2000 Factbook

National Institute on Aging

Intramural Research Program

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Contents

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 nine 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 Brain Physiology and Metabolism Section and the newly created Laboratory of Neurogenetics 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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Biography: Dr. Lakatta received his M.D. from Georgetown University School of Medicine, Washington, D.C. in 1970. His postdoctoral training included an internship and residency in medicine at Strong Memorial Hospital, University of Rochester School of Medicine, cardiology fellowships at Georgetown and Johns Hopkins University Hospitals, and basic research training at NIH and at the Department of Physiology, University College, London, England. He was section chief of the Cardiovascular Laboratory in the Clinical Physiology Branch from 1976 to 1985, at which time he founded the Laboratory of Cardiovascular Science.

Keywords:

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

Recent Publications:

Xiao RP, et al. *Circ Res* 1999; 84(1): 43-52.

Kuschel M, et al. *Circulation* 1999; 99: 2458-2465.

Long X, et al. *J Geront* 1999; 54: B23-B27.

Nussbacher A, et al. *Am J Physiol* 1999; 277: H1863-H1871.

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 hypertension. 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.

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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, he worked at the NIA as a Visiting Associate.

Keywords:

Na, K-ATPase
endogenous inhibitors
hypertension
protein kinases

Recent Publications:

Bagrov AY, et al. *Hypertension* 1998; 31: 1097-1103.

Lopatin DA, et al. *J Hypertens* 1999; 17: 1179-1187.

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

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

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; Ricardo Garay, M.D., Ph.D. INSERM Unite 600 Creteil, France; Denis A. Lopatin, M.D., Sechenov Institute of Evolutionary Physiology and Biochemistry and Research Institute of Obstetrics and Gynecology, St. Petersburg, Russia.

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Biography: Dr. Jerome Fleg received his M.D. from the University of Cincinnati in 1970. After completing training in Internal Medicine and Cardiovascular Disease at Washington University in 1977, he assumed his current position in NIA's Laboratory of Cardiovascular Science. His research interests include normative aging changes in cardiovascular structure and function, silent myocardial ischemia, and congestive heart failure.

Keywords:

aging
exercise physiology
silent ischemia
heart failure

Recent Publications:

Rywik TM, et al. *J Appl Physiol* 1999; 87: 2136-2142.

Nussbacher A, et al. *Am J Physiol* 1999; 277: H1863-H1871.

Rywik TM, et al. *Circulation* 1998; 97: 2117-2122.

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

Effects of Age, Gender, Lifestyle and Disease on Cardiovascular Structure and Function: Advancing age in humans is accompanied by significant changes in the cardiovascular system and, all too often, by the development of cardiovascular disease. A major challenge undertaken by our laboratory is to define normative aging changes in cardiac and vascular structure and function and their modulation by lifestyle variables and disease. To accomplish this ambitious task, we utilize a wide variety of noninvasive testing methodologies at rest and during exercise.

Early M-mode echocardiographic studies in our laboratory, pioneered by Drs. Gary Gerstenblith and Edward Lakatta, demonstrated that normative aging was accompanied by a thickening of the left ventricular (LV) muscular wall and a reduction of early mitral value closure slope analogous to the findings in mild hypertension. These findings have led us to conceptualize that aging is a muted form of hypertension. In industrialized societies, a 20-30 mmHg rise in systolic blood pressure (SBP) typically occurs across the adult lifespan in subjects who remain normotensive by clinical criteria. The etiology of this SBP rise involves a gradual replacement of elastic fibers in the vascular media by less distensible collagen and calcium. Recent studies in our laboratory using pulse wave velocity and applanation tonometry of the large arteries have demonstrated a 200-500% increase in arterial stiffness across the adult life span. Two-dimensional echocardiographic determination of LV mass in these same subjects has revealed that the late systolic augmentation of arterial pressure quantified by applanation tonometry is an independent determinant of LV mass, beyond the effect of SBP. These studies, therefore, support the hypothesis that age-associated increases in arterial stiffness are responsible in part for the mild LV hypertrophy and substantial reduction in early diastolic LV filling rate seen with aging. Cross-sectional studies in our laboratory have

demonstrated both lesser arterial stiffness and enhanced endothelium dependent arterial dilation in endurance trained athletes correspond to their sedentary age peers. To further examine the hypothesis that arterial stiffness can be reduced, we have designed short-term drug interventions and longer-term exercise training interventions, both in normal older subjects and individuals with congestive heart failure. Although the exercise training studies are still in progress, a recently completed study has shown that acute infusion of the vasodilator sodium nitroprusside to normal older subjects dramatically reduced their resting arterial stiffness and improved their LV performance during exhaustive cycle exercise to levels typical of unmedicated young individuals.

Another major goal of our laboratory is to determine the mechanisms for the well known decline in maximal aerobic capacity (VO_{2max}) seen with aging. In an early study, we found that normalization of treadmill VO_{2max} for total body muscle mass nearly eliminated the age-associated reduction in VO_{2max}, inferring that the loss of muscle tissue with age contributes importantly to the decline in VO_{2max}. We have employed gated cardiac blood pool scans with the isotope technetium-99m to quantify LV performance at rest and during maximal upright cycle exercise and its modulation by age, gender, lifestyle variables and cardiovascular disease. Our initial investigation using this techniques demonstrated that stroke volume at peak exercise was preserved across age by a greater reliance on LV dilatation to compensate for reduced systolic emptying. More recently we have found that this age-associated LV dilatation during exercise is more prominent in men than women despite similar impairment in emptying. Endurance trained older subjects utilize both larger end-diastolic LV volumes and enhanced LV emptying to augment stroke volume during exercise to a greater degree than untrained individuals.

Simultaneous monitoring of oxygen consumption throughout these exercise cardiac blood pool scans has allowed us to examine the relative importance of cardiac versus peripheral factors in the age-associated decline in aerobic capacity and its modulation by endurance training. A recent investigation using this methodology suggests that declines in cardiac output and arteriovenous oxygen difference contribute nearly equally to this decline in aerobic capacity with aging. Similarly, the marked augmentation of peak VO_2 in endurance trained older subjects relative to their sedentary peers is accomplished to a similar extent by enhanced cardiac output and peripheral oxygen extraction.

We have also utilized pharmacological probes to further define mechanisms for the decline in maximal exercise cardiac performance with age and their potential for modulation. For example, beta adrenergic blockade during exhaustive cycle ergometry in younger subjects markedly reduced their maximal heart rates and systolic emptying and augmented their exercise-induced LV dilatation, producing a profile similar to that of older unmedicated subjects. These data support our hypothesis that an important mechanism for the age-associated reduction in maximal cardiac performance is reduced beta adrenergic responsiveness.

Our laboratory has utilized the electrocardiographic and Thallium scintigraphic response to treadmill exercise to predict the development of future coronary events in clinically healthy adults. Although we and others have demonstrated the increased coronary risk associated with ischemic ST segment depressions that develop during treadmill exercise, we recently showed that such ST depression beginning during recovery has similar adverse prognostic significance. In another study, we found that exercise-induced silent ischemia by electrocardiogram and Thallium scan was strongly associated with increased carotid artery intimal-medial thickness, an indicator of carotid atherosclerosis.

Collaborators: Edward Shapiro, M.D., Gary Gerstenblith, M.D., Lewis Becker, M.D., Steven Schulman, M.D., Johns Hopkins University; Leslie Katzel, M.D., Andrew Goldberg, M.D., University of Maryland at Baltimore; James Hagberg, Ph.D., Stephen Porges, Ph.D., University of Maryland, College Park; Yoji Nagai, M.D., Edward G. Lakatta, M.D., LCS, NIA; E. Jeffrey Metter, M.D., LCI, NIA.

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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:

Lundberg MS, et al. *J Mol Cell Cardiol* 1998; 30: 2377-2389.

Blystone SD, et al. *J Cell Biol* 1999; 145: 889-897.

Ekheterae D, et al. *Circ Res* 1999; 85: e70-e77.

Senzaki H, et al. *Circ Res* 2000; 86: 807-815.

Vascular Smooth Muscle 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.

With some of the collaborators listed below, we are also currently examining the role of ARC in skeletal muscle, particularly its role in skeletal muscle atrophy associated with disuse, cachexia, age-related disorders such as sarcopenia, and musculoskeletal diseases. We hypothesize that changes in the activation and/or expression of ARC is a common mechanism for regulating not only cell death but also cell growth (hypertrophy/atrophy) in skeletal muscle. One experimental model of skeletal muscle disuse currently under investigation is that of the sarcoglycan-deficient mouse. Sarcoglycan forms an essential connection between the contractile apparatus and cell membrane, linking contraction to work and potentially to intracellular signaling pathways through membrane proteins, such as integrins. The muscles of the deficient mouse are chronically “unloaded” and undergo severe atrophy. Our preliminary analysis indicates that apoptosis is elevated in such muscle cells, that it is “regionalized” to certain nuclei within the multinucleated fiber, and is correlated with reduced ARC expression. Experiments are underway to determine if forced ARC expression can prevent “regionalized” apoptosis within sarcoglycan-deficient single muscle fibers and reverse muscle fiber atrophy in this disease model. Experiments are also underway to examine ARC expression in aging muscles and in myoblast stem cells from these muscles, which exhibit an age-associated decline in proliferative potential and increased susceptibility to apoptosis.

Collaborators: Richard Kitsis, Albert Einstein School of Medicine, Bronx, NY; Charlotte Peterson, University of Arkansas, Little Rock, AR; Thomas Rando, Stanford University, Stanford, CA; H. Lee Sweeney, University of Pennsylvania, Philadelphia, PA; Larry Denner, Texas Biotechnology Corporation, Houston, TX; Edward G. Lakatta, M.D., LCS, NIA.

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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:

Koban MU, et al. *Cardiovasc Res* 1998; 37: 405-423.

Boateng SY, et al. *J Mol Cell Cardiol* 1998; 30: 2683-2694.

Ribadeau-Dumas A, et al. *Cardiovasc Res* 1999; 43(2): 426-436.

Santalucía T, et al. *J Biol Chem* 1999; 274: 17626-17634.

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 embryonic 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.

Collaborators: Professor Magdi H. Yacoub, Imperial College School of Medicine, United Kingdom; Professor Antoon F.M. Moorman, University of Amsterdam, The Netherlands; Professor Alan Williams, Imperial College School of Medicine, United Kingdom; Dr. Kenneth MacLeod, Imperial College School of Medicine, United Kingdom; Prof. Tony Lai, Cardiff, United Kingdom; Prof. Antonio Zorzano, University of Barcelona, Spain; Dr. Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany; Dr. Edward G. Lakatta, LCS, NIA.

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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:

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

Stern MD, et al. *J Gen Physiol* 1999; 113(3): 469-489.

Cheng H, et al. *Biophys J* 1999; 76(2): 606-617.

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

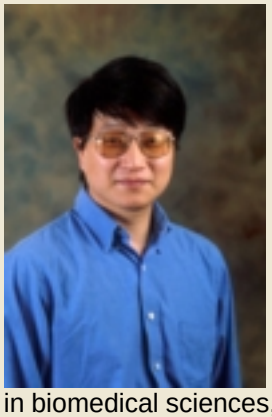
means that the trigger for CICR is not whole cell calcium, but rather the local calcium microdomain generated in the neighborhood of the triggering channel. We have shown mathematically that the interaction between the stochastic gating of individual channels and the fluctuating calcium microdomains 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 importantly, 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
intermolecular Ca^{2+} signaling
excitation-contraction coupling

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Sham JS, et al. *Proc Natl Acad Sci USA* 1998; 95: 15096-15101.

Zhou Y-Y, et al. 1999; *J Physiol* 521: 351-361.

Cheng H, et al. *Biophys J* 1999; 76: 606-617.

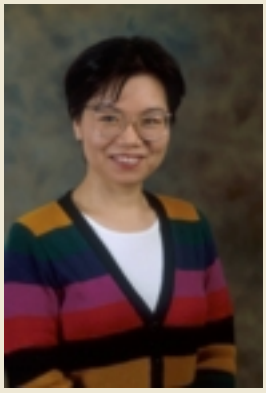
Shirokova N, et al. *J Gen Physiol* 1999; 113: 377-384.

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.

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.

Collaborators: James S. K. Sham, Division of Pulmonary and Critical Care Medicine, Johns Hopkins Medical Institutions; Weinian Shou, Department of Molecular Physiology and Biophysics, Baylor College of Medicine; Hector H. Valdivia, Department of Physiology, University of Wisconsin Medical School, Madison; Eduardo Rios, Department of Molecular Physiology and Biophysics, Rush University; Joel E. Keizer, Institute of Theoretical Dynamics, University of California; James T. Russel, Laboratory of Cellular and Molecular Neurophysiology, National Institute of Child Health and Human Development, NIH; Collaborators at LCS: Edward G. Lakatta, Michael D. Stern, Rui-Ping Xiao, Kenneth Boheler.



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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
cAMP dependent protein kinase
pertussis toxin-sensitive G proteins
cardiac contractility

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Kuschel M, et al. *Circulation* 1999; 99: 2458-2465.

Kuschel M, et al. *J Biol Chem* 1999; 274: 22048-22052.

Dual Coupling of Cardiac $ß_2$-Adrenergic Receptor to G_s and G_i Proteins: $ß_1$- and $ß_2$-adrenergic receptor (AR) are among the most extensively characterized members of the G protein coupled receptor family. Stimulation of these receptors by catecholamines plays a pivotal role in regulating cardiovascular function. Dr. Xiao's previous studies have suggested that, despite their marked structural and functional similarities, $ß_1$AR and $ß_2$AR play distinct functional roles in cardiac myocytes. Her recent studies have been concentrated on signaling mechanisms underlying different functionality of $ß_2$AR versus $ß_1$AR subtype in healthy and diseased hearts, and reshaped the conceptual framework of cardiac ß-AR signal transduction. While the classical linear G_S-adenylyl cyclase-cAMP-protein kinase A signaling (PKA) cascade has been corroborated for $ß_1$AR stimulation, the $ß_2$AR signaling pathway bifurcates at the very first post-receptor step, the G protein level. In addition to G_S, $ß_2$AR couples to pertussis toxin (PTX)-sensitive G_i proteins, G_{i2} and G_{i3}. The coupling of $ß_2$AR to G_i proteins mediates, to a large extent, the differential actions of the ß-AR subtypes on cardiac Ca^{2+} handling, contractility, cAMP accumulation, and PKA-mediated protein phosphorylation. There is an apparent dissociation of $ß_2$AR-induced augmentations of intracellular Ca transient and contractility from cAMP production and PKA-dependent cytoplasmic proteins phosphorylation. This can be largely explained by G_i-dependent functional

compartmentalization of the ß$_2$AR-directed cAMP/PKA signaling to sarcolemmal microdomain. This compartmentalization allows the common second messenger, cAMP, to perform selective functions during ß-AR subtype stimulation.

