancer cells.

We are studying the molecular deficiencies in human premature aging disorders using cell biological approaches and biochemistry. These hereditary progeriod disorders serve as model systems to study human aging and age-related diseases including cancer. In particular, the laboratory is studying DNA helicases, ATPases and exonucleases, such as the Werner syndrome, Bloom syndrome and Cockayne syndrome proteins. These enzymes are also essential in maintaining genomic instability and we are investigating their function at a biochemical level and their interactions with other proteins. A major goal is to understand the role of these proteins in important DNA metabolic processes and to clarify their role in important pathways. We are interested in understanding the role of these proteins in the normal aging process.

In the laboratory we are generally interested in a better understanding of the processes that lead to genomic instability. Aside from the DNA repair process, which clearly is of importance in maintaining genomic stability, we are interested in the role of DNA polymerases in causing mutation. Recently, a number of new DNA polymerases have been discovered and some of these have low fidelity which can lead to mutation. Somatic hypermutation is a distinct process, which is central to the normal immune response. We are interested in the mechanism and how it relates to DNA repair, and whether it changes with age.

An interesting DNA structure that may arise in certain parts of the genome is the triple helix, which can lead to genomic instability. In addition these structures can be used to mediate gene targeted DNA damage and this is being studied in the laboratory.

We are involved with a number of studies using material from the Baltimore Longitudinal Study on Aging (BLSA). In DNA samples from individuals in this study, we are examining various aspects of genomic instability and how they function in aging and premature aging disease. We are interested in the prevalence of genetic polymorphism in genes involved in DNA repair and in premature aging.

The best therapeutic intervention against age-associated disease is caloric restriction. We study calorically restricted rodents with the aim of exploring whether this condition is associated with changes in the formation or repair of oxidative DNA lesions.

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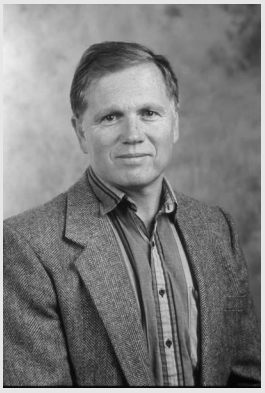

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Biography: Dr. Bohr received his M.D. in 1978, Ph.D. and D.Sc. in 1987 from the University of Copenhagen, Denmark. After training in neurology and infectious diseases at the University Hospital in Copenhagen, Dr. Bohr did a postdoctoral fellowship with Dr. Hans Klenow at the University of Copenhagen, Denmark. He then worked with Dr. Philip Hanawalt at Stanford University as a research scholar from 1982-1986. In 1986 he was appointed to the National Cancer Institute (NCI) as an investigator, becoming a tenured Senior Investigator in 1988. Dr. Bohr developed a research section in DNA repair at the NCI. In 1992 he moved to the NIA to become Chief of the Laboratory of Molecular Genetics, now Laboratory of Molecular Gerontology. Dr. Bohr has conducted clinical studies (infectious diseases and oncology), but worked most extensively in basic research. His main contributions have been in the area of DNA repair. He has worked on many aspects of DNA damage and it's processing in mammalian cells. He developed a widely used method for the analysis of DNA repair in individual genes and found that active genes are preferentially repaired. This observation was a major advance in the understanding of the tight interaction between DNA repair and transcription, a process termed transcription-coupled repair. In recent years numerous papers from his laboratory have focused on mechanisms of DNA damage processing, mainly on the pathways of nucleotide excision, transcription coupling and base excision. A main interest now is to elucidate how these processes change in relation to aging. Another focus of Dr. Bohr's research is the area of premature aging disorders such as Werner and Cockayne syndrome. His laboratory has studied cellular, molecular and biochemical functions in cells from afflicted individuals. Recent studies have focused on biochemical properties of the purified proteins, defective in these disorders.

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DNA repair
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Cockayne syndrome
Werner syndrome
mitochondria

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DNA Repair Processes: Several types of DNA lesions have been observed in mammalian DNA. They are removed by a number of different DNA repair pathways. One is nucleotide excision repair (NER), which removes and replaces bulky lesions, such as UV-light induced pyrimidine dimers. Another important DNA repair pathway is base excision repair (BER), which removes single damaged bases as free bases, and replaces them. Base excision repair removes a large number of minor lesions from DNA, many of which are caused by oxidative modification. Other pathways of DNA repair include mismatch repair, homologous recombination and non-homologous recombination.

Oxidative DNA Damage and Mitochondrial Functions: Reactive oxygen species are generated in cells as by-products of cellular metabolism. They are products of the metabolic processes in each cell, and reactive oxygen species react with proteins, lipids, and DNA to generate oxidative damage. Oxidative DNA damage results from various forms of cellular stress, including exogenous exposures and endogenous metabolic processes. Oxidative damage is thought to contribute to carcinogenesis,

mitochondrial dysfunction, and aging. Because most reactive oxygen species are generated by the oxidative phosphorylation processes that occur in mitochondria, it is of great interest to understand the oxidative DNA damage processing mechanisms in these organelles. Mitochondrial DNA is not protected by histones and lies in close proximity to the free radical producing electron transport chain. Oxidative DNA damage that arises in mitochondrial DNA can cause mutations, gene inactivation, or deletions. These changes are commonly found in the mitochondrial genome in association with aging and cancer. Because mitochondrial DNA is subjected to high amounts of oxidative damage, mitochondria need efficient DNA repair activity to remove oxidative damage from their DNA. Although the notion has prevailed for many years that mitochondria cannot repair DNA damage (including the highly mutagenic lesion, 8-oxo-G) recent studies from our group and elsewhere have shown that a number of lesions are efficiently repaired from mitochondrial DNA.

We have established several assays for the study of DNA repair in mitochondrial extracts. Mammalian mitochondrial repair enzymes have been purified and characterized. We have also studied DNA repair changes with aging in the mitochondria. Whereas nuclear DNA repair declines with age, mitochondrial repair increases, perhaps to handle increased oxidative stress.

Premature Aging Syndromes: A number of rare mutations and disorders in humans are associated with premature aging. The patients prematurely have many signs and symptoms associated with normal aging. We are particularly interested in Cockayne syndrome (CS) and in Werner syndrome (WS), which are good model systems for molecular studies of human aging. The WRN gene, defective in WS, has been cloned. The WRN gene, the CS gene, and other genes mutated in premature aging syndromes encode putative helicases. Therefore, further understanding of the molecular defects in these disorders is a high and achievable priority in the understanding of normal aging. The functions of the CS protein, which is mutated in CS, and of the WRN protein, which is mutated in WS, appear to be at the crossroads of aging, DNA transcription, replication, and repair, thereby nicely affording a combination of our interest in DNA function with our interest in aging.

DNA Repair and Aging: The accumulation of unrepaired damage to DNA contributes to cellular senescence. DNA repair efficiency may decline in normal human aging. This decline may be subtle and may reflect changes in specific DNA repair pathways. We are studying DNA repair pathways and transcription in cells from patients with premature aging (segmental progeroid disorders) to identify which specific repair pathway may be defective.

Werner's Syndrome (WS): Werner's syndrome is a homozygous recessive disease characterized by early onset of many characteristics of normal aging, such as wrinkling of the skin, graying of the hair, cataracts, diabetes, and osteoporosis. The symptoms of WS begin to appear around the age of puberty, and most patients die before age 50. A hallmark defect in WS is genomic instability characterized by karyotypic abnormalities including inversions, translocations, and chromosome losses. Because of the acceleration of aging in WS, the study of this disease will hopefully shed light on the degenerative processes that occur in normal aging.

The molecular basis of genomic instability in WS remains to be defined. Our laboratory is using several approaches to identify and characterize the molecular defect in WS cells. One approach is to compare the DNA metabolic activities of WS and normal cells. WS cells are not hypersensitive to treatment with most DNA damaging chemicals, with the exception of one carcinogen, 4-nitroquinoline. Some WS cells are defective in transcription coupled DNA repair, but no other DNA repair defects have been demonstrated. Experiments with intact cells and cell extracts suggest that WS cells may have a defect in basal transcription. Cells from WS patients grow more slowly and become senescent at an earlier population doubling than age-matched normal cells, possibly because the WS cells appear to have accelerated losses of the telomeric ends of their chromosomes. Telomeric shortening is an established marker of cellular senescence.

WS cells have a high level of genomic instability, with increased amounts of DNA deletions, insertions, and rearrangements. These effects could potentially be the result of defects in DNA repair, replication, and/or recombination, although the actual biochemical defect remains unknown.

The gene defective in WS, the WRN gene, is a member of the RecQ helicase family. Helicases play roles in a number of DNA involving processes: transcription, replication, and DNA repair and chromatin structural organization. We have purified the WRN protein for use in a number of basic and complex biochemical assays. The protein has helicase and exonuclease catalytic activities. It interacts with replication protein A (RPA), both physically and functionally. RPA enhances the helicase activity when unwinding larger DNA duplex structures. WRN protein interacts with the Ku heterodimer, which stimulate its exonuclease activity, and this suggests that WRN may be involved in non homologous endjoining, the pathway in which Ku exerts its main function. WRN also interacts with p53, possibly in the pathway of apoptosis, since WS cells have attenuated apoptosis. Further, we have recently discovered that WRN

protein interacts functionally and physically with Flap endonuclease 1 (FEN-1), a protein involved in DNA replication and DNA base excision repair. This suggests that WRN protein plays a role in one or both of those processes.

Although much progress is being made, the nature of the defect(s) in WS is still a mystery, as is the nature of the processes that occur in cellular senescence and normal human aging. Our ongoing and future studies will be directed towards elucidation of the causes of the accelerated aging phenotype in WS, with hope that this knowledge can also be applied to our current understanding of both the aging of cells and organisms in general.

Oxidative DNA Damage: One major theory of aging holds that oxidative damage to cellular components, such as proteins, lipids and DNA, accumulates with age, leading to the cellular dysregulation that result in the process of aging experienced by the organism. We are interested in understanding how exogenous and endogenous sources of reactive species produce oxidative damage in DNA, how that damage is processed in human cells, and the effects of unrepaired damage. Reactive oxygen species produce a wide variety of products in DNA. They are then repaired by a different DNA repair pathway, the main one being base excision repair. We introduce well-defined oxidative lesions into DNA in cells *in vivo* and study the DNA repair reactions of cell extracts *in vivo* or purified proteins *in vitro* with the damaged DNA.

Mitochondrial DNA Repair. It has been suggested that oxidative DNA damage accumulates in the mitochondrial DNA because these organelles have deficient DNA repair mechanisms and repair proficiency. Over the past decade, it has been shown that mammalian mitochondria possess efficient base excision repair (BER), and are able to remove many different base adducts from their genome. In addition, we have recently demonstrated that the 8-oxo-dG glycosylase/AP lyase (mtODE) activity increases with age in rat liver and heart mitochondria. The specific increase in this activity, compared to the decline in nuclear DNA repair, suggests an induction of the mitochondrial pathway.

It is challenging to understand the mechanisms involved in the mitochondrial DNA repair process. We take several approaches to this. In one, we are studying the repair in *in vitro* mitochondrial extracts, and here we can determine the role of various individual proteins by use of specific antibodies or by addition of the purified proteins to the extracts. In another approach, we use transgenic animals that are defective in specific DNA repair genes involved in nucleotide excision repair or base excision repair to study the function of these gene products.

DNA Repair in Alzheimer's Disease. Recent work from other laboratories has suggested that cells from Alzheimer's disease patients are defective in the processing of DNA lesions induced by irradiation with fluorescent light. We are assessing various pathways of DNA repair in Alzheimer's cells to characterize this possible defect which could be etiologically linked to the disorder.

Cockayne Syndrome (CS): Cockayne syndrome is a rare human disease characterized by arrested post-natal growth and resulting in premature aging and death. Complementation studies demonstrated that two genes, designated CSA and CSB, are involved in CS. Cells from CS individuals are abnormally sensitive to killing by ultraviolet radiation as well as certain so-called UV-mimetic chemicals, such as 4-nitroquinoline-1-oxide and N-acetoxy-2-acetylaminofluorene. CS cells are defective in the enhanced rate of repair of the template (transcribed) strand relative to the coding (non-transcribed) strand of transcriptionally active genes. In recent experiments from this laboratory, we have demonstrated that mutations in the CSB gene are the cause of the transcription coupled repair defect.

The complex clinical phenotype of CS, however, suggests that DNA repair may not be the only defect. Moreover, recent evidence from our laboratory demonstrated that CSB cells are defective in RNA polymerase II (Pol II) transcription. Studies of transcription *in vitro* in a plasmid-based system demonstrate a significant transcription defect in CSB cells.

We have generated stable human cell lines with functional domain knockout of different regions of the CSB gene. Mutations are introduced by site-directed mutagenesis directed at various motifs in the ATPase or helicase domain of the gene. The phenotypical alterations caused by these mutations are then examined, and studies are also carried out using cell extracts from these cell lines. Further, the wild type CSB and mutated recombinant proteins are made from baculovirus constructs and studied biochemically. Mutations in the ATPase domain do not appear to affect the potential for oxidative DNA damage repair whereas certain mutations in the helicase domain markedly affect the capacity for DNA repair of oxidative DNA base lesions. These results demonstrate that the CSB protein plays a role in base excision repair of oxidative DNA damage. Thus, this protein has several roles in DNA metabolism, it is involved in transcription, DNA repair, apoptosis and chromatin assembly. Studies are now aimed at further structure/function analysis of CSB protein and aimed at further clarification of its function in these pathways.

The function of the CSB protein is also investigated with microarray studies of gene expression. Here we find that several genes are under expressed in mutated CS cells, and that some of these confirm a substantial role for CSB protein in transcription and apoptosis.

Collaborators: E.C. Friedberg, University of Texas, Dept. of Pathology, Southwestern Medical Center, Dallas, Texas; J. Hoeijmakers, Erasmus University Rotterdam, Rotterdam, The Netherlands; C.C. Harris, Laboratory of Human Carcinogenesis, NCI; K.H. Kraemer, NCI; A.P. Grollman, State University of New York at Stony Brook; I. Hickson, Inst. Molecular Medicine, Oxford, United Kingdom; George Martin, University of Washington, Seattle, Washington; Erling Seeberg, University of Oslo, Norway.

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Keywords:

immunoglobulin
somatic hypermutation
DNA polymerases
aging

Recent Publications:

Winter DB, et al. *Philos Trans R Soc Lond (Biol)* 2001; 356(1405): 5-11.

Rosner K, et al. *J Clin Immunol* 2001; 21(2): 102-115.

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Frank EG, et al. *EMBO J* 2001; 20(11): 2914-2922.

DNA Polymerases in Somatic Hypermutation of Immunoglobulin Variable Genes: Somatic hypermutation of variable genes, which encode a portion of immunoglobulin molecules, occurs at a frequency that is a million times greater than mutation in other genes. The molecular mechanism that introduces these mutations is unknown. Evidence points to a process that involves DNA repair events at sites of targeted strand breaks. In vertebrate cells, there are many recently identified DNA polymerases that inaccurately copy templates. One or more of these are potential candidates for enzymes that introduce base changes during hypermutation. We are studying the roles of DNA polymerases zeta, eta, and iota in the mechanism.

Polymerase Zeta: This enzyme creates errors at a frequency of 5×10^{-4}/bp when copying undamaged DNA and may function in extending synthesis past mismatched bases that are introduced during repair of lesions. In collaboration with R. Wood, we disrupted the catalytic subunit of polymerase zeta and made gene-deficient mice. However, the mice died during mid-gestation, suggesting that the enzyme is critical for embryonic development. Current studies are focused on conditional expression of the gene in order to obtain viable animals.

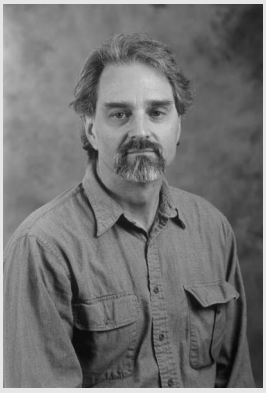
Polymerase Eta: The polymerase synthesizes errors at a frequency of 3×10^{-2}/bp on non-damaged DNA and helps cells tolerate damage by inserting bases opposite DNA lesions. The enzyme is defective in people with xeroderma pigmentosum variant disease. We sequenced variable genes from three patients and found that their frequency of hypermutation was normal, but the types of base changes were different. Polymerase eta-deficient clones had a three-fold decrease in the proportion of mutations at

A and T with a concomitant rise of mutations at G and C. It is notable that this shift in mutation pattern is consistent with the specificity of the polymerase when copying non-damaged DNA *in vitro*. This finding implies that polymerase eta is an A-T mutator in hypermutation, and another polymerase acts at G and C nucleotides. We are currently trying to identify proteins that interact with polymerase eta during hypermutation.

Polymerase Iota: This polymerase makes errors at an extraordinary overall frequency of 3×10^{-1}/bp on undamaged templates and allows it to synthesize several bases past the site of DNA lesions. In collaboration with R. Woodgate, we have studied the specificity of polymerase iota on DNA substrates that might be formed during hypermutation. The overall fidelities of the polymerase are 10-fold lower when it fills a template at a DNA terminus compared to when it fills a longer template. Since mutations occur in variable genes near strand breaks, the polymerase may well function as a mutator during hypermutation. Its actual involvement needs to be tested in mice that are deficient for the polymerase.

Hypermutation in Old Humans: To determine if aging alters the frequency and pattern of hypermutation, we compared 1000 mutations in variable genes from young and old humans. The frequency, location, and types of substitutions were similar between the young and old groups. However, the ratio of replacement to silent mutations was much higher in the complementarity-determining regions from old people, which suggests that their B cells were recently selected by antigen. Analyses of the data for the third complementarity-determining region showed that mutated genes had a shorter length than non-mutated genes. A smaller region would allow more room in the antibody binding pocket for antigen to interact with other parts of the antibody and increase the overall affinity. We also showed that the Werner helicase is probably not involved in hypermutation since variable genes from a Werner syndrome patient who is defective in the helicase had a normal frequency of mutation.

Collaborators: R.D. Wood, Ph.D., University of Pittsburgh, PA, and R. Woodgate, NICHD, NIH, Bethesda, MD.



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Keywords:

gene targeting
DNA triple helix
DNA repair

Recent Publications:

Puri N, et al. *J Biol Chem* 2001; 276(31): 28991-28998.

Opresko PL, et al. *J Biol Chem* 2001; 276(48): 44677-44687.

Brosh RM, et al. *J Biol Chem* 2001; 276(5): 3024-3030.

Lin FL, et al. *J Biol Chem* 2000; 275(50): 39117-39124.

Cellular Response to DNA Damage: We are interested in the response of cells to targeted DNA damage and the application of site specific targeting for modulating genomic sequences.

Gene Targeting: Current approaches for manipulating genomic sequences rely on homologous recombination. In these procedures relatively lengthy DNA fragments are introduced into cells and via an enzymologically driven process engage in a search for homologous sequences in the chromosome. After a recombinational intermediate forms, the process is completed in a series of additional enzymatic steps. The procedure is inefficient and time consuming. Given the marked increase in sequence data from the genome project there is a clear utility in having a more efficient and less cumbersome process.

We are developing oligonucleotides, that can form a three-stranded DNA structure called a triple helix. The third strand lies in the major groove of an intact double helix and is stabilized by hydrogen bonds between the bases in the third strand and the purine bases in the duplex. These structures are quite stable and very stringent with respect to sequence specificity. The oligonucleotides can be linked to DNA reactive compounds and site-specific modification of DNA with these oligo-reagent conjugates has been demonstrated by many groups. Although these structures have been studied for many years, there have been relatively few accounts of biological applications.

Recently we and our colleagues constructed an oligonucleotide linked to psoralen (a photoactive DNA crosslinker), which was designed to form a triplex with a sequence in the well-known cellular housekeeping gene HPRT (hypoxanthine guanine phosphoribosyl transferase). This gene encodes an enzyme engaged in purine salvage. There is a simple selection

procedure for cells, which lack the enzyme, consequently, the gene has become the most commonly used mutation marker gene in mammalian cells. The oligo was introduced in cells in culture and after photoactivation the cells were processed according to a standard mutation selection protocol. Mutations were found at the target site, and sequence analysis demonstrated that the majority were small deletions. This was the first evidence that chromosomal targets are accessible to triplex forming oligonucleotide reagents.

In more recent work we have examined the influence of novel sugar modifications on the activity of triplex forming oligonucleotides. We have identified the nature and distribution of these derivatives in oligonucleotides that support robust activity in gene knockout assays. We are now using these new reagents in additional gene knockout studies and as probes of cellular chromatin structure.

This approach can now be used to deliver additional DNA reactive compounds to specific genomic locations and we are in the process of developing those reagents. We are also looking at the influence of DNA repair deficiencies on the targeted mutation frequencies. This will tell us which DNA repair activities are active in repair of the directed lesions, and lead to the development of strategies designed to inhibit these functions during the time of oligo introduction. Eventually this approach will be used to modulate genomic sequences with targeted gene knockout as a specific application.

Collaborators: Dr. Paul Miller, Johns Hopkins; Dr. Peter Glazer, Yale University; Dr. Gordon Hayer, National Cancer Institute, NIH.

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Biography: Dr. Robert Brosh received his Ph.D. in molecular-cell biology from the University of North Carolina at Chapel Hill in 1996 and his M.S. in biochemistry from Texas A&M University in 1988. He obtained postdoctoral training at NIH before assuming his present position in the Laboratory of Molecular Gerontology, NIA.

Keywords:

helicase
genomic instability
Werner syndrome
DNA repair
replication

Recent Publications:

Brosh RM, et al. *EMBO J* 2001; 20(20): 5791-5801.

Brosh RM, et al. *J Biol Chem* 2001; 276(37): 35093-35102.

Brosh RM, et al. *J Biol Chem* 2001; 276(5): 3024-3030.

Brosh RM, et al. *J Biol Chem* 2000; 275(31): 23500-23508.

Brosh RM, et al. *Nucleic Acids Res* 2000; 28(12): 2420-2430.

Roles of DNA Helicases in Genomic Stability: The growing number of DNA helicases implicated in human disease suggests that these enzymes have vital specialized roles during replication, DNA repair, recombination, and transcription. RecQ DNA helicases are of particular interest because the human hereditary disorders Werner syndrome (WS), Bloom syndrome, and Rothmund-Thomson syndrome all arise from mutations in genes of the RecQ helicase family. We have focused our efforts on understanding the cellular and molecular defects of WS, a premature aging disorder characterized by genomic instability. Defining the biochemical functions of DNA helicases will help us to better understand the molecular defects associated with cancer and aging.

WRN Helicase as Caretaker of the Genome: The defects observed in WS cells may result from the inability to resolve alternate DNA structures. One hypothesis is that Werner syndrome protein (WRN) functions to resolve structures that impede progression of the replication fork. Replication defects observed in WS are consistent with this notion. Recently we have shown that WRN unwinds a number of alternate structures including triplexes, tetraplexes, and Holliday junctions. The cellular defects and genomic instability of WS may arise from persistent DNA structures that fail to be resolved by WRN or certain RecQ helicases. My group is currently investigating the reaction mechanism for WRN-catalyzed DNA unwinding and the action of WRN on important DNA substrate intermediates of replication, DNA repair, and recombination. The goal of this work is to elucidate the role of WRN protein in pathways of DNA metabolism necessary for the maintenance of genomic stability.

Protein Interactions of WRN and BLM Helicase: To understand the molecular functions of DNA helicases, we are interested in protein interactions of WRN. Defining the protein interactions of WRN will help to elucidate cellular processes to maintain genome integrity. Our studies have demonstrated that WRN physically and functionally interacts with a number of important cellular proteins that include RPA, Ku, and p53. These interactions modulate the catalytic activities of WRN, and are likely to be important in DNA metabolic pathways that confer genome stability. Recently we demonstrated that WRN physically interacts with human flap endonuclease 1 (FEN-1), a structure-specific nuclease implicated in DNA replication and repair, and dramatically stimulates the cleavage activity of FEN-1. We are presently exploring mechanistic aspects of the WRN-FEN-1 interaction and the functional importance of the WRN-FEN-1 interaction *in vivo*. Ongoing studies in this area will hopefully shed light on the potential importance of the WRN-FEN-1 interaction to genome stability that is perturbed in WS.

Collaborators: Dr. Vilhelm Bohr, NIA; Dr. Michael Seidman, NIA; Dr. Ian Hickson, University of Oxford; Dr. Mark Kenny, Albert Einstein Cancer Center; Dr. Curt Harris, NCI; Dr. Dmitry Gordenin, NIEHS; Dr. Robert Bambara, University of Rochester; Dr. Donald Jerina, NIDDK.

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Since 1986, our lab has had a very simple philosophy: find the genes and mutations which cause neurological disease; take those genes and mutations into cells; make animal (transgenic mouse) models of them to better understand the disease processes; and, use those models to test therapies. This simple philosophy underpins the current organization of the lab. However, for many diseases we have been interested in, particularly Alzheimer's disease (AD), all the genes involved in the simple forms of the disease have been identified (the amyloid gene and presenilin 1 and 2). For Parkinson's disease some of the 'simple' genes have been identified (synuclein and parkin) but others remain undefined, and for frontal temporal dementia, the tau gene has been identified but there are likely to be others. Increasingly for these diseases, and also for other diseases we are interested in, such as stroke, we will be searching for risk factor genes such as the apolipoprotein E gene in late onset Alzheimer's disease. A major focus of work in the lab will be to develop and use strategies designed to find such risk factor genes.

In Alzheimer's disease, our work and that of others, suggested that mutations that led to disease signposted a pathologic biochemical pathway which lead to disease pathogenesis. In AD, this pathway seems to be the "amyloid cascade." We think it is likely that this type of relationship exists between the different gene products in other diseases and this belief informs the cell biology work we undertake. Thus, we will be continuing to work on Alzheimer's disease cell biology, both the presenilins and amyloid precursor protein (APP), and with other pathogenic gene products as we and others identify them. This philosophy will also underpin our work on the cell biology of Parkinson's disease and the other diseases we are interested in.