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.

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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:

Vila-Petroff MG, et al. *Circ Res* 1999; 84(9): 1020-1031.

Srivastava RK, et al. *Mol Cell Biol* 1999; 19(8): 5659-5674.

Zorov DB, et al. *J Exp Med* 2000; 192: 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-Lue 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.

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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.

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

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

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

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) Vascular Permeability Effect of Adenovirus Mediated Vascular Endothelial Growth Factor Gene Transfer to the Rabbit and Rat Skeletal Muscle: Vascular endothelial growth factor (VEGF) has been used in preclinical and phase 1 and 2 clinical trials as a potent mediator of therapeutic angiogenesis; however, its ability to enhance the vascular permeability might be a source of potential complications. The objective of this work was to evaluate the effects of the intramuscular injection of an adenovirus vector coding for the 121 amino acid form of VEGF ($Ad.VEGF_{121}$) on vascular permeability and edema development in rabbits and rats. Different concentrations of $Ad.VEGF_{121}$ ranging from 10^5 to 10^{10} pfu/ml ($3x10^6$ to $3x10^{11}$ particles/ml) were injected into hindleg or frontleg muscles of Wistar rats or rabbits. The size of scrotum and circumferences of limbs, as well as concentration of VEGF in the serum, was measured daily after injection. The injection of different $Ad.VEGF_{121}$ into the hindleg muscles of rabbits led to a dose - dependent scrotal edema in rabbits at concentrations higher than 10^7 pfu/ml (p=0.002). The edema developed slowly, reached its maximum level six days after the injection, and spontaneously resolved thereafter. At concentrations higher than 10^9 pfu/ml, the scrotal edema was accompanied by skin necrosis (p=0.001). No scrotal edema was observed in rats. Therefore, excessive increase of vascular permeability after treatment with $AdVEGF_{121}$ is species specific. Results of our animal experimentations suggest that the potential for side effects of VEGF therapy due to increased vascular permeability is not very alarming in generally healthy patients and may not cause a significant clinical problem for treatment of peripheral vascular diseases.

C) 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.

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: Dr. David E. Anderson received his Ph.D. from the University of Oregon in 1966. He developed his career interest in the environmental and behavioral origins of hypertension and on the nature of the mediating physiological mechanisms at The Johns Hopkins University School of Medicine (1968-1981) and the University of South Florida (1981-1987). During that period, he was a recipient of an NIH Research Career Development Award (1983-1987) and the Pavlovian Award for Biological Science in 1985. He came to the National Institute on Aging in 1987, and was appointed Chief of the Behavioral Hypertension Section of the Laboratory of Cardiovascular Science in 1997.

Keywords:

blood pressure
carbon dioxide
endogenous digitalis-like factors
hypertension
sodium

Recent Publications:

Anderson DE, et al. *J Hypertens* 1998; 16: 1015-1022.

Anderson DE, et al. *Biol Psychol* 1998; 49: 151-163.

Anderson DE, et al. *J Hypertens* 1999; 17: 1073-1080.

The Environmental Influence in Hypertension: Epidemiological studies confirm that environmental factors can participate significantly in the development of human hypertension. The prevalence of hypertension is low in cultures with low sodium intake or those with traditional life styles, but increases when individuals from those cultures migrate to industrialized societies with high sodium intake and/or chronic stress. A number of pharmacological interventions in hypertension have been developed. One recent study presented at the American Society of Hypertension reported, however, that the magnitude of decreases in resting blood pressure that can be obtained simply by decreasing dietary intake of sodium and fats was comparable to that of any drug. However, many individuals are not sensitive to dietary sodium, and, in any event, a number of questions remain about how stress might impact on regulation of sodium, potassium and other nutrients. The goal of this Section is to clarify interactions between stress and salt intake in the development of hypertension, which remains a major risk factor for stroke, congestive heart failure, and coronary disease.

Behavioral Stress Effects on pCO_2 and Blood Pressure Regulation: It is generally acknowledged that chronic behavioral stress can contribute to the development of human hypertension, but the mediating mechanisms remain to be clarified. Behavioral stress, per se, can activate the sympathetic nervous system, increasing cardiac output and blood pressure, and decreasing renal excretion of sodium acutely. However, anticipation of behavioral stress may impact on blood pressure regulation via another mechanism, involving sustained suppression of breathing, which increases pCO_2 and decreases renal sodium excretion, perhaps via changes in acid-

base balance and increases in renal sodium-hydrogen exchange. Experimental studies with dogs have shown that progressive hypertension can be generated over days by a combination of high sodium intake and intermittent behavioral stress that suppresses respiration and increases pCO_2.

Parallel studies with humans have shown that voluntary hypoventilation which increases pCO_2 for 30 min is accompanied by decreases in renal sodium excretion and increases in endogenous digitalis-like factors that are stimulated by expansion of plasma volume. Substantial individual differences in resting end tidal CO_2 ($PetCO_2$) have been observed in normotensive humans. Those with high resting $PetCO_2$ report a greater tendency to worry and feel vulnerable to the environment than those with lower resting $PetCO_2$. In addition, humans with high resting $PetCO_2$ show greater blood pressure sensitivity to high sodium intake than others. It is of interest, therefore, that resting $PetCO_2$ is higher in African Americans than in Caucasian Americans, since the former are slower to excrete a salt load, are more salt sensitive, and have a higher prevalence of hypertension.

Recent studies have found that high resting $PetCO_2$ is an independent predictor of high resting systolic blood pressure in female participants in the Baltimore Longitudinal Study of Aging. This association was stronger in older than in younger women. In addition, it was observed in women with low expression of anger, but not in women with high anger expression. No such relationships were observed in men. Whether the observed gender differences are related to the fact that pCO_2 of women remains stable over the life span, while that of men decreases with age, remains to be determined.

Finally, high resting $PetCO_2$ has been found to be an independent predictor of carotid artery intima-media thickness and wall to lumen ratio in women, but not in men. The association with elevated blood pressure was statistically significant, but showed that the association is partially independent of the accommodative effects of elevated pressure in this normotensive population. The extent to which the association is specific to intima, linked to atherosclerosis, or media, which is increased in hypertension, is unknown.

Ongoing Studies: Experiments are in progress to develop a rodent model of hypertension which involves potentiation of sensitivity to dietary sodium induced by behavioral or other procedures which increase pCO_2. Studies with humans are assessing endocrine correlates of high resting $PetCO_2$ in participants in the Baltimore Longitudinal Study of Aging, including endogenous digitalis-like factors, atrial natriuretic factors, the

renin-angiotensin system and nitric oxide. A study is in progress to investigate the effects of endogenous estrogen on blood pressure sensitivity to high sodium intake in postmenopausal women. This study also assesses the relationship of high end tidal CO_2 to vasodilatory capacity. Finally, a study is being designed to assess the role of ambulatory breathing habits in blood pressure sensitivity to high sodium intake and to psychological factors known to be involved in the development of some forms of human hypertension.

Collaborators: Margaret A. Chesney, Ph.D., University of California at San Francisco; Edward G. Lakatta, M.D., Olga V. Fedorova, Ph.D., Alexei Y. Bagrov, M.D., Ph.D., Angelo Scuteri, M.D., Ph.D., Laboratory of Cardiovascular Science, NIA.

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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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Keywords:

cellular stress
growth regulation
apoptosis
signal transduction
gene regulation
cDNA microarray analysis

Recent Publications:

Wang X, et al. *J Biol Chem* 1999; 274: 29599-29602.

Potapova O, et al. *Mol Cell Biol* 2000; 20: 1713-1722.

Wang X, et al. *J Biol Chem* 2000; 275: 14624-14631.

Huang Y, et al. *J Biol Chem* 2000; 275: 18234-18242.

Cell Stress and Aging Section Program: This research program focuses on cellular responses to stress and how they become altered with aging. The rationale for such studies is as follows: Aging is characterized by a general decline in most physiologic functions, and in particular, by a decreased capacity to maintain homeostasis during episodes of stress. These changes are believed to reflect the accumulation of damage to cells and tissues resulting from a variety of toxic factors, either produced endogenously during normal growth and metabolism, or derived from the environment. Normal function and survival are dependent on the cell's ability to resist or adapt to such stress and to repair or replace damaged molecules. Genetic systems have evolved to detect specific forms of damage and to activate the expression of genes whose products increase the resistance of the cell to damage or aid in its repair. The continued effectiveness of these genetic responses to environmental insults is likely to be a major factor in the resistance to disease and aging, and may be an important determinant of longevity.

Signal Transduction Pathways Mediating the Response to Genotoxic/ Oxidative Stress and Consequences for Cell Survival: A number of distinct pathways can be activated in response to stress, and together these serve to coordinate the cellular response to a given stimulus and ultimately determine the cell's fate. These include, but are not limited to, the tumor suppressor protein p53, the heat shock response, mitogen-activated protein kinase (MAPK) cascades, PI-3 Kinase/Akt pathway, NF*k*B, and the unfolded protein response. Recent work has focused on the activation of the various pathways in response to cell stresses such as genotoxins, oxidant injury and perturbations to the endoplasmic reticulum. Our efforts are concentrated on 1) identifying the initiating events and critical

mediators involved in the response, 2) examining the crosstalk between different signaling pathways, 3) determining the consequences of activation of particular pathways for cell survival and 4) identifying downstream targets of the signaling cascades that influence cell survival.

Stress-Induced Gene Products: Numerous stress-regulated genes have been identified in mammalian cells, which are presumed to play an important role in determining cell fate. Depending on the particular stress or cell type examined, the response can range from proliferation or transformation, to growth arrest or programmed cell death. Our research in this area examines specific genes that are believed to mediate these differential effects, the goal being to understand their regulation and determine their function during the stress response. Particular genes of interest include the the transcription factor *c-jun* and cyclin-dependent kinase inhibitor *p21/Waf1/Cip1*, both of which are up-regulated during stress, and *cyclin D1*, which is repressed in response to adverse stimuli. Another long-standing interest of ours is the growth arrest and DNA damage-inducible gene *GADD153*, a C/EBP-related transcription factor implicated in the induction of both growth arrest and cell death following stress, particularly that involving perturbations of the endoplasmic reticulum. More recently we have initiated investigations using serial analysis of gene expression (SAGE), and cDNA microarray technology to examine global changes in patterns of gene expression during the cellular response to stress, and identify novel players in the process.

Age-Related Alterations in the Stress Response: Aged cells and tissues exhibit a reduced ability to respond to environmental stresses. Studies in this project area are focused on identifying the causes for this altered responsiveness. Using primary hepatocytes derived from young adult and aged rats as a model system, we have shown that aged hepatocytes display reduced activation of ERK MAPK in response to both proliferative signals and stress stimuli including hydrogen peroxide, sodium arsenite, and heat shock. This results in reduced expression of ERK-regulated genes and is associated with decreased survival following oxidant injury. Activation of ERK and induction of heat shock proteins in response to heat are also attenuated in aged cells. Current studies are addressing other mitogen- and/or stress-activated pathways that may contribute to the aged cells' altered responsiveness to external stimuli. In addition, cDNA microarray analysis is being employed to investigate age-related changes in the global patterns of gene expression in hepatocytes, both basally and following stress. The overall goal is to better understand the basis for the age-related decline in stress responsiveness so that we might be able to devise strategies to up-regulate these homeostatic responses in aged cells.

Collaborators: Dr. Dan Mercola, Sidney Kimmel Cancer Center; Dr. Thomas Franke, Columbia University; Drs. Nicholas Dean and William Gaarde, ISIS Pharmaceuticals; Dr. Sergey V. Anisimov, Laboratory of Cardiovascular Sciences, NIA; Dr. Graham Carpenter, Vanderbilt University School of Medicine; Dr. Vincent Cristafalo, Lankenau Medical Research Center; Dr. Tak Yee Aw, Louisiana State University Medical Center; Dr. Kevin Becker, Research Resources Branch, NIA; and Drs. Patrice Morin, Myriam Gorospe, Yusen Liu and Ronald Wange, Laboratory of Cellular and Molecular Biology, NIA.

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Keywords:

mRNA turnover
von Hippel-Lindau
stress response
cell cycle

Recent Publications:

Gorospe M, et al. *Mol Cell Biol* 1998; 18(3): 1400-1407.

Gorospe M, et al. *Mol Cell Biol* 1999; 19: 1289-1300.

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

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

Cellular Response to Stress and Gene Expression: Aging is characterized by a general decline in the ability of individuals to adequately respond to different stresses, either environmental or endogenously generated. Stressful signals are transduced through various signalling pathways, ultimately resulting in alterations in gene expression. Many such 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 splicing, transport, subcellular localization, stability and translation, as well as post-translational events such as protein processing, transport, phosphorylation and degradation. Our long-term interest is to explore post-transcriptional processes that govern gene expression during the stress response.

Post-transcriptional Control of Cell Cycle Regulatory Genes: We and others have shown that expression of the inhibitor of cyclin-dependent kinases p21 (also known as Cip1, Waf1 and Sdi1) is highly induced by various stresses and this enhances cell survival. In response to short-wavelength ultraviolet light (UVC), we previously showed that this induction was due to the stabilization of the p21 mRNA, and have recently demonstrated that this stabilization events requires the association of the RNA-binding protein HuR with the proximal region of the p21 mRNA. Our latest efforts have led us to the identification of cyclin A mRNA and cyclin B1 mRNA as additional targets of HuR binding activity. Our studies have revealed that HuR's subcellular localization varies throughout the cell division cycle, with its highest cytoplasmic presence seen during the S phase, and that HuR contributes to time-dependent alterations in stability of mRNAs encoding cyclins A and B1 (which display greatest stability during S phase). Our long-term aim is to continue to identify RNA regions and proteins involved in regulating the stability of genes involved in growth control and cell cycle regulation. This analysis involves both *in vitro* and *in vivo* determinations of RNA binding and RNA degradation, the identification of the RNA-binding proteins involved, and the signalling pathways that modulate their activities.

Functional Analysis of the von Hippel-Lindau (VHL) Tumor Suppressor Gene: Absence of functional von Hippel-Lindau (VHL) tumor suppressor protein leads to the development of neoplasias characteristic of VHL disease, including renal cell carcinomas (RCCs). The VHL protein is believed to function in modulating gene expression at the levels of transcription elongation, mRNA stability and protein degradation. Our current efforts are centered around the identification of mRNAs whose expression is altered by VHL by using SAGE (*s*erial *a*nalysis of *g*ene *e*xpression). We are also examining the functional significance of pVHL's association with several partners identified by a yeast two-hybrid screen. This part of the program aims at identifying the mRNAs and proteins whose expression is altered by VHL and to understand how VHL prevents tumorigenesis.

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Keywords:

neurodegeneration
Alzheimer's disease
amyloid
glutamate

Recent Publications:

Bai G, et al. *J Biol Chem* 1998; 273: 1086-1091.

Krainc D, et al. *J Biol Chem* 1998; 273: 26218-26224.

Naruse S, et al. *Neuron* 1998; 21: 1213-1221.

Luo J-J, et al. *J Neurosci Res* 1999; 55: 629-642.

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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Biography: Dr. Yusen Liu received his Ph.D. degree in 1991 from Hiroshima University in Japan, and then served as Assistant Professor for a year in the Faculty of Engineering in the same University. He did his postdoctoral training at the National Institute on Aging before becoming an Investigator in the Laboratory of Biological Chemistry in 1996 (renamed Laboratory of Cellular and Molecular Biology, 2000). His research has focused on signal transduction pathways involved in the stress response and their implications to the aging process.

Keywords:

signal transduction
MAP kinase
stress response
aging

Recent Publications:

Liu Y, et al. *Exp Cell Res* 1998; 240: 40-48.

Chen W, et al. *Mol Cell Biol* 1998; 18: 5178-5188.

Hutter D, et al. *J Gerontol: Biol Sci* 2000; 55A; B125-B134.

Signal Transduction Pathways Involved in the Stress Response: Over the past several years, increasing evidence has emerged from studies in lower eukaryotic organisms that extended longevity is frequently associated with an enhanced resistance to stress. Therefore, investigation of the signaling pathways through which cells detect stressful conditions and activate their defense machinery is of critical importance for understanding the basic mechanisms involved in the aging process. Information gained from the study of the stress response could be exploited for the development of strategies to improve the quality of life for the increasing aged population.

Exposure of eukaryotic cells to harmful environmental conditions evokes alterations in gene expression. Altered gene expression can, at least in part, account for the variable phenotypic changes cells undergo after stress. Almost immediately after exposing cells to genotoxic agents, an increase in the activities of numerous proteins can be detected. Activation of these proteins initiates protein phosphorylation cascades leading to the activation of a group of mitogen-activated protein (MAP) kinases including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase/Stress-activated protein kinase (JNK/SAPK) and p38 MAP kinase.

These MAP kinases are responsible for the phosphorylation of a variety of transcription factors leading to changes in gene expression. While activation of MAP kinases is achieved through phosphorylation by MAP kinase kinases, attenuation of the MAP kinase activities is accomplished through dephosphorylation by a group of MAP kinase phosphatases. 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 MAP kinase signaling pathways.

We have previously demonstrated that stressful treatments can differentially activate ERK, JNK and p38 MAP kinases. Recent studies have focused on the potential role of growth factor receptors in mediating ERK activation in response to extracellular stresses. Using arsenite as a model stress agent, we have shown that arsenite treatment results in the rapid activation of epidermal growth factor receptor (EGFR), tyrosine phosphorylation of the Shc adaptor protein, and the formation of EGFR-Shc-Grb2 complexes in rat pheochromocytoma PC-12 cells. These events, as well as activation of ERK, were all drastically reduced by treatment of cells with either a selective inhibitor of EGFR, or down-regulation of EGFR expression. These results demonstrate that the EGFR and Shc are critical mediators in the activation of the Ras/ERK signaling cascade by stress and suggest that arsenite acts as a tumor promoter largely by usurping this growth factor signaling pathway.

MAP kinase phosphatase-1 (MKP-1) is the archetypical member of the MAP kinase phosphatase family, whose expression can be rapidly induced by a variety of growth factors and cellular stress. Since MKP-1 protein localizes in the nucleus, it has been suggested to play an important role in the feedback control of the MAP kinase-regulated gene transcription. Recently, it has been demonstrated that the interaction of several cytosolic MAP kinase phosphatases with MAP kinases can trigger the catalytic activation of the phosphatases. It is unclear whether such a regulatory mechanism can apply to nuclear MAP kinase phosphatases and serve as an additional apparatus for the feedback control of MAP kinase-mediated gene expression. Here we have shown 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 MAP kinase

phosphatase through direct binding to a MAP kinase, suggesting that such a regulatory mechanism may play an important part in the feedback control of MAP kinase signaling in the nuclear compartment.

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 on 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:

ovarian cancer
ß-catenin
SAGE
gene expression

Recent Publications:

Morin PJ, et al. *Science* 1997; 275: 1787-1790.

He TC, et al. *Science* 1998; 281: 1509-1512.

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

Morin PJ, et al. *Bioessays* 1999; 21: 1021-1030.

Furlong MT, et al. *Gynecol Oncol* 2000; 77: 97-104.

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.

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Keywords:

T lymphocyte activation
signal transduction
protein kinases
lipid phosphatases

Recent Publications:

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

Ortaldo JR, et al. *J Immunol* 1999; 163: 5269-5277.

Shan X, et al. *J Biol Chem* 1999; 274: 29323-29330.

Baroja ML, et al. *J Immunol* 1999; 162: 2016-2023.

Griffith CE, et al. *J Biol Chem* 1998; 273: 10771-10776.

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.

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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 **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 recently formed **Hematology/Oncology Section (H/OS)** has as an overall goal 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 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 are currently 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. With this work we hope to both improve the application of currently available anti-cancer therapy in the aging population as well as 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 and the Body Mass Index for obesity) and the subsequent development of complications that are strongly related to the diseases. The BLSA and the Follow-up Study of the National Health and Nutrition Examination Survey-I have provided unparalleled data sources 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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Eric Westin	Staff Clinician

Metabolism Section

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Irene Vasilios	Secretary
Denis Muller	Comp Program Analyst
Howard Baldwin	Chemist
Mary Bannon	Biologist
John Sorkin	IPA Agreement
Dariush Elahi	Special Volunteer
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Olga Carlson	Laboratory Manager
Cheng Zhang Shi	Research Associate
Zhenguo Zhang	Research Fellow
Evgeny Kobrinsky	Special Expert
Shari Ling	Staff Clinician
Michael Theodorakis	Clinical Fellow

Nuclear Magnetic Resonance Unit

Richard Spencer	Senior Investigator
Kenneth Fishbein	Chemist
Craig Galban	IRTA Fellow
Erik Petersen	Special Volunteer
Patrick McConville	Visiting Fellow
Scott Ellis	Pre-IRTA Fellow
Leila Laouar	Special Volunteer
Alexei Khmelnitski	Visiting Fellow
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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 became Clinical Director and Chief of the Laboratory of Clinical Investigation 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:

Jones DS, et al, *Clin Pharmacol Ther* 1999; 65: 408-412.

Andrawis N, et al, *J Am Geriatr Soc* 2000; 48: 193-198.

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

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

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 diminished ability to relax their blood vessels in response to acetylcholine, which causes relaxation via the activation of this enzyme. 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; Martin Morad, Ph.D., Georgetown University; Janice Schwartz, M.D., Northwestern University; Jane Freedman, M.D., Georgetown University; David Flockhart, M.D., Ph.D., Georgetown University; Irving Wainer, Ph.D., Georgetown University; Stephen Donahue, M.D., Bristol-Myers Squibb Research Institute.

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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
programmed cell death

Recent Publications:

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

Pandey SK, et al. *Biochemistry* 1999; 38: 14667-14675.

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

Insulin Receptor Structure and Function: The wide array of metabolic and mitogenic responses of insulin are initiated by the binding of insulin to its cell surface receptor. The insulin receptor (IR) is a heterotetrameric glycoprotein with intrinsic tyrosine kinase activity in its ß-subunit cytoplasmic domain whose function can be modulated by thiol-modifying agents. We and others have demonstrated the presence of reactive cysteine residues within the cytoplasmic domain of the human IR ß-subunit whose role in insulin signaling has not been elucidated. It is well recognized that transmission of the insulin signal requires the association between the IR and several proximal proteins and adaptor molecules. However, it is unclear whether reactive IR cytoplasmic thiols contribute to this association. We have recently showed that the human IR contains a unique nucleophilic cysteine residue whose reactivity was required for covalent cross-linking between the receptor ß-subunit and a thiol-reactive membrane-associated protein, termed TRAP. We are currently designing strategies to purify TRAP which we believe will have significant role in the transmission of insulin signal. In addition, we are studying the cellular compartmentalization and regulation in the formation of the ß-subunit•TRAP complex in intact cells in response to pharmacological manipulations of intracellular reduction/oxidation (redox) potential, microfilament/microtubule network, and many of the proximal kinases and adaptor molecules implicated in insulin signaling.

Insulin Receptor Signaling and Apoptosis: 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. Diabetes is characterized by increasing oxidative stress within the cell. In recent years, evidence accumulated to indicate that insulin has antiapoptotic properties, in part, by activating transcription factors that are known to be involved in the control of the apoptotic process. However, it was unclear which signaling pathway(s) participated in this action of insulin. We have recently shown that isoprenylation of short-lived proteins may play an important role in mediating insulin's antiapoptotic effects. We are extending these studies to identify those farnesylated proteins that we believe will have a major role in control of cell proliferation and apoptosis and possibly other processes. In addition, we very recently demonstrated that, like insulin, antioxidants induced protection against apoptosis in our experimental cell model. Because of the pivotal role of the transcription factor NFκB in inducing genes involved in cell survival, we are studying many of the proximal kinases and adaptor molecules implicated in the phosphorylation and subsequent degradation of its inhibitory protein, IκBα, in response to insulin and antioxidants.

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: Dr. Ashok K. Srivastava, University of Montreal, Canada; Dr. Lance Macaulay, CSIRO Division of Biomolecular Engineering, Australia; Dr. Cecil Yip, University of Toronto, Canada; Dr. Motoyoshi Sakaue, Kobe University School of Medicine, Japan.

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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
islets of Langerhans

Recent Publications:

Greig NH, et al. *Diabetologia* 1999; 42: 45-50.

Zhou J, et al. *Diabetes* 1999; 48: 2358-2366.

Stoffers DA, et al. *Diabetes* 2000; 49: 741-748.

Szayna M, et al. *Endocrinology* 2000; 141: 1936-1941.

Aging and Type II 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. The approach is to take the agents that have been first tested in beta cell lines into animal models of aging and diabetes, and with the information gained from the animal models, go as quickly as possible directly into the human situation.

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 II 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. 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 was 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 NIA in May 2000. His research interest from fellowship training was in the role of proto-oncogenes in control of hematopoietic cell differentiation with subsequent focus on 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 and how these processes may be modulated by chemotherapeutic and other signaling agonists or antagonists. From this we hope to develop studies within the clinical research unit to directly test the therapeutic potential of interactions found.

Keywords:

c-*myb*
hematopoiesis
breast cancer
proliferation
differentiation

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 potentially 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 and risk of intervention becomes increasingly narrow with age.

***MYB* and 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 has focused on the human c-*myb* gene and its function in hematopoiesis and more recently 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 with significant potential effects on c-*myb* protein function. Interesting recent observations from this laboratory related to hematopoiesis include the definition of sequences that limit transactivation potential of a dominant/negative splice form of *myb* published previously to those amino acids contributed by the alternative exon and the finding that the dominant/negative potential of this form of *myb* may be promoter dependent with suppression of c-*myc* promoter activity while retaining transactivation potential for constructs containing multiple *myb* binding sites in conjunction with a minimal promoter. 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*. This laboratory has

subsequently begun to examine mechanisms regulating *myb* expression as well as consequences of expression in both ER+ and - tumor cell lines. 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. Thus, c-*myb* would be expected to be expressed uniformly unless regulation was occurring by a post-transcriptional mechanism without precedence in hematopoietic models of *myb* regulation. This is also true of decreases and increases in steady state c-*myb* expression in response to estrogen withdrawal and restimulation in MCF-7 cells. If the regulatory mechanism involving attenuator control of c-*myb* expression in hematopoietic model systems examined to date represents the “normal” mechanism, absence of attenuator function in breast tumor cell lines that do or do not express *myb* at detectable levels could indicate that an early defect in breast epithelial cell transformation may be development of a defect in attenuator function by either *cis* or *trans* mechanisms. The avenues of investigation of mechanisms regulating *my*b expression suggested by these findings will be a focus of future studies once a role for this expression in breast epithelial proliferation has been established. This will also complement and potentially reinforce ongoing studies in hematopoietic cell systems.

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.

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. 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, as a HHMI Research Associate. He cloned the first human L-type calcium channel from fibroblasts and investigated its genomic structure. Since 1993 he has worked as an Assistant 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 an Adjunct Associate Professor at Georgetown University.

Keywords:

calcium signaling
human L-type calcium channel
calcium sensor

Recent Publications:

Soldatov NM, et al. *J Biol Chem* 1998; 273: 957-963.

Soldatov NM, et al. *Hum Hered* 1998; 48: 241-244.

Soldatov NM, et al. *J Membr Biol* 2000; 177: 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. 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. 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. Further investigation of this region by NMR spectrometry and electron diffraction will allow us to compare involved structural determinants which may lead to development of new drugs.