Finally, we will be continuing to use this genetic information to help us build animal models of disease which will be useful, for developing an understanding of the pathogeneses of the disease, and for developing treatments for these devastating disorders.

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Biography: Dr. John Hardy is a human geneticist and molecular biologist whose research interests focus on neurological disease. Dr. Hardy received his B.Sc. (Hons) degree from the University of Leeds, UK (1976) and his Ph.D. from Imperial College, London, UK where he studied dopamine and amino acid neuropharmacology. Dr. Hardy performed his postdoctoral training at the MRC Neuropathogenesis Unit in Newcastle upon Tyne, England and then further postdoctoral work at the Swedish Brain Bank in Umea, Sweden where he started to work on Alzheimer's disease. He became Assistant Professor of Biochemistry at St. Mary's Hospital, Imperial College, London in 1985 and initiated genetic studies of Alzheimer's disease there. He became Associate Professor in 1989 and then took the Pfeiffer Endowed Chair of Alzheimer's Research at the University of South Florida, in Tampa in 1992. In 1996 he moved to the Mayo Clinic in Jacksonville, Florida, as Consultant and Professor of Neuroscience. He became Chair of Neuroscience in 2000 and moved to NIA as Chief of the Laboratory of Neurogenetics in 2001. He has won the MetLife, the Allied Signal and the Potamkin Prize for his work in describing the first genetic mutations, in the amyloid gene in Alzheimer's disease, in 1991.

Keywords:

neurogenetics
Alzheimer's disease
Parkinson's disease
neurodegeneration

Recent Publications:

Lewis J, et al. *Science* 2001; 293(5534): 1487-1491.

Morgan D, et al. *Nature* 2000; 408(6815): 982-985.

Myers A, et al. *Science* 2000; 290(5500): 2304-2305.

The **Laboratory of Neurogenetics (LNG)** will perform genome screens for both our programs in neurodegenerative diseases including stroke, as well as provide the underpinning of this work in terms of bioinformatics and sample handling for the laboratory in general. In addition, our own research focus will be on the dementias, particularly late onset Alzheimer's disease. In this disease, apolipoprotein E is known to be a risk factor locus, but linkage studies suggest that there are a handful of other genes still to be identified.

However, more generally, it is our intention to reach out to the extramural community and work with colleagues, both within the United States and abroad, to act as a resource for those who have identified interesting neurological syndromes whose elucidation may provide more general insights. For example, we have worked extensively on the Parkinson's Dementia Complex of Guam over the last five years, and this is the type of work we wish to engage in more actively over the next period. We intend to have an 'open lab' policy towards collaborators who have identified interesting family material so that we can facilitate the process of finding genes to those who do not have access to 'state of the art' genetics and bioinformatics facilities.

Collaborators: Andrew Lees, M.D., Martin Rossor, M.D., Huw Morris, M.D., Nick Wood, M.D., Henry Houlden, M.D., Rohan DeSilva, Ph.D., University of London; Ron Peterson, M.D., Ph.D., Demetrius Maraganore, M.D., Mayo Clinic; Alison Goate, Ph.D., Washington University; Mike Owen, M.D., Ph.D., University of Wales.; Dave Morgan, Ph.D., University of South Florida; Karen Duff, Ph.D., New York University.

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Biography: Dr. Andrew Singleton is a human geneticist whose research interests focus on the genetics of neurological disease. Dr. Singleton received his B.Sc. (Hons) degree from the University of Sunderland, UK and his Ph.D. from the University of Newcastle upon Tyne, UK where he studied genetic causes and contributors to dementia. Dr. Singleton performed his postdoctoral training at the Mayo Clinic in Jacksonville, Florida, studying the genetic basis of neurological diseases such as dystonia, ataxia, essential tremor, dysautonomia, stroke and Parkinson's disease. In 2001 he joined the NIA as an Investigator within the newly created Laboratory of Neurogenetics. Dr. Singleton's group investigates the genetic and cellular mechanisms underlying simple-Mendelian and complex neurological diseases.

Keywords:

neurogenetics
X-linked dystonia
parkinsonism
ataxia
stroke
dementia
hyperhidrosis

Recent Publications:

Gwinn-Hardy K, et al. *Arch Neurol* 2000; 58(2): 296-299.

Farrer M, et al. *Hum Mol Genet* 2001; 10(17): 1847-1851.

Hardy J, et al. *Neurobiol Dis* 2000; 7(2): 65-69.

Singleton AB, et al. *Brain* 2000; 123(Pt 12): 2467-2474.

In recent years, an extremely successful approach to understanding disease has arisen from the study of rare familial forms of disorders related to more common "sporadic" disease. This is a research paradigm that was successful in Alzheimer's disease (AD). The identification of the APP, PS-1 and PS-2 mutations as causal of rare forms of early-onset familial AD led to a huge increase in our knowledge of the pathogenic mechanisms underlying the common late-onset form of AD. We are applying this approach to a number of disorders. Lubag or X-linked recessive dystonia parkinsonism (XDP) is a rare inherited movement disorder; however, given the clinical phenotype associated with this disorder, delineation of the disease process in XDP will be informative for Parkinson's disease, dystonia and related movement disorders. We are currently involved in a positional cloning project aimed at identifying this gene defect.

Our group, in collaboration with that of Dr. Gwinn-Hardy's of NINDS, employs two clinical research coordinators who recruit and expand families. In addition to XDP, we have actively recruited >300 families with a history of various neurological diseases, including but not limited to parkinsonism, dystonia, stroke, diffuse Lewy body disease, ataxia, essential tremor and hyperhidrosis. Once again the aim of this family collection is to aid in the identification of genes important in the pathogenesis of disease.

With the beginning of the new millennium, we are entering the post genome era. Now the vast majority of human genes have been sequenced and their sequences will be available on the web. In the last 10 years, the application of molecular genetics has led to the unraveling of the

etiologies of many of the single gene disorders that lead to neurodegenerative disease, but has barely begun to allow the dissection of the more complex genetics of most neurodegenerative disease which do not show simple patterns of inheritance. The only genes that have been unambiguously identified as risk factors for non-mendelian disorders are the prion gene in iatrogenic and idiopathic Creuzfeldt Jakob disease (Collinge et al. 1991: Palmer et al. 1991), the apolipoprotein E gene in Alzheimer's disease (Corder et al. 1993) and the tau gene in progressive supranuclear palsy (Baker et al. 1999): in the cases of both the prion and tau genes, there were good genetic or pathologic reasons for suspecting their involvement in disease etiology (Hsiao et al. 1989, Flament et al. 1991); thus, apolipoprotein E is the only neurodegenerative risk-factor gene found, in part, through positional genetic analysis (Pericak-Vance et al. 1990).

It is to be expected that over the next decade, the application of molecular genetic techniques will promote dissection of the etiologies of non-mendelian neurodegenerative diseases in general; however, the problems of identifying risk factor loci for diseases with complex modes of inheritance and in particular oligogenic (10 genes) and polygenic (>10 genes) disease are formidable. Given the huge socio-economic impact of some of the disorders of this nature such as Parkinson's disease and Alzheimer's disease, it is of paramount importance to design a viable strategy for the delineation of genetic predisposition in complex traits.

We are tackling the problems of complex disease in a number of ways. First, we are studying rare familial forms of disease and then extrapolating the function of genes involved to related conditions (as outlined above). One of the methodologies we are using to reach this goal is expression analysis using microarray technology. In collaboration with Dr. Mark Cookson, we are analyzing the effects of TorsinA, known to be mutated in certain forms of idiopathic torsion dystonia, on the genomic expression pattern within neuronal cells. The idea of this approach is really two-fold; first, to give us an idea of pathologically relevant interactions/pathways and second, to provide us with candidate genes for other positional cloning efforts. A second technique that is aimed at simplifying complex traits and identifying genetic linkage is the use of population isolates to simplify complex traits. A similar paradigm has been used by DeCode Genetics, Inc. in Iceland to examine a number of diseases. Other methodologies currently in use in the general genetic community include sib-pair analysis, candidate gene association studies, whole genome association studies and linkage disequilibrium mapping. We employ some aspect of all

of these techniques in our sample series and it seems clear that rather than using one approach, a complimentary battery of techniques is likely to yield success. Furthermore, as the contribution of an individual genetic defect to disease decreases, geneticists will have to rely increasingly on biology rather than statistics to prove pathogenicity.

Collaborators: Don Cleveland, Ph.D., University of California, San Diego; Matthew Farrer, Ph.D., Mayo Clinic, Jacksonville; Karen Parko, M.D., Shiprock Medical Center; Virgilio Evidente, M.D., Mayo Clinic, Scotsdale; Sub Subramony, M.D., University of Mississippi Medical Center; Horacio Kaufmann, Mount Sinai School of Medicine; Katrina Gwinn-Hardy, M.D., NINDS, NIH.

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Biography: Dr. Mark R. Cookson is a cell biologist whose current research interests include the effects of mutations in the genes associated with neurodegeneration at the cellular and molecular level. His laboratory efforts are directed at finding the underlying pathways that lead to neuronal dysfunction and cell death. Dr. Cookson received both his B.Sc. and Ph.D. degrees from the University of Salford, UK in 1991 and 1995, respectively. His postdoctoral studies included time spent at the Medical Research Council laboratories and at the University of Newcastle, Newcastle, UK. He joined the Mayo Clinic, Jacksonville, Florida, as an Assistant Professor in 2000 and moved to the NIA in February 2002. Within the Laboratory of Neurogenetics, Dr. Cookson's group will continue to work on movement disorders such as Parkinson's disease and dystonia, attempting to understand mechanisms leading to neuronal damage.

Keywords:

Parkinson's disease
neurons
cell culture models

Recent Publications:

O'Farrell C, et al. *Mol Brain Res* 2001; 97(1): 94-102.

Fray AE, et al. *Mol Brain Res* 2001; 94(1-2): 131-136.

Anneser JMH, et al. *Exp Neurol* 2001; 171(2): 418-421.

The group of neurodegenerative disorders collectively known as **movement disorders** include a diverse set of conditions selectively affecting groups of neurons along the neuraxis of the CNS. Several of these diseases have rare forms which are inherited in a Mendelian fashion, and there are often several different genes which, when mutated, can cause a similar phenotype in patients. The challenge is to understand how each gene product acts to produce neuronal damage and to identify pathways leading to neurodegeneration in human disease. This is not only intellectually challenging but may, one day, be used to underpin new treatments for neurological illness.

In **Parkinson's disease (PD)**, there is a striking, although not entirely selective, loss of dopaminergic neurons in the substantia nigra. There are several reported linkages to PD in different families, with two causal genes identified. The routes by which dominant mutations in α-synuclein produce cell loss are being explored. The "revealing illusion of selective vulnerability," where different neuronal groups are lost at different rates, is also of specific interest. In PD, we are concerned with the selectivity of dopaminergic cells to synuclein-mediated damage. The specific relationship between cell damage induced by dominant mutations in α-synuclein and the function of the recessive gene product, Parkin, are also being investigated. Identification of substrates for Parkin (an E3 ligase linked to the ubiquitin-proteasome system) might lead us to better understand the downstream pathway leading to cell death.

This group is also attempting to understand the nature of dominant mutations in torsinA, a gene associated with the related disorder **torsion dystonia**. In contrast to Parkinson's disease, there is evidence in dystonia of cellular dysfunction without underlying pathology. Therefore, a major effort is to understand the way in which torsinA mutations alter cellular physiology in terms of synaptic function. In both dystonia and Parkinson's disease, we are interested in the relationship between formation of intracellular protein aggregates and cell dysfunction, how these two phenomena interact and to what extent they can be dissociated.

There are a number of related neurological conditions, which are being explored in a collaborative manner. For example, **amyotrophic lateral sclerosis**, where there is loss of motor neurons in the spinal cord and motor areas of the cortex, is an area that we are beginning to explore. As in PD, there are several genes which, when mutated, can lead to this phenotype and genes associated with dominant (SOD1) and recessive (ALSIN) forms have been identified. One of the reasons for doing this is that there are likely to be common mechanisms late in the neurodegenerative pathway that are similar in different disorders. Identification of which parts of the neurodegenerative pathway are similar, and which are different, may give us a better understanding of the nature of specificity of neuronal damage in these diseases.

We are interested in applying specific techniques to the identification of pathways leading to neurodegeneration. One specific project that we have started is to apply **microarray technology** to cell culture models of neurodegeneration. By examining altered patterns of gene expression in the presence of several different genes that produce similar phenotypes, we aim to clarify the contribution of multiple gene products to neurodegeneration and to describe the likely order of each in a common pathway.

Collaborators: Dr. Christopher Eckman, Associate Consultant, Mayo Clinic, Jacksonville; Dr. Matthew J. Farrer, Associate Consultant, Mayo Clinic, Jacksonville; Dr. J. Timothy Greenamyre, Neurology, Emory University; Dr. Mark Mattson, Chief, Laboratory of Neurosciences, National Institute on Aging, NIH; Dr. Diane D. Murphy, National Institute of Neurological Disorders and Stroke, NIH; Dr. Leonardo Petrucelli, Instructor, Mayo Clinic, Jacksonville; Dr. Benjamin Wolozin, Associate Professor, Loyola University.

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The aging process in the nervous system shares many mechanisms with the aging process in other organ systems. At the biochemical and molecular levels such age-related changes include: increased oxidative damage to proteins, DNA and lipids; perturbations of energy metabolism; and alterations in the regulation of cell proliferation and death. At the functional level, both speed and accuracy of a range of behaviors, including cognition and control of body movements, are impaired. Due to improved preventative and therapeutic measures for cardiovascular disease and cancers, the average age of our population continues to increase. Unfortunately, accompanying the increase in life span there has been a progressive increase in the numbers of persons with age-related neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and stroke. Two major goals of research at the **Laboratory of Neurosciences (LNS)** are to understand normal aging of the nervous system at the cellular and molecular levels, and to identify the mechanisms responsible for age-related neurodegenerative disorders. Knowledge gained in such basic research is then being used by LNS investigators in preclinical studies to develop approaches (diet, lifestyle, drugs and cell therapy) for preventing and treating these disorders.

The organization of the research projects being performed by LNS scientists is as follows:

Oxidative Stress and Calcium Regulation: Studies by LNS investigators have provided evidence that excessive increases of oxygen free radicals and intracellular calcium levels are major factors contributing to neuronal dysfunction and degeneration in many different neurodegenerative disorders of aging. Novel approaches to measuring and manipulating free radicals and intracellular calcium levels are being developed, and incorporated into studies of experimental animal models of neurodegenerative disorders, in order to identify key alterations that result

in damage to neuron in humans with the disorders. Information gained from these studies is being used to develop treatments aimed at suppressing oxyradical production and stabilizing calcium homeostasis in neurons.

Apoptotic and Neuroprotective Signaling Pathways: A stereotyped biochemical cascade of events occurs in neurons that die in many different age-related neurodegenerative disorders. Such “programmed cell death” or “apoptosis” involves activation of proteolytic enzymes called caspases, mitochondrial dysfunction and nuclear DNA fragmentation. LNS researchers have shown that genetic mutations that cause Alzheimer’s disease and amyotrophic lateral sclerosis predispose neurons to apoptosis. Ongoing work is identifying the specific molecular triggers and executioners of neuronal apoptosis in different neurodegenerative disorders with the aim of developing drugs that interact with and block the cell death cascade. The fact that some individuals are able to age successfully with little or no evidence of neuronal degeneration in their brains suggests that the brain possesses cellular signaling mechanisms that protect neurons against adversity. A major effort of LNS investigators involves the identification of such neuroprotective signaling pathways.

Neural Regulation of Energy Metabolism and Stress Responses: The lifespan of organisms ranging from worms to mammals can be increased by genetic and/or dietary manipulations that affect energy metabolism. For example, mutations in the insulin signaling pathway increase the lifespan of *C. elegans*, and caloric restriction extends lifespan and enhances insulin sensitivity in rodents and monkeys. Studies by LNS scientists suggest that these same genetic and dietary factors can increase the resistance of the organism to stress, and may protect neurons in experimental models of neurodegenerative disorders. Recent findings of LNS investigators suggest that the brain can control energy metabolism and lifespan. Studies have shown that insulin signaling in the nervous system controls lifespan in *C. elegans*, and that neurotrophic factor signaling in the brain controls peripheral glucose metabolism in mice. Current studies are aimed at establishing the specific neural circuits involved in the regulation of stress responses and energy metabolism. The abilities of genetic and pharmacological manipulations of these pathways to modify neuronal damage and behavioral outcome in animal models of neurodegenerative disorders are being tested.

Synaptic Signaling and Plasticity: Signaling at the synapse plays fundamental roles in both immediate brain functions such as visual recognition and responses, and body movements, and long-term changes such as learning and memory. Recent findings by LNS investigators suggest that alterations in synaptic signaling occur very early in the course

of Alzheimer's disease and other age-related neurodegenerative disorders. The impact of oxidative stress, neurotrophic factor and cytokine signaling, and genetic aberrancies on synaptic physiology are being examined. By studying synaptic physiology, molecular biology and biochemistry in normal aging and in animal models of neurodegenerative disorders, LNS scientists hope to identify the specific alterations underlying neurodegenerative disorders.

Stem Cell Biology: Within the developing and adult brain, cells exist that are capable of proliferating and differentiating into neurons and glial cells. Such "neural stem cells" hold great promise for understanding brain development and plasticity, and for implementing novel approaches to maintaining or replacing neurons in the aging brain. LNS investigators are currently working to: 1) understand fundamental mechanisms that control stem cell proliferation and differentiation; 2) determine whether abnormalities in neural stem cell regulation occur in aging and neurodegenerative disorders; and 3) determine whether stem cell therapy approaches will have beneficial effects in animal models of neurodegenerative disorders.

Telomerase: Telomerase is an enzyme activity that prevents chromosome shortening and may counteract the adverse effects of aging on cellular DNA. LNS scientists have recently discovered that telomerase serves a neuroprotective function in experimental models relevant to Alzheimer's disease and stroke. These findings suggest the possibility that restoration of telomerase in neurons in the adult brain may protect against age-related neurodegeneration. Ongoing research is aimed at identifying the specific mechanisms whereby telomerase affects the function and survival of neurons. The work involves production of transgenic mice that overexpress the catalytic subunit of telomerase, and molecular studies aimed at identifying signals that can stimulate the telomerase gene.

Invertebrate Genetics: Fundamental mechanisms of aging have been highly conserved during evolution, and many aspects of aging are influenced greatly by genetics. Therefore, it is important to identify genes that either promote or hinder successful aging of the nervous system. The discovery of such genes, and the establishment of their normal functions and involvement in aging and disease, can be greatly facilitated by invertebrate molecular genetic approaches in species such as the fly *Drosophila melanogaster* and the roundworm *C. elegans*. The LNS aims to take advantage of the power of such invertebrate systems to identify new genes involved in aging and neurodegenerative disorders. Once the genes are identified, their human homologues will be cloned, and their normal functions and possible roles in neurodegenerative disorders elucidated in mammalian systems.

Inflammatory Processes: Inflammation-like changes occur in the brain during aging and in neurodegenerative disorders. These changes may include both innate (intrinsic) and acquired (involving circulating immune cells) immune responses. Work at the LNS suggests that some signaling pathways involved in the inflammatory process may be beneficial for neurons, whereas others may be detrimental. The mechanisms for activation of such inflammatory processes, and how such processes affect neuronal function and survival, are being examined. Based upon the knowledge gained from this work, novel preventative and therapeutic strategies for Alzheimer's disease and related disorders are being developed.

Behavior: Difficulties with learning and memory, motor problems, and anxiety and depression are among the most prominent problems that result from age-related alterations in brain function. In an effort to understand the biochemical and molecular alterations responsible for such behavioral disorders of aging, LNS investigators are developing technologies for quantifying various relevant behaviors in rodents and monkeys. Tests of learning and memory and motor function are being used to determine changes in these behaviors that occur during usual aging, and in animal models of Alzheimer's and Parkinson's diseases. Gene array technology is being used to identify genes that exhibit increased or decreased expression in association with age-related or disease-specific behavioral deficits.

Diet and Lifestyle: It is becoming increasingly appreciated that diet and daily habits can have a major impact on both risk for and severity of neurodegenerative disorders. A major effort at the LNS is aimed at identifying dietary and lifestyle factors that may either promote or ward-off neurodegenerative disorders of aging. LNS investigators have discovered that when rats and mice are maintained on a dietary restriction regimen (reduced calorie intake with maintenance of micronutrient levels), neurons in their brains are more resistant to dysfunction and degeneration in experimental models of Alzheimer's disease, Parkinson's disease, Huntington's disease and stroke. Ongoing projects are elucidating the molecular and cellular basis of this beneficial effect of dietary restriction. Recent findings indicate that dietary restriction induces increases in the levels of neurotrophic factors and "stress proteins" in brain cells. In related projects, the effects of "environmental enrichment" and physical activity on gene expression and neuronal vulnerability in experimental models of neurodegenerative disorders is being examined.

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Biography: Dr. Mattson received his Ph.D. in Biology from the University of Iowa in 1986. After 3 years of postdoctoral studies in Developmental Neuroscience at Colorado State University, Dr. Mattson took a faculty position at the Sanders-Brown Research Center on Aging at the University of Kentucky Medical Center where he was promoted to Full Professor in 1997. Dr. Mattson is currently Chief of the Laboratory of Neurosciences at the National Institute on Aging, and Professor of Neuroscience at Johns Hopkins University. He is Editor-in-Chief of the Journal of Molecular Neuroscience, and a Managing or Associate Editor of the Journal of Neuroscience, Journal of Neurochemistry and Journal of Neuroscience Research. In addition, he has edited 7 volumes in the areas of mechanisms of cell death, aging and age-related neurodegenerative disorders. Dr. Mattson has received numerous awards including the Metropolitan Life Foundation Award and the Alzheimer's Association Zenith Award. He is considered a leader in the area of cellular and molecular mechanisms underlying neuronal plasticity and neurodegenerative disorders, and has made major contributions to understanding of the pathogenesis of Alzheimer's disease, and to its prevention and treatment. Dr. Mattson has published more than 270 original research articles and more than 60 review articles.

Keywords:

neurodegenerative disorders
calcium and oxyradicals
signal transduction
synaptic plasticity

Recent Publications:

Glazner G, et al. *J Neurosci* 2000; 20(10): 3641-3649.

Kruman I, et al. *J Neurosci* 2000; 20(18): 6920-6926.

Prolla TA, et al. *Trends Neurosci* 2001; 24: S21-S31.

Cheng A, et al. *J Biol Chem* 2001; 276(46): 43320-43327.

A multifaceted array of experimental models of aging and age-related neurodegenerative disorders are being employed in Dr. Mattson's laboratory in order to establish the molecular and biochemical changes that occur during aging and in disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and stroke. Data obtained in these experimental models are integrated with data obtained in studies of both normal elderly humans and patients with neurodegenerative disorders to arrive at conclusions as to why neuronal dysfunction and degeneration occur in the disorders. In addition to identifying the molecular and cellular alterations that lead to neuronal degeneration in age-related neurological disorders, investigators are elucidating the cellular signaling mechanisms that allow successful brain aging.

Although specific brain regions are more severely affected in a given age-related neurodegenerative disorder (e.g., hippocampus in AD and substantia nigra in PD), each disorder appears to involve similar biochemical and cellular cascades that ultimately lead to dysfunction and death of the neurons. Specific components of such cascades include oxidative damage to proteins, lipids and DNA; metabolic compromise resulting from impaired glucose metabolism and mitochondrial dysfunction; and overactivation of glutamate receptors and disruption of neuronal calcium homeostasis. Each of these cascades is implicated in the

pathogenesis of AD, PD and stroke. Dr. Mattson's laboratory has played a major role in elucidating such neurodegenerative cascades, and is currently working to advance our understanding of the molecular and biochemical underpinnings of age-related neurodegenerative disorders. They have shown that genetic mutations that cause AD predispose neurons to apoptosis. Ongoing work is identifying the specific molecular triggers and executioners of neuronal apoptosis in different neurodegenerative disorders with the aim of developing drugs that interact with and block the cell death cascade. Several different experimental models have proven valuable in elucidating cellular and molecular mechanisms, and in developing novel preventative and therapeutic strategies. Models of AD being employed include transgenic mice that have been engineered to express mutant genes known to cause early-onset inherited AD, models of PD include administration of the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), and models of stroke include transient occlusion of the middle cerebral artery in rats and mice.

Perhaps of equal importance to knowledge of the molecular and cellular mechanisms that result in neuronal dysfunction and death in age-related neurodegenerative disorders, is a better understanding of successful brain aging at the cellular and molecular levels. It is clear that such "anti-aging" signaling mechanisms exist because some individuals can live for more than a century with very little decline in their cognitive or motor capabilities. A major goal of research in Dr. Mattson's laboratory is to identify the cellular signaling mechanisms that promote the survival and plasticity of neurons during aging. They have shown that signaling pathways activated by neurotrophic factors and certain cytokines can increase resistance of neurons to degeneration in experimental models of neurodegenerative disorders. The specific molecular and biochemical changes that participate in such beneficial signaling mechanisms are currently under study.

Synapses are sites of where neurotransmission and trophic factor signaling occurs. Synaptic signaling pathways play fundamental roles in both immediate brain functions such as visual recognition and responses, and body movements, and long-term changes such as learning and memory. Recent findings in Dr. Mattson's laboratory suggest that alterations in synaptic signaling occur very early in the course of AD and other age-related neurodegenerative disorders. The impact of oxidative stress, neurotrophic factor and cytokine signaling, and genetic lesions on synaptic physiology are being examined. Work is currently focussing on synaptic physiology, molecular biology and biochemistry in experimental animal models of neurodegenerative disorders.