Molecular Determinant of the Voltage-dependence of the Channel Availability: Voltage-dependent channel availability is controlled by the inner mouth of the pore. Previously it has been shown (Soldatov, *Proc. Natl. Acad. Sci. USA 89: 4628, 1992*) that the A752T replacement at the cytoplasmic end of transmembrane segment IIS6 of the α_{1C} channel rarely occurs due to a single nucleotide $g^{2254}\rightarrow a$ conversion. Comparative quantification of steady state availability of the current carried by the mutated $\alpha_{1C,94}$ and conventional $\alpha_{1C,77}$ channel showed that A752T mutation prevented a large (≈25%) fraction of the current carried by Ca^{2+} or Ba^{2+} from fully inactivating. The data suggest that Ala752 at the cytoplasmic end of IIS6 is a new molecular determinant of the Ca^{2+} channel inactivation, critical primarily for the voltage-dependence of its availability. This site is subject to naturally occurring mutation that may lead to Ca^{2+}-overload related human defects. Therefore we plan to study it in a transgenic model with selective induction in the heart and in the brain. It may enhance our knowledge about calcium-related cardiac and neuronal disorders and their possible link to the naturally occurring mutation of the channel in humans.

Conditional Age-dependent Switch of Ca^{2+} Channel Isoforms: The human L-type Ca^{2+} channel pore-forming α_{1C} subunit gene is composed of 56 identified exons (Soldatov, *Genomics*, **22**, 77, 1994) ten of which are subject to alternative splicing. Expression of α_{1C} splice variants is controlled by a variety of poorly investigated biochemical stimuli which may underlie physiological and metabolical abnormalities associated with diseases. Exon 21 of the α_{1C} gene contributes to weaker inhibition of Ca^{2+} channel by isradipine at negative potentials (Soldatov et al., *J. Biol. Chem. 270: 10540, 1995*). Our data suggest that expression of the exon 21-isoform of α_{1C} occurs in response to suppression of proliferative signals in an age-dependent manner and is in part sensitive to hormonal stimuli known to control intracellular signaling in vascular smooth muscle cells. A possible link between the exon 21-isoform of α_{1C} expression and age-related alteration of proliferation as well as Werner's syndrome is the subject of our forthcoming research.

Functional Architecture and Regulation of Human L-type Ca^{2+} Channel : 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 University of Cologne, Germany (Dr. Hescheler) to investigate the role of amino acids and motifs of the pore-forming α_{1C} subunit in regulation, membrane targeting, assembly and clasterization of the human L-type Ca^{2+} channel using recombinant channel isoforms and mutants stably or transiently

expressed in *Xenopus* oocytes and human embryonic kidney cells. Various Living Color GFP variants fused to the termini of the expressed proteins allowed us to visualize targeting, clusters formation and some molecular interactions in the channel. Our data obtained by state-of-the-art single channel recordings, two-photon FRET (Fluorescence Resonance Energy Transfer) and single molecule two-photon fluorescence spectroscopy 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. We plan to extend this study to investigate the assembly of the channel from differentially labeled subunits as well as molecular motion underlying the channel activity. 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; Olga Carlson, Ph.D., NIA; Zhenguo Zhang, M.D., NIA; Chengzhang Shi, 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; Martin Morad, Ph.D., Georgetown University, DC; Gerhard Meissner, Ph.D., University of North Carolina, NC.

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Biography: Dr. Fleg received his medical degree from the University of Cincinnati in 1970 and was an internal medicine intern at Baltimore City Hospitals from 1970-1971. After serving 2 years as an Air Force Flight Surgeon, he completed his medical residency at Barnes Hospital in St. Louis, Missouri from 1973 to 1975, followed by a fellowship in Cardiovascular Disease at the Washington University School of Medicine from 1975 to 1977. He joined the Laboratory of Cardiovascular Science in 1977 and has been Head of the Human Cardiovascular Studies Unit since 1992. He became the Interim Director of the Baltimore Longitudinal Study of Aging and Acting Chief, Longitudinal Studies Section in July 1998.

Keywords:

aging
cardiovascular
exercise

Recent Publications:

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

Tracy BL, et al. *J Appl Physiol* 1999; 86: 195-201.

Conwit RA, et al. *Muscle Nerve* 1998; 21: 1338-1340.

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

The **Longitudinal Studies Section** (LSS) is responsible for the operation of the Baltimore Longitudinal Study of Aging (BLSA). Research has focused primarily on the BLSA in the following areas:

Physical Activity and Aging: Increasing age is accompanied by a reduction in leisure time physical activity (LTPA), especially high intensity activity. Such a decrease in PA is a risk factor for development of cardiovascular disease, the most common cause of death and morbidity in older adults. In addition, reduced LTPA is typically accompanied by accelerated loss of aerobic exercise capacity, which adversely influences the ability to perform everyday activities and hence the quality of life. With the collaboration of Dr. Laura Talbot of Johns Hopkins University, we have examined the relationship between self-reported LTPA and aerobic capacity in healthy BLSA subjects ages 18-95 years (*Med Sci Sports Exerc 2000; 32: 417-425*). In addition, we are determining the relative importance of LTPA versus aerobic capacity in predicting coronary events and total mortality in such individuals. In light of the recent widely publicized recommendations from the Surgeon General and several medical organizations to increase activity levels, we are characterizing both secular trends in LTPA from the 1960's through 1990's and longitudinal change in LTPA during followup. With the increasing recruitment of minority participants into the BLSA, a current goal is to examine racial differences in LTPA and their impact on aerobic capacity.

Prostate Growth and Disease: Our work is defining anatomic and physiologic characteristics that distinguish normal prostate growth with age and the development of prostate disease; characterizing the development and normal progression of benign and cancerous prostate disease; identifying hormonal changes important in the diseases; characterizing markers (serum and genetic) that identify high risk groups; and improve diagnostic strategies for prostate cancer detection and prevention.

We plan to continue to use knowledge of the natural history of prostate growth to improve diagnostic acumen, and raise awareness that some men with low PSA levels may not need intensive screening, while other men are at high risk. We are currently examining PSA blood levels as a general risk factor for prostate cancer rather than as a specific diagnostic test. We plan to explore dietary issues that may affect prostate cancer or BPH risk, as well as their impact on PSA levels. Two studies have been developed to examine genetic factors contributing to prostatic disease. The first study examines specific genes associated with prostatic cancer. The initial focus is on four genes: (1) the μ-class glutathione S-transferase, (GST) gene GSTM1, (2) the π-class GST gene GSTP1, (3) the human androgen receptor gene hAR, and (4) the inherited prostate cancer susceptibility gene PRCA. The second study will identify genes associated with prostatic growth.

Neuromuscular Changes with Aging: Our goal is to understand the time course of strength loss, factors that contribute to the loss, and the degree that exercise response differs between old and young individuals. Our research has three main components.

1. Descriptive cross-sectional and longitudinal characterization of neuromuscular and functional changes with age: We are examining the relationship between a variety of clinical, physiological and genetic factors and their contributions to age-associated changes in muscle strength and muscle mass. Of particular interest are changes that begin during mid-life. A better understanding of the contributors can lead to better preventive measures that may allay the marked changes that occur in late life. We are currently looking at the effect of changing serum testosterone, DHEA, and DHT levels on muscle strength in men. In addition, we are exploring whether muscle related genes are associated with age-associated strength changes.

To study the relationship between strength and functional performance, we have established a collaboration with Dr. William Paloski of NASA to examine balance in the BLSA using the Equitest equipment used to study the effects of space flight on balance in astronauts. As a result of the Glenn flight, NASA wants to know the impact of age on balance performance. The BLSA data will be compared to the astronaut data to (a) examine the implications of increasing age on potential long term space flight, and (b) document the sequence and magnitude of changes in various elements of the balance system during normal aging. In addition, we will examine the relationship between muscle strength, cardiovascular fitness, leisure time physical activity on balance performance, and the association between balance performance, gait and falls.

2. Comparison of exercise response to resistive strength training in young and old subjects: We have recently completed an exercise intervention study with the Department of Kinesiology of the University of Maryland, College Park, comparing the response to resistive training in old and young men and women. Using knowledge gained from the intervention, we are now working with the School of Nursing at Johns Hopkins University to examine the effects of electromyostimulation versus an educational intervention to increase leisure time physical activity in elderly subjects with moderate to severe osteoarthritis of the knees. The alternative strategies being tested were selected with the hope of overcoming the need for a more comprehensive strength training program, which is usually not well accepted and maintained in older adults.

3. Examination of the motor unit and its relationship to muscle strength and exercise response: The goal of this project is to understand the changes that occur in motor units with aging, the effects of these changes on muscle strength and how these changes affect the exercise response. We have developed a protocol to examine motor unit size as well as firing characteristics at different levels of muscle exertion in the vastus medialis. We are currently examining the effects of aging and motor neuron disease on motor unit physiology. A protocol has been developed with NASA researchers that will examine the motor unit physiology before and after 17 weeks of absolute bed rest. These problems are of importance to health and independence of the elderly and to the effects of extended bed rest. A protocol has been developed with Dr. Christine Asper and Dr. Laura Talbot, School of Nursing, Johns Hopkins University, to examine the recovery of muscle strength in rats following hind limb suspension.

Cerebrovascular Changes with Aging: This project is studying carotid and intracerebral arteries using Doppler ultrasonographic techniques in BLSA participants. The goal is to determine whether differences in arterial structure and function explain racial and gender differences in cerebrovascular disease (CVD), and whether changes in arterial characteristics are associated with fitness and frailty. These findings and previous LCS findings that stiffness properties of the central arteries are inversely related to cardiovascular fitness (VO_2max) raise new questions about the potential value of arterial properties in characterizing the risk of CVD in apparently healthy subjects. We plan to continue this work by examining the following questions. (1) What is the relationship between intimal medial thickness and arterial stiffness? (2) Are there relationships between carotid intimal medial thickness (IMT), arterial stiffness and cerebrovascular flow characteristics? (3) How do longitudinal changes in IMT affect arterial stiffness? (4) Does IMT, arterial stiffness, or their interactions predict hard endpoint including myocardial infarction, stroke or CVD death?

A large body of evidence supports the importance of diet in the prevention of cardiovascular disease. The primary focus has been on alteration of fat intake, but evidence exists for the importance of antioxidants, folate, B12, and other nutrients. Clinical trials have demonstrated decreased risk through diet, drugs, and exercise. However, the relationship between diet and arterial structure and function is less clear. This leads to: (5) Do dietary factors, e.g. lipid intake, antioxidant intake, B12, or folate affect IMT or arterial stiffness?

Collaborators: Edward Shapiro, M.D., Gary Gerstenblith, M.D., Lewis Becker, M.D., Steven Schulman, M.D., Laura Talbot, Ph.D., Johns Hopkins University; Leslie Katzel, M.D., Andrew Goldberg, M.D., University of Maryland at Baltimore; James Hagberg, Ph.D., Ben Hurley, Ph.D., University of Maryland, College Park; Yoji Nagai, M.D., E. Jeffrey Metter, M.D., NIA.

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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:

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

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.

Lynch NA, et al. *J Appl Physiol* 1999; 86: 188-194.

Health Evaluation in the Baltimore Longitudinal Study of Aging (BLSA): A clinical evaluation unit, under my supervision, is responsible for the health evaluations in the BLSA. The characterization of the health status of all subjects is important to many of the researchers and projects within the study. Starting in 1985, the BLSA health evaluation has undergone major changes to improve medical information collection. The most substantial change occurred between 1988 and 1990, when we began to use nurse practitioners and physician assistants (NP/PA) to perform the history and physical examinations, rather than medical staff fellows. Subsequently, revisions have occurred in health questionnaires, medication and diagnosis listing. We continually try to improve the quality of the clinical evaluation. We continue to assess quality assurance across the questionnaires, maintain staff training, and monitor and improve staff cooperation so that reliability and consistency of the clinical evaluation remains at a high level over time. This effort seems successful as over the past several years, staff has turned over, and new staff have easily adjusted and adapted to the unique needs of the BLSA. As new research questions are developed by scientific staff, we add new dimensions to the evaluation. We try to do this so that existing questionnaires are not changed, to maximize the longitudinal capabilities of the health data.

The unit is also responsible for the day to day health requirements of the participants during their visit. The unit tries to maintain and improve as necessary the high level of nursing and technical support, and to maximize the good will between the staff and the BLSA participants. The technical support includes health screening for a number of research protocols and

assisting researchers in project development as it applies to unit interaction with the research. To meet these ends, the NP/PA and nursing staff have established quality assurance in the evaluation program. They have regularly scheduled meetings to discuss evaluation problems and related issues. A protocol manual was prepared describing most of the procedures and questionnaires. Ongoing efforts are designed to maximize the participant well-being, and to optimize forms, records, and protocols.

Prostate Aging and Disease: The 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 1000 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 still 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 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 is being 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. The study has been completed, and analyses are currently underway. 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 Investigators 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:

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.

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

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, recently recommended by the American Diabetes Association, 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.

Interactions of Aging, Obesity, and Mortality: There is continuing controversy over recommended weight-for-height in men and women and whether or not these standards need to be age-specific. The NHANES I Follow-up Study provides an unparalleled data set to examine the association between Body Mass Index at age 55-74 years at entry being and subsequent mortality over the next 20 years in white and black men and women. Additionally, in collaboration with the Longitudinal Studies Section, some 40 years of anthropometric measurements have been used to generate equations for the computation of percent body fat using DEXA scanning as the gold standards.

Collaborators: Dr. Dariush Elahi, Massachusetts General Hospital; Dr. Elizabeth Barrett-Connor, University of California, San Diego; Dr. Katherine Flegal, National Center for Health Statistics; Drs. John Sorkin and Andrew Goldberg, University of Maryland; Dr. Jordan Tobin, Case-Western Reserve University, Cleveland; 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:

Horská A, et al. *Am J Physiol* 1999; 276: E766-E773.

Spencer RGS, et al. *J Magn Reson* 2000; 142: 120-135.

Velan SS, et al. *Mag Reson Med* 2000, 43: 804-809.

Potter K, et al. *Arthritis Rheum* 2000, 43: 1580-1590.

Nuclear Magnetic Resonance Unit: The interests of the Nuclear Magnetic Resonance (NMR) Unit are primarily in imaging (NMRI) 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 investigating the effects of biologic response modifiers on neocartilage development. Using MRI, we have found that matrix proliferation from human articular chondrocytes is accelerated by addition of the combination of insulin like growth factor-1 (IGF-1) and transforming growth factor-ß (TGF-ß), or addition of the combination of IGF-1 and connective tissue growth factor, to the growth medium. Studies of the interactions of these growth factors and cytokines are ongoing.

Angiogenesis in Rats as a Function of Age, and in Response to Gene Therapy: Atherosclerosis is a critical factor in the development of both peripheral vascular disease and cardiac ischemia. One approach to treatment of ischemic vascular disease is the application of angiogenic factors delivered through genetically altered viral vectors. Therefore, we have utilized NMR spectroscopy (NMRS) methods to measure high energy phosphate metabolites in muscle distal to femoral artery resection in rats.

In our first series of experiments, we investigated angiogenesis as a function of animal age and days after femoral artery resection without addition of growth factor. NMR spectra of the gastrocnemius muscle of the anesthetized rat were collected at rest, during a period of intense muscle stimulation, and during recovery from stimulation. We have found that over a period of weeks following femoral artery resection, 2 month old rats recover muscle metabolic reserve significantly more rapidly than 20 month old rats. This likely reflects loss of angiogenic potential with age.

Modulators of angiogenesis have vast potential for treatment of arterial vascular disease. Accordingly, we have performed a set of experiments involving application of vascular endothelial growth factor (VEGF) prior to femoral artery resection. Distal muscle bioenergetics was then assessed over a period of weeks. All NMRS measurements incorporated physiologic stress in order to probe vascular reserve. We found that VEGF acted to help normalize the pattern of high energy phosphate response to muscle stimulation and recovery, indicating an increase in the rate of development of perfusing vessels. These results were consistent with concomitant studies of blood flow using contrast angiography and blood pressure measurements.

Extensions of this work which are underway include variations in the timing and other important elements of VEGF therapy delivery. We also plan to implement NMR imaging methods to more directly look at increased blood flow to the ischemic limb.

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.

Laboratory of Genetics

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The **Laboratory of Genetics (LG)** was established in Autumn, 1997 by David Schlessinger, with a Human Genetics Unit, a Transcription Remodeling and Regulation Unit initiated 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. Five 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. 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.

4. 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.

5. 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 Cardiovascular Sciences (Edward Lakatta and Angelo Scuteri), Personality and Cognition (Paul Costa and Alan Zonderman), and EDB (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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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:

Huber R, et al.*Proc Natl Acad Sci USA* 1999; 96: 616-621.

Mazzarella R, et al. *Genome Res* 1998; 8: 1007-1021.

Pengue G, et al. *J Biol Chem* 1999; 274(37): 26477-26484.

Ciccodicola A, et al. *Hum Mol Genet* 2000; 9(3): 395-401.

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

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

Human Genetics Unit: The program is designed to complement studies by many groups in lower animal models and in fibroblast senescence with corresponding studies of embryonic 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). 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 correlated developmental work, systematic studies are beginning of gene cohorts specifically expressed during the development of the ovary follicles, and comparative studies of gene expression in the testis.

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 IGF2 and related features of growth hormone action. Tests and extensions of this hypothesis are based on developmental studies, including the generation of a mouse model and the study of other 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 150 inherited ectodermal dysplasias.

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. 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 ATP-dependent chromatin-remodeling complexes, histone deacetylase complexes, and a helicase complex involved in the Werner premature aging syndrome.

Keywords:

chromatin-remodeling
deacetylase
SWI/SNF
helicase

Recent Publications:

Wang W, et al. *Proc Natl Acad Sci USA* 1998; 95: 492-498.

Zhao K, et al. *Cell* 1998; 95: 625-636.

Xue Y, et al. *Mol Cell* 1998; 2: 851-861.

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

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

The establishment and maintenance of transcriptionally active and inactive chromatin structure in higher eucaryotes is key for global gene regulation during development, differentiation and adaptation to environmental stimuli. Evidence accumulated during the last two decades indicates that chromatin structures are remodeled when multipotent precursor cells develop into terminally-differentiated cells. However, the underlying mechanism of chromatin remodeling is poorly understood, primarily because molecules that remodel chromatin structures have been discovered only recently. These complexes can be classified into two different families: one, the histone acetyltransferase or deacetylase complexes which alter the chromatin structure by covalently modifying the tails of histones; the other, the ATP-Dependent Chromatin-Remodeling (ADCR) complexes which use the energy of ATP to disrupt non-covalent DNA-histone contacts. The main focus of our lab is to purify and characterize mammalian ADCR complexes.

Structural and Functional Studies of 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 Drosophila the complex is required for control of important developmental regulators, such as homeotic genes and segmentation genes. In mammals, the SWI/SNF-related complexes appear to be involved not only in gene regulation, but also in targeting of HIV integration, and in tumor suppression by interacting with Rb protein. Mutation of the hSNF5 subunit has been shown as a cause for pediatric rhabdomyosarcoma. We have completely purified several distinct mammalian SWI/SNF-related complexes. By microsequencing, we have cloned all 10 subunits from a

major complex of human KB cells. Six of these belong to five different multigene families. In one case, three members of the same gene family have different tissue expression patterns, suggesting the existence of tissue-specific chromatin remodeling complexes.

NURD, a Novel Complex with both ATP-dependent Chromatin-remodeling and Histone Deacetylase Activities: ADCR 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 which is usually associated with transcriptional repression. Our results suggest that ATP-dependent chromatin-remodeling can participate in transcriptional repression by assisting repressors in gaining 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.

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, which include the Werner's Syndrome gene (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, Bloom's Syndrome and ATR-X (*a*-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 our better understanding of the human aging process.

Identification of a Human Chromatin-remodeling Complex that is related to Yeast Rsc rather than SWI/SNF: In yeast, there are two distinct chromatin-remodeling complexes, SWI/SNF and Rsc. They resemble each other in structures and catalytic activities, but are distinct in their functions. We have previously purified two different human chromatin-remodeling machines, SWI/SNF-A and SWI/SNF-B, and have identified most of their components. The two human complexes share as many as 8 identical components, making it difficult to distinguish their structures and function. Now we have identified the unique subunits of each complex. We found that human SWI/SNF-A has a set of biochemical markers conserved only in yeast SWI/SNF, whereas SWI/SNF-B contains

markers conserved only in Rsc. Our data suggest that human SWI/SNF-A and yeast SWI/SNF represent one subfamily of chromatin-remodeling complexes, whereas human SWI/SNF-B and yeast Rsc represents the other family. We are now investigating how each complex is targeted to their specific loci.

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 will use the facility to identify new proteins important in gene regulation and aging.

Collaborators: Dr. Jacques Cote, Laval University Cancer Research Center; Dr. Bradley Cairns, Harvard Medical School; Dr. Jiemin Wong, Baylor College of Medicine; Dr. Xiao-Long Zhang, Smith-Kline Pharmaceuticals.

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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.

Keywords:

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

Recent Publications:

Ko MSH, et al. *Hum Mol Genet* 1998; 7: 1967-1978.

Nakashima H, et al. *Genomics* 1999; 60: 152-160.

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

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

Kargul GJ, et al. *Genome Res* 2000; 10: 916-923.

The major goal is to understand the fundamental mechanisms for cellular commitment to mortality. 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 will utilize the potential of a systematic genomic approach to analyze early mammalian development. The approach includes the construction of cDNA libraries from a small number of cells followed by large-scale cDNA sequencing, cDNA mapping on the mouse and human genomes, in situ hybridization to mouse embryonic and fetal preparations, and simultaneous gene expression analyses by DNA microarray technologies. This is a powerful route to characterize cells and tissues and their differential functions. Although the approach can be applied to any biological phenomenon, we will focus primarily on two developmental systems in mouse: differentiation of extraembryonic cells and differentiation of germ line cells.

1. **Differentiation of Extraembryonic Cells:** The first differentiation event in mammalian embryos generates two distinct lineages: the trophectoderm (TE) and the inner cell mass (ICM). The ICM will eventually become most of the embryo proper, while the TE will

eventually become the extraembryonic tissues such as placenta. The mechanism for this transition of cellular state is not well understood. However, ICM cells of the 3.5-days post coitum (dpc) mouse blastocyst have a feature characteristic of immortal cells: namely, cells from the ICM can be propagated indefinitely in appropriate cell culture conditions as Embryonic Stem (ES) cells. Once cells are differentiated into the TE, however, their life span is set and they cannot be propagated indefinitely. In this sense, TE cells are mortal. Therefore, to look for genes that turn on or off to initiate differentiation of the TE is to look for genes that transform immortal cells into mortal cells.

2. **Differentiation of Germ Line Cells:** Germ line cells are often viewed as immortal, because they provide continuity from generation to generation and do not seem to age. In fact, the embryonic germ (EG) cells established from 8.5-dpc primordial germ cells show the same pluripotent stem cell phenotypes as the ES cells. Therefore, this is another relevant system to look for genetic determinants of cellular immortality.

Collaborators: Dr. Kuniya Abe, Kumamoto University, Japan; Dr. John Schimenti, Jackson Laboratories, Bar Harbor, Maine; Dr. Janet Rossant, Mount Sinai Hospital, Toronto, Canada; Dr. Ryuzo Yanagimachi, University of Hawaii, Hawaii.

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:

Baek JY, et al. *Cytogenet Cell Genet* 2000; 89(1-2): 6-7.

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

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

Tedla N, et al. *Clin Exp Immunol* 1999; 117(1): 92-99.

Nilsson G, et al. *Blood* 1999; 93(9): 2791-2797.

Wolff EA, et al. *J Biol Chem* 1999; 274(4): 2518-2524.

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. With the advent of life-prolonging therapies such as anti-retrovirals and drugs for opportunistic infections, an increase in life expectancy post HIV exposure has been observed worldwide with approximately 580 million HIV-infected subjects over 50 years of age in 1999 compared to approximately 1 billion people expected in 2020. With this increased incidence in aged populations, it is important to determine if AIDS pathology, the HIV infection cycle, and mode of viral transmission are distinct in susceptible cells and/or subjects of differing age groups.

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. We have observed a similar phenomenon using T-helper 1 (Th1) versus T-helper 2 (Th2) populations as well as and Bcl-2 and Bcl-xl-transfected T cells. HIV-1-infected Th1 clones have demonstrated a higher degree of viral susceptibility and death compared to Th2 clones isolated from the same individuals. This differential activity may be partially explained by poor expression of the anti-apoptotic proteins, bcl-2 and bcl-xl, within Th1 clones as well as the differential expression of various HIV co-receptors (chemokine receptors) on their cell surface. 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.

Alterations in Cytokine and Immune Subset Profiles within Aged Hosts: It is well known that the ability to mount effective immune responses often declines with age. There are several, possibly interconnected shifts in T cell phenotype and function that may be either a cause or consequence of immune changes which occur in aging. The best described changes are a shift from “naive” to “memory” phenotype among both CD4 and CD8 T cells, and a decline in IL-2 production relative to cells from young subjects. The mechanisms involved in these shifts are largely unknown. To design experiments that can start to define such mechanisms, it is necessary to be able to define T cell subsets to understand the relationships among them, and what factors regulate the transition from one stage to the next. Several key cytokines, which influence the development and cytokine profile of memory T cells, are IL-2, IL-4, IFN-γ, and IL-12, as well as TGFβ. The unregulated expression of various inflammatory cytokines, including IL-1, IL-6, IL-8, TNF, and many CXC and CC chemokines has been shown to be involved in several disease states such as inflammation, autoimmunity, and some hematopoietic malignancies. Several studies using aged rodent and primate T cells for differential cytokine production have yielded variable results. Additional studies have demonstrated a significant shift from Th1-Th0 cells to Th2 cells over the course of aging while others have observed no phenotypic Th switch. Various studies have reported on the hyperproduction of IFN-γ and TNF-α in aged versus younger animals and that these differences may relate to alterations in circulating immune cell subsets. While several human studies have also yielded variable cytokine expression results, we have initiated studies to examine such cytokine alterations (as well as the gene and protein expression of other related molecules) using several unique molecular methodologies currently in place including cytokine promoter studies, kinetic-based microarray cDNA analysis of young versus old immune cell subsets in various states of activation, RNAse protection assays, quantitative RT-PCR, nuclear binding protein analysis using DNA mobility shift analysis, and DNA methylation analysis. Using these various methods in combination, we should be able to determine if there are any alterations in cytokine production during normal and advanced accelerated (but not poor) aging (e.g., frailty) and if these changes correlate with alterations in a subject’s immune status.

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; Robert Strieter, M.D., University of California, Los Angeles (UCLA); Richard Horuk, Ph.D., Berlex Pharmaceuticals; Milan Fialo, M.D., UCLA; Stefan Brocke, Ph.D., NINDS, NIH; Francis Ruscetti, Ph.D., William Murphy, Ph.D., NCI, NIH, James Lillard, Ph.D., University of Alabama at Birmingham.

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Biography: After completing medical school at the University of Missouri, Columbia and internal medicine training at the Peter Bent Brigham Hospital and Harvard Medical School in Boston, he obtained fellowship and laboratory training at NIH and has been here for 22 years. Before becoming Scientific Director, NIA in 1995, Dr. Longo was the Director, Biological Response Modifiers Program, and Associate Director, Division of Cancer Treatment, National Cancer Institute, Frederick, Maryland. He is the author of over 600 articles and book chapters. He is an editor of *Harrison's Principles of Internal Medicine*, and *Cancer Chemotherapy and Biotherapy*. He is an associate editor of *Blood*, *Journal of the National Cancer Institute*, and *Clinical Cancer Research* and he sits on the editorial boards of six other peer-review journals. He has been cited by *Good Housekeeping* as one of the "Best Cancer Doctors in America" and listed in *Best Doctors in America*.

Keywords:

lymphocyte
immunosuppression
p53
cancer
CD28
aging
cell cycle
lymphoma
SAP
cadherin
catenin

Recent Publications:

Murphy W, et al. *Immunol Today* 2000; 21: 211-213.

Srivastava RK, et al. *J Exp Med* 1999; 190(2): 253-265.