In studies aimed at identifying preventative and therapeutic strategies for neurodegenerative disorders, the laboratory has shown that rats and mice maintained on a dietary restriction (DR) regimen exhibit increased resistance to degeneration of hippocampal neurons in models of AD, increased resistance of substantia nigra dopaminergic neurons in models of PD, and increased resistance of cortical and striatal neurons in stroke models. Interestingly, DR increases neurogenesis in the hippocampus which may possibly contribute to enhanced cognitive function and resistance to injury. The cellular and molecular mechanisms that mediate the beneficial effects of DR on brain plasticity and resistance to injury are being studied.

DNA damage increases in brain cells during aging and may be an important trigger of cell death in neurodegenerative disorders. A better understanding of mechanisms of DNA damage and repair may therefore provide a foundation for developing novel approaches for preventing neuronal degeneration. Investigators in Dr. Mattson's laboratory have identified genetic and environmental factors that may promote or prevent DNA damage and its adverse consequences in the nervous system. An example of recent findings include the demonstration that folic acid deficiency can sensitize neurons to DNA damage and death in experimental models of Alzheimer's disease and Parkinson's disease. Low levels of dietary folic acid result in an elevation of homocysteine levels. Homocysteine impairs the ability of neurons to repair DNA damage resulting in increased uracil misincorporation and oxidatively modified DNA bases. In another set of studies LNS scientists have shown that telomerase, a reverse transcriptase that prevents chromosome shortening in mitotic cells, can protect neurons against DNA damage-induced apoptosis. Additional studies have established roles for telomerase in brain development where it appears to promote neuroblast proliferation and the survival of early postmitotic neurons. Telomerase is not normally expressed in neurons in the adult brain, but LNS scientists have generated transgenic mice that do express the telomerase protein in neurons and are testing the hypothesis that their neurons will be protected against damage in experimental models of age-related neurodegenerative disorders.

Stroke is the major neurological cause of disability and death worldwide. Research in Dr. Mattson's laboratory is revealing the molecular mechanisms responsible for neuronal death after a stroke, and is developing novel therapeutic strategies for improving outcome in stroke patients. A mouse stroke model in which the middle cerebral artery is occluded resulting in highly reproducible damage to the cerebral cortex and associated sensory-motor dysfunction is employed in combination

with studies of cultured brain cells. Two examples of ongoing major efforts are projects that target the tumor suppressor protein p53 and mitochondrial ATP-sensitive potassium (Mito-KATP) channels. Using molecular and biochemical analyses it has been established that p53 plays a critical role in a form of programmed cell death that occurs in neurons after a stroke. In collaboration with the Drug Design and Development Section, a panel of chemical inhibitors of p53 has been synthesized and screened for efficacy in protecting neurons against ischemic injury in culture and against stroke-induced damage in mice. Several highly effective p53 inhibitors have been identified, two of which readily cross the blood-brain barrier and are effective when given intraperitoneally. The lead agent is being moved toward phase I clinical trials. In a second project, it was discovered that a drug called diazoxide, which opens Mito-KATP channels, is very effective in reducing brain damage and improving functional recovery following a stroke in mice. This drug has already been approved by the FDA for other indications, and it is therefore hoped that it can be used in clinical trials in human stroke patients. By studying mice with targeted disruption of specific genes believed to play a role in the pathogenesis of stroke, investigators are working to identify additional therapeutic targets.

A major effort is underway to determine whether abnormalities in the process of neurogenesis, the production of new nerve cells from neural stem cells, occur in aging and age-related neurodegenerative disorders. The proliferation, differentiation and survival of neural stem cells in the hippocampus and subventricular zone/cerebral cortex are being assessed in mouse models of Alzheimer's disease, Parkinson's disease and stroke. Studies of transgenic mice expressing mutant forms of amyloid precursor protein and presenilin-1, which cause inherited forms of Alzheimer's disease in humans, exhibit defects in neurogenesis. These abnormalities appear to result from increased production of the amyloid beta-peptide and perturbed calcium regulation in the neural stem cells and their progeny. In other studies, the signals that regulate the differentiation and survival of neural stem cells are being elucidated. Investigators in the Cellular and Molecular Neurosciences Section have shown that nitric oxide and brain-derived neurotrophic factor can promote neurogenesis. Interestingly, neurogenesis can be affected by diet – caloric restriction and dietary supplementation with folic acid stimulate neurogenesis suggesting a mechanism whereby dietary factors may modify brain aging and risk of neurodegenerative disorders.

Sphingomyelin and cholesterol are important components of the plasma membrane of neurons where it functions in cellular signal transduction and cellular responses to stress. By analyzing spinal cord and brain tissues

from human patients and mouse models, investigators in this section of the LNS have shown that profound abnormalities in sphingomyelin metabolism occur in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. The alterations, which include accumulation of long-chain ceramides and cholesterol esters, occur prior to neuronal degeneration and functional deficits in the mouse models. Moreover, agents that inhibit sphingomyelin synthesis or metabolism can protect neurons from being damaged and killed in experimental models of ALS and Alzheimer's disease, suggesting that the abnormalities in lipid metabolism are central to the disease process.

Collaborators: George Martin, M.D., University of Washington; Junying Yuan, Ph.D., Harvard University; Tej Pandita, Ph.D., Columbia University; Joseph D. Buxbaum, Ph.D., Mount Sinai School of Medicine; Frank LaFerla, Ph.D., University of California Irvine; Jonathan Geiger, Ph.D., University of Manitoba; William Markesbery, M.D., University of Kentucky; D. Alan Butterfield, Ph.D., University of Kentucky; Don Gash, Ph.D., University of Kentucky; James Herman, Ph.D., University of Cincinnati.

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Biography: Dr. Wolkow received her Ph.D. in 1997 in molecular biology and genetics from the Johns Hopkins University School of Medicine where she studied the regulation of transposition target site selection in bacteria. Moving to Boston, she carried out postdoctoral research as a research fellow of the Leukemia and Lymphoma Society with joint appointments at the Massachusetts General Hospital and Harvard University. During this period, Dr. Wolkow investigated longevity control by insulin-like signaling in C. *elegans*. This work forms the basis for current studies into the neuronal pathways under control of insulin-like signaling in C. *elegans*. She is also expanding her research program to investigate genes necessary for successful nervous system aging.

Keywords:

lifespan control
insulin/insulin-like signaling
nervous system aging
gerontogene

Recent Publications:

Wolkow C, et al. *Science* 2000; 290(5489): 147-150.

Genetics of Longevity in *C. elegans*: The nematode, *C. elegans*, is quickly becoming a favorite organism for studying the genetics of longevity. Under laboratory conditions, wild-type *C. elegans* adults have a two-week lifespan. Genetic mutations have been identified which allow worms to live up to three times longer. The molecular identification and characterization of the genes responsible for these mutant phenotypes has provided new insights into pathways controlling lifespan. In particular, we have learned that multiple pathways control longevity in *C. elegans*. Some of these lifespan pathways interact, while others function independently of the rest. In addition, *C. elegans* lifespan can be lengthened by caloric restriction, as has been documented for other species. Humans and nematodes share many of the same genes, so it is likely that human longevity will be controlled by some of the same genes that control *C. elegans* lifespan. Thus, studies of genetic control of longevity in *C. elegans* will help to reveal mechanisms that also control human longevity.

Insulin Control of Longevity: Mutations disrupting insulin-like signaling in *C. elegans* dramatically increase lifespan and enhance stress resistance. Animals lacking a functional insulin receptor or PI(3)K, encoded by the genes *daf*-2 and *age*-1, respectively, live two to three times longer than wildtype. Insulin-like control of longevity has been documented in other species as well. Fruitflies with defective insulin-like signaling survive longer than wild-type and mice lacking growth hormone display extended longevity.

The components of insulin-like signaling pathways have been conserved from *C. elegans* to humans. The *C. elegans* genome contains 37 genes encoding insulin-like genes, all potential ligands for the DAF-2/insulin receptor. Once activated, the DAF-2/insulin receptor transduces signals intracellularly via IST-1, a homolog of vertebrate IRS1-4, and via AGE-1/AAP-1, comprising the p110 catalytic subunit and p55 adaptor subunit of PI(3)K. The lipid products of AGE-1 activate downstream S/T kinases, PDK-1 and AKT-1 and –2. DAF-18, a homolog of the vertebrate PTEN lipid phosphatase, antagonizes DAF-2 signaling. Signaling downstream of DAF-2 antagonizes the activity of the forkhead transcription factor DAF-16. When DAF-2 signaling is disrupted, DAF-16 is active and can activate the expression of target genes required for long lifespan. Two candidate DAF-16 target genes have been identified, *ctl*-1, encoding a cytosolic catalase, and *sod*-3, encoding Mn-SOD. One hypothesis is that *ctl*-1 and *sod*-3 expression enhance stress resistance in *daf*-2 mutants, thereby extending lifespan. Consistent with this hypothesis, *ctl*-1 is required for long lifespan in *daf*-2 mutants.

Insulin-like signaling in neurons is required for normal lifespan in *C. elegans*. Animals with insulin-like signaling restricted to non-neuronal cell types live as long as *daf*-2 pathway mutants with no insulin-like signaling at all. A major project in this laboratory is to define how neurons control longevity. We will determine whether specific neurons control lifespan and identify downstream pathways of insulin-like signaling in the nervous system.

A second goal of the IMG unit is to determine the roles of *sod*-3 and *ctl*-1 in increasing longevity and to identify the cell types where these genes function. In collaboration with Koen Houthfood and Jacques R. Vanfleteren, Ph.D. of Gent University Department of Biology, Belgium, the metabolic parameters of long-lived insulin pathway mutants are being characterized. The goal of these metabolic studies is to provide insight into the physiologic traits that may distinguish long-lived animals. The roles of other gene products which may impinge upon stress resistance pathways in neurons are also being assessed. For example, the function of a worm uncoupling protein homolog is being studied in collaboration with Eric Bachman, M.D., Ph.D. and Bradford B. Lowell, M.D., Ph.D. of the Beth Israel Deaconess Medical Center Division of Endocrinology and with Kaveh Ashrafi, Ph.D. and Gary Ruvkun, Ph.D. of the Massachusetts General Hospital Department of Molecular Biology.

An independent, but related, research direction is the identification of new components of insulin-like signaling pathways. Many signaling pathways important in the human nervous system utilize the same pathway components as does insulin-like signaling in the worm. By using the worm, new components of these pathways can be quickly identified. A genetic screen for mutations suppressing a developmental arrest phenotype of *age*-1/PI(3)K(-/-) mutants was used to identify nearly 40 independent mutations in genes that may normally function to antagonize insulin-like signaling. We are actively pursuing molecular identification of these genes.

Successful Nervous System Aging: The Worm's Tale: In humans, nervous system function declines significantly as a consequence of aging. Even healthy aged individuals display losses of nervous system function, for example, the progressive loss of sensory and motor function. To understand the changes that accompany aging in the nervous system, it is important to identify the critical components of the cellular machinery mediating nervous system function. Our strategy for contributing to this effort is to use the worm to identify genes whose function is required for successful nervous system aging.

Relatively little is known about nervous system aging in *C. elegans*. Members of the IMG unit will first characterize longitudinally how *C. elegans* nervous system function changes during aging and then use these findings to design genetic screens for mutations disrupting successful nervous system aging. Cloning and characterizing these genes will enable us to identify the critical components for successful nervous system aging.

C. elegans can also be used to identify genes that are critical in neuronal degenerative processes. Strategies for inducing neuronal degeneration in other models, such as MPTP treatment or induction of oxidative stress, will be examined for their effects on nematode nervous system function. Again, genetic screens will be used to identify mutants affecting the animal's sensitivity to these treatments in order to identify genes that are critical for resistance to these stresses.

Finally, the IMG unit will use *C. elegans* as a tool for rapidly screening compounds that can mimic longevity extension of caloric restriction. Several studies have documented the fact that *C. elegans* lifespan is extended by dietary restriction, as has been shown for other species of

invertebrates and vertebrates. In addition, caloric restriction has been shown to enhance stress resistance in nematodes and other species and may therefore aid in successful aging. However, it may be difficult to convince the human population to submit to dietary restriction voluntarily. An alternative strategy is to identify chemical compounds which are non-toxic and mimic the effects of caloric restriction. In collaboration with other labs in the LNS, chemical compounds will be rapidly screened for lifespan-extending effects in *C. elegans* and lead compounds identified in such screens will be further characterized for effects on mammalian aging phenotypes.

Summary: The research program of the IMG unit is targeted to provide a comprehensive understanding of how aging affects the nervous system. Studies of neuronal insulin-like control of longevity will identify factors that determine longevity and help us understand how lifespan could be increased. These studies also investigate the role of stress resistance in longevity control. Nervous system aging in normal and challenged backgrounds will reveal gene products critical for successful aging. Nematodes will also be useful for rapidly identifying chemical compounds affecting longevity that may offer therapeutic potential in humans. Together, this work will provide insight into challenges confronting the aging nervous system as well as strategies for coping with them.

Collaborators: Koen Houthoofd and Jacques R. Vanfleteren, Ph.D., Gent University, Department of Biology, Belgium; Eric Bachman, M.D., Ph.D. and Bradford B. Lowell, M.D., Ph.D., Beth Israel Deaconess Medical Center, Division of Endocrinology; Kaveh Ashrafi, Ph.D., and Gary Ruvkun, Ph.D., Massachusetts General Hospital, Department of Molecular Biology and Harvard University, Department of Genetics.

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Biography: Dr. Mahendra S. Rao received his M.D. from Bombay University in India and his Ph.D. from the California Institute of Technology in 1991. After completing postdoctoral training with Dr. S. Landis at Case Western Reserve and Dr. D. J. Anderson at California Institute of Technology, he joined the University of Utah as an Assistant Professor in 1994. He was promoted to an Associate Professor and awarded tenure in 1999. At Utah he began a new line of investigation which was to define the molecular events that underlie differentiation of the central and peripheral nervous system. In 1999 he was honored by the American Association of Anatomists as the C.J. Herrick Young Investigator and the University of Utah recognized his abilities by awarding him early tenure and promotion to Associate Professor. Dr. Rao recently accepted a position as the Head of a newly established stem cell group in NIA's Laboratory of Neurosciences.

Keywords:

stem cells
aging
neurobiology
ES cells
neurons
strocytes
oligodendrocytes

Recent Publications:

Mujaba T, et al. *Dev Biol* 1999; 214: 113-127.

Stark M, et al. *Mech Dev* 2000; 93: 195-200.

Yang T, et al. *Proc Natl Acad Sci USA* 2000; 97(24): 13366-13371.

Coskun V, et al. *Int J Dev Neurosci* 2001; 19(2): 219-227.

Overview: A fundamental breakthrough in our understanding of nervous system development was the identification of multipotent neural stem cells (neurospheres) about ten years ago. Dr. Weiss and colleagues showed that EGF (epidermal growth factor) dependent stem cells could be harvested from different brain regions at different developmental stages and that these could be maintained over multiple passages *in vitro*. This initial finding has lead to an explosion of research on stem cells, their role in normal development and their potential therapeutic uses. Many investigators have entered this field and the progress made has been astounding.

My group in the Laboratory of Neurosciences, Stem Cell Biology Unit focuses on the cellular and molecular mechanisms that regulate the proliferation, differentiation and survival of neural progenitor cells in the brain and spinal cord during development and in the adult. This research is based firmly on the concept that the same signaling mechanisms that regulate development and plasticity of the nervous system are altered during aging and in age-related neurodegenerative disorders. Accordingly, an understanding of developmental mechanisms is likely to lead to novel approaches to preventing and treating neurological disorders of aging. Ongoing research is divided into four interrelated areas: 1) Signal transduction mechanisms regulating the proliferation, differentiation and survival of embryonic stem cells and pluripotent neural stem cells. 2) Cellular and molecular alterations that occur in neural stem cells during

aging and in age-related neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. 3) Elucidation of the mechanisms whereby environmental factors such as diet, and intellectual and physical activity affect neural stem cells. 4) Development of novel stem cell therapy-based approaches for repairing the aging and diseased nervous system.

Molecular Mechanisms That Regulate Stem Cell Differentiation into Neurons and Glia: We are working to define the molecular and cellular interactions that instruct pluripotent cells to differentiate into cells restricted to a particular phenotype. The current focus is on characterizing the neuroepithelial precursor cell that gives rise to the central and peripheral nervous system in mammals. These precursor cells differentiate from the epithelium and form the neural plate that subsequently folds to form the neural tube. At or around the time of neural tube closure some neuroepithelial cells are excluded from the neural tube and form the neural crest. Available evidence suggests that the neuroepithelial precursors may be heterogeneous in terms of trophic dependence and developmental potential. We are examining the properties of the neuroepithelial precursor that is present in the caudal neural tube and generates the spinal cord and neural crest cells of the trunk region. Differentiation into spinal cord and neural crest cells has been studied in many different species including chick, xenopus, and mouse using transplantation, cell culture and single cell injections. Our data suggest that individual cells in the neural tube are pluripotent and can give rise to neural tube cells as well as to crest cells. Furthermore, the repertoire of responses of individual precursor cells becomes progressively restricted during development. Thus, neural development appears to involve a sequence of events in which multipotent stem cells undergo progressive developmental restriction to give rise to terminally differentiated phenotypes. Our studies of neuroepithelial precursors are focused on addressing the following questions: 1) What are the environmental signals or factors that specify the phenotypic fate of neural precursor cells? 2) What stage of development do these factors regulate? 3) Can we identify the earliest phenotypic and antigenic changes that distinguish a restricted precursor cell from more pluripotent cells? 4) Are these factors involved in known disorders of the nervous system and neural crest development? We have chosen to address these questions by analyzing neuroepithelial development in mice to take advantage of known mutants and recently generated transgenic mice. We plan to focus on two different lineages that arise from the caudal neural tube, namely, the neural crest stem cell and the central nervous system stem cell. Our strategy in both systems is to combine in vitro culture and clonal analysis experiments and in vivo expression and perturbation studies to identify environmental signals that influence differentiation. Some of our recent findings are as follows:

Differentiation of CNS Stem Cells. We have begun studying differentiation by establishing culture conditions that maintain stem cells in an undifferentiated state and thereby allow initiation of the differentiation process by removal of the proliferative signals. To date we have identified neuronal restricted precursors (NRP1s) and glial restricted precursors (GRP1s) that can be isolated from fetal and adult tissue using cell surface markers. We have used degenerate PCR to identify cell-specific molecules expressed at specific stage of development. Preliminary results have identified several novel genes that are present in subsets of early neural precursor cells. We have begun to establish precursor cell lines and overexpress candidate molecules in cell lines to identify their role in development. To date we have identified a glial restricted precursor cell line and a neuronal precursor cell line. We will use these cell lines for large-scale genomic screens to identify novel genes that may be involved in the process of differentiation. We are also studying the interactions of identified factors with other transcriptional regulators of stem cell development. Our current focus is on HLH proteins and POU homeodomain proteins.

Neural Crest Differentiation. We have established mass and clonal culture assays to determine how a single neural crest cell differentiates into neurons, glia, melanocytes, smooth muscle and cartilage. We have generated stage-specific markers to distinguish stages of differentiation. Clonal cell lines that recapitulate normal development have also been established. We have begun to use these tools to define the factors that regulate differentiation.

Embryonic Stem Cell Differentiation. Embryonic stem (ES) cells are totipotent cells of the blastocyst that are capable of forming any cell type in the body. Our laboratory examines ES cell cultures to determine whether normal embryonic development can be recapitulated *in vitro*. We have shown that neural stem cells, NRP cells and GRP cells can be directly isolated from ES cell cultures and that these cells appear similar to cells isolated from later stages of development. Current work is focused on isolating other lineages and determining if similar strategies can be used to isolate more differentiated precursors from human ES cell cultures.

In order to critically evaluate the roles of specific genes in the regulation of neural stem cell proliferation, differentiation and/or survival, we are initiating a major effort in which ES cell lines are derived from mice lacking expression of individual genes. For example, we are studying the role of the transcription factor NF-κB in neural stem cell fate determination by analyzing ES cells isolated from mice lacking the p65 subunit of NF-κB.

Identifying factors that regulate neural precursor cell differentiation is important because there are many prominent neurological disorders for which such knowledge may provide new avenues for prevention and treatment. Identifying instructive and trophic molecules and their stage-specific roles in regulating normal development will provide important information both in diagnosing neurological disorders and in suggesting possible therapeutic strategies. In a more general sense, we hope that the principles of differentiation we elucidate will be applicable to other developmental stages and locations where phenotypic restriction occurs.

Changes in Neural Stem Cells During Aging and in Neurodegenerative Disorders: Despite the fact that the adult brain and spinal cord contain neural stem cells, there is virtually no information available concerning the impact of aging and neurodegenerative disorders on these stem cell populations. In collaboration with investigators in the Cellular and Molecular Neuroscience Section, we are performing a series of studies aimed at understanding the cellular and molecular changes that occur in neural stem cells during aging and in age-related neurodegenerative disorders including Alzheimer's, Parkinson's and Huntington's diseases, stroke, and amyotrophic lateral sclerosis. Using a battery of cell culture and animal models, in combination with studies of postmortem brain and spinal cord tissues from human patients, alterations in stem cell populations are being identified, and the molecular basis of the alterations ascertained.

Impact of Diet and Lifestyle on Neural Stem Cells: Investigators in the Cellular and Molecular Neuroscience Section have recently discovered that neural stem cells in the brains of rats and mice can be influenced by dietary factors. Specifically, it was found that dietary restriction (a reduced calorie diet) can enhance the survival of newly generated neural cells in the hippocampus of rats and mice (Lee et al., J. Mol. Neurosci. 15: 99-108, 2000). Other investigators have shown that raising rodents in an enriched environment, or under conditions where their level of exercise is increased, results in increased neurogenesis in their brains. We are currently performing studies aimed at identifying the underlying cellular and molecular mechanisms. Interestingly, the data available to date suggest that calorie restriction induces a mild cellular stress response in neural cells that results in increased production of neurotrophic factors (particularly brain-derived neurotrophic factor) and certain stress proteins (including HSP-70 and GRP-78). This may account for the increased survival of neural stem cells, as well as the increased resistance of neurons

to injury in experimental models of neurodegenerative disorders. We are currently evaluating the status of several other candidate signaling pathways including those activated by insulin-like growth factors, opioids and cytokines.

Transplantation- and Signaling-Based Therapeutic Approaches for Neurodegenerative Disorders: Much of the recent excitement surrounding stem cell research is due to the potential use of stem cells for replacing dysfunctional cells within a tissue, or even entire organs. Recovery of function has been recently reported in animal models of spinal cord trauma and demyelinating disease. We are employing transplantation technologies in order to understand fundamental mechanisms of neural stem cell biology, on the one hand, and to develop therapeutic interventions, on the other hand. Stem cells from transgenic mice that express reporter genes (green fluorescent protein or beta-galactosidase) are transplanted into the brain or spinal cord of wild-type mice, and their fate followed. Reporter mice are being crossed with gene knockout mice to obtain embryonic stem reporter cells that lack a single gene. The function of that gene in stem cell behavior can then be studied. The ability of selected stem cell populations to repopulate normal and diseased brains (mouse models of Alzheimer's, Parkinson's and Huntington's diseases, stroke and ALS) is being evaluated. As additional information accumulates, we hope to select population of human cells for cell replacement therapy.

Collaborators: Mark Mattson, Laboratory of Neurosciences, NIA, NIH; William Freed, National Institute on Drug Abuse, NIH; Nicolas Maragakis, Jeffrey Rothstein, Johns Hopkins Medical School, Baltimore, MD; Steve Goldman, Columbia University, New York, NY; Itzhak Fischer, MCP Hanneman, Philadelphia, PA.

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Biography: Dr. Furakawa received his M.D. degree from Yamagata University in Japan in 1988 and his Ph.D. in Neuroscience from Tohoku University School of Medicine in 1992. He performed postdoctoral studies with Dr. Mark Mattson at the Univesity of Kentucky Medical Center and was an Assistant Research Professor at the University of Washington in Seattle. His work in Seattle focused on mutations in the microtuble-associated protein tau that cause an inherited form of dementia. Dr. Furukawa moved back to Japan in 1998 to work on his clinical medicine practice as a neurologist and to continue his research on tau mutations. He joined the Laboratory of Neurosciences in 2001 as a tenure-track investigator responsible for developing a Synaptic Physiology Unit.

Keywords:

Alzheimer's disease
electrophysiology
calcium
ion channels
patch-clamp
brain slice recording

Recent Publications:

Furukawa K, et al. *Neuroreport* 2000; 11(1): 57-60.

Shimizu H, et al. *Tohoku J Exp Med* 1999; 189(3): 203-211.

Overview: A unique feature of the central nervous system, that is largely responsible for both the speed and complexity of inter-cellular signaling in this organ system, is the synapse. Synapses are the sites where various neurotransmitters, neuropeptides, and neurotrophic factors act to regulate neuronal development and survival. Signaling at synapses controls all of our intellectual, sensory, motor, and neuroendocrine activities. Alterations in synaptic structure and function occur during normal aging, and increasing evidence suggests that abnormalities in synaptic signaling play major roles in the pathogenesis of age-related neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, spinocerebellar degenerations, and stroke. The **Synaptic Physiology Unit** aims to understand the molecular basis for synaptic dysfunction and degeneration in aging and age-related neurological disorders. The two major types of technologies employed are electrophysiological recordings using patch-clamp methods and imaging of fluorescent probes for ions and second messengers. Current and planned projects can be divided into four interrelated areas: 1) Mechanisms whereby genetic mutations and polymorphisms affect synaptic plasticity. 2) The roles of biochemical cell death cascades in synaptic plasticity and degeneration. 3) Interactions of oxidative and metabolic stress with membrane voltage-dependent and ligand-gated ion channels. 4) The impact of dietary and other environmental factors on synaptic function in relation to aging and neurodegenerative disorders.

Impact of Genetic Aberrancies on Synaptic Function and Ion Homeostasis: We have and are continuing to examine the effects of genetic mutations that cause Alzheimer's disease on ion channel function and synaptic plasticity in cell culture and animal models. Mutations in the amyloid precursor protein (APP) may cause Alzheimer's disease by increasing the production of neurotoxic forms of amyloid β-peptide and by decreasing production of a secreted form of APP (sAPP). Our whole-cell patch clamp analyses of ion currents in cultured hippocampal neurons have shown that amyloid β-peptide can enhance currents through glutamate receptor channels and voltage-dependent calcium channels. On the other hand, sAPP suppresses neuronal excitability by activating high-conductance potassium channels. Mutations in presenilin-1 are responsible for many cases of early-onset autosomal dominant Alzheimer's disease. We have found that presenilin-1 mutations perturb neuronal calcium homeostasis by enhancing calcium release from the endoplasmic reticulum. The alterations in calcium regulation conferred by presenilin-1 mutations render neurons vulnerable to excitotoxicity and apoptosis. More recently, we have been examining the effects of mutations in the microtubule-associated protein tau that cause fronto-temporal dementia and parkinsonism on neuronal ion homeostasis. The tau mutations result in increased calcium influx through voltage-dependent channels when neural cells are subjected to growth factor deprivation. We are now examining the effects of APP, presenilin-1 and tau mutations on synaptic correlates of learning and memory in hippocampal slice cultures from transgenic and knock-in mice.

In addition to Alzheimer's disease, several other age-related neurodegenerative disorders can be caused by genetic defects. For example, some cases of Parkinson's disease are caused by mutations in α-synuclein and others by mutations in parkin. Huntington's disease is a purely inherited disorder resulting from trinucleotide expansions in the huntingtin gene resulting in polyglutamine repeats in the huntingtin protein. We are currently assessing the impact of these mutations on ion channel activity and synaptic plasticity in dissociated cell cultures and brain slices from transgenic mice expressing mutant forms of α-synuclein and huntingtin.

Modulation of Ion Channels by Cell Death Cascades and Neuroprotective Signal Transduction Pathways: LNS investigators have recently shown that biochemical cascades that mediate a form of programmed cell death called apoptosis can be activated locally in

synaptic terminals. Interestingly, we have identified several ion channel subunits as substrates for caspases, proteases that play a pivotal role in apoptosis. For example, subunits of the AMPA subtype of ionotropic glutamate receptor are cleaved by one or more caspases resulting in a suppression of channel activity. Cleavage of the glutamate receptor subunits by caspases appears to serve the function of ensuring that the neuron dies by apoptosis rather than excitotoxic necrosis. Interestingly, we also have evidence from studies of hippocampal slices that caspase-mediated cleavage of glutamate receptors may function in the regulation of synaptic plasticity under physiological conditions.

A major effort in the LNS has been to identify, characterize and manipulate signal transduction pathways that promote neuronal survival and plasticity. We have found that several such trophic factors act, at least in part, by modulating neuronal excitability via transcription-dependent mechanisms. One transcription factor of interest is NF-κB which exerts a strong anti-apoptotic effect in neurons. NF-κB can modulate the expression of certain ion channel subunits and thereby modify long-lasting changes in synaptic strength (long-term potentiation and long-term depression). We are determining the roles for such signaling pathways in the pathogenesis of neurodegenerative disorders, and are also using the knowledge gained from these studies to develop novel preventative and therapeutic strategies for neurodegenerative disorders.

Oxidative Stress and Synaptic Function: Levels of oxidative stress and oxidative damage to proteins, nucleic acids and membrane lipids increase during aging of the nervous system. Moreover, oxidative damage to neurons is widely recognized as a major contributing factor to the dysfunction and death of neurons in a range of age-related neurodegenerative disorders. Despite the fact that perturbed cellular ion homeostasis is implicated in the same neurodegenerative disorders, very little information has been obtained on the impact of oxidative stress on neuronal ion homeostasis and synaptic function. LNS investigators have discovered several novel mechanisms whereby oxidative stress affects neuronal excitability, and have shown how these mechanisms are involved in the pathogenesis of Alzheimer's disease, amyotrophic lateral sclerosis and stroke. For example, we have found that an aldehyde called 4-hydroxynonenal, which is produced when membrane lipids are attacked by free radicals, can covalently modify proteins and impair their function. In this way, 4-hydroxynonenal promotes excessive influx of calcium into

neurons. We are currently determining the roles of specific oxyradicals and antioxidants on neuronal ion channels in patch clamp studies, and on synaptic plasticity in brain slice preparations.

Impact of Dietary Factors on Synaptic Physiology: A major focus of LNS investigators is to establish the cellular and molecular mechanisms whereby diet influences risk of neurodegenerative disorders. In this regard major progress has recently been made in establishing neuroprotective effects of dietary restriction in experimental models of Alzheimer's, Parkinson's and Huntington's diseases and stroke. This work has led to the hypothesis that dietary restriction reduces risk of age-related neurodegenerative disorders by inducing a mild cellular stress response in which neurons upregulate the expression of genes that encode cytoprotective proteins including heat-shock proteins and neurotrophic factors. Because dietary restriction can also enhance learning and memory, we are determining the effects of dietary restriction on synaptic physiology. Additional dietary factors may influence risk of neurodegenerative disorders. For example, LNS investigators have recently provided evidence that folic acid deficiency can endanger neurons in experimental models of Alzheimer's and Parkinson's diseases. We are therefore determining the effects of folic acid deficiency on synaptic function.

Collaborators: Professor Gerard D. Schellenberg, University of Washington, Seattle, WA; Professor Thomas D. Bird, University of Washington, Seattle, WA; Professor Yasuto Itoyama, Tohoku University, Sendai, Japan; Dr. Akihiko Takashima, Riken Institute, Wako, Japan.

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Biography: Dr. Ingram was trained in psychology and gerontology at the University of Georgia where he received his Ph.D. in 1978. From 1978-79 he served as a National Institute of Mental Health-supported postdoctoral fellow in behavior genetics at the Jackson Laboratory. He came to NIA in 1980 as a Staff Fellow in the Laboratory of Behavioral Sciences and then moved to the Laboratory of Cellular and Molecular Biology in a tenured position in 1985. He was appointed Chief of the Behavioral Neuroscience Section in 2000. His work has concerned development of behavioral assays of aging in rodents and in primates with focus on motor and memory performance as well as assessment of various pharmacologic, genetic, and nutritional interventions that affect brain aging. Dr. Ingram currently serves on the editorial boards of several journals, including the *Neurobiology of Aging*, *Experimental Aging Research*, *Journal of Anti-Aging Medicine*, and *CNS Drug Reviews*, and he is an editor for *Gerontology*. He has also served in numerous positions within the Biology Section of the Gerontological Society of America, and he is a past president of the American Aging Association.

Keywords:

brain aging
behavioral performance
memory
neurotransmitters

Recent Publications:

Ingram DK, et al. *Ann NY Acad Sci* 2001; 928: 316-326.

Ingram DK, et al. *Exp Gerontol* 2001; 36(7): 1025-1034.

Umegaki H, et al. *Neuroscience* 2001; 103(1): 27-33.

Jucker M, et al. *Exp Gerontol* 2000; 35(9-10): 1383-1388.

Ogawa O, et al. *Neuroreport* 2000; 11(4): 743-748.

Behavioral Neuroscience of Aging: Aging occurs at multiple levels of biological organization. Behavior represents the integration of multiple aging processes that reflect the functional capacity of the organism. We have developed a battery of cognitive and motor tests to assess neurobiological mechanisms of age-related behavioral impairments in rodents and to evaluate interventions that purport to alter these impairments.

Regarding age-related decline in memory performance, we have focused on the cholinergic and glutamatergic systems and their interaction. For cholinergic interventions, we have collaborated with Dr. Nigel Greig of the Laboratory of Neurosciences to develop a novel class of cholinesterase inhibitors, that are long-acting, highly specific for acetylcholinesterase, with a wide range of therapeutic efficacy and low toxicity within this range. For glutamatergic interventions, we are examining manipulations of the glycine and polyamine sites on the N-methyl-D-aspartate (NMDA) glutamate receptor as well as generators of nitric oxide (NO) that is activated through the NMDA receptor. We have found that combinations of the glycine agonist, D-cycloserine, and the polyamine agonist, spermine, can act synergistically to improve learning performance. NO donors are also being assessed to overcome age-related learning impairments. In collaboration with Dr. Hideki Kametani, age-related changes in NMDA-stimulated NO release are being assessed using *in vivo* microdialysis. Collaborating with Dr. Peter Mouton, we are examining the

role of estrogen in preserving memory and reducing glia-mediated inflammation in a mouse model of Alzheimer's disease. In addition to the behavioral analysis, the latter project is part of a larger collaboration with Drs. Peter Mouton and Mathias Jucker that involves quantitative morphometrics using unbiased stereology in a variety of mouse models. Specifically, we are assessing age-related changes in the numbers of neurons, synapses, and glia, in the hippocampus of mice from different genders and strains including transgenics and knock-outs. The objective is to relate specific neuromorphometric parameters to age or treatment-induced changes in cognitive performance. In collaboration with Drs. Nan-Ping Weng and Dan Longo of the Laboratory of Immunology, we are using microarray technology to identify genes involved in memory formation and possible age-related changes in gene expression. Several candidate genes have been identified that show little expression in the hippocampus of learning-impaired rats compared to higher levels of expression in young rats.

Regarding age-related motor impairment, we have focused on the loss of striatal dopamine D_2 receptors. Collaborating with Drs. George Roth, Hiroyuki Ikari, and Hiroyuki Umegaki, we have developed an adenoviral vector that can mediate genetic transfer of the D_2 receptor into rat brain and produce functional changes due to this receptor. We are currently using positron emission tomography (PET) to image vector-mediated production and decline of D_2 receptors in rat brain.

In collaboration with Drs. Joseph Rifkind and Dan Longo of the Laboratory of Immunology, we are examining the long-term cognitive effects of various regimens of chemotherapy in female rats. Specifically, we are assessing the effects of cyclophosphamide and 5-fluorouracil on various hematological parameters that could indicate erythrocyte damage resulting in impaired blood flow and maze learning. We are also examining the effects of erythropoietin, which stimulates red blood cell production, on maze performance of aged rats.

Thus, our research program applies a range of approaches from molecular biological techniques to behavioral analysis for examining possible mechanisms of age-related neurobiological changes that reduce functional capacity at advanced ages and for identifying possible treatments.

Collaborators: Hiroyuki Ikari, M.D., Ph.D., Hiroyuki Umegaki, M.D., Nagoya University School of Medicine, Japan; Mathias Jucker, Ph.D., University of Basel, Switzerland; Hideki Kametani, Ph.D., Fukuoka Prefectural University, Japan; Peter Mouton, Ph.D., Stereology Resource Center; Dan Longo, M.D., Joseph Rifkind, Ph.D., George Roth, Ph.D., Nigel Greig, Ph.D., NIA.

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Biography: Dr. Lane received his Ph.D. from Penn State University in 1991 as a predoctoral NIA training fellow at the Penn State Gerontology Center. Dr. Lane came to the National Institute on Aging, Intramural Research Program as an IRTA postdoctoral fellow to pursue his interests in interventions targeting basic mechanisms of aging and age-related disease. Following his postdoctoral fellowship, Dr. Lane remained at the NIA where he is a tenure track investigator. His research work focuses on nutritional modulation of aging using rodent and nonhuman primate models. Particular emphasis is placed on elucidation of the biological mechanisms that underlie the diverse effects of caloric restriction (CR) on aging. Work on possible mechanisms of CR focuses on the possible role of insulin signal transduction in aging and its modulation by CR and the development of interventions that could possibly mimic the effects of CR without the need to reduce food intake. Dr. Lane is also the principal investigator of the NIA project on caloric restriction in aging where his research interests are focused on primate models of aging, biomarkers of aging in primates, and the effects of CR on aging and age-related disease in primates. Dr. Lane is the immediate past President of the American Aging Association and serves on the association scientific advisory board. He is also a member of the Gerontological Society of America and was the recipient of the society's Nathan Shock New Investigator Award in 1998.

Keywords:

calorie restriction
nonhuman primates
biomarkers
insulin signaling

Recent Publications:

Vaitkevicius PV, et al. *Proc Natl Acad Sci USA* 2001; 98(3): 1171-1175.

Black A, et al. *Bone* 2001; 28(3): 295-302.

Roth GS, et al. *J Clin Endocrinol Metab* 2001; 86(7): 3292-3295.

Lane MA, et al. *J Nutr* 2001; 131(3): 820-827.

Calorie Restriction in Primates: Among gerontologists calorie restriction (CR) is widely recognized as the only intervention proven to consistently extend lifespan and maintain physiological function in many systems at more youthful levels. CR also delays the onset and slows the progression of many age-related diseases, including cancer. This nutritional intervention is among the most powerful and versatile experimental tools for the study of aging processes and age-related diseases in experimental animal models, and possibly humans. The diverse beneficial effects of CR have been extensively documented in short-lived species including rats, mice, hamsters, spiders, flies, and fish. However, the effects of CR on longer-lived species more closely related to humans are not known. If it is shown the CR has beneficial effects in longer-lived species similar to those reported in rodents, the implications for human aging are significant.

With colleagues George Roth, and Donald Ingram, Chief, of the Behavioral Neuroscience Section, a main project of the laboratory involves studies of CR in long-lived nonhuman primates with an aging colony of nearly 200 rhesus and squirrel monkeys. Monkeys in several age groups representative of the species life span are being studied. Experimental groups are approximately equally divided between freely

eating controls and monkeys receiving 30% less calories per day. The main hypothesis being tested is whether, as extensively reported in rodents and other short-lived species, CR will extend lifespan and slow aging in longer-lived species more closely related to humans. Another major focus of the laboratory is investigation of the biological mechanisms that mediate the anti-aging and anti-disease effects of CR.

Previous work in the laboratory helped to establish the safety and efficacy of this model in nonhuman primates. Briefly, caloric intake can be reduced by 30% in both rhesus and squirrel monkeys with no apparent adverse effects. CR monkeys do not exhibit any signs of increased stress such as elevated blood pressure, lethargy, loss of appetite or increased stereotypical behavior. We have also shown that with few exceptions effects of CR on primate physiology agree with previous reports in rodents. For example, monkeys on CR weigh less and have less body fat and lean mass. CR monkeys also show reduced body temperature, a transient reduction in metabolic rate, and glucoregulatory changes that are consistent with findings in rodents. Recent findings suggest that morbidity related to the major classes of age-associated diseases is reduced in CR monkeys. It will be several more years until definitive data regarding effects of CR on lifespan are known; however, to date findings from the monkey study suggest that this intervention will likely have beneficial effects in this long-lived primate model. Current work in the laboratory focuses on possible metabolic mechanisms of CR and effects of CR on age-related disease including bone loss and menopause. A second major focus involves assessment of the impact of CR on physiological function that may impact upon quality of life. For example, new studies are being initiated to assess function in several body levels including immune, sensory, skeletal, cardiovascular, endocrine and the brain and nervous systems among others.

Metabolic Mechanisms of CR: Even if CR is proven to extend lifespan in primates, it is unlikely that 30% reduction in caloric intake will become a widespread practice in humans. However, elucidation of underlying biological mechanisms of CR could make possible novel interventions with beneficial effects on aging and age-related diseases that are not dependent on reduced food intake. Studies in the laboratory related to possible mechanisms of CR utilize both monkey and rodent model systems.

Studies in primate models continue to explore metabolic adaptations during CR. These involve studies of metabolic hormones and key regulatory enzymes during relatively short exposures to CR (2-4 yr.). We are also utilizing microarray analyses of gene expression to study effects

of short-term CR in both young and old monkeys. A second primate study involves administration of the metabolic inhibitor 2-deoxyglucose. Previous work in our group reported that metabolic inhibition by 2-deoxyglucose "mimics" certain metabolic effects of CR. Dr Mattson's group subsequently demonstrated that this compound, like CR, protects neurons from certain toxic insults. The primate study will investigate the metabolic and neuroprotective effects of both CR and 2-deoxyglucose.

Studies in rodent models focus in two areas. One line of investigation is examining the possible role of insulin signaling in CR-induced enhancement of stress tolerance. In addition to *in vivo* studies of stress responsiveness during CR, Rafael deCabo in our group has developed an *in vitro* model of CR. In brief, this model has suggested that enhanced protection from heat and oxidative stress during CR may be mediated through factors in the serum. This *in vitro* stress response model is being used to investigate signaling pathways involved in the CR-induced enhancement of stress tolerance. In addition, work is now focusing on the possible role of neuroendocrine factors in the underlying biological mechanisms of CR. *In vivo* studies have revealed that several genes in the insulin pathway are altered during CR both at baseline and during insulin stimulation. Future work will focus on further investigation of these and other insulin signaling genes during aging and CR.

A second major focus of the rodent studies involves investigation of candidate CR mimetic compounds. Our previous work with 2-deoxyglucose led to development of this CR mimetic approach. Specifically, that it may be possible through metabolic inhibition or other interventions to "mimic" certain metabolic and protective effects of CR without reducing food intake. Work in this area focuses on the possible protective effect of 2-deoxyglucose and other candidate mimetic compounds on heat stress and tumor growth. In addition several candidate compounds will be investigated for lifespan effects. The *in vitro* stress response model described above will also be used in future studies to rapidly screen potential CR mimetic compounds.

Biomarkers of Aging: Noninvasive biomarkers of aging are being developed to test whether or not the rate of aging has been altered in monkeys on CR. In addition to their utility in our CR studies, noninvasive markers of primate aging could be employed to evaluate a broad spectrum of anti-aging strategies in humans and other species. The recent popularity of anti-aging therapies, such as dehydroepiandrosterone sulfate ($DHEA_S$) and melatonin, underscores the need for objective criteria by which to evaluate the efficacy of proposed treatments related to aging processes. We have established a strategy for evaluating candidate markers and have

identified several that may prove useful in a variety of species. These include serum markers such as $DHEA_S$ and pentosidine, a collagen cross-link product measured in skin samples. Several other markers are currently under study. Recently, we have shown that CR slows the age-related decline in serum $DHEA_S$ levels and studies of pentosidine accumulation in rhesus monkeys on CR are underway. In collaboration with several primate research centers, the NIA Biology of Aging Program and the National Center for Research Resources, a primate aging database has been established for developing and evaluating candidate markers. Although still under development, the database contains data on several species and has yielded several candidate markers that are under evaluation.

Collaborators: Steven Spindler, Ph.D., University of California-Riverside; Richard Weindruch, Ph.D., University of Wisconsin-Madison; James Nelson, Ph.D., University of Texas Health Science Center-San Antonio; Janko Nikolich-Zugich, Oregon Health Sciences Center; Gerald Schatten, Magee Women's Research Institute; Abraham Aviv, School of Medicine and Dentistry of New Jersey; John Matochik, National Institute of Drug Abuse, NIH; Judy Cameron, Oregon Regional Primate Center and University of Pittsburgh.

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Biography: Nigel Greig was trained as a pharmacologist with a background in medicinal chemistry and physiology and gained his Ph.D. from the University of London, England. Leaving the Cancer Chemotherapy Department of the Imperial Cancer Research Fund, London, he joined NIA in 1982. His initial studies focused on optimizing the delivery to and action of drugs within the brain. This resulted in the development of drug candidates for the treatment of brain tumors, and cancers of the breast, lymphatics and ovaries, as well as agents for the treatment of drug abuse and technology for the delivery of neuropeptides, antisense oligonucleotides and proteins to the brain. Leaving NIA in 1989, Dr. Greig was involved in the initiation of the successful California biotechnology company, Athena Neurosciences, now Elan Pharmaceuticals.Returning to NIA as a tenured scientist in 1991, his research has evolved into his present interest, the design and development of drugs and diagnostics for the treatment of Alzheimer's and other neurodegenerative diseases and of type 2 diabetes. This has resulted in the development of several agents from concept in the laboratory, through the required U.S. Government regulatory requirements to the bedside. Patents covering a variety of novel compounds of clinical interest have now been licensed from the NIA to industry and are in preclinical and clinical development.

Keywords:

drug design
acetylcholinesterase
butyrylcholinesterase
Alzheimer's disease
type 2 diabetes
apoptosis

Recent Publications:

Doyle ME, et al. *Endocrinology* 2001; 142(10): 4462-4468.

Shaw KT, et al. *Proc Natl Acad Sci USA* 2001; 98(13): 7605-7610.

Culmsee C, et al. *J Neurochem* 2001; 77(1): 220-228.

Zhu X, et al. *Tetrahedron Lett* 2000; 41: 4861-4864.

Design of Drugs and Diagnostics: The goal of the Drug Design and Development program is to develop novel agents against rate-limiting steps involved in the pathophysiology of nervous system diseases, with particular interest in Alzheimer's disease (AD).

Alzheimer's Disease: Although the neuropathological quantification of ß-amyloid plaques and neurofibrillary tangles in the AD brain is the basis for confirming disease diagnosis after death, it is the neocortical synapses rather than the plaques and tangles that correlate best with psychometric indices of cognitive performance in AD. The loss of cholinergic synaptic markers in selected brain regions remains one of the earliest events leading to AD, with the cholinergic system being the most affected of the neurotransmitters and intimately involved in memory processing.

One of our efforts has focused on augmenting the cholinergic system, but maintaining the normal temporal pattern of neurotransmitter release by selectively inhibiting the enzyme acetylcholinesterase (AChE), acetylcholine's degrading enzyme, in brain. Extensive studies involving chemistry, X-ray crystallography, biochemistry and pharmacology resulted in our development of "selective cholinesterase inhibition technology" (SCIT). This has provided us the basis for the development of novel drugs to selectively and reversibly inhibit either AChE or butyrylcholinesterase

(BChE) in the brain for an optimal time duration for the potential treatment of AD, age-associated memory impairment and other dementias. In addition, incorporation of charged moieties to restrict the brain entry of resulting compounds has provided drug candidates for potential treatment of myasthenia gravis and glaucoma.

The targeting of selective and site-directed drugs to specific enzymes rather than to receptors is a conceptually attractive method to optimize drug action. The reason for this is that formation of reversible drug/enzyme complexes allows selective enzyme inhibition over a long time duration, which is independent of the pharmacokinetic half-life of the drug. Once the drug has formed a slowly reversible drug/enzyme complex to inhibit its function, the presence of free drug is no longer required for continued action. In contrast, drug/receptor stimulation requires the continued presence of drug, and its time-dependent maintenance at the target receptor for continued activity. Our use of the former method, targeted enzyme inhibition, enhances specificity and reduces toxicity, and has resulted in several novel compounds with dramatic sustained cognitive action for once daily dosing with wide therapeutic windows and minimal toxicity. For example, the novel drug, phenserine, a long-acting and brain-directed, selective AChE inhibitor is now in clinical assessment and appears to be well tolerated in elderly individuals. Other novel agents from SCIT are presently being developed as the first available reversible, nontoxic and brain-directed selective inhibitors of the enzyme BChE. BChE, unlike AChE and most other enzymes in the AD brain, has been found elevated early in the disease process, particularly in brain regions associated with AD. The association of BChE with the AD neurotoxic peptide, ß-amyloid, has been shown to dramatically amplify the toxicity of the peptide. In addition, a mutant variant of BChE, the K form, when found together with the ApoE 4 allele, is associated with an increased susceptibility of sporadic AD. Hence, inappropriate BChE activity can increase the risk of AD and accelerate the disease process. Our novel selective inhibitors of BChE, are highly potent and up to 5000-fold selective. Being the first in this class of compounds, they will test the new hypothesis that central nervous system BChE inhibition is of value in the treatment of AD, and a representative of this novel class of compounds will be ready for clinical assessment within 2 years.

Another of our focuses to develop therapeutics for treating AD relates to reducing the production and secretion of ß-amyloid, a toxic peptide that derives from the misprocessing of the normal endogenous protein, ß-amyloid precursor protein (ß-APP), that is found in brain and throughout the body. In this regard, we have developed and are presently optimizing a pharmacophore that binds to and regulates the production of ß-APP (in

collaboration with Jack Rogers, Ph.D., Harvard, MA) both in tissue culture and in the brain of rodents. Recent collaborative studies (Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Mayo Clinic, FL) have demonstrated that these reductions lead to reduced synthesis and secretion of ß-amyloid peptide. Yet other agents are being developed as potential imaging probes, to quantitate lowered AChE and elevated BChE levels associated with the AD brain, as early diagnostic tools.

Further studies are elucidating the mechanism by which nicotine protects neuronal cells from the toxicities associated with insults, such as from ß-amyloid and gp120. In this regard, novel subtype-selective nicotinic receptor channel modulators are being developed in collaborative studies with John Daly, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Studies also are elucidating the mechanism by which HIV-infected immune cells cross the blood-brain barrier to gain access to and infect the brain, to characterize potential targets for treatment of AIDS dementia complex.

Drug Abuse: Among its many roles, BChE is a critical and rate-limiting enzyme in the metabolism of a number of drugs, including cocaine. In collaborative studies with Charles Schindler, Ph.D., and colleagues at the National Institute on Drug Abuse, (NIDA), we have demonstrated that we can increase the metabolism of cocaine, both *in vitro* and *in vivo*, by manipulating plasma BChE levels to increase its clearance and alter its metabolic profile to favor less toxic metabolites. Furthermore, we can substantially reduce cocaine's psychomotor stimulatory action by exogenous BChE administration. Collaborative studies with Amy Newman, Ph.D., and colleagues, NIDA, are additionally elucidating mechanisms to reduce cocaine's euphoric actions by inhibiting its binding to the dopamine reuptake transporter with novel tropane analogues, which, likewise, are being developed as potential therapeutics for the treatment of cocaine abuse.