Srivastava RK, et al. *Mol Cell Biol* 1999; 19(8): 5659-5674.

Srivastava RK, et al. *Proc Natl Acad Sci USA* 1999; 96(7): 3775-3780.

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-kappa B transcription factor, shortened half-lives for a number of cellular proteins such as TCR-zeta 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-gamma, 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 beta-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 via CD28: The CD28-mediated costimulatory signal plays a pivotal role in the outcome of many immune responses including cytolytic responses in tumor and autoimmune diseases. Depending on the primary stimulation, CD28 can initiate multiple intracellular signaling pathways including signaling pathway insensitivity towards immunosuppressive drug, Cyclosporin A (CsA). This CsA-insensitive pathway is believed to be involved in graft-vs-host disease (GVHD) during allogeneic bone marrow transplantation. Our current objectives are to characterize the CsA-resistant costimulatory pathway and determine the physiological role of this signaling pathway in normal immune responses. Our initial studies have focused on the mechanism of activation of the IL-2 promoter in a CsA-resistant manner. Based on our preliminary data, we will focus our efforts on the role of protein phosphatase 2A (PP2A) instead of calcineurin in the activation of nuclear factor of activated T cells (NFAT) and Jun N-terminal kinase (JNK). Transient transfection in fresh peripheral blood lymphocyte will be used to examine the transcriptional activation of the IL-2 promoter through a CD28-responsive element. In addition, we will also investigate the role of PP2A in the CsA-resistant synergistic effects of IL-12 and CD28 on T cell activation. Furthermore, we propose to dissect the CsA-sensitive component of an allogeneic mixed lymphocyte reaction from the CsA-resistant patients using different phosphatase inhibitors and neutralizing antisera (e.g., anti-IL-2, IL-12 and anti-IFN-ϒ). We believe that further elucidation of this signaling pathway may assist in the identification of novel therapeutics to prevent GVHD.

Role of SAP in Lymphocyte Subpopulations: X-linked lymphoproliferative disease (XLP) is an inherited immunodeficiency characterized by increased susceptibility to Epstein-Barr virus (EBV). Following infection with EBV, patients with XLP exhibit a vigorous uncontrolled polyclonal expansion of T and B cells, which may account for a severe or fatal infectious mononucleosis, acquired hypogammaglobulinemia, and/or malignant lymphoma observed in various subjects. The recent identification of a mutated form of the gene, SH2D1 or SAP has provided great insight into our current understanding of XLP and EBV infections. In an effort to define a physiological role for the XLP gene, we examined various normal human tissues for SAP mRNA expression. A high level of SAP mRNA expression was observed in the thymus and lung with modest levels in the spleen and liver and low levels in heart, placenta, muscle, kidney and pancreas. Additional studies using various primary and transformed human lymphocyte populations have demonstrated the differential expression of the SAP protein within the

nuclear and cytosolic fractions of these cells during cellular activation. Studies are ongoing to determine the role of SAP in cellular activation and effector functions, which in turn may provide a better understanding of the pathophysiology of XLP.

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Keywords:

lymphocyte differentiation
immunological memory
telomere
telomerase
immune senescence
learning and memory
aging

Recent Publications:

Weng N, et al. *Clin Immunol* 1999; 92(1): 1-2.

Liu K, et al. *Proc Natl Acad Sci USA* 1999; 96: 5147-5152.

Son NH, et al. *J Immunol* 2000; 165: 1191-1196.

Weng N, et al. *J Clin Immunol* 2000; 20: 257-267.

Research Interests: The research interests of this laboratory are focused on two fields: immunology and neurobiology. In the field of immunology, we are studying 1) the molecular and cellular mechanisms of lymphocyte differentiation from naïve to effector and/or memory lymphocytes, and 2) regulation of telomere length and telomerase activity in human lymphocytes during aging. These studies will enhance our understanding on issues such as how naïve lymphocytes differentiate to become memory cells, what is the molecular basis of long-lived memory cells, and how age affects lymphocyte functions. In the field of neurobiology, we are studying 1) the molecular basis of learning and memory formation, and 2) the mechanisms of age influence on the learning and memory process. We use rat maze learning as a model and analyze temporal and spatial changes of gene expression in hippocampus during maze learning. The analysis allows us to identify and to characterize genes that are involved in the normal maze learning and are regulated during aging. The information obtained from both immunological and neurobiological studies will serve as a rational basis for developing strategies of experimental and clinical intervention in the future.

Regulation of Telomere Length and Telomerase Gene Expression in Human Lymphocyte Development, Differentiation, Activation and Aging: Telomere, the terminal structure of chromosomes, has captivated considerable attention recently for its newly discovered function involving the regulation of cellular replicative lifespan. Every telomere consists of an array of tandem hexamer repeats, $(TTAGGG)_n$ and the binding proteins. 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. 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. 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 that do not express telomerase. In contrast, germline and malignant cells which have infinite lifespan and maintain telomere length by expressing telomerase. 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. 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 Naïve and Memory Lymphocytes: Immunological memory is one of the defining features of the immune function, yet its underlying mechanisms are not completely understood. Human $CD4^+$ naïve and memory T cells are distinct both phenotypically, naïve T cells express CD45RA and memory T cells express CD45R0, and functionally, naïve T cells require two-signals for activation and memory T cells require only one signal. In an attempt to elucidate the molecular mechanisms of immunological memory, we analyzed difference at the molecular level between $CD4^+$ naïve and memory T cells using cDNA microarray. Approximately half of the estimated total human genes (~55,000) were analyzed and found that naïve and memory $CD4^+$ T cells expressed similar number of genes (~20% of total genes). *In vitro* activation induced more than one hundred genes. Some of these activation-induced genes are known for their immune functions and many others are unknown in functions. Currently, we are investigating their functions in T cell activation.

Analysis of Gene Expression in Rat Hippocampus in Maze Training and Aging: Learning and memory formation is a complex neurological process that consists of acquisition, storage and/or retrieval of information. Hippocampus is one of essential components of brain that is responsible for long-term memory formation, which requires de novo RNA, and protein synthesis, and declines with increase of age. Although progresses have 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 in this process is largely unknown. In an attempt to dissect the memory process at the molecular level, we used cDNA microarray to analyze the changes of rat hippocampal gene expression before and after maze training (T-stone and water maze) and between young- and old-rats. After analyzing over 16,000 unique cDNA mouse clones, we found several genes that were up-regulated in maze trained hippocampus in both young and old rats and identified differentially expressed genes between young and old rats. Currently, we are characterizing the structure and function of these genes.

Collaborators: Richard J. Hodes, M.D., National Cancer Institute and National Institute on Aging; 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, NIA.

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The **Laboratory of Molecular Genetics (LMG)** investigates DNA related mechanisms such as genomic instability, DNA repair, DNA replication, and transcription. We consider the increased DNA damage accumulation in senescence as the 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 thus to understand the underlying mechanisms involved in DNA damage formation and its processing as well as the changes that take place with aging and that make aging cells susceptible to cancer. DNA repair is likely to play a critical role, and we have a special interest in the fine structure of DNA repair, which includes the study of the DNA repair process in individual genes. We are investigating the molecular mechanisms involved in DNA repair and in genomic instability in normal, senescent and cancer cells. We are investigating nucleotide excision repair and base excision repair *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 alterations.

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 also in cancer cells.

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.

We are studying the molecular deficiencies in human premature aging disorders using cell biological approaches and biochemistry.

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 processes of mutation and the role of DNA polymerases in this process. 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 this process and how it relates to DNA repair and other processes and whether it changes with age.

The Laboratory is studying DNA helicases and exonucleases such as that of the Werner syndrome protein. 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.

An interesting DNA structure that may arise in certain parts of the genome is the triple helix. These can lead to genomic instability and are of significant biological relevance. In addition, these structures can be used to mediate gene targeted DNA damage, and this approach is established in the laboratory.

We are involved with a number of studies using material from the Baltimore Longitudinal Study of Aging (BLSA). In DNA samples from individuals in this study, we are examining various aspects of genomic instability and how it changes with aging and premature aging diseases.

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Biography: Dr. Bohr received his M.D. in 1978, Ph.D. in 1987, 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. His main contributions have been in the area of DNA repair. He has worked on many aspects of DNA damage and its 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 clarification 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, particularly on nucleotide excision repair and transcription coupling. A main interest now is to elucidate how these processes change in relation to aging.

Keywords:

DNA repair
oxidative damage
Cockayne syndrome
Werner syndrome
mitochondria

Recent Publications:

Cooper MP, et al. *Genes Dev* 2000; 14: 907-912.

Dianov GL, et al. *J Biol Chem* 2000; 275: 11809-11813.

Anson RM, et al. *FASEB J* 2000; 14(2): 355-360.

Prasad R, et al. *J Biol Chem* 2000; 275(6): 4460-4466.

Balajee AS, et al. *Oncogene* 2000; 19: 477-489.

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. Damaged bases are removed as nucleotides, typically as oligonucleotide fragments. This pathway involves several of the xeroderma pigmentosum DNA repair proteins. 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. A third important pathway of DNA repair is mismatch repair, which occurs during DNA replication. Finally, a fourth pathway is recombination repair.

We mainly focus on NER, BER and mismatch repair. We are interested in some of the subcomponent NER and BER DNA repair pathways: gene-specific DNA repair and transcription-coupled repair (TCR). TCR reflects the tight interaction between DNA repair and transcription that leads to the highly efficient removal of lesions from the transcribed strand of active genes. Gene-specific DNA repair occurs at the nuclear matrix, where a number of repair proteins are recruited early in the repair process. Research

questions that are being addressed include: What is the signal for transcription coupled repair? Which DNA lesions are repaired by this pathway? Can oxidative DNA damage, thought to accumulate with aging, be repaired by this pathway? To address these questions, we are taking a number of approaches. DNA damage is induced by exposure of cells or purified DNA to various types of DNA damage or cellular stress. DNA repair is studied in intact cells, in situ, in tissue culture, in cell extracts, or using purified components.

DNA Repair and Aging: DNA damage accumulates with senescence. This could be due to defects in DNA repair. The question of whether or not DNA repair declines with aging is central in our research. This decline may be subtle and may reflect changes in the repair of actively transcribed DNA.

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. Mitochondrial DNA contains a higher steady state amount of oxidative DNA damage than nuclear DNA. Oxidative DNA damage that arises in mitochondrial DNA might give rise to the mutations, gene inactivation, or the type of deletions that are commonly found in the mitochondrial genome in association with aging and cancer. Because mitochondrial DNA is subjected to high amounts of oxidative damage, it seems that mitochondria would need an 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, there is now evidence to the contrary. Studies from our group and elsewhere have shown that a number of lesions are efficiently repaired from mitochondrial DNA. This includes the highly mutagenic lesion, 8-oxo-G.

Studies on mitochondrial DNA damage and repair have traditionally required the purification of mitochondrial DNA. This purification is laborious, and may introduce oxidative lesions in the DNA. As an

alternative approach, we have used a gene specific repair assay that does not require the isolation of mitochondrial DNA. We have established an assay using a repair enzyme that detects 8-oxo-G, and it appears that this lesion is repaired very efficiently from both mitochondrial and nuclear DNA.

We have partially purified and characterized some mitochondrial repair enzymes that recognize specific oxidative DNA lesions, 8-oxo-G and thymine glycols. These appear to be unique but to be homologues of nuclear repair enzymes. Interestingly, the enzyme that recognizes 8-oxo-G is up regulated with age in rats from 6 to 24 months of age. Thus, there is no decline, but instead an increase in mitochondrial DNA repair activity with age. This finding is contrary to current notions of mitochondrial decline and is being pursued further experimentally.

We have established novel experimental conditions for the study of DNA repair in mitochondrial extracts. For example, we have developed an assay for DNA nicking activity in mitochondrial extracts from rats. The assay can detect nicking activity on plasmids containing different types of DNA damage. We plan to determine what types of DNA lesions that are recognized in mitochondria as a way to better understand which DNA repair pathways operate in these organelles. A particular focus is whether there are any nucleotide excision repair or recombinational repair pathways. Mitochondrial repair studies have suffered from a lack of availability of *in vitro* systems for biochemical study. We are purifying components and antibodies to many proteins involved in nucleotide excision repair and base excision repair, and these will be tested for their effect on mitochondrial DNA incision. We will determine whether the mechanism of mitochondrial DNA repair differs from that of nuclear DNA repair, whether mitochondrial DNA repair declines with age, and whether local DNA repair defects in mitochondria lead to DNA deletions.

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's syndrome (WS), which we believe represent good model systems for molecular studies of normal 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 proteins with helicase (DNA unwinding) activity. 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 affording a combination of our interest in DNA function with our interest in aging.

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Keywords:

DNA damage
DNA repair
cancer
adaptive response
senescence
reactive oxygen species
cataracts

Recent Publications:

Gorospe M, et al. *J Free Radical Biol Med* 2000; In press.

Kumaravel TS, et al. *Neoplasma* 1999.

Bohr VA, et al. *NATO Meeting Proceedings* 1998.

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:

immunoglobulin
somatic hypermutation
DNA repair
age-related mutation

Recent Publications:

Winter DB, et al. *Mol Immunol* 2000; 37: 125-131.

Duncan MD, et al. *J Gastrointest Surg* 2000; 4: 290-297.

Alrefai RH, et al. *Biochem Biophys Res Commun* 1999; 264: 878-882.

Phung QH, et al. *J Immunol* 1999; 162: 3121-3124.

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. Our project has three aims.

DNA Repair and Hypermutation: The first goal is to determine if DNA repair either introduces or removes hypermutations in variable genes. Nucleotide substitutions are likely inserted by an error-prone DNA polymerase during replication or repair, and the mismatch repair pathway may process the mispairs before they are replicated into both DNA strands. We have studied the frequency and pattern of mutations in mice defective for the mismatch repair proteins PMS2, MSH2, and MLH1. Although the frequency of mutation in the repair-deficient mice was similar to that of wild-type mice, the pattern was altered. The results suggest that the hypermutation pathway frequently introduces tandem mutations, which are then corrected by a PMS2-dependent repair process, and frequently mutates G:C basepairs, which are then corrected by an MSH2-dependent pathway. The MLH1 protein had no direct effect on the mutational spectrum. We also studied the mutation pattern in mice deficient in nucleotide excision repair ($XPA^{-/-}$) and base excision repair ($OGG1^{-/-}$), and found no effect on hypermutation.