Neurodegeneration: Collaborative studies with Mark Mattson, Ph.D., (Chief, Laboratory of Neurosciences) are focused on modifying the course of apoptotic cell death. Apoptosis is a major form of cell death that involves a stereotyped sequence of biochemical and morphological events. Inhibition of rate limiting biochemical steps within this cascade of events can halt and rescue cells from a variety of physiological and pharmacological insults that induce cell death via apoptosis. Studies have

focused on the design, synthesis and assessment of a novel series of potent compounds that inhibit the intracellular protein, P53. These compounds protect cells of neuronal origin from toxic concentrations of a variety of insults, including ß-amyloid peptide, in tissue culture, and largely protect the brain from ischemic insults in *in vivo* rodent studies.

Type 2 Diabetes: Collaborative studies with Josephine Egan, M.D., (Diabetes Section, LCI, NIA) are being undertaken on type 2 diabetes, a disease prevalent in the elderly that is caused by a relative refractoriness of the insulin receptor to its ligand and a deficiency in its normal release. The focus of these studies has been to optimize the performance of pancreatic islet cells both *in vitro* and in rodent diabetic models with peptides that stimulate insulin release to develop novel therapeutics. Extensive studies have been undertaken on the peptide, exendin-4 (Ex-4), which bears a 52% homology to the endogenous insulinotropic peptide, glucagon like peptide-1 (GLP-1). GLP-1 is released from the gastrointestinal tract during eating to stimulate pancreatic insulin release to lower blood glucose levels. Like other endogenous hormones, it is short acting. In contrast, Ex-4 has a duration of action of some 16 hours, is more potent than GLP-1 and maintains blood glucose levels chronically without toxicity. Our studies have supported the transition of this peptide from the laboratory and into phase I and II clinical trials as an experimental therapeutic for type 2 diabetes. Initial studies in humans indicate that the peptide appears to be both safe and effective in controlling blood glucose levels in afflicted subjects. Current studies in the laboratory are focused at understanding the mechanism of action of Ex-4 and analogues, and further optimizing their action.

Collaborators: Mark Mattson, Ph.D. and Donald Ingram, Ph.D., LNS, NIA; Josephine Egan, M.D., Diabetes Section, LCI, NIA; Arnold Brossi, Ph.D., University of North Carolina, Chapel Hill, NC; Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Mayo Clinic, Jacksonville, FL; Jack Rogers, Ph.D., Harvard, Boston, MA; Judith Flippen-Anderson, Ph.D., Naval Research Center, Washington D.C.; John Daly, Ph.D., NIDDK, NIH; Amy Newman, Ph.D. and Charles Schindler, Ph.D., NIDA, NIH.

Laboratory of Personality and Cognition

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The fundamental scientific paradigm guiding research in the **Laboratory of Personality and Cognition (LPC)** is the analysis of individual differences. Few phenomena are more basic than the fact that human beings differ—in health, in rates of aging, in cognitive ability, in personality, in happiness, and in life satisfaction.

The Laboratory of Personality and Cognition (1) conducts basic and clinical research on individual differences in cognitive and personality processes and traits; (2) investigates the influence of age on these variables and their reciprocal influence on health, well-being and adaptation; and (3) employs longitudinal, experimental, and epidemiological methods in the analysis of psychological and psychosocial issues of aging, including health and illness, predictors of intellectual competence and decline, models of adult personality, and correlates of disease risk factors.

The Personality, Stress, and Coping Section conducts basic and applied research on personality as it relates to aging individuals including studies of stress and coping, mental and physical health risks and outcomes, adaptation and well-being. Basic research has centered on a taxonomic model of personality traits and its assessment.

The Cognition Section conducts studies that attempt to distinguish pathological from healthy, age-related cognitive changes in a broad range of cognitive tasks including short-term and long-term memory, visuo-spatial rotation, attention and decision tasks. In addition, structural and functional brain changes are examined using MRI and PET. Studies are performed on regional structural brain changes, especially the hippocampus, and their relationship to cognitive performance and dementia. Regional differences in cerebral blood flow derived from PET studies at rest and during cognitive challenge are related to aging and patterns of cognitive change.

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Biography: Dr. Costa received his undergraduate degree in Psychology from Clark University and his doctorate in Human Development from the University of Chicago. After academic positions at Harvard and the University of Massachusetts at Boston, he joined NIA to inaugurate a Stress and Coping Section. Since 1985 he has been Chief of the Laboratory of Personality and Cognition. His research interests include adult development, personality assessment, and Alzheimer's disease.

Keywords:

personality assessment
Alzheimer's disease
five-factor model
personality
genetics

Recent Publications:

Costa PT Jr., et al. *J Pers Soc Psychol* 2001; 81(2): 322-331.

McCrae RR, et al. *J Pers* 2001; 69(2): 154-174.

Trobst KK, et al. *J Pers* 2000; 68: 1233-1252.

McCrae RR, et al. *J Pers Soc Psychol* 2000; 78(1): 173-186.

Herbst JH, et al. *Am J Psychiatry* 2000; 157(8): 1285-1290.

A major obstacle to progress in personality psychology for many decades was the inability of psychologists to agree on a taxonomy of traits that would offer a comprehensive yet manageable set of trait constructs. Since 1983, this Laboratory has contributed to a worldwide consensus that the Five-Factor Model points to such a taxonomy. The broad factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness appear to encompass most specific traits, and offer a framework for systematic literature reviews and research designs.

Basic Research in Personality - The Five-Factor Model: One focus of research has been a comparison of the NEO-PI-R system with alternative operationalizations of the Five-Factor Model and alternative taxonomies. A popular psychobiological model has been proposed by C. Robert Cloninger and colleagues who assert that there are independent temperament dimensions corresponding to chemically-coded neural networks or brain systems: dopaminergic neurons regulate the dimension of novelty seeking, serotonergic neurons regulate harm avoidance, and norepinephrinergic neurons regulate reward dependence. At the biological level, they argue that the temperament traits are associated with neurochemical substrates that have a genetic basis. One implication of this theory is that genes associated with neurotransmitters should be related to the hypothesized temperament traits. Another implication is that traits hypothesized to have a shared genetic basis should covary at the phenotypic level. According to Cloninger and colleagues, the psychobiological model, as measured by the Temperament and Character Inventory (TCI), accounts for the genetic basis of the personality phenotype, whereas alternative models of personality like the five-factor model comprise genetically and environmentally heterogeneous factors. In a study of 946 male and female participants in the BLSA to whom the TCI was administered, 587 were genotyped for a polymorphism in the

dopamine D4 receptor (D4DR) and 425 were genotyped for a polymorphism in the serotonin transporter (5-HTT) linked promoter region. Results indicated no significant association between D4DR polymorphisms and novelty seeking, and no significant association between 5-HTTLPR polymorphisms and harm avoidance. Furthermore, the factor structure of the TCI did not reveal the hypothesized phenotypic seven-factor structure. This study produced no support for the temperament and character model at either the biological or psychological level.

Personality Changes at Midlife: Past research has demonstrated high levels of stability of adult personality over long time intervals in men. However, few studies here or elsewhere have examined the long-term stability of personality of women; one of the exceptions (the Mills Longitudinal Study of about 100 women) reports appreciable change that invites replication. In collaboration with colleagues at the UNC Alumni Heart Study and Duke University Medical Center, a recently completed study on 495 women and 1,779 men in their 40's and retested after 6 to 9 years, tested hypotheses about the plateauing of rank-order stability and mean-level maturational changes in personality trait levels. Results confirmed previous longitudinal findings confirming basic stability for both women and men at the mid-life: rank-order stability coefficients were high, mean-level changes were small, and life events had only very specific influences on personality. Personality was shown to be resilient in that it was unchanged by the sheer occurrence of reported life events, whether positive or negative; but subjective appraisals of negative life circumstances did show limited effects on personality. Promising directions of future research suggest that events that affect central aspects of one's identity, such as loss of a job or changes in marital status, be a central focus. For both women and men, being fired from a job (vs. promoted) appears to increase Neuroticism (negative affect) and lower aspects of Conscientiousness. Effects of changing marital status differed for men and women: Divorce seemed to be liberating for women, but demoralizing for men.

Applied Research: Stress, Coping, and Psychopathology: Personality traits are important determinants of the ways in which people deal with stress. For example, Extraversion is associated with forms of coping that involve humor, talking about feelings, and seeking support; Agreeableness is associated with stoic and compliant attitudes in the face of stress. Our perspective integrates stress-and-coping research into the broader field of psychology, linked to normal adaptation, psychopathology, and the personality dimensions that affect all these. Traditionally, normal and abnormal psychology were held to be distinct and qualitatively different.

Our research has shown that in many respects they are closely related, and thus that knowledge from one field is relevant to the other. For example, some of our research has focused on depression. We have shown that depressive symptoms are related to the normal personality disposition Neuroticism, can be predicted years in advance from personality traits, and can themselves predict psychiatric diagnoses noted in hospitalization records. Perhaps most important, we have also shown that depressive symptoms and the personality traits that predispose people to depression do not increase as a normal consequence of aging. Most older people are not depressed, and those that are should receive appropriate treatment.

Several studies have examined the potential of the five-factor model of personality to describe and differentiate various health risk behaviors among HIV and AIDS related patient groups. Perceived risk of contracting HIV has been theoretically and empirically linked to the likelihood of engaging in HIV risk behaviors; however, little is known regarding the determinants of risk perceptions and perceived risk of contracting HIV. A recent study examined the extent to which perceptions of risk are determined by HIV-related knowledge, history of engaging in HIV risk behaviors, and personality variables. Consistent with previous research from this laboratory linking low Openness to Experience (O) to defensive denial, individuals who engage in unsafe sex and deny any risk for contracting HIV had lower O scores than individuals who engage in unsafe sex and accept that they are at risk. Low O may facilitate minimization or even denial of risk as relatively closed individuals have difficulty imagining that these consequences apply to them and are closed to the feelings involved in dealing with a sense of vulnerability. Another study investigated how FFM personality traits are related to adherence to highly active anti-retroviral therapies (HAART) for HIV. Preliminary results suggest that individuals endorsing personality traits associated with high conscientiousness, openness and agreeableness report greater adherence to HAART; traits associated with neuroticism (e.g., depression) and extraversion (e.g., high excitement-seeking) were related to less than medically necessary adherence; and greater levels of angry hostility, lower gregariousness and lower positive emotions were associated with higher viral loads. These findings have direct implications for psychosocial interventions designed to sustain or improve adherence to HAART among HIV+ individuals.

Axis II of the DSM-IV is used for the diagnosis of personality disorders, which are defined as inflexible and maladaptive personality traits. It is reasonable to ask whether these traits are the same as or different from those encountered in non-psychiatric populations. Several recent studies on this question have concurred in finding strong and replicable links

between scales measuring personality disorders and the five factors in both normal and clinical populations. The potential of the five-factor model of personality to describe and differentiate personality disorders was suggested by research in North American samples of patients and psychiatrically normal individuals. Relatively little research has examined relations between the FFM and personality disorders in psychiatric patient populations in other cultures. Former Visiting Scientist Dr. Jian Yang, in collaboration with investigators from the PSCS and the Hunan Medical University, conducted a multi-center study of over 2,000 psychiatric inpatients and outpatients throughout the People's Republic of China. Results showed that both personality traits and personality disorders can be reliably measured by Chinese translations of American instruments, and that the pattern of correlations between personality traits and disorders appears similar in China to that which has been reported in the US (cite). The results of these studies suggest that conceptions and measures of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) personality disorders are cross-culturally generalizable to Chinese psychiatric populations, and both personality disorders and personality traits may reflect biologically-based individual differences common to the human species as a whole. This is one of over 50 studies linking normal personal dimensions and personality disorders together they have led to a fundamental reconceptualization of the field of personality and psychopathology: Personality disorders do not correspond to discrete psychiatric entities, rather they are better construed as a systematic collection of problems in living associated with different dimensions of personality.

Collaborators: R. Michael Bagby, Ph.D., Jian Yang, M.D., Ph.D., University of Toronto; Krista K. Trobst, Ph.D., Jerry S. Wiggins, Ph.D., York University; Michael H. Bond, Ph.D., Chinese University of Hong Kong; Sampo V. Paunonen, Ph.D., University of Western Ontario; Gergorio H. del Pilar, Jean-Paul Rolland, Ph.D., University of Paris X Nanterre; Wayne D. Parker, Ph.D., Stephanie V. Stone, Ph.D., Peter Fagan, Ph.D., O. Joseph Bienvenu, M.D., Ph.D., Thomas Brashers-Krug, M.D., Gerald Nestadt, Ph.D., Johns Hopkins University; Fritz Ostendorf, Ph.D., Alois Angleitner, Ph.D., University of Bielefeld; Margarida P. de Lima, Ph.D., Antoino Simoes, Ph.D., University of Coimbra; Iris Marusic, Ph.D., Denis Bratko, Ph.D., University of Zagreb; Gian Vittorio Caprara, Ph.D., Claudio Barbaranelli, Ph.D., University of Rome; Joon-Ho Chae, Ph.D., Sogang University; Ralph L. Piedmont, Ph.D., Loyola College of Maryland; Mark R. Somerfield, Ph.D., American Society of Clinical Oncology; Thomas A. Widiger, Ph.D., University of Kentucky; Henry L. Masters III, M.D., AIDS Healthcare Foundation, Los Angeles CA; Neil Schneiderman, Ph.D., University of Miami.

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Biography: Dr. McCrae received a B.A. in Philosophy from Michigan State University, and a Ph.D. in Personality Psychology from Boston University. After three years at the Normative Aging Study in Boston, he joined the NIA to become Research Psychologist and Senior Investigator in the Personality, Stress, and Coping Section, Laboratory of Personality and Cognition. His work has been centered on studies of personality structure (the Five-Factor Model) and assessment (the Revised NEO Personality Inventory) and applications in health and aging.

Keywords:

personality structure
longitudinal studies
openness to experience
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Recent Publications:

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McCrae RR, et al. *Dev Psychol* 1999; 35: 466-477.

Personality traits are dimensions of individual differences in the tendencies to show consistent patterns of thoughts, feelings, and actions. Traits are important because their influence is pervasive: They affect personal interactions and social support, health habits and somatic complaints, attitudes and values, ways of coping, occupational and recreational interests, and much more. For the past 17 years, research in this laboratory has utilized a particular version of trait structure, the Five-Factor Model, and an instrument developed to assess 30 specific traits that define the five factors, the Revised NEO Personality Inventory (NEO-PI-R). Work in the past year has emphasized basic research on the generalizability of the model and its development in adulthood across cultures.

Cross-Cultural Studies of the Five-Factor Model: Cross-cultural studies are of immense importance in personality psychology, because the major variables thought to affect personality development—genetic inheritance, early family environment, and social structural variables such as class, political climate, and religious traditions—cannot feasibly or ethically be manipulated. Personality psychologists must depend on natural experiments, and many of these are provided by comparing individuals across cultures.

Since the publication of the NEO-PI-R in 1992, researchers outside the U.S. have translated the instrument into over 30 different languages, and many have collected data for their own research purposes. In collaboration with these investigators, we have recently conducted cross-cultural studies

of personality structure and development. In the first of these we reported an analysis of personality structure in Hong Kong Chinese and Japanese samples. Using statistical methods developed in part in this Laboratory, we showed that the Five-Factor Model is well replicated in both these non-Indo-European languages. Subsequent research has extended this finding to several other languages—in fact, to date no study using an authorized translation, adequate sample size, and appropriate analysis has failed to replicate the five-factor structure of the NEO-PI-R. These data suggest that the Five-Factor Model may be a human universal.

American studies of adult personality development can be summarized by saying that three of the factors (Neuroticism, Extraversion, and Openness) decrease, whereas the other two (Agreeableness and Conscientiousness) increase with age; most of the change occurs between age 18 and age 30. These cross-sectional differences might reflect cohort effects attributable to the historical experience of different generations of Americans. But other nations have had very different histories during the same period, and if age differences are due to cohort effects, it is unlikely that the same kinds of age differences would emerge in cross-sectional studies in those countries. However, reanalysis of data provided by collaborators in twelve countries (including Portugal, Russia, Turkey, Croatia, and South Korea) show very similar patterns of age differences, suggesting that these may perhaps best be interpreted as effects of intrinsic maturation.

In the first half of this century, anthropologists attempted to assess the modal personality of various groups and relate personality to features of culture. In an updating of this endeavor, recent analyses have examined the mean levels of personality traits across cultures. Preliminary results suggest that personality profiles obtained in different languages or versions are comparable to the original, that subgroups (men and women, students and adults) from the same culture have similar personality profiles, and that culture-level analyses of personality traits show the same Five-Factor structure seen in analyses at the individual level.

The Origins of Personality - Behavior Genetics: According to Five-Factor Theory, personality traits are endogenous basic tendencies. Genetic factors are expected to play a major role in their origin and development, whereas environmental factors like culture should play a minor role. In collaboration with Swedish researchers, we published one of the first

studies on the heritability of Openness to Experience, and we collaborated with John Loehlin and Oliver John to reanalyze the classic National Merit Twin Study data for all five factors. A collaboration with behavior geneticists in Canada and Germany suggests that the five factors are strongly heritable in both these two cultures. In addition, that study demonstrates that more narrow and specific facet-level traits are also substantially heritable. Thus, it appears that there is a genetic basis for many of the details of personality, as well as the broad outlines.

Genetic covariance analyses are used to examine the origins of covariation between traits. In previous research, it has been claimed that the phenotypic structure is unaffected by shared environmental influences, but is mirrored by both genetic influences and non-shared environmental influences. However, non-shared environmental influences are estimated as a residual term that includes measurement bias. When we supplemented Canadian and German twin data with cross-observer correlations from American samples, measurement bias was reduced, and the phenotypic structure appeared to be due only to genetic influences.

Studies of Openness to Experience: Openness to Experience is the least well understood of the five personality factors. Different versions of the factor have been labeled Culture, Inquiring Intellect, Imagination, and Independence of Judgment. As assessed by the NEO-PI-R, Openness is seen in Fantasy, Aesthetics, Feelings, Actions, Ideas, and Values, and is thus much broader than labels such as Intellect suggest. Correlational studies in the BLSA have shown that Openness is empirically related to a wide variety of constructs, including Jung's Intuition, Hartmann's Thin Boundaries, Tellegen's Absorption, and Murray's Need for Sentience, as well as to corresponding factors in alternative measures of the Five-Factor Model (e.g., Goldberg's Intellect). It shows smaller, if still significant, correlations with measures of intelligence and divergent thinking ability.

This body of empirical findings has been used to develop a conceptualization of Openness with both motivational and structural aspects. Although Openness is essentially a matter of differences in the internal processing of experience, it has far-reaching consequences in social interactions. A review of the literature showed that Openness or related constructs were important for understanding cultural innovation, political ideology, social attitudes, marital choice, and interpersonal relations.

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Keywords:

heart period variability
spectral analysis
anxiety

Recent Publications:

Thayer JF, et al. *Psychophysiology* 2000; 37: 361-368.

Uijtdegaage SH, et al. *Clin Auton Res* 2000; 10: 107-110.

Thayer JF, et al. *Ann NY Acad Sci* 2001; 930: 452-456.

Heart Period Variability as an Index of Neurovisceral Integration: One aspect of our research program is to develop, elaborate, and apply a model of neurovisceral integration in the context of normal and pathological functioning. This model uses heart period variability (HPV) to index the functioning of central-peripheral feedback mechanisms that produce goal-directed behavior. We have related HPV to attentional regulation and affective regulation in humans. These studies suggest that autonomic, attentional, and affective regulation are coordinated in the service of system adaptability and goal-directed behavior.

Autonomic Characteristics of Anxiety and Mood Disorders: Anxiety and depression are disorders associated with somatic symptoms such as tachycardia, rapid breathing, and disturbed sleep. Moreover, anxiety and depression are risk factors for cardiovascular morbidity and mortality. Our research has focused on the autonomic characteristics on these disorders to investigate their physiological and psychological concomitants with an eye toward understanding their development, course, and treatment. Research to date indicates that these disorders are associated with a relative decrease in vagally mediated cardiovascular control. This lack of cardiac vagal control is associated with poor affective and attentional regulation. Importantly, these deficits normalize with therapeutic intervention.

Cardiovascular Variabilities and Health: We are examining the relationship between HPV and cardiovascular system control. This research suggests that HPV and blood pressure variability (BPV) are inversely related in the healthy, intact organism and serves to maintain adequate blood pressure control. In spinal cord injury, the relationship between HPV and BPV can become dysfunctional, leading to poor blood pressure regulation and increased risk for cardiovascular disorders.

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Keywords:

individual differences
age-associated cognitive decline
mild cognitive impairment
risk factors and protective factor for AD
cognitive decline and Alzheimer's disease
behavioral genetics

Recent Publications:

Kawas C, et al. *Neurology* 2000; 54: 2072-2077.

Resnick SM, et al. *Cereb Cortex* 2000; 10: 464-472.

Moffat SD, et al. *Neurology* 2000; 55: 134-136.

Moffat SD, et al. *Arch Int Med* 2000; 160: 2193-2198.

Distinguishing Pathological from Normal Cognitive Aging: Research in the Cognition Section focuses on distinguishing pathological from normal cognitive aging. The purpose of this research is to identify predictors of cognitive morbidity, and to identify which cognitive processes are preserved with aging and which processes are vulnerable to disease. The primary effort of research in the Cognition Section is focused on longitudinal research in the Baltimore Longitudinal Study of Aging (BLSA). Cognitive tests have been administered to participants in the BLSA since 1960. Some individuals presently in the study have as many as seven repeated assessments beginning in the 1960's.

The cognitive tests administered to participants in the BLSA reflect our primary interest in pathological cognitive impairments, especially Alzheimer's disease (AD). The cognitive testing program is divided into two batteries, one for longitudinal prediction and another for cognitive and neuropsychological outcomes. The longitudinal repetitions of these tests distinguish typical changes in performance associated with aging from changes in performance which may be associated with disease when combined with neurological and neuropsychological outcomes and clinical diagnoses of AD.

An increasingly important area of research in the Cognition Section focuses on factors that reduce the risk of cognitive declines. An example of this focus is the finding that nonsteroidal anti-inflammatory drugs reduce the risk of Alzheimer's disease. Another example of this focus is based on recent findings that estrogen replacement therapy reduces the

risk for both AD and cognitive declines in post-menopausal women. In an intervention study testing the effects of hormone replacement on cognition, we are examining the effects of estrogen and testosterone in older women and men in conjunction with structural and functional neuroimages.

Cognitive Declines in Aging Subjects Free of Dementing Diseases: In people with no signs of dementia, some cognitive abilities resist decline while other abilities show characteristic age-related changes beginning in the 50's or 60's. Research by investigators in the Cognition Section has shown that vocabulary scores generally resist declines, and may increase slowly over time until there are small decreases after the eighth or ninth decades. Immediate visual memory shows a much different pattern of change. We found that errors in immediate visual recall increased exponentially with increased age in both cross-sectional and longitudinal analyses.

We also found that there were different rates of change in separate types of errors over time. Distortions, rotations, perseverations and mislocations were the most frequent errors across all ages. Although older participants made significantly greater errors regardless of error type, the greatest age differences were found for distortions and omissions. Men and women showed similar patterns of age-associated increases in errors, but there was a significant interaction between gender and error type indicating that women across all ages made more omissions and rotations, not other types of errors. Longitudinal analyses showed that distortions, omissions and rotations increased with age. Although women made more omission errors, men showed steeper increases with age.

Long-Term Predictions of Cognitive Impairment and Dementia: The onset of cognitive impairment is either a discrete event or a gradual process that manifests over time. We asked whether changes in previous test performance predict evidence of cognitive impairment assessed by the Mini-Mental Status Examination (MMSE) over relatively long intervals. We hypothesized that visual memory administered prior to the MMSE would significantly account for cognitive impairment after controlling for age at mental status exam and vocabulary score (a measure highly related to general intelligence). The correlations between visual memory and MMSE over 6-8 and 9-15 years were .36 and .34 ($p < .05$). These results provide preliminary evidence that mental status can be predicted, at least in part, by earlier performance on cognitive tests. Although the present findings are limited to only these cognitive tests, they provide important evidence that early signs of dementia may be detectable as many as 6-15 years prior to noticeable decline on mental status tests.

Six-year changes in immediate visual memory predicted Alzheimer's disease (AD) prior to its onset. Individuals with diagnoses of AD had larger changes in immediate memory performance over the six-year interval prior to the estimated onset of their disease than subjects without AD. Six-year longitudinal change in immediate visual memory performance also predicted subsequent cognitive performance 6-15 and 16-22 years later, even after adjusting for the influences of age, general ability, and initial immediate memory. These results provide evidence that change in immediate visual memory performance has long-term prognostic significance. These results further suggest that change in recent memory performance may be an important precursor of the development of the disease.

Analyses comparing BLSA participants who developed dementing illnesses with nondemented participants also showed that particular errors in visual memory may be more sensitive markers of impairment than others. More than 5 years before the onset of illness, demented individuals made more distortion errors than participants who did not develop dementing illnesses. In addition, individuals with signs of dementia had significantly greater rates of change in perseverations, rotations, and size errors compared with nondemented participants. These findings suggest that immediate visual memory is an important test for distinguishing normal from pathological cognitive decline and that specific types of errors in short-term memory may be important early markers of dementia.

Risks and Protective Factors for Cognitive Decline: If cognitive decline is an important predictor of pathological cognitive aging then it seems reasonable to investigate factors that decrease or increase the risk of cognitive decline. Estrogen replacement therapy (ERT) is increasingly recommended for postmenopausal women due to its potential beneficial effects on physical health in older women. The possibility of a protective effect on cognitive function has also been suggested. In the BLSA, women receiving hormone treatment at the time of testing made significantly fewer errors in immediate visual recall than women who were not on hormone therapy. Less memory change was found in women who started hormone therapy between examinations than women who never received hormone therapy. These findings support the notion that estrogen has a beneficial role on cognitive functioning in aging women.

We continue to extend our present studies on the risks and protective factors for cognitive declines and dementias. In particular, as we gather additional repeat data on which to base reliable measures of cognitive trajectories, we will relate apoE and other genotypic and genomic measures to determine whether there are critical periods of decline. In addition, we will examine the role of modulators of cognitive decline such as hypertension and hormone replacement therapy, particularly in conjunction with MRI anatomical and PET functional assessments. We will also examine chronicity of hypertension, adequacy of blood pressure control, and differential effects and interactions with other known risks such as apoE genotype.

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Keywords:

memory aging
Magnetic Resonance Imaging
Positron Emission Tomography
estrogen and cognition

Recent Publications:

Resnick S, et al. *Cereb Cortex* 2000; 10(5): 464-472.

Maki PM, et al. *Neurobiol Aging* 2000; 21(2): 373-383.

Brain Changes as Predictors of Cognitive and Memory Decline: The goal of our research program is to identify brain changes which may predict declines in memory and other cognitive functions in older individuals. We use magnetic resonance imaging (MRI) to measure the structure of the brain and positron emission tomography (PET) to measure changes in regional cerebral blood flow (rCBF) during the performance of memory tasks and over time. A variety of risk and protective factors for cognitive impairment and dementia are examined.

Early Markers of Alzheimer's Disease - Brain Changes in the Baltimore Longitudinal Study of Aging (BLSA): We are performing a longitudinal neuroimaging study involving annual MRI and PET scans and neuropsychological evaluations in selected BLSA participants aged 55 and older. This longitudinal design provides a sensitive to way to investigate the relationship between changes in brain structure and physiology and decline in memory and cognition. Furthermore, using the wealth of prior psychological and medical information available for BLSA participants, we are able to examine trajectories of cognitive aging in relation to individual differences in the brain years later. To date, approximately 155 individuals (90 men, 65 women) have enrolled in the brain imaging study and most have completed their fifth neuroimaging assessment.

The specific goals of this study are: to determine the rate of brain changes with age, including increases in brain atrophy and ischemic/demyelinating white matter abnormalities; to determine the association between trajectories of memory and cognitive change and changes in brain structure and function; and to determine whether risk and protective factors, such as genetic susceptibility factors, hormone replacement therapy, use of non-steroidal anti-inflammatory agents, and vitamins, modulate these relationships. An understanding of the associations between brain and neuropsychological changes, as well as early detection of these changes, will be critical in identifying individuals likely to benefit from new interventions in preventing and treating Alzheimer's disease and other memory problems in the elderly.

MRI data from the first 2 years of our longitudinal brain imaging study have been published. A great deal of effort in our laboratory has focused on the development and validation of an image processing approach that provides sufficient accuracy for longitudinal studies. Quantitative analysis of MRI volumes, including separate estimates of gray and white tissue volumes and cerebrospinal fluid (CSF), for 116 subjects who have completed 2 evaluations reveals significant effects of age and sex on brain volumes and ventricular volumes. The cross-sectional findings from the Year 1 MRI scans indicate less gray and white matter volume and more ventricular CSF in older compared with younger participants; the magnitude of these findings is different across frontal, parietal, temporal and occipital brain regions. Consistent with previous studies, men have greater ventricular CSF volumes. There are no detectable changes in lobar brain volumes over a one-year period, but there was a small but significant increase in the volume of the ventricles.

We have also examined the effect of Apolipoprotein E genotype on hippocampal volumes and rates of longitudinal hippocampal volume loss. Neuroimaging study participants without dementia who carry the e4 allele (e4+) did not differ from those negative for the e4 allele (e4-) at initial evaluation. In contrast, e4+ individuals showed a faster rate of hippocampal volume loss than age, sex and education matched e4- individuals. Because both the presence of the e4 allele and hippocampal volume loss are risk factors for Alzheimer's disease (AD), our findings suggest one mechanism by which e4 genotype may confer an increased risk for AD.

In addition to morphologic predictors of cognitive impairment and AD, we are investigating the utility of early blood flow changes as predictors of cognitive and memory change. PET-rCBF studies are performed annually as part of our BLSA neuroimaging study. These scans are obtained under three conditions: during rest and the performance of verbal and figural delayed recognition tasks. This procedure is conceptualized as a cognitive stress test to examine age-associated changes in rCBF during increased demand. Our memory tasks produce robust patterns of CBF activation, with increased blood flow in prefrontal cortex (right > left), bilateral insula and visual association areas during memory recall. In addition, voxel-based maps of the associations between age and resting rCBF (normalized for global CBF) demonstrate significant negative correlations between age and CBF in the insular and superior temporal regions, and in visual association cortex (Areas 18 and 19) bilaterally for both men and women. To our knowledge, this sample represents the largest study of associations between age and regional CBF studied with PET and provides a detailed map of age differences in blood flow during a period of accelerating cognitive and memory decline.

Effects of Hormones on Cognitive Decline:

Hormone Replacement Therapy: A major focus of our research program is the investigation of the potential modulatory role of hormone replacement therapy on risk for Alzheimer's Disease and cognitive and memory decline in older women. We have shown that women in the BLSA who had ever used estrogen replacement therapy had a reduced risk of developing Alzheimer's Disease in comparison with women who had never used hormone therapy. We have also shown that nondemented women in the BLSA who were using estrogen replacement therapy performed better on a test of short-term memory for designs compared with never-users. In a small subgroup of women with memory assessments prior to and following initiation of hormone treatment, the estrogen therapy appeared to protect against age-associated decline in memory. We have also compared ERT users and nonusers who participate in our longitudinal imaging study. ERT users and nonusers showed significant differences in the patterns of brain activation during the performance of memory tasks. Most recently, we reported that ERT users compared with nonusers showed greater relative increases over a 2 year period in CBF in the hippocampus, entorhinal cortex, posterior parahippocampal gyrus, and portions of the temporal lobe. Interestingly, these regions overlap substantially with those showing physiologic abnormalities in early AD and in individuals at increased genetic risk for AD.

As our published studies to date have been observational and relied on women who choose to take estrogen as part of their regular medical care, we have initiated an ancillary study to the Women's Health Initiative randomized clinical trial. This study, the Women's Health Initiative Study of Cognitive Aging (WHISCA), examines the effects of hormone replacement therapy on longitudinal change in memory and other cognitive functions within the context of the large randomized intervention trial.

DHEA and Cognition: Dehydroepiandrosterone (DHEA) is a widely available hormone marketed as an anti-aging dietary supplement beneficial for physical and cognitive health. We have examined the associations of plasma concentrations of DHEA sulfate (DHEAS) and longitudinal changes in DHEAS with cognitive changes in older men in the BLSA. In this large sample, there were no associations between DHEAS concentrations or longitudinal changes in DHEAS and multiple measures of cognitive change. These data offer no support for the hypothesized relationship between endogenous DHEA levels and cognitive health.

Future Directions: Our future work will emphasize continuation of the longitudinal neuroimaging study, including continued acquisition of annual evaluations, further analyses of existing imaging and neuropsychological data, development of new approaches for longitudinal analyses of functional images, and examination of modulating factors on the relationship between brain and neuropsychological changes. The data collected over the first 2 years of the study indicate only small changes over one year in regional brain volumes and ventricular CSF. In contrast, the cross-sectional age differences between younger and older participants are 5 to 7% in frontal and temporal volumes and 51% in ventricular volume. It will be critical to continue repeated evaluations to examine the relation between brain and cognitive changes as the number of individuals with cognitive decline increases over the duration of the study.

Another important area of future research, which has only recently received attention in the brain imaging literature, is the role of modulatory factors on brain morphology and function. We are examining suggested risk and protective factors in relation to brain changes, neuropsychological changes and their association. For example, data on family history for Alzheimer's disease, apolipoprotein E genotype, head trauma, history of hypertension, use of estrogen replacement therapy, and circulating

hormones (DHEA, testosterone, cortisol) are being investigated as potential modulators of the relationship between brain and neuropsychological changes. The neuroimaging study has been expanded to younger adults to determine whether our observations of sex differences in the brain reflect group differences or differential aging for men and women. Ongoing and future work will include intervention studies to examine suggested protective agents, such as estrogen and testosterone, on brain structure and function. In collaboration with Dr. Pauline Maki, we are performing studies of regional brain morphology and functional activity within the context of double-blind placebo-controlled studies of estrogen and testosterone effects on cognition and mood in older women and men, respectively.

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Biography: Dr. Maki received her Ph.D. in Experimental Psychology from the University of Minnesota and completed a postdoctoral fellowship in the Dementias of Aging at Johns Hopkins University School of Medicine. Following her postdoctoral fellowship, she held joint appointments as a National Research Council (NRC) Fellow in the Laboratory of Personality and Cognition, NIA and as an Instructor in the Department of Psychiatry and Behavioral Sciences at Johns Hopkins. In 1999, she joined the Laboratory as an Investigator. She studies the neuropsychology of memory and the effects of sex steroid hormone on memory, other cognitive abilities, and brain functioning. She leads a number of clinical intervention trials that focus on the effects of estrogen, selective estrogen receptor modulators (SERMs), and testosterone on cognition and brain functioning in older adults.

Keywords:

estrogen
cognition
hormone
memory
brain activation

Recent Publications:

Maki PM, et al. *Neuropsychologia* 2002; 40(5): 518-529.

Maki PM, et al. *Neurobiol Aging* 2000; 21(2): 373-383.

Effect of Sex Steroid Hormones on Cognition and Brain Functioning: The effect of aging on cognition and brain functioning varies from one individual to the next. Recent studies suggest that one of the factors influencing individual differences in cognitive aging is the use of hormone replacement therapy (HRT). For example, recent findings from the Baltimore Longitudinal Study on Aging (BLSA) suggest that the use of estrogen replacement therapy (ERT) protects against age-associated declines in memory and against the development of Alzheimer's disease. One limitation of such studies is that the women who participated in them chose to receive ERT. Research suggests that such women tend to be better educated and to receive better health care than women who do not receive ERT. The bias is termed the "healthy user bias."

To address this bias, we are recruiting postmenopausal women who are not currently receiving hormone replacement therapy, and we are investigating their cognitive functioning at two points, after receiving ERT for 3 months and after receiving placebo for 3 months. We are conducting a parallel study on men, age 65 and older. Men experience a gradual loss of testosterone, about 1% a year after age 40. Little is known about how testosterone replacement therapy affects cognition in older men, though there is some suggestion that the hormone may enhance spatial abilities. Both studies examine both cognitive test scores and neuroimaging outcomes. We use positron emission tomography (PET) to examine brain functioning and magnetic resonance imaging (MRI) to examine brain structure.

The overall aim of the study is to determine whether estrogen and testosterone enhance cognitive functioning and mood in healthy older adults, and to identify the neural correlates of the expected changes. By studying the same woman on and off of ERT, we can extend our previous findings to all women, not just those who typically choose to go on ERT. By overcoming the healthy user bias of previous studies, we can more strongly support the hypothesis that ERT improves memory and verbal abilities in women. Moreover, by extending this area of inquiry to the study of testosterone replacement, we can begin to address whether HRT offers similar benefits to men.

Finally, to address the healthy user bias in the context of a large-scale randomized clinical trial, we recently initiated the Women's Health Initiative Study of Cognitive Aging or WHISCA, a 6-year longitudinal study assessing cognitive outcomes in 2900 women assigned randomly to receive active treatment (estrogen replacement therapy or estrogen and progesterone) or placebo. WHISCA is an ancillary study to the Women's Health Initiative (WHI) Randomized Hormone Replacement Trial and is the largest randomized trial of hormone replacement therapy on cognitive outcomes. WHISCA will provide invaluable data on the effects of hormone treatments on cognitive aging.

Effects of Endogenous Estrogens on Cognition: Reproductive events such as menarche, pregnancy, and menopause influence a woman's risk for a number of diseases. For example, the incidence of breast cancer is higher in women who have longer estrogen exposure due to early menarche, late menopause, or nulliparity. Conversely, a naturally high exposure to estrogen over a lifetime may decrease the chance of developing osteoporosis. Little is known about the effects of endogenous estrogens on cognition across the lifespan. We are currently examining this in the BLSA cohort and in other cohorts.

Natural fluctuations in ovarian hormones across the menstrual cycle allow for noninvasive studies of the effects of estrogen on cognition in young women. Studies indicate that fluctuations in estradiol underlie a reliable pattern of cognitive change across the menstrual cycle. Increases in estrogen are associated with improvements on tests in which women typically outperform men such as verbal fluency and decreases on tests in which men typically outperform women such as mental rotations. We are examining cognitive function in women across the menstrual cycle to see if the effects of endogenous estrogen in young women are similar to the effects of exogenous estrogen (i.e., estrogen replacement therapy) in older women.

Effects of Selective Estrogen Receptor Modulators (SERMs) on Cognition and Brain Functioning: We recently extended our research on hormones and cognition to a newer class of estrogen agents, selective estrogen receptor modulators (SERMs). SERMs have mixed estrogen agonist-antagonist properties, acting as agonists on bone and antagonists on certain reproductive tissues. The two most commonly prescribed SERMs are tamoxifen and raloxifene. There have been no observational or clinical intervention trials comparing the effects of tamoxifen and raloxifene on cognition, nor any observational or clinical intervention studies comparing the effects of SERMs and common HRT regimens on cognitive aging. The effect of tamoxifen on cognition is unknown, and the only published study on the effects of raloxifene on cognition showed a small, transient benefit to memory. Such studies take on great importance, because raloxifene is being offered as an alternative to HRT and tamoxifen is being recommended for primary prevention of breast cancer in women who have only a moderate increase in risk for this disease. If one or both of these SERMs act as estrogen agonists in brain, they may be beneficial to cognitive functioning. In contrast, if one or both act as antagonists, they may be detrimental to cognitive functioning. In the face of potential widespread use of SERMs in healthy women, information on the effects of these agents on memory and other cognitive functions is essential.

To better understand the effects of SERMs on cognition and brain functioning, we are conducting a series of observational and clinical trials. One clinical trial examines the effects of tamoxifen on cognition and brain functioning in women with breast cancer. Another clinical trial examines and compares the effects of tamoxifen, raloxifene, and estrogen on cognition and brain functioning in healthy postmenopausal women. We are conducting parallel observational studies that involve women who take tamoxifen for prevention of breast cancer and women who take raloxifene for prevention of osteoporosis. Finally, we are conducting an ancillary study to the National Cancer Institute initiated the Study of Tamoxifen and Raloxifene (STAR), a multi-center, 5-year, randomized clinical trial comparing the two drugs in 22,000 women at increased risk for breast cancer. The ancillary study, called STAR-Cog, will involve neuropsychological assessments in 1800 STAR volunteers, age 65 and older, randomly assigned to raloxifene or tamoxifen. The aims of the study are to address the long-term effects of raloxifene and tamoxifen on cognitive aging and the long-term cognitive effects of tamoxifen and raloxifene in comparison to those of ERT and ERT + progesterone.

Collaborators: Jason Brandt, Ph.D., JHUSOM, Department of Psychiatry and Behavioral Sciences; Adrian Dobs, M.D., JHUSOM, Department of Endocrinology; Michael A. Kraut, M.D., Ph.D., JHUSOM, Department of Radiology; Jill B. Rich, Ph.D., Department of Psychology, York University, Ontario; Lorraine Dennerstein, University of Melbourne.

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The **Brain Physiology and Metabolism Section (BPMS)** studies brain phospholipid metabolism in intact animals and humans, as well as synaptic integrity and function in aging and Alzheimer's disease. Methods involve *in vivo* tracer studies, chemical analytical techniques quantitative autoradiography, and positron emission tomography (PET). Studies are related to neuroplasticity and signal transduction, central action of drugs, and nutritional regulation of brain fatty acid metabolism.

(1) Brain Phospholipid Metabolism in Signal Transduction and Neuroplasticity: Radiolabeled long chain fatty acids are injected intravenously into awake rodents. By mathematical modeling, rates of incorporation into brain phospholipids, recycling and half lives are determined. Short half-lives (minutes to hours) and high turnover rates within brain phospholipids reflect their active participation in signal transduction and membrane remodeling. Brain incorporation from plasma of labeled arachidonic acid, an important second messenger, is increased in response to cholinergic and dopaminergic agonists in rat models of Alzheimer's disease (chronic unilateral lesion of nucleus basalis) and Parkinson disease (chronic unilateral lesion of substantia nigra), respectively, reflecting upregulation of phospholipase A_2 mediated signal transduction. Upregulated signaling may be imaged in the human brain using positron emission tomography (PET) and [C^{11}]arachidonic, and may help in the early diagnosis and understanding disease mechanisms of neurodegenerative disorders.

The fatty acid model can elucidate targets for centrally acting drugs with indeterminate modes of action. For example, the model has shown that lithium, used to treat manic depressive (bipolar) disorder reduces turnover of arachidonate within brain phospholipids by 80%, by downregulating gene expression (mRNA level) and enzyme activity of an arachidonate-specific phospholipase A_2. With this information, we may design drugs less toxic and with a wider therapeutic window than lithium for treating bipolar disorder. The model has demonstrated that the brain responds to

nutritional deficiency of the polyunsaturated essential fatty acid, docosahexaenoic acid, by reducing its turnover and metabolism within brain phospholipids, thus helping to retain it. Brain lipid composition and myo-inositol (involved in phosphoinositide metabolism) levels are reduced in Down syndrome, in relation to Alzheimer's disease.

(2) Synaptic Dysfunction in Aging and Alzheimer's Disease: *In vivo* imaging methods involving positron emission tomography (PET) were developed to examine brain blood flow and metabolism at rest and during activation in patients with Alzheimer's disease and in healthy control subjects. The activation, or stress paradigm, was shown to quantify synaptic integrity, which was shown to decline with dementia progression in Alzheimer's disease in two stages, the first potentially reversible and sensitive to synaptic enhancing drugs (e.g. physostigmine), the second irreversible and associated with mitochondrial and synaptic dropout.

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Biography: Dr. Rapoport received his M.D. from Harvard Medical School, Boston, interned in Medicine at Bellevue Hospital, New York, and received post-doctoral training at the Department of Physiology, University of Uppsala, Sweden, and at the Laboratory of Neurophysiology, National Institute of Mental Health (NIMH). He was appointed as a tenured scientist at NIMH in 1968, and 1978-1999 was Chief of the Laboratory of Neurosciences, NIA. He currently is Chief of the Section on Brain Physiology and Metabolism, NIA. He is a Fellow of the American College of Neuropsychopharmacology, the American Academy of Neurology and the Gerontological Society of America.

Keywords:

phospholipid metabolism
arachidonate
imaging
lithium
brain
fatty acids
Alzheimer's
synapses

Recent Publications:

DasGupta SF, et al. *Mol Cell Biochem* 2001; 221(1-2): 3-10.

Pietrini P, et al. *J Nucl Med* 2000; 41: 575-583.

Rapoport SI, et al. *Proc Natl Acad Sci USA* 2000; 97: 5696-5698.

Rapoport SI, et al. *Ann NY Acad Sci* 1999; 893: 138-153.

Rapoport SI, et al. *Neurochem Res* 1999; 24: 1403-1415.

Brain Phospholipid Metabolism in Relation to Signal Transduction and Neuroplasticity: Phospholipids are major constituents of cell membranes and participate in neuroplastic remodeling and signal transduction. We developed in rats an *in vivo* method and model to localize and quantify brain phospholipid metabolism and turnover of fatty acids within specific sites of brain phospholipids. A radiolabeled long chain fatty acid (unsaturated arachidonate or docosahexaenoate, saturated palmitate) is injected intravenously and its rate of incorporation into brain is measured using quantitative autoradiography and chemical analysis. With this model, we showed that lithium, used clinically to treat manic depressive disorder, reduces arachidonate turnover by some 80% without affecting turnover of docosahexaenoate and palmitate, and thus likely acts on phospholipase A_2. Additionally, C^{11}-labeled fatty acids were synthesized, in collaboration with the PET Department at NIH, and are used to image phospholipid metabolism of monkey brain with PET (tracer uptake was independent of blood flow) and to initiate a clinical PET protocol on healthy controls at rest and during activation. We plan to extend this protocol and related animal protocols to image phospholipase A_2-mediated signal transduction involving the brain, cholinergic, serotoninergic and dopaminergic systems.

Synaptic Dysfunction in Aging and Alzheimer's Disease: *In vivo* imaging methods involving positron emission tomography (PET) were developed to examine brain blood flow and metabolism at rest and during activation in patients with Alzheimer's disease and in healthy control subjects. The activation, or stress paradigm, was found to quantify synaptic integrity. Synaptic integrity was shown to decline with dementia progression in Alzheimer's disease in two stages, the first potentially reversible and sensitive to synaptic enhancing drugs (e.g. physostigmine), the second irreversible and associated with mitochondrial and synaptic dropout.

Collaborators: William Eckelman, PET Department, Clinical Center, NIH; Norman Salem, Laboratory of Membrane Biochemistry and Biophysics, NIAAA; Joseph Deutsch, School of Pharmacy, Hebrew University, Jerusalem, Israel; Pietro Pietrini, University of Pisa, Italy; Harald Hampel, University of Munich, Germany; Kimmo Hatanpaa, Yale University, New Haven; Gene Alexander, University of Arizona, Phoenix.

Molecular Dynamics Section

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The **Molecular Dynamics Section (MDS)** focuses on the interplay between structure and dynamics and how these influence biological function. The section is presently involved in studying the structural and dynamic factors in hemoglobin which regulate the binding of oxygen, the uptake and release of nitric oxide as well as autoxidation with its associated release of superoxide. The finding that autoxidation of hemoglobin is appreciably enhanced at reduced oxygen pressures, has lead to the proposal of a novel method for producing oxyradicals under hypoxic conditions. Studies are being performed on erythrocytes, interaction of erythrocytes with other tissues and with whole animals to determine to what extent this mechanism contributes to the pathophysiology of aging.

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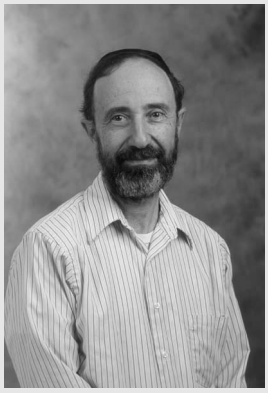
Somasundaran Ramasamy	Visiting Fellow
James Butzow	Special Volunteer
Gunther Eichhorn	Scientist Emeritus
Omoefe Abugo	Special Volunteer



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Biography: Dr. Joseph M. Rifkind received his Ph.D. in Physical Chemistry from Columbia University in 1966. He obtained postdoctoral training in protein chemistry at the University of Minnesota and joined the Gerontology Research Center of what was then part of National Institute of Child Health and Human Development (NICHD) in1968. He is the chief of the Molecular Dynamics Section. He is a member of the American Chemical Society, the Biophysical Society, the American Association for the Advancement of Science, the Gerontological Society of America, the International EPR (ESR) Society, and the International Society on Oxygen Transport to Tissue.

Keywords:

protein structure
oxyradical damage
oxygen transport
heme proteins

Recent Publications:

Abugo OO, et al. *Artif Cells Blood Substit Immobil Biotechnol* 2001; 29(1): 5-18.

Demehin AA, et al. *Free Radic Res* 2001; 34(6): 605-620.

Nagababu E, et al. *Biochem Biophys Res Commun* 2000; 273(3): 839-845.

Mendelman A, et al. *Brain Res* 2000; 867(1-2): 217-222.

Ajmani RS, et al. *Neurobiol Aging* 2000; 21(2): 257-269.

Nagababu E, et al. *Biochemistry* 2000; 39(40): 12503-12511.

Nagababu E, et al. *Free Radic Biol Med* 2000; 29(7): 659-663.

Molecular Dynamics Section: The Molecular Dynamics Section under the direction of Joseph Rifkind is studying the role of oxygen in biological systems and how it influences the aging process. Our current focus is on the detrimental effects of oxyradicals produced in erythrocytes under hypoxic conditions as well as the beneficial and deleterious effects of nitric oxide reactions with erythrocytes. This program is being pursued simultaneously on three different levels.

1. We are studying the mechanism whereby oxyradicals are produced under hypoxic conditions. Using electron paramagnetic resonance combined with visible spectroscopy, fluorescence spectroscopy and molecular dynamics simulations, we are studying the hemoglobin autoxidation process which produces oxyradicals. Enhanced protein fluctuations for partially oxygenated hemoglobin results in the nucleophilic displacement of oxygen as a superoxide. This superoxide formed in the heme pocket can (i) pick up an additional electron from nearby amino-acids producing protein radicals, (ii) react with the heme resulting in a damaged heme with altered geometry leading to the formation of heme degradation products, or (iii) leak out of the globin.

2. We are studying how these processes produce cellular damage despite the presence of antioxidants and the enzyme systems designed to protect from oxidative stress. Under hypoxic conditions, there is an enhanced affinity of hemoglobin for the erythrocyte membrane. The superoxide that is liberated from hemoglobin bound to the membrane is relatively inaccessible to cytoplasmic superoxide dismutase and ideally located to damage the red cell membrane. This hypothesis is supported by the

formation of protein cross-links and a decrease in red cell deformability when red cells are incubated under hypoxic conditions. An additional source for membrane damage is the accumulation of hydrophobic heme degradation products in the membrane. The hemoglobin membrane binding site is on the membrane band 3, which is also the anion channel, capable of transporting superoxide out of the red cell where it can damage lipoproteins and endothelial cells. We are studying these reactions and have found that red cells do induce oxidation of low density lipoproteins. These modified lipoproteins were shown to induce aortic smooth muscle cell proliferation, suggesting a possible relationship to the pathophysiology of the atherosclerotic process.

3. Impaired red cell deformability found to be induced under hypoxia is also associated with subject aging. We are very interested in understanding altered deformability in the aged as well as other decrements in blood rheology. Our studies suggest a link with oxidative stress which could originate in hypoxic induced oxyradical production. Recent results indicate greater oxidation in venous blood than arterial blood confirming the production of oxyradicals as blood passes through the capillary bed at reduced oxygen pressures. Our studies on erythrocytes have been extended to include the role of erythrocytes in carrying nitric oxide and the possible coupling of NO release to hemoglobin autoxidation under hypoxic conditions. These changes can influence the ability of the organism to maintain an adequate supply of oxygen resulting in possible functional decrements. We are investigating the relationship between decrements in blood rheology and function using subjects from the Baltimore Longitudinal Study of Aging. In collaboration with the LSS, we are investigating the relationship between blood flow in the brain and our hemorheological measurements. Studies are also being initiated with LCS to determine the effect of exercise on changes in blood rheology.