Hypermutation in Old Humans: The second goal is to analyze hypermutation in variable genes from old humans. As described above, we have recently correlated several patterns of hypermutation in mice with proteins in the mismatch repair pathway. By studying the frequency and pattern of hypermutation in old people, it will be possible to determine if the hypermutation and/or mismatch repair pathways have decreased. Genes were cloned from RNA made from peripheral blood lymphocytes taken from old and young humans, and were sequenced to identify mutations. The results show that antibodies from old people have the same frequency of mutation as those from young people, indicating that old people have high affinity antibodies that can bind to various pathogens. Both the pattern of mutation and increase in microsatellite instability in old humans suggests that mismatch repair declines with age, which may lead to formation of cancers. However, the ratio of replacement to silent mutations was much higher in the complementarity-determining regions from old people, which indicate that they were producing high affinity antibodies in response to antigens. In addition, tandem mutations, which have been associated with defective DNA mismatch repair in mice, were frequently observed in some individuals. Microsatellite variability in DNA, which is caused by impared mismatch repair, was then measured, and there was a strong correlation between the frequency of tandem mutations and microsatellite alterations. The data suggest that individuals vary in their mismatch repair capacity, and tandem mutations in human variable genes are related to impaired mismatch repair.

Mechanism of Hypermutation: The third goal is to identify genes that are involved in hypermutation. Since mutations are likely introduced by a DNA polymerase, we will eliminate the expression of different DNA polymerases in mutating cells to see if mutation decreases. Other genes involved in the process will be identified on DNA microarrays by comparing RNA from mutating cells against RNA from nonmutating cells. Candidate genes that are unique to mutating cells will then be transfected as antisense cDNA into mutating cells to see if mutation decreases.

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Keywords:

gene targeting
DNA triple helix
DNA repair

Recent Publications:

Faruqi F, et al. *Mol Cell Biol* 2000; 20: 990-1000.

Canella KA, et al. *Mutat Res Rev* 2000; 450: 61-73.

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Tobi SE, et al. *Carcinogenesis* 1999; 20: 1293-1301.

Chan PP, et al. *J Biol Chem* 1999; 274: 11541-11548.

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.

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.

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Keywords:

helicase
DNA repair
genomic instability
Werner syndrome
Bloom syndrome

Recent Publications:

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

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

Cooper MP, et al. *Genes Dev* 2000; 14: 907-912.

Brosh RM, et al. *J Biol Chem* 1999; 274: 18341-18350.

Brosh RM, et al. *Mol Biol Chem* 1999; 10: 3583-3594.

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. We and others have shown that both Werner (WRN) and Bloom (BLM) gene products are helicases, suggesting that basic defects in DNA metabolic pathways give rise to the aberrant cellular and clinical phenotypes of the premature aging disorder Werner Syndrome (WS) and cancer predisposition disorder Bloom Syndrome (BS). Cellular studies and biochemical data are consistent with this notion; however, the precise molecular functions of the sequence-related WRN and BLM proteins remain to be defined. Defining the biochemical functions of DNA helicases will help us to understand molecular defects associated with aging and cancer.

Helicases as Caretakers of the Genome: The defects observed in WS and BS cells may result from the inability to resolve alternate DNA structures. DNA structures such as hairpins, triplexes, or tetraplexes may deter replication or repair and contribute to genome instability. One hypothesis is that WRN or BLM helicases may function to resolve structures that impede progression of the replication fork. Replication defects observed in WS and BS cells are consistent with this notion. Recently we have shown that WRN and BLM enzymes unwind a DNA triple helix. Since triplex structures are known to be recombinogenic, it is reasonable to suggest that triplex structures might be more persistent in WS and BS cells, and could contribute to the genomic instability characteristic of both syndromes. Our current studies address the catalytic activities of WRN and BLM helicases on other types of novel DNA structures that may arise during replication or repair.

Protein Interactions of WRN and BLM Helicases: To understand the molecular functions of DNA helicases, we are interested in protein interactions of WRN and BLM. Defining the protein interactions of WRN and BLM helicases will help to elucidate cellular processes to prevent chromosome breaks or correct DNA damage in order to maintain genome integrity. We demonstrated the first physical and functional interactions of WRN and BLM helicases with the single stranded DNA binding protein Replication Protein A (RPA). The presence of RPA stimulates the helicases to unwind long DNA duplexes, an activity that may be important in replication or some other pathway of DNA metabolism. Ongoing studies in this area explore other protein interactions of WRN and BLM that are important to genome stability.

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The Molecular Defect Responsible for Premature Aging of Werner's Syndrome Patients

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Keywords:

Werner syndrome
DNA repair
genomic instability
premature aging

Recent Publications:

Machwe A, et al. *Nucleic Acids Res* 2000; 28: 2762-2770.

Bohr VA, et al. *Exp Gerontol* 2000; 35: 695-702.

Cooper MP, et al. *Genes Dev* 2000; 14: 907-912.

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Balajee AS, et al. *Mol Biol Cell* 1999; 10(8): 2655-2668.

Werner's Syndrome (WS) 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, heart disease, and osteoporosis. The symptoms of WS begin to appear around the age of puberty, and most patients die before age 50. Because of the acceleration of aging in WS, the study of this disease may shed light on the degenerative processes that occur in normal aging.

A hallmark defect in WS is genomic instability characterized by karyotypic abnormalities including inversions, translocations, and chromosome losses.

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 that is defective in WS, the WRN gene, has recently been identified.

The amino acid sequence suggests that the WRN gene is a member of a large family of helicases with the putative ability to unwind DNA or RNA duplexes. 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 activity and exonuclease function. Interestingly, it interacts with replication protein A (RPA), both physically and functionally. RPA enhances the helicases activity in the unwinding of larger DNA duplex structures. It interacts with the Ku heterodimer proteins, which stimulate the exonuclease activity of the Werner protein.

Although progress is being made, the true nature of the biochemical 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.

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Oxidative DNA Damage Processing

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Keywords:

DNA repair
8-oxodeoxyguanosine
oxidative damage
oxidative stress

Recent Publications:

Dianov GL, et al. *Prog Nucl Acids Res and Molecular Biol* 2000; In press.

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Anson RM, et al. *FASEB J* 2000; 14(2): 355-360.

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Stierum RH, et al. *Nucleic Acids Res* 1999; 27: 3712-3719.

One 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. Differences in how these lesions are processed have made the repair of oxidative damage in DNA difficult to understand. In addition to the complex chemistry of the reactions of these reactive species with DNA and the multiple pathways involved in their repair, at least two of these species also act as intracellular messengers affecting the control of cellular processes. We seek to tease apart these complexities by introducing well-defined oxidative lesions into DNA in cells *in vivo* or by studying the reactions of cell extracts or purified proteins with DNA containing well-defined lesions *in vitro*.

Age-Associated Effects in the Repair of Oxidative Damage By Human Cell Extracts: Previously, 8-oxodeoxyguanosine has been studied as the prototypical oxidative lesion in association with aging. We are examining the repair capacity of extracts derived from subjects with a range of ages. From this study, we expect to determine the variation in the repair of one type of oxidative damage in the normal population and discern any age-associated effect on these pathways.

Mitochondrial DNA Repair: It has been suggested that oxidative DNA damage accumulates in the mitochondrial DNA because these organelles are poor in 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 does not decrease, but

rather increases with age in rat liver and heart mitochondria. The specific increase in this activity, in contrast to an overall change in DNA metabolizing enzymes, suggests a possible up-regulation of this pathway.

It is challenging to understand the mechanisms involved in the mitochondrial DNA repair process. We take two approaches to this. In one, we are studying the repair in *in vitro* mitochondrial extracts, and here we can assess 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 knockout for various specific DNA repair genes involved in nucleotide excision repair or base excision repair.

DNA Repair Defect in Alzheimer's Disease: Recent work in other laboratories, using an indirect technique reflective of DNA repair capability, 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 using more traditional measures of DNA repair to assess the relative repair capacity of cells from normal and Alzheimer's disease patients for various types of oxidative damage.

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Transcription and DNA Repair in Cockayne Syndrome Cells

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Keywords:

aging
transcription
repair
enzymes

Recent Publications:

Balajee AS, et al. *Nucleic Acids Res* 1999; 27: 4476-4482.

Balajee AS, et al. *Oncogene* 2000; 19: 477-489.

Brosh RM, et al. *Mol Biol Cell* 1999; 10: 3583-3594.

Cockayne Syndrome (CS) 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. This cellular phenotype prompted extensive studies on the ability of CS cells to carry out nucleotide excision repair both in intact cells and in cell-free systems. 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. In hamster cells homologous to CSB, we can transfect a normal CSB gene and complement the 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 intact or permeabilized 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. This defect may be related to oxidative damage or structural changes in the DNA that somehow affect the transcription in CSB cells but not in normal cells. Experiments in intact CSB cells also demonstrate a defect in basal transcription, which can be complemented by transfection with the normal CSB gene. Further, these experiments suggest that CSB cells may have a defect in the assembly of the higher order chromatin structural organization in conjunction with transcription and DNA repair. This is supported by the observation that CSB chromatin is much more sensitive to detergent than normal chromatin.

We have generated some stable cell lines with functional domain knockout of different regions of the CSB gene. In one of these cell lines, we have mutated the ATPase/helicases domain of the gene. These cells have a phenotype similar to CSB cells. This includes a marked increase in apoptosis. Thus, this region of the gene is very important for its function. We are analyzing the phenotypes of cell lines with mutations in other regions of the gene.

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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.

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:

Guo Q, et al. *Nature Med* 1999; 5: 101-106.

Duan W, et al. *Ann Neurol* 1999; 46: 587-597.

Bruce-Keller AJ, et al. *Ann Neurol* 1999; 45: 8-15.

Mattson MP, et al. *Trends Neurosci* 2000; 23: 222-229.

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.

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. 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 Molecular Physiology and Genetics Section 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 recently in primates with focus on motor and memory performance as well as assessment of various pharmacologic, genetic, and nutritional interventions that affect brain aging.

Keywords:

brain aging
behavioral performance
memory
neurotransmitters

Recent Publications:

Hengemihle JM, et al. *Neurobiol Aging* 1999; 20(1): 9-18.

Ingram DK, et al. *Neurobiol Aging* 1999; 20(2): 137-145.

Long JM, et al. *J Gerontol A Biol Sci Med Sci* 1999; 54(10): B407-B417.

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. 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 Yongquan Luo 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.

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: M.G. DiSimone, Ph.D., Mario Negri Institute of Pharmacology, Italy; 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., Johns Hopkins University, School of Medicine; Dan Longo, M.D., Joseph Rifkind, Ph.D., George Roth, Ph.D., Dennis Taub, 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 pre-doctoral 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 of Neurosciences. 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 current 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:

Lane MA, et al. *J Anti-Aging Med* 1999; 1(4): 327-337.

Roth GS, et al. *J Am Ger Soc* 1999; 47(7): 896-903.

Lane MA, et al. *Mech Ageing Dev* 2000; 112(3): 185-196.

Lane MA, et al. *J Am Aging Assoc* 2000; 23(1): 1-7.

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, effects of CR on age-related disease including bone loss and menopause, and the development of primate biomarkers of aging.

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. We have demonstrated that reductions in metabolic rate, body temperature and glucoregulation are among the earliest changes to occur during CR. Ongoing studies of these metabolic adaptations involve several cohorts of young and old monkeys and focus on assessment of metabolic rate, body temperature, glucoregulation, and endocrine regulation of metabolism.

Studies in rodent models are focused in two areas. One line of investigation focuses on insulin signaling during CR. Recent studies in nematodes have suggested the possible relationship between regulation of

lifespan in this species and genes homologous to components of the insulin-signaling pathway in mammals. Preliminary findings suggest that CR alters at least one of these mammalian genes in this pathway. Future work will focus on further investigation of this pathway during aging and CR. A second approach involves identifying intervention that can mimic effects of CR without reducing food intake. Studies in rodent models are focused in two areas. In a recently published study we showed that administration of a glucose analogue to rats induced several physiological effects known to occur during CR. Specially, administration of 2 deoxy-D-glucose in the diet reduced body weight, body temperature, and fasting insulin levels without significantly reducing food intake. Future studies will involve assessment of the effect of glucose analogues on aging processes and lifespan and on the development of additional "CR mimetic" agents.

Effects of CR on Age-Related Disease: Recent work has focused on nutritional modulation of risk factors associated with several age-related diseases including diabetes, cardiovascular disease, menopause and osteoporosis. Our group and others have reported that CR lowers fasting glucose and insulin levels and increases insulin sensitivity, suggesting that this intervention may have beneficial effects in preventing diabetes. We recently reported that CR lowered serum triglycerides and increased the levels of a high density lipoprotein subfraction ($HDL2_b$) that is protective against cardiovascular disease in humans. More in-depth studies of both diabetes and cardiovascular disease are underway including investigation of the effect of CR and high salt intake on arterial stiffness and other risk factors. Preliminary studies in older (> 18 yr.) monkeys suggest that even when initiated in older animals CR may have beneficial effects on several risk factors such as hyperinsulinemia, hypertriglyceridemia, and obesity/ central obesity.

Little is known regarding the effects of this nutritional intervention on osteoporosis or menopause. However, rodents on CR have lower bone density, but remain reproductively capable longer and do not exhibit significant bone loss in old age. In humans, reduced body weight and intake may be related to lower bone mass and altered reproductive cycling. Current findings show that CR does not lower peak bone mass and that bone density is slightly lowered at selected skeletal locations examined. Our findings also show that CR does not alter menstrual cycling or reproductive hormones and that markers of calcium metabolism and bone turnover are not disturbed. Ongoing studies will determine if CR alters the timing of menopause or the rapid acceleration of bone loss that occurs

after menopause in humans. Studies are ongoing to focus on the relationship of body weight to bone density in this model by simulating increased biomechanical stress to compensate for the reduction in body weight seen in CR monkeys.