Collaborators: P.T. Manoharan, Ph.D., Indian Institute of Technology, Madras, India; V.J. Sharma, University of California, La Jolla, California; Avraham Mayevsky, Ph.D., Bar Ilan University, Israel; Victor McDonald, Ph.D., Walter Reed Army Institute of Research; David Danon, M.D., Weissman Institute, Rechovot, Israel; Jerome Fleg, M.D., NHLBI, NIH; Jeffrey Metter, M.D., Longitudinal Studies Section, Laboratory of Clinical Investigation, NIA.

Research Resources Branch

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The **Research Resources Branch (RRB)** provides centralized research resources and research support services essential to the productive conduct of biomedical research by the Intramural Research Program. Personnel in the Research Resources Branch represent a wide variety of talents, skills, and expertise for supporting Intramural investigators.

The Branch is divided into eight Sections that focus on particular specialties or types of service. The Sections are Central Laboratory Services, Comparative Medicine, Data Management Services, Instrumentation, Design and Fabrication, Library and Information Services, Network, Computing, and Telephony, Photography and Arts, and Statistical and Experimental Design.

Central Laboratory Services is subdivided into the Clinical Core Laboratory, Confocal Microscopy, DNA Array Facility, Flow Cytometry, Genotyping Services, and Mass Spectrometry.

The Comparative Medicine Section includes animal husbandry for a variety of species, producing transgenic and knockout rodents, and the breeding, weaning, and mating of rodents consistent with the genetic model from which they derived.

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Biography: Dr. Dennis D. Taub received his Ph.D. from the Department of Microbiology and Immunology at Temple University School of Medicine in Philadelphia in 1991. He subsequently entered the laboratory of Dr. Joost J. Oppenheim as a staff fellow at the National Cancer Institute in Frederick, Maryland. From 1994-1997, Dr. Taub headed the vaccine monitoring laboratory within the Clinical Services Program at the National Cancer Institute. In early 1997, he moved to NIA's Laboratory of Immunology and has held a joint appointment in the Research Resources Branch as Director of the Clinical Core Laboratory since February 1998 and as Chief of the Central Laboratory Services Section since July 1999.

The **Central Laboratory Service Section (CLSS)** offers investigators specialized support to help them succeed in today's fast-paced and complex scientific environment. Established by NIA's Office of the Scientific Director and the Chief of the Research Resources Branch in 2000, this Section provides specific expertise, new technologies, and experienced staff to enhance the research efforts of all NIA investigators. High-throughput, cutting-edge analysis capabilities that can be found within CLSS include advanced sequencing, imaging, cell sorting, genetics, genomics, and proteomics technologies. The primary goal of the CLSS is to support the research interests and ongoing projects of various Laboratories within the GRC as well as to provide the expertise necessary to assist in the proper performance of specialized experiments and in the interpretation of obtained data. In addition to their service duties, some CLSS Unit Heads also perform hypothesis-driven, defined research projects within their laboratories.

The CLSS is currently divided into 6 service units:

(1) The **Clinical Core Laboratory Unit (CCLU)** was developed in February of 1998, to assist in the development of scientific research in regards to the aging process. Its fundamental purpose is to assist researchers in analyzing data to better understand the predictors and risk factors for specific diseases that occur among individuals of different ages and changes that occur with the passing of time. The laboratory is CLIA certified.

The CCLU collects, analyzes, and prepares for long-term storage of blood and tissue samples. The CCLU also performs or arranges for DNA extractions, as well as cell transformations in preparation for creating renewable cell lines. Services provided by this Unit include tissue handling and preservation, DNA extraction, and inventory management of stored samples.

This laboratory offers a wide range of clinical testing services, information and laboratory management expertise. CCLU supports new test development for research, particular infectious disease, genetic disorders, immunological and degenerative diseases.

(2) The **Confocal Imaging Unit (CIU)** provides investigators with state-of-the-art 3D optical confocal microscopy facilities for imaging of living and fixed cells and tissues and computational resources for visualization and extraction of quantitative information from images.

(3) The **DNA Array Facility (DAF)** provides support and training spanning the entire microarray process, from sample preparation through data analysis. Several GRC arrays are available for use within this Unit including the GRC Human 15K cDNA array and the Laboratory of Genetics 15K cDNA murine embroyonic array. This Unit also provides support in the production of custom arrays based on investigator specifications and provides cDNA templates for spotting. New state-of-the-art instruments and software have greatly expanded available services and capabilities of this facility.

(4) The **Flow Cytometry Unit (FCU)** provides cell sorting and enhanced fluoragraphic analysis in support of research at the GRC. In addition, the Shared Service technologist and Unit Head provide consultation to investigators in design and interpretation of flow cytometry and cell sorting studies. Various uses of this facility include measurements of antigen or ligand density, apoptosis, enzyme activity, DNA and RNA content, membrane potential, cytokine receptors and its synthesis, phagocytosis and viability obtained from cells, changes in cell cycle, intracellular pH, intracellular calcium, intracellular glutathione and oxidative burst.

(5) The **Genotyping Service Unit (GSU)** offers genotyping services to investigators conducting typing and mapping studies with inbred strains of mice. Purified DNA samples are obtained from mouse tails and subsequently genotyped according to the investigator’s specifications

using PCR primers specific for the gene of interest or specific mouse markers. This Unit also possesses the expertise to assist investigators with the design of specific primer sets. In addition, this Unit has initiated genotyping of human DNA samples (under IRB approval) for various gene polymorphisms of interest within the Program.

(6) The **Mass Spectrometry Unit (MSU)** was formed in 2000 in response to a demand for high-sensitivity amino acid sequencing of purified and blotted proteins. The scope of this service Unit has been expanded to include amino acid sequencing, MALDI-TOF mass spectrometry, and the phosphopeptide mapping of proteins from various cellular populations.

In addition to these services, we are also currently creating a Bioinformatics Service Unit within CLSS which will offer services in bioinformatic technology for both information management and the detailed analysis of genomic, proteomic, imaging, and clinical/ epidemiological data.

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Biography: Dr. Juhaszova received her M.S. and Ph.D. in the Slovak Republic. She obtained her postdoctoral training at the University of Maryland at Baltimore. In 1994 she became a Research Associate and in 1996 an Assistant Professor at the University of Maryland at Baltimore. She joined NIA's Intramural Research Program, Research Resources Branch in 1999.

Keywords:

imaging
confocal microscope
fluorescence
mitochondria
mitochondrial ATP-sensitive K channels
mitochondrial permeability transition (MPT)
ischemic preconditioning

Recent Publications:

Juhaszova M, et al. *Eur J Neurosci* 2000; 12(3): 839-846.

Platoshyn O, et al. *Am J Physiol (Cell Physiol)* 2000; 279(5): C1540-1549.

Barrett T, et al. *Neurobiol Dis* 2001; 8(5): 822-833.

Research Interests: Role of Mitochondrial ATP-sensitive Potassium channels in Ischemic Preconditioning.

Ischemic preconditioning (PC) is the most powerful known form of myocardial protection in which brief episodes of ischemia and reperfusion before a prolonged ischemic event attenuate subsequent myocardial cellular damage. The mechanisms of this important phenomenon are largely unknown. Several recent studies have reported that, (1) the mitochondrial ATP-dependent K^+ (mitoK_{ATP}) channel is an effector of PC, and (2) PKC activity is important in mitoK_{ATP} channel mediated protection. We study the effect of the mitochondrial K_{ATP} channels modulators and PKC modulators on the **Mitochondrial Permeability Transition** (MPT) in freshly isolated rat cardiac myocytes. Our work is designed to understand the mechanism by which the mitoK_{ATP} channel and trafficking of PKC δ and $\in$ control the susceptibility to MPT induction. We showed that activation of the mitoK_{ATP} channel, acting via specific regulation of certain PKC isoforms, produces an increased cellular resistance to the deleterious induction of the MPT pore by reactive oxygen species (i.e., ROS, which are produced in great abundance during prolonged myocardial ischemia/reperfusion).

Collaborators: Steven J. Sollott, M.D., Laboratory of Cardiovascular Science, National Institute on Aging.

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Biography: Dr. Becker attended Emory University as an undergraduate graduating with a BSc. in Biology. He received a Masters degree from the Johns Hopkins University in Business. Thereafter, Dr. Becker received his Ph.D. in Molecular Biology and Genetics from the Johns Hopkins University School of Medicine in 1989. He did fellowships at the NIH in gene regulation and complex gene expression in the National Institute of Child Health and Human Development, National Institute of Neurological Disorders and Stroke, and the National Human Genome Research Institute. He began the DNA Array Unit at the NIA in November of 1998.

Keywords:

cDNA microarray
bioinformatics
autoimmunity
gene expression
genetic linkage

Recent Publications:

Becker KG, et al. *BioInformatics* 2000; 16(8): 745-747.

Tanaka TS, et al. *Proc Natl Acad Sci USA* 2000; 97(16): 9127-9132.

Cho YS, et al. *Proc Natl Acad Sci USA* 2001; 98(17): 9819-9823.

Konu O, et al. *Brain Res* 2001; 909(1-2): 194-203.

Becker KG, et al. *Nat Rev Neurosci* 2001; 2(6): 438-440.

The **DNA Array Unit** is involved in the design, assembly, application, and analysis of cDNA arrays and related gene expression systems. Three main areas of research include; a) applications in gene expression; b) technology development in array based assays; and c) genomic bio-informatic applications that integrate genetic and gene expression studies.

Gene expression studies using cDNA arrays this past year have included antisense treatment of prostate cells, nicotine administration in rats, studies in human schizophrenia, human asthma and rhinitis, and studies of human carotid artery occlusions, among others.

Efforts in technology development of cDNA arrays include projects in large scale development of high-density nylon membrane/ radioactive based cDNA arrays in multiple species including mouse, human, among others.

Bioinformatic development and applications include the development of a WWW-based relational database of biological pathways (http://bbid.grc.nia.nih.gov). This database is used to relate gene expression studies with complex biological processes. A second bioinformatic project in the DNA Array Unit includes a WWW-based database of the genetics of common complex diseases.

Collaborators: Dr. Jim Eberwine, University of Pennsylvania; Dr. Thomas DeGraba, NINDS, NIH; Dr. Kathleen Barnes, Johns Hopkins Medical Institutions; Dr. Yoon Cho-Chung, NCI, NIH; Dr. Ming Li, University of Tennessee; Dr. Mark Vawter, University of California; Dr. William Freed, NIDA, NIH.

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Biography: Dr. Robert Wersto received his Ph.D. from the Department of Biochemistry and Biophysics, Loyola University of Chicago in 1982. Dr. Wersto did his postdoctoral work in the Departments of Pathology and Hematology at the University of Rochester using the first commercially available flow cytometers and sorters. From 1985 until 1989, he was Assistant Professor of Pathology, Albert Einstein College of Medicine in the Bronx and Head of Flow Cytometry and Analytical Cytology. After a brief stay in industrial biotechnology, Dr. Wersto joined the Pulmonary Branch, National Heart, Lung, and Blood Institute (NHLBI) and played a seminal role in the first human gene therapy trial for cystic fibrosis. Most recently, he headed the flow cytometry laboratory in the non-human primate gene transfer program within the Hematology Branch, NHLBI. In mid 1999, he moved to the Flow Cytometry Unit, Research Resources Branch at the National Institute on Aging.

Keywords:

gene therapy
adenovirus
proliferation specific antigens
bone marrow progenitors
flow cytometry
cell cycle

Recent Publications:

An DS, et al. *J Virology* 2000; 74(3): 1286-1295.

Donahue RE, et al. *Blood* 2000; 95(2): 445-452.

Wersto RP, et al. *Cytometry* 2001; 46(5): 296-306.

Donahue RE, et al. *Mol Ther* 2001; 3(3): 359-367.

Cell Cycle Progression and Aging: The effects of aging on T-cell cycle progression and arrest is the subject of an on-going investigation utilizing multiparameter flow cytometry. Age-related cell cycle properties of human T cells are assessed using simultaneous measurements of DNA content and KI-67 protein expression following co-stimulation with immobilized CD3 antibody and soluble CD28. In T-cells from elderly individuals, there is increased G_0 cell cycle arrest that cannot be overcome following subsequent exposure to IL-2. Based on mitotic blocking, the delayed cell cycle entry in T-cells from older donors appears to be independent of early activation events.

Flow Cytometry Applications: Debris and aggregates can be prominent components of DNA histograms affecting the accuracy and reproducibility of cell cycle estimates. Debris originates from the damage and disintegration of cells following apoptosis or the fragmentation associated with the slicing of cells or nuclei during mechanical disaggregation. Aggregates can be composed of large clusters of cells or nuclei or two or more $G_{0/1}$ (2N) events adhered together ($G_{0/1}$ doublets) that are indistinguishable from particles with 4N, 6N, or 8N DNA content. Strategies to separate overlapping $G_{0/1}$ doublets from the G_2+M population have utilized the gating of correlated measurements of integral DNA fluorescence pulse (» area) with either peak pulse height or pulse duration (width), gating on the G_2+M cells that lack cyclin B1 protein expression, and computer algorithms to model aggregate probability distributions in

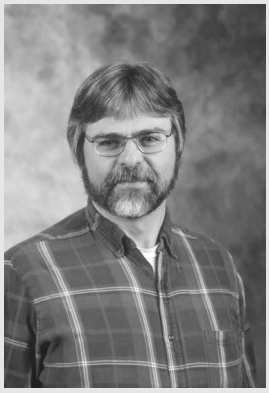
DNA histograms. While $G_{0/1}$ doublets are easily discernable from G_2+M singlets in cells or nuclei that are generally spherical in shape, doublet discrimination based on pulse processing or cyclin B1 measurements is nonconcordant in epithelial cells following cell cycle arrest. Significant differences in $G_{0/1}$ doublet estimates is observed in breast tumor specimens, with estimates based on pulse width twice those of pulse height and nearly five times computer estimates. Differences between techniques is attributed to increasing uncertainty between the boundaries of suspected $G_{0/1}$ doublets and G_2+M singlet populations in biologically heterogeneous specimens. The laboratory has a strong interest in molecular cytometry such as single cell PCR sorting, and the development of new techniques that permit multiparameter analysis of both cell function and proliferation-restricted proteins.

Adenovirus-Based Gene Therapy: Based on the tropism of wild-type adenovirus (Ad) for the respiratory epithelia and its ability to infect nonreplicating cells, replication-defective Ad vectors were thought to be the ideal approach by gene therapy to correct the physiological defects in the airways of individuals having the inherited human disease cystic fibrosis (CF). Culminating in human clinical trials, Ad vectors have become the prototype for other gene therapy protocols targeting cancers, inherited metabolic deficiencies, and cardiovascular disease. First-generation Ad vectors that had been rendered replication defective by removal of the E1 region of the viral genome (ΔE1)or lacking the Ad E3 region in addition to E1 sequences (ΔE1E3) induce G2 cell cycle arrest and inhibit traverse across the G1/S boundary in primary and immortalized human bronchial epithelial cells, independent of the cDNA contained in the expression cassette. Arrest is associated with the inappropriate expression and increase in cyclin A, cyclin B1, cyclin D, and cyclin-dependent kinase p34cdc2 protein levels. In some instances, infection with ΔE1 or ΔE1E3 Ad vectors produces aneuploid DNA histogram patterns and induces polyploidization resulting from successive rounds of cell division without mitosis. Cell cycle arrest was absent in cells infected with a second-generation ΔE1 Ad vector in which the entire early region E4 was deleted except for the sixth open reading frame. Current research focuses on the individual proteins encoded by the open reading frames in the E4 viral gene region and their interactions with cellular regulators of proliferation (signal transduction, transcription factors, oncogenes).

Bone Marrow Progenitor Identification: Gene transfer to hematopoietic stem cells (HSCs) has been hampered by their low frequency, the lack of positive selection markers, and the reduced potential for self-renewal and multi-lineage differentiation following *ex vivo* retroviral gene therapy. In

mammalian bone marrow stained with the dye Hoechst 33342, bivariate flow cytometric analysis of blue and red fluorescence identifies a small cell population, termed SP cells, that constitute primitive HSCs via a mechanism thought to involve *mdr* P-glycoprotein. Using unfractionated non-human primate and murine bone marrow, SP cell staining was found to be an energy-dependent process involving dye efflux, consistent with the hypothesis that this phenomena is mediated by a member of the ATP Binding Cassette family of transporters. However, dye efflux was specifically inhibited by probencid or sulfinpyrazone, implicating involvement of other multi-drug resistance associated proteins or membrane transporters. Cells having the identical staining characteristics and responses as those of bone marrow SP cells are present in cultures of the HL-60 promyleocytic cell line and exhibited a dependence on $G_{0/1}$ entry. SP cells are therefore not unique to bone marrow, but reflect multidrug resistance protein (MRP) functional expression that is present in a small fraction of quiescent cells. Understanding the basis for Hoechst 33342 staining and subsequent discrimination of SP cells from other blood elements provides insights into the functional characteristics of primitive multipotent hematopoietic that may be advantageous for future primate gene transfer protocols.

Collaborators: Donna Armentano, Ph.D., Genzyme Corporation; Eugene Rosenthal, Ph.D., Office of the Director, NIH; Edward Gabrielson, M.D., Johns Hopkins; Francesco Turturro, M.D., Human Gene Therapy Research Institute; Robert Donahue, D.V.M., National Heart, Lung, and Blood Institute, NIH; Tony Eissa, M.D., Baylor College of Medicine.



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Biography: Dr. Rowley received a B.S. in Chemistry from Eastern Illinois University where he did inorganic chemistry research in the synthesis of the first pan group VI transition metal complex: $(OC)_5WPPh(CH_2CH_2PPh_2Mo(CO)_5)$-$(CH_2CH_2PPh_2Cr(CO)_5$. He received a Ph.D. in Biology from University of Maryland Baltimore County working on the genetic and physical analysis of the growth rate dependent regulation of Escherichia coli zwf expression. His first post-doctoral project at Uniformed Services University of the Health Sciences was on virulence gene induction in Shigella flexneri 2a. His second post-doctoral experience at the United States Department of Agriculture involved the cloning and sequencing of soybean oleosin genes. At University of Maryland College Park, his work involved phenotypic expression of Pseudomonas syringea avr genes in E. coli.

Keywords:

PCR
genotyping

Recent Publications:

Duncan MD, et al. *J Gastrointest Surg* 2000; 4(3): 290-297.

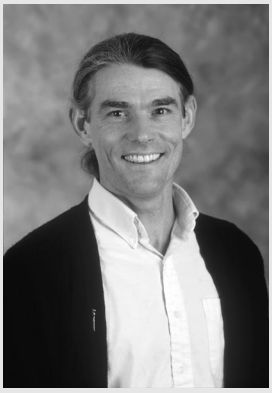
Barrett T, et al. *Neurobiol Dis* 2001; 8(5): 822-833.

Zhou L, et al. *Mammalian Genome* 2001; 12(10): 772-778.

Services Available: The **Genotyping Services Unit** (GSU) has been in operation since 7/31/00. Dr. Rowley currently has over 65 separate genotyping reactions working in the lab. Among them are several that he designed for two mouse dopamine transporter knockouts, a rat dopamine transporter transgenic construct, a Mu-opioid receptor LoxP knockin construct, an E. coli Beta-galactosidase sequence used in transgenic expression vectors, a transgenic adenoviral construct that causes a tissue specific tumor in mice, and a Prostate Apoptosis Response protein. The design of new genotyping reactions by GSU for GRC labs is considered both a service and a collaborative effort.

Research Interests: There are two main research collaborations in the lab. The first involves a sequence comparison of hypervariable regions of the Mu-opioid receptor gene in mouse strains that are known to exhibit different behavior profiles in response to morphine. The second involves screening of BLSA participants for their ApoE3/4 alleles; one of the genes implicated in Alzheimer's disease. Future directions include the influence of genetics on behavior.

Collaborators: Drs. Alan Zonderman, David Donovan, Kevin Becker, Research Resources Branch, NIA; Dr. Dennis Taub, Laboratory of Immunology, NIA.



Peter Gasper, D.V.M., Ph.D., Staff Scientist/Facility Head
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Biography: Dr. Gasper received his D.V.M. from The Ohio State University in 1980. He was awarded an National Research Service Award from the National Cancer Institute in 1981 to investigate the pathogenesis of a strain of feline leukemia virus that causes aplastic anemia in cats. In 1984, he completed his residency in veterinary pathology, received his Ph.D. from and joined the faculty of the Pathology Department in the College of Veterinary Medicine at Colorado State University. Drs. Gasper and Mary Anna Thrall established the Marrow Transplant Laboratory and performed 120 allogeneic and nine autologous bone marrow transplants in cats between 1984 and 1994. In 1994, Dr. Gasper accepted a position as a hematopathologist at the University of Maryland, College Park and extended his investigations into utilizing fetal blood stem cells as targets for gene therapy for FIV infections in cats. Dr. Gasper commenced as NIA's Animal Program Director and Chief, Comparative Medicine Section, RRB, in 2000.

Keywords:

hematopoiesis
stem cell
marrow
transplantation

Recent Publications:

Gasper PW. *The Hemopoietic System*. The Fifth Edition of Schalm's Veterinary Hematology, Lippincott Williams & Wilkins, Philadelphia, 2000; 63-101.

Gasper, PW, et al. *Pathogenic Yersinae*. Infectious Diseases of Wild Mammals, Iowa State Press, Ames, Iowa, 2001; 313-329.

Watson R, et al. *Vet Pathol* 2001; 38(2): 165-172.

Research Interests: Comparative hematopoiesis, particularly the mechanics of lymphohematopoietic engraftment following stem cell transplantation in cats and mice. The comparative biology of aging from the perception of mammals as mobile marine environments, individual communities of trillions of cells assembled in tissues and organs sustained by blood which serves as a life-sustaining internal ocean.

Current Projects:

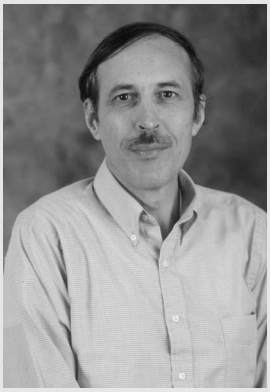
Embryonic Stem Cells as Hematopoietic Stem Cells: Investigations are being performed to determine if embryonic stem cells can be used to reconstitute the lymphohematopoietic system of mice following total body irradiation and stem cell transplantation.

Role of the Macrophage in the Pathogenesis of Plague (*Yersinia pestis* infection) in Cats: Cats are unique among mammals in developing bubonic, pneumonic, and septicemic plague. In contrast to all most all other carnivores, cats allow *Yersinia pestis* to proliferate early following exposure that leads to the fatal lesions of plague. As part of completing her Ph.D., Ms. Rowena Watson is investigating the role of macrophages in this failure to contain the infection.

Mathematical Modeling of the Pathogenesis of Feline Leukemia Virus-induced Erythroid Aplasia: We are seeking to understand the pathogenesis of the naturally occurring and experimentally inducible erythroid aplasia by utilizing archival data of hematopoietic colony from normal and anemic cats. As part of completing her Ph.D., Ms. Bren Ewen has developed an accurate simulation of normal feline erythropoiesis and she is challenging this model to gain insight into the mechanisms involved in this model system of retrovirus-induced anemia.

Intracellular Protection of Feline Fetal Blood Stem Cells as Therapy for FIF in Cats: We are using the feline-model feline immunodeficiency viruse (FIV) of HIV to determine whether decreasing retrovirus burden by myeloablation followed by transplantation of a life-long source of blood cells that are prophylactically protected against retrovirus infection might provide a new therapy for individuals infected with HIV. In addition to the virologic similarities between FIV and HIV, the feline host is a particularly attractive species for transplantation therapy of retrovirus disease. To date, the utility of HSC gene therapy has been hampered by the small number of HSCs available for transfection. We are poised to overcome these impediments by using fetal hematopoietic cells which are rich in immunologically-naive stem cells—thereby increasing the cell target numbers for transfection and transplantation. We are collecting feline HSCs from tissues that are normally discarded by local veterinarians who spay cats who happen to be pregnant at the time of surgery. We are simultaneously developing ribozyme-based antiviral gene therapy against FIV infection in cats by targeting the regulatory gene rev and its cognate recognition sequences, rev response element (RRE), which are critical for virus replication. Antiviral sequences against rev and RRE will be ultimately delivered into cats using retroviral vectors by way of fetal hematopoietic cells.

Collaborators: Ayalew Mergia, University of Florida; Nazareth Gengozian, University of Tennessee; Carol Pontzer, University of Maryland; Mark Carter, Mark Mattson, Zhihong Guo, and Melvin Ware, Jr., NIA, NIH.



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Biography: Dr. Larry J. Brant received his B.S in Mathematics in 1968 from Frostburg State College, Frostburg, Maryland. He received his M.A. in 1972 in Mathematics from the Pennsylvania State University, University Park, Pennsylvania, and his Ph.D. in 1978 from The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland.

Keywords:

biometry
longitudinal studies
mathematical modeling
statistical computing
statistical consultation

Recent Publications:

Morrell CH, et al. *The American Statistician* 2000; 54: 1-4.

Rabins PV, et al. *JAMA* 2000; 283: 2802-2804.

Scuteri A, et al. *Ann Intern Med* 2001; 135: 229-238.

Research Interests: Development of Statistical Methods (in particular, multiple comparisons), Development of Models for Biological Processes, Longitudinal Studies, Aging, Health Screening, Epidemiology of Circumpolar Health, and Combinatorics

The **Statistical and Experimental Design Section** is responsible for providing statistical and experimental design expertise appropriate to studies of aging and gerontology. Statistical methodology, including the use of Bayesian, maximum likelihood, and numerical computing methods, is applied and developed for longitudinal studies and other studies of aging. A major emphasis is on the development and application of methods that provide cogent, yet easily understood results.

The research and development of the Section currently focuses on several types of statistical models. These include 1) longitudinal multi-level models, which use empirical Bayesian methods to analyze the repeated measurements for all individuals in the study population as a function of the between- and within-subject variance estimates, 2) mixture models for describing and identifying high risk or preclinical disease groups of patients based on the distribution of changes in biological markers over time, 3) survival analysis techniques for studying risk factors in follow-up studies, 4) multiple comparisons for addressing the issue of multiplicity in the testing of group differences in experimental or observational designs, and 5) issues of power, sample size, and other experimental design issues.

Recent efforts in longitudinal data analysis include the development of a piecewise nonlinear mixed effects model to describe the transition of a biological marker from a normal to a disease state, a graphical method for studying the natural heterogeneity of a population by graphing the estimates of the individual random effects from a mixed-effects model, a method for detecting and modeling residual serial correlation in linear

mixed models, and a heterogeneous random effects model to aid in the detection of preclinical disease. Also, an imputation method has been proposed using estimates from a linear mixed-effects model to correct for measurement error bias in traditional risk factor analyses. Methods developed by the Section have been applied in studies of prostate cancer, pulmonary function, cardiovascular science, long-term caloric restriction in rats, and genome-wide mapping in mice.

Collaborators: Dr. Harry A. Guess, Dr. Jay D. Pearson, Epidemiology Department, Merck Research Laboratories; Dr. Emmanuel Lesaffre, Dr. Geert N. Verbeke, Biostatistical Center for Clinical Trials, Catholieke Universiteit, Belgium; Dr. Alena Horska, Department of Radiology, Johns Hopkins University School of Medicine; Dr. H. Ballentine Carter, Dr. Patrick C. Walsh, Department of Urology, Johns Hopkins University School of Medicine.

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Index of Principal Investigators

Index of Keywords

Invited Speaker Seminars - 2001

JANUARY

Matthew D. Ringel, M.D., Assistant Professor of Medicine, Uniformed Services University of the Health Sciences, Co-Director, Laboratory of Molecular Endocrinology, MedStar Research Institute, Staff Endocrinologist, Washington Hospital Center. “The Role of Akt Signaling in Thyroid Neoplasia.”

Jerrel L. Yakel, Ph.D., Leader, Ion Channel Physiology Group, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina. “Neuronal Nicotinic Receptors in Rat Hippocampus; Functional and Molecular Characterization.”

Richard Gibbons, MA, D.Phil, FRCP, Hon. Consultant in Clinical Genetics, University Lecturer in Clinical Biochemistry, Nuffield Department of Clinical Laboratory Science, University of Oxford, Oxford, United Kingdom. “ATR-X Syndrome: A Chromatin Remodeling Disease.”

Jean Lud Cadet, M.D., Clinical Director and Chief of the Molecular Neuropsychiatry Section, National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland. “The Involvement of Free Radicals and Apoptosis in Methamphetamine Induced Neurotoxicity: Implications for Parkinsonism.”

Jiemin Wong, Ph.D., Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas. “Transcriptional Regulation by Thyroid Hormone Receptor: the Tale of Coactivators and Corepressors.”

Mark Cookson, Ph.D., Assistant Professor of Pharmacology, Neurogenetics Laboratory, Mayo Clinic, Jacksonville, Florida. “Modeling Familial Neurodegenerative Disease in Cultured Cells.”

Robert A. Marciniak, M.D., Ph.D., Instructor in Medicine, Harvard Medical School, Postdoctoral Fellow, Massachusetts Institute of Technology, Cambridge, Massachusetts. “Werner Syndrome: Implications for Telomere Maintenance in Aging and Cancer.”

Professor Gerhard Meissner, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. “Redox Modulation of Ca2 + Release Channels (Ryanodine) Receptors.”

Sanjay Katiyar, Ph.D., Department of Urology, University of Tennessee, Memphis, Tennesse. “Role of p53 Tumour Suppressor Gene During Cervical Carcinogenesis.”

Dale A. Ramsden, Ph.D., UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina. “DNA Double Strand Break Repair: Smarter Than You Think.”

Alfred Geller, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Stem Cell Properties.”

Lily Moy, Ph.D., Professor, Rutgers University, New Jersey. “Role of Dopamine and Reative Oxygen Species in Malonate-Included Neuronal Damage.”

Zhen Pang, Ph.D., Research Scientist, Merck, Scotch Pines, New Jersey. “From Yeast to Mouse: Searching for the Function of Cerebellin/ Precerebellin.”

Weihong Song, Ph.D., Research Scientist, Harvard Medical School, Boston, Massachusetts. "Regulation of APP Processing and Notch Signaling by Presenilins."

Gang Yu, Ph.D., Professor, University of Toronto, Canada. "Presenilin Complex, APP Processing & Cell Signaling."

FEBRUARY

Andrew Singleton, Ph.D., Instructor in Biochemistry/Molecular Biology, Laboratory of Neurogenetics, Mayo Clinic, Jacksonville, Florida. "Searching for the Gene Defect Responsible for X-linked Dystonia Parkinsonism (lubag)."

Miyong To Kim, Ph.D., R.N., Assistant Professor, Johns Hopkins University School of Nursing, Baltimore, Maryland. "Cardiovascular Disease in the Korean Population."

Reid Huber, Ph.D., Applied Biotechnology, DuPont Pharmaceuticals Company, Wilmington, Delaware. "Practical Application of Expression Profiling Technology to Pharmaceutical Research."

Peter Bregestovoski, Ph.D., Director of Molecular and Cellular Neurobiology, Institute Pasteur, Paris, France. "Glycine Receptor Channels in Vertebrates: Structure, Function and Modulation by Calcium."

John David Carpten, Ph.D., Prostate-Cancer Investigation Group, Laboratory of Cancer Genetics, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland. "Identification of Genes Involved in Hereditary Prostate Cancer: Observations of Population Specific Variance."

Catherine Wolkow, Ph.D., Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts. "Youth Signals from the Brain: C. elegans Longevity is Controlled from Neurons."

John R. Atack, Ph.D., Senior Investigator, Merck, Merck Neuroscience Centre, Terlings Park, England. "Benzodiazepines: The Next Generation."

Christoph Romanin, Ph.D., Institute for Biophysics, University of Linz, Linz, Austria. "Regulation of L-type Ca2+ channel in a Mammalian Expression System."

Sakina Farzana Hussain, Ph.D., Department of Cell Biology and Molecular Genetics, University of Maryland at College Park, Maryland. "A Functional and Biochemical Characterization of Memory and Effector CD4 T cells."

Balan Venkatesh, Ph.D., Osaka University, Osaka, Japan. "Conformational Changes in Partially Liganded Hemoglobin Intermediates."

Graham F. Carpenter, Ph.D., Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee. "Recent Studies of ErbB-4 and Phospholipase C-gamma 1."

Aziz Sancar, M.D., Ph.D., Department of Biochemistry & Biophysics, University of North Carolina, Chapel Hill, North Carolina. "Technical Discussions with Laboratory of Molecular Gerontology."

Steen Kolvraa, Ph.D., Institute for Molecular and Structural Biology, Aarhus University, Arhus, Denmark. "Technical Discussions with Laboratory of Molecular Gerontology."

Daniel Turnbull, Ph.D., Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York. "Imaging Development and Disease in the Mouse CNS with Ultrasound and Magnetic Resonance Microscopy."

Hae-Young Chung, Ph.D., Research Scientist, Pusan National University, Korea. "The Inflammation of Hypothesis of Aging – Molecular Modulation by Calorie Restriction."

MARCH

Julia A. Segre, Ph.D., National Human Genome Research Institute, NIH, Bethesda, Maryland. "Molecular Mechanisms, Underlying the Epidermal Barrier Function."

Jeanine A. Harrigan, Ph.D., SUNY at Buffalo, New York. "Bioactivation of Benzo (a) pyrene & DNA adduct Formation in Rat Liver and Lung Slices."

Susan L. Swain, Ph.D., Director and Edward C. Brewster Chair, Trudeau Institute, Saranac Lake, New York. "The Impact of Aging Defects in CD4 T Cells on Immunity."

Colin L. Stewart, D.Phil., Chief, Laboratory of Cancer and Developmental Biology, National Cancer Institute, Frederick Cancer Research Development Center, Frederick, Maryland. "Changes in Nuclear Organization in Development and Diseases."

Joe G. N. Garcia, M.D., Dr. David Marine Professor of Medicine (Endowed Chair), Johns Hopkins University School of Medicine, Baltimore, Maryland. "Mechanisms of Endothelial Barrier Regulation."

Litsa Kranias, Ph.D., Professor and Director, Cardiovascular Biology, Department of Pharmacology & Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio. "Women in Science: Choices, Choices, Choices!"

Marc R. Blackman, M.D., Chief, Endocrine Division, Professor, Department of Medicine, Program Director, General Clinical Research Center, Johns Hopkins Bayview Medical Center, Baltimore, Maryland. "Endocrinology of Aging."

James Herman, Ph.D., Department of Psychiatry, University of Cincinnati Medical Center, Ohio. "Glucocorticoid Receptor Signaling in Aging Brain: Don't Get Around Much Anymore."

Sally Richards, Ph.D., Biomolecular Structure Unit, London, United Kingdom. "Inhibiting the Transcription of MOM2 using triplex forms Oligonucleotides."

APRIL

Hua Lu, Ph.D., M.D., Department of Biochemistry and Molecular Biology, Oregon Health Science University, Portland, Oregon. "Regulation of p53 by an hSpt16-SSRP1-CK2 Complex."

David MacLennan, Ph.D., University of Toronto, Canada. "Calcium Signaling and Muscle Disease."

Judith Keen, Ph.D. Candidate, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland. "Role for NFATc in Increased IL-4 Gene Expression"

Michel Sadelain, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center, New York. “The Genetic Treatment of ß Thalassemia.”

Marie Mancini, Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland. “Function of Caspases: A Matter of Location.”

En Li, Ph.D., Assistant Professor, Department of Medicine, Harvard Medical School, Boston, Massachusetts. “Regulation and Function of DNA Methylation in Mammalian Development.”

William Bonner, Ph.D., Chief, Chromatin Structure and Function Group, National Cancer Institute, Bethesda, Maryland. “Chromatin Remodeling in Double Strand Break Repair.”

Alan Meeker, Ph.D., Department of Urology, Johns Hopkins University School of Medicine, Baltimore, Maryland. “Telomere Dynamics and Androgen Regulation of Telomerase Enzymatic Activity in Normal and Pathological States of the Prostate.”

Chang-Tai (John) Hsieh, Ph.D., Department of Biochemistry and Molecular Biophysics, Washington University at St. Louis, Missouri. “Characterization of the W250A mutant of the E.coli Rep Helicase.”

Mario R. Capecchi, Ph.D., Professor of Human Genetics, Investigator of the Howard Hughes Medical Institute, Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah. “Gene Targeting: Where Is It Going?”

MAY

Jeff Stuart, Ph.D., Research Associate, Department of Emergency Medicine, Carolinas Medical Center, North Carolina. “Functional Studies of Mitochondrial Uncoupling Proteins.”

James L. Weber, Ph.D., Director, Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, Wisconsin. “Maximizing Genetic Information using Genome Polymorphism Scans.”

Thomas F. Franke, Ph.D., Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York. “Apoptosis Suppression by Akt.”

Mark Kenny, Ph.D., Department of Radiation Oncology, Montefiore Medical Center, Bronx, New York. “The Pluses and Minuses of DNA Replication and Repair.”

Kristin Abraham, Ph.D., Department of Microbiology and Immunology, University of Maryland, Baltimore, Maryland. “Regulation of Thymopoiesis by Src-family Tyrosine Kinases: From Stromal Cell Activation to TCR Signaling.”

Andrew Arai, M.D., Investigator, Laboratory of Cardiac Energetics, National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland. “Contrast Enhanced Cardiovascular MRI of Atherosclerosis.”

Joseph H. Nadeau, Ph.D., Professor, Genetics Department, Case Western Reserve University School of Medicine, Cleveland, Ohio. “Genomic Approaches for Dissecting the Genetic Basis of Complex Traits.”

Ahmed Zaid, Ph.D., Department of Biochemistry and Biophysics, Stockholm University, Sweden. “Transcriptional Regulation of Some Nuclear Encoded Mitochondrial Genes.”

Jingli Cai, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Stem Cell Properties.”

Jeffrey Lee, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Role of NGN-3 in Regulating Cell Fate.”

Ying Liu, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “PS1 and Astrocyte Differentiations.”

Tahmina Mujtaba, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Generation of Cell Lines.”

Yuan Yuan Wu, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Glial Lineages in the CAN.”

Haipeng Xue, Ph.D., Research Scientist, University of Utah, Salt Lake City, Utah. “Generating CRE Recombinase Vectors.”

Min Zhu, Ph.D., Professor, Research Institute for Children, Louisiana. “Insulin/IGFs Axis Regulatory System for Diabetes Mellitus.”

JUNE

Susan E. Lyons, M.D., Ph.D., National Human Genome Research Institute, NIH, Bethesda, Maryland. “Hematopoietic Studies in the Zebrafish Model.”

Giorgio Ascoli, Ph.D., George Mason University, Virginia. “The Algorithmic Beauty of Neurons.”

Tobi Limke, Ph.D., Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan. “Mechanisms of Methyl Mercury-induced Neurotoxicity in Rat Cerebella Granule Neurons.”

Roland A. Owens, Ph.D., Senior Investigator, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland. “The Roles of Rep Protein Recognition Sequences in Adeno-Associated Virus Type 2 Replication, Gene Regulation and Integration.”

Ken Blumer, Ph.D., Professor of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri. “Regulation of G Protein Signaling by RGS Proteins in the Heart.”

Nicholas Lukacs, Ph.D., University of Michigan Medical School, Ann Arbor, Michigan. “Roles of Cytokines and Chemokines in Airway Inflammation.”

Ching-Yi Chen, Ph.D., Department of Pharmacology, University of California, San Diego. “Mechanisms and Regulation of mRNA Turnover in Mammalian Cells.”

Lorraine Dennerstein, Ph.D., Director, Office for Gender and Health, Department of Psychiatry, The University of Melbourne, Victoria, Australia. “Mood, Memory and the Menopausal Transition.”

Kevin Gardner, M.D., Ph.D., Investigator, Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland. “The Biochemistry of p300 Co-activator Complexes in the Activated T-cell Nucleus.”

JULY

Susan Everson, Ph.D., M.P.H., Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan. “Negative Emotions and CVD: What Are the Biologic Pathways?”

David Sheps, M.D., MSPH, Professor of Medicine, Associate Chief, Division of CVD Medicine, University of Florida College of Medicine, Gainesville, Florida. "Psychological Stress and Coronary Disease."

Christopher J. Earley, M.D., Ph.D., Associate Professor of Neurology, Johns Hopkins School of Medicine, Johns Hopkins Bayview Medical Center, Baltimore, Maryland. "Iron Dysregulation and Restless Legs Syndrome."

James Herman, Ph.D., Professor, University of Cincinnati, Ohio. "Glucocorticoid Receptor Signaling in Aging Brain: Don't Get Around Much Anymore."

AUGUST

Liangtang Wu, Ph.D., Biotechnology Research Institute, Montreal, Quebec, Canada. "Functional Relationship: p59 Fyn Kinase, Adaptor Protein SKAP55, and PTPase CD45 in T Lymphocytes."

Michael J. Quon, M.D., Ph.D., Investigator, Cardiology Branch, National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland. "Complexities in Insulin Signaling: From Cells to Humans."

Yinhua Yang, Ph.D., Department of Genetics, Yale University School of Medicine, New Haven, Connecticut. "Nucleolar Localization of hTERT Protein is Required for Telomerase Activity and Function."

Allan J. McLean, M.D., Ph.D., Professor of Geriatric Medicine, University of Sydney, Australia. "Pseudo-capillarisation of the Hepatic Sinusoidal Endothelium, A Specific Aging Lesion with Implications for Tissue Energetics and Metabolism, Pathogenesis, and Specific Therapies."

SEPTEMBER

Theresa Shapiro, M.D., Ph.D., Director, Division of Clinical Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland. "DNA Topoisomerases: A New Twist for Antitrypanosomal Therapy."

Martha Ogilvie, Ph.D., Scientific Application Specialist, Celera Genomics. "An Introduction to the Celera Discovery System."

Fumio Hanaoka, Ph.D., Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan. "Translesion DNA Synthesis by Human Pol Eta, the Xerorma Pigmentosum Variant (XPV)-responsible Gene Product."

Aravinda Chakravarti, Ph.D., Director, McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University, Baltimore, Maryland. "Genetic Dissection of Hirschsprung Disease."

Junichi Sadoshima, M.D., Ph.D., Associate Professor, University of Medicine and Dentistry, New Jersey Medical School and Hackensack University, New Jersey. "The Role of Glycogen Synthase Kinase 3-Beta in Cardiac Hypertrophy."

Michael J. Shamblott, Ph.D., Assistant Professor, Department of Gynecology & Obstetrics, Division of Developmental Genetics, Johns Hopkins School of Medicine, Baltimore, Maryland. "Human Pluripotent Stem Cells: A New Paradigm for Regenerative Medicine."

Juan S. Bonifacino, Ph.D., Chief, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland. "Protein Sorting in the Endosomal-Lysosomal System."

Richard Wahl, M.D., Director, Division of Nuclear Medicine, Department of Radiology, Johns Hopkins University, Baltimore, Maryland. “PET Scanning Applications in Oncology: Clinical and Research.”

Rudolph E. Tanzi, Ph.D., Professor of Neurology (Neuroscience), Director, Genetics and Aging Research Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. “Alzheimer’s Disease: From Genes to Drugs in the 21st Century.”

OCTOBER

Olaf Pongs, Ph.D., Director, Institute of Neuronal Signaling, Center of Molecular Neurobiology, Hamburg, Germany. "Mice with Disrupted BK Channel B1 Subunit Gene Feature Abnormal Ca^{2+} Spark/STOC Coupling and Elevated Blood Pressure."

David M. Wilson, Ph.D., Senior Biomedical Scientist, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California. "Mammalian Nucleases - Mechanisms and Role in Disease Susceptibility."

Michael K. Lee, Ph.D., Department of Pathology and Neuropathology, Johns Hopkins University School of Medicine, Baltimore, Maryland. "Mutant Alpha-synuclein Transgenic Mice: Pathology and Dopaminergic Phenotype."

Zhigang Wang, Ph.D., Associate Professor, Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky. "DNA Repair and Mutagensis: Mechanisms and Relevance to Aging Research."

Paul Insel, M.D., Professor of Pharmacology and Medicine, University of California at San Diego, California. "Critical Determinants of G-protein Mediated Signaling."

Juan Rivera, Ph.D., Chief, Molecular Inflammation Section, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland. "The Relationship of the Adapters LAT and Gab2: A New Paradigm in Fc Receptor Signaling."

Grigory L. Dianov, Ph.D., Senior Scientist, MRC Radiation and Genome Stability Unit, Medical Research Council, Oxfordshire, United Kingdom. "Co-ordination of Base Excision Repair Pathways."

Rita Sung-Yun Cha, Ph.D., Post Doctoral Fellow, Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts. "Role of an ATRIATM Homolog, MECI, in DNA Replication and Genome Stability."

Dr. Terry Hebert, Assistant Professor, Montreal Heart Institute, Montreal, Quebec, Canada. "G Protein-Coupled Receptor Heterodimers: GABA-B and Beta-adrenergic Receptors as Paradigms"

Anand Jacob, Ph.D., Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma. "Crosstalk Between the P1 3-Kinase Pathway and the Ras Pathway in the B Cell Antigen Receptor Signaling."

NOVEMBER

John D. Gearhart, Ph.D., C. Michael Armstrong Professor of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland. "Human Pluripotent Stem Cells: The End of the Beginning."

Peter Lansdorp, M.D., Ph.D., British Columbia Cancer Research Center, Terry Fox Laboratory, Vancouver, British Columbia, Canada. "Maintenance and Repair of Telomeric DNA."

Jennifer J. Manly, Ph.D., Assistant Professor, Columbia University, New York. "Deconstructing Race and Education - Lessons from Cross-cultural Neuropsychology."

John E. Gerich, M.D., Director, Endocrine Unit, University of Rochester Medical Center, General Clinical Research Center, Rochester, New York. "Role of the Kidney in Glucose Metabolism."

Michael Fasullo, Ph.D., Associate Professor, Center for Immunology and Microbial Disease, Albany Medical College, Albany, New York. "Cell Cycle Checkpoint Control of Homologous Recombination in Yeast."

Susan L. Hamilton, Ph.D., Professor and Interim Chair, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas. "Calmodulin and Excitation-Contraction Coupling."

Karin Drotschmann, Ph.D., Visiting Fellow, National Institute of Environmental Health Services, Research Triangle Park, North Carolina. "Muts Homologs in Mismatch Repair."

Jeffrey S. Friedman, M.D., Ph.D., Instructor in Pediatrics, Dana Farber Cancer Institute and The Children's Hospital, Boston, Massachusetts. "Effects of Oxidative Stress and Aging on Blood Cell Development and Function in Mice."

Pamela J. Yao, Ph.D., Department of Neurobiology and Anatomy, Center for Aging and Developmental Biology, University of Rochester, New York. "Synaptic Vesicle Trafficking and Alzheimer's Disease."

Irene Ginis, M.D., Ph.D., Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland. "Is Oxygen a Factor in Embryonic Stem Cell Differentiation?"

Ilya Goldberg, Ph.D., Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts. "Quantitative Microscopy and Image Informatics: Using Imaging to Solve Biological Problems from Large-scale Morphological Screens to Details of Biological Mechanisms."

Nathan Dascal, Ph.D., Professor of Physiology, Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel. "The N-terminus of Cardiac Ca Channel: Role in Gating, Modulation by PKC, G Proteins, and Calmodulin."

DECEMBER

Janko Mikolich-Zugich, M.D., Ph.D., Professor and Senior Scientist, Vaccine and Gene Therapy Institute, and the Oregon Regional Primate Research Center, Oregon Health and Science University, Portland, Oregon. "The Role of Age-related CD8 T-cell Clonal Expansions in Immune Deficiency of the Old Age."

Gary Fiskum, Ph.D., Professor and Research Director, Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, Maryland. "Mechanisms of Mitochondrial Outer and Inner Membrane Permeability Changes Mediated by Apoptotic Proteins."

Liang-Peng Yang, Ph.D., National Cancer Institute, NIH, Bethesda, Maryland. "Genomics and Proteomics: Mining Novel Genes by Computational and Experimental Anaylsis."

Qing Yang, Ph.D., Naval Medical Research Center, Bethesda, Maryland. "Chromatographic Purification and Refolding of Recombinant Proteins."

Invited Speaker Seminars - 2002

JANUARY

Dr. Donald E. Mager, Department of Pharmaceutical Sciences, School of Pharmacy, SUNY-Buffalo, New York. "Pharmacokinetic/ Pharmacodynamic Modeling of Target Mediated Drug Disposition."

Dr. Alan Bank, Associate Professor, Cardiovascular Division, University of Minnesota Medical School and Director of Research, St. Paul Heart Clinic, Minnesota. "Fatigue and the Biomechanics of Atherosclerotic Plaque Rupture."

Pu Paul Liu, M.D., Ph.D., Senior Investigator, Head, Oncogenesis and Development Section, National Human Genome Research Institute, NIH, Bethesda, Maryland. "CBFB, An Important Player in Leukemogenesis, Hematopoiesis, and Osteogenesis."

Olli-P Kallioniemi, M.D., Ph.D., Senior Investigator, Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, Maryland. "Biochip Technologies: Beyond cDNA Microarrays."

Ilya B. Bezprozvanny, Ph.D., Assistant Professor, Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas. "Targeting of Synaptic Calcium Channels."

G. Tong, M.D., Ph.D., Department of Neurology, University of California at San Diego, California. "Synaptic Plasticity and Alzheimer's Disease."

FEBRUARY

Professor John Gordon, MRC Centre for Immune Regulation, The University of Birmingham, United Kingdom. "Life and Death of Burkitt's Lymphoma Cells."

Gerard D. Schellenberg, Ph.D., Departments of Neurology and Pharmacology, University of Washington, Seattle, Washington. "Tau and Neurodegenerative Disease: Genetic and Transgenic Animal Approaches."

Camille Pierre Granvil, Ph.D., Laboratory of Metabolism, National Cancer Institute, NIH, Bethesda, Maryland. "The Use of Transgenic Mice Expressing Human Cytochrome P450s as *In Vivo* Model for Studying Drug Disposition and Metabolism."

Wayne D. Bowen, Ph.D., Chief, Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland. "Sigma-2 Receptors Activate a Novel Pathway to Apoptosis."

Johannes D. Veldhuis, M.D., Professor Internal Medicine, University of Virginia Health System, Charlottesville, Virginia. "Aging of the Human Male Gonadotropic Axis."

Jennifer L. Balfour, Ph.D., Center for Social Epidemiology and Population Health, University of Michigan, Ann Arbor, Michigan. "Aging in Challenging Neighborhood Environment: Research Evidence and Methodology."

MARCH

Yunbiao Lu, Ph.D., Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut. “mPak4, a New Regulator of Raf-1 in KGF/KGFR Signal Transduction Pathway.”

Howard J. Worman, M.D., Associate Professor of Medicine and Anatomy and Cell Biology, College of Physicians and Surgeons of Columbia University, New York. “Inner Nuclear Membrane Proteins and Human Disease.”

Anna M. Wobus, Ph.D., In Vitro Differentiation Group, Institute of Plant Genetics, Gatersleben, Germany. “Comparative Embryonic and Somatic Stem Cell Differentiation.”

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