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: Joseph Kemnitz, Ph.D., University of Wisconsin Regional Primate Center; Richard Weindruch, Ph.D., University of Wisconsin; James Nelson, Ph.D., University of Texas Health Science Center, San Antonio; Richard Feures, Ph.D., National Center for Toxicological Research; Vincent Monnier, Ph.D. and David Sell, Ph.D., Case Western Reserve University; Steven Spindler, Ph.D., University of California.

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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:

Yu QS, et al. *J Med Chem* 1999; 42: 1855-1861.

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

Greig NH, et al. *Diabetologia* 1999; 42: 45-50.

Szayna M, et al. *Endocrinology* 2000; 141: 1936-1941.

Shaw KT, et al. *Neuroreport* 1999; 10: 53-56.

Carmona GN, et al. *Drug Metabol Dis* 2000; 28: 367-371.

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:

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.

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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
cross-cultural research

Recent Publications:

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

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

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.

Collaborators: Kerry Jang, Ph.D., and W. John Livesley, M.D., Ph.D., University of British Columbia; Fritz Ostendorf, Ph.D., Alois Angleitner, Ph.D., and Rainer Riemann, Ph.D., University of Bielefeld; Robert P. Archer, Ph.D., Eastern Virginia Medical School; Jennifer Fontaine, Ph.D., Virginia Consortium for Professional Psychology; Oliver P. John, Ph.D., University of California at Berkeley; John Loehlin, Ph.D., University of Texas at Austin; Margarida P. de Lima, Ph.D., and Antoino Simoes, Ph.D., University of Coimbra; Iris Marusic, Ph.D., and Denis Bratko, Ph.D., University of Zagreb; Gian Vittorio Caprara, Ph.D., and Claudio Barbaranelli, Ph.D., University of Rome; Joon-Ho Chae, Ph.D., Sogang University; Ralph L. Piedmont, Ph.D., Loyola College of Maryland; Martina Hrebickova, Ph.D., Academy of Sciences of the Czech Republic; Maria Avia, Ph.D., Jesus Sanz, Ph.D., and Maria Sanches-Bernardos, Ph.D., Universidad Complutense de Madrid; Peter B. Smith, Ph.D., University of Sussex; Thomas A. Martin, Ph.D., Susquehanna University; Valery Oryol, Ph.D., Ivan Senin, Ph.D., and Alexey Rukavishnikov, Ph.D., Yaroslavl State University; Yoshiko Shimonaka, Ph.D., Katsuharu Nakazato, Ph.D., Yasuyuki Gondo, Ph.D., and Midori Takayama, Ph.D, Tokyo Metropolitan Institute of Gerontology; Juri Allik, Ph.D., University of Tartu.

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Biography: Dr. Thayer received a B.A. in Psychology from Indiana University, and Master's and Ph.D. degrees from New York University. After academic positions at Penn State University and the University of Missouri, he joined NIA to initiate a program on Emotions and Quantitative Psychophysiology. His research interests concern biological and psychological adaptation and flexibility in the context of dynamical systems models with applications to psychopathology, pathophysiology, and health. This work utilizes indices of autonomic nervous system function derived from cardiac variability measures to probe whole organism systems.

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.

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.

Collaborators: Thomas D. Borkovec, Penn State University; Jos F. Brosschot, University of Leiden, The Netherlands; Bruce H. Friedman, Virginia Tech University; Arve Asbjornsen, Kenneth Hugdahl, Bjorn Helge Johnsen, Jon Christian Laberg, University of Bergen, Norway; Richard D. Lane and Geoffrey L. Ahern, University of Arizona; Richard A. Tyrrell, Clemson University.

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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.

Maki PM, et al. *Am J Psych* 2000; In press.

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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Biography: Dr. Resnick received her Ph.D. in Differential Psychology and Behavioral Genetics from the University of Minnesota and completed a postdoctoral fellowship in Neuropsychology and Neuroimaging at the University of Pennsylvania. She was Research Assistant Professor of Psychology in Psychiatry at the University of Pennsylvania prior to joining the Laboratory of Personality and Cognition, NIA in 1992. She studies brain-behavior associations in health and disease and is currently the principal investigator of the brain imaging component of the Baltimore Longitudinal Study of Aging (BLSA). This longitudinal neuroimaging study focuses on early structural and physiological brain changes that may be predictors of memory and cognitive change in older individuals. Through this study and others in the BLSA, she has also been examining the hormonal modulation of age-associated cognitive and brain changes.

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 hormone replacement therapy 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. *Am J Psych* 2000; In press.

Maki PM, et al. *Neurobiol Aging* 2000; 21: 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 elected on their own 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 effect."

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: Antonio C. Wolff, M.D., Johns Hopkins University School of Medicine (JHUSOM), Department of Oncology; Nancy E. Davidson, M.D., JHUSOM, Department of Oncology; Dave M. Yousem, M.D., JHUSOM , Department of Radiology; Michael A. Kraut, M.D., Ph.D., JHUSOM, Department of Radiology; Adrian Dobs, M.D., JHUSOM, Department of Endocrinology; Jason Brandt, Ph.D., JHUSOM, Department of Psychiatry and Behavioral Sciences; Jill B. Rich, Ph.D., Department of Psychology, York University, Ontario.

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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:

Pietrini P, et al. J Nucl Med 2000; 41: 575-583.

Rapoport SI, et al. Ann NY Acad Sci 1999; 893: 138-153.

Rapoport SI, et al. Neurochem Res 1999; 24: 1403-1415.

Rapoport SI, et al. Proc Natl Acad Sci USA 2000; 97: 5696-5698.

Rintala J, et al. Neuroreport 1999; 10: 3887-3890.

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 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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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:

Nagababu E, et al. *Biochem Biophys Res Commun* 2000; 273(3): 839-845.

Rifkind JM, et al. *Life Sci* 1999; 64(4): 237-247.

Ramadas N, et al. *Biophys J* 1999; 76(4): 1796-1811.

Risby TH, et al. *J Appl Physiol* 1999; 86(2): 617-622.

Mendelman A, et al. *Brain Res* 2000; 867(1-2): 217-222.

Ajmani RS, et al. *Neurobiol Aging* 2000; 21(2): 257-269.

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. 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 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. 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.

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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, Genotype 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 Chief of the Central Laboratory Services Section since July 1998.

The **Central Laboratory Services Section (CLSS)** collects, analyzes, and prepares for long-term storage of blood and tissue samples. CLSS also performs or arranges for DNA extractions, as well as cell transformations in preparation for creating renewable cell lines. Services provided by CLSS include phlebotomy, centrifugation and other tissue handling and preservation, DNA extraction, and inventory management of stored samples.

The GRC **Clinical Core Laboratory (CCL)** was developed in July 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.

It offers a wide range of clinical testing services, information and laboratory management expertise. CCL supports new test development for research, particular infectious disease, genetic disorders, immunological and degenerative diseases.

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Biography: Dr. Juhaszova received her M.S. from Faculty of Sciences Commenius University, Slovakia in 1978 and Ph.D. from Slovak Academy of Sciences in 1987. 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 Research Resources Branch in 1999.

Keywords:

imaging
confocal microscope
fluorescence
mitochondria
mitochondrial ATP-sensitive K channels
mitochondrial permeability transition (MPT)
ischemic preconditioning

Recent Publications:

Slodzinski MK, et al. *Methods Enzymol* 1999; 314; 313-323.

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.

Research Interests: Mitochondrial ATP-sensitive K channels modulate cardiac mitochondrial function.

1) In the **Confocal Imaging Unit** 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. We employed immunocytochemical and immunoblotting techniques to determine the effect of these modulators on activation and translocation of PKC δ and PKC $\in$. Our results suggest that MPT-induction susceptibility is controlled by the activity of the mito K_{ATP} channel via a PKC dependent mechanism-likely the major mechanism of cardiac protection by ischemic preconditioning.

We proposed a novel means of subcellular localization of PKC signaling based on the prototype model of the mito-K_{ATP} channel: RACK (receptor for activated PKC) affinity modulation. The gating of mito K_{ATP} channel may modulate specific PKC δ and $\in$ RACK- affinities which, in turn, recruits specific patterns of PKC activation and translocation to mitochondria and controls end effector signaling.

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.

Whitney LW, et al. *Ann Neurol* 1999; 46(3): 425-428.

Becker KG, et al. *Diabetes* 1999; 48(7): 1353-1358.

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 testis development in Drosophila, nicotine administration in rats, studies of murine experimental autoimmune encephalomyelitis, 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, Drosophila, among others. Glass-based protein microarrays are also being developed using an enzyme-linked immunosorbent assay (ELISA).

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. This is being developed in collaboration with Donna Maglott at the National Center for Biotechnology Information/NIH.

Collaborators: Dr. Jim Eberwine, University of Pennsylvania; Dr. Thomas DeGraba, NINDS, NIH; Dr. Kathleen Barnes, Johns Hopkins Medical Institutions; Dr. Cory Teuscher, University of Illinois.

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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: 1286-1295.

Donahue RE, et al. *Blood* 2000; 95: 445-452.

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 (*Δ*E1)or lacking the Ad E3 region in addition to E1 sequences (*Δ*E1E3) 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 *Δ*E1 or *Δ*E1E3 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 *Δ*E1 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. He joined the NIA Intramural Research Program to work with Dr. Dave Donovan in the Transgenic and Knockout Facility.

Keywords:

PCR
genotyping

Recent Publications:

Duncan MD, et al. *J Gastrointest Surg* 2000; 4(3): 290-297.

Services Available: The **Genotyping Services Unit** (GSU) has been in operation since 7/31/00. Dr. Rowley currently has over 35 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, with Dr. Dave Donovan, involves a sequence comparison of hyper variable 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.

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Biography: Dr. Salvatore Sechi received his Ph.D. in biology from the University of Padua in Italy in 1990. He joined the National Cancer Institute as a visiting fellow in 1990 where he was trained in protein chemistry. During this period, he contributed to the cloning of several novel genes and has patented several unique methods for protein sequence analysis. In 1995, he joined the Laboratory of Mass Spectrometry headed by Dr. Brian T. Chait at the Rockefeller University as a Research Associate. During this time, Dr. Sechi improved the laboratory methodologies of protein identification by peptide mapping and developed a novel approach for the characterization of the carboxy-terminal ends of proteins. In 1998/99, he was appointed principal investigator in the Proteomic Laboratory at Dupont and Adjunct Professor in Methodology in Biochemistry at the University of Padua. Dr. Sechi recently joined the National Institute on Aging as Head of the Mass Spectrometry Unit (MSU).

Keywords:

ESI
MALDI
mass spectrometry
protein
protein structure
protein sequencing
proteomics
post-translational modifications

Recent Publications:

Sechi S, et al. *Anal Chem* 2000; 72(14): 3374-3378.

The **Mass Spectrometry Unit** works primarily with polypeptides and complex proteins. The unit supports the work of intramural investigators by applying novel methods for protein identification and characterization. When required, new methodologies are developed and implemented. The laboratory is equipped with a MALDI-TOF instrument and with an ESI ion trap coupled to a capillary HPLC for LC-MS and LC-MS-MS analysis. In the near future, we hope to implement large-scale two-dimensional gel electrophoresis and other protein profiling methodologies.

Proteomics and Protein Identification: Genomic studies have been progressing at a rapid pace with the completion of over 39 genome sequences including three Eukaryota. Sequencing of the human and mouse genomes appear to be at the final stages of completion. In contrast, very little is known about the human and murine proteomes. The proteome can be defined as the complete set of proteins expressed by a given cell at certain time intervals and is far more complex than the genome. The higher complexity of the proteome is due to post-translational modifications of expressed proteins and cell to cell variations in protein expression within the same organism. Typically, proteomic studies are performed in two stages: protein profiling and protein identification. For protein profiling, the most commonly utilized methodology is two-dimensional gel electrophoresis, while for the protein identification, mass spectrometry has proven to be the most rapid and efficient means of analysis. We currently identify unknown proteins via MALDI peptide mapping and LC-MS-MS. Protein profiling will soon be implemented.

Projects and collaborations with investigators from several laboratories requiring identification of unknown proteins associated in macromolecular complexes are in progress.

Protein Characterization: The protein amino acid sequence is so diverse that its chemical physical properties are usually unique. In addition to the amino acid sequence, many post-translational modifications such as phosphorylation, glycosylation and acetylation, just to mention a few, make the characterization of the primary structure of a protein a complex study. Mass spectrometry is an essential tool in protein characterization. For example, an accurate MALDI-TOF measurement of the peptide masses deriving from the proteolytic digest of a protein could lead to the characterization of the primary structure of a large protein. From the interpretation of an MS-MS spectra of a peptide, we can identify a phosphorylation site within a large protein. In the past, this type of analysis required several months of intensive study and, in many cases, the results proved unapproachable. Today, using mass spectrometry, we might be able to identify several phosphorylation sites in a very short time. Mass spectrometry can also be utilized in characterizing non-covalent interactions and compact folding domains within given proteins and polypeptides. The Mass Spectrometry Unit is currently collaborating with several GRC investigators on the characterization of several proteins with compact folding domains and non-covalent interactions.

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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. Assoc. Ed. Hemopoiesis Section: The Fifth Edition of Schalm's Veterinary Hematology, WB Saunders, Philadelphia, 2000.

Watson R, et al. *Vet Path* 2001; In press.

Intracellular Protection of Feline Fetal Blood Stem Cells as Therapy for FIV in Cats: Mammals produce an estimated 1.0 billion leukocytes, 2.5 billion erythrocytes, and 2.5 billion thrombocytes per kilogram every day of their lives. Lymphohemopoietic cells have the highest rate of mitosis, continuous differentiation, and protein synthesis of all of the 40-60 trillion cells in the body. This unique characteristic makes cells derived from hematopoietic stem cells (HSCs) particularly vulnerable to cell-entry and replication of retroviruses. Human immunodeficiency virus (HIV) preferentially infects and survives in actively dividing lymphohemopoietic cells. Moreover, because of a short generation time, variable antigenicity, and a large number of infective virions, HIV exhibits an extraordinary capacity to survive and, therefore, is confounding existing therapeutic strategies of combinations of sophisticated anti-viral pharmaceutical compounds. Hemopoietic stem cell gene therapy offers a fresh approach. The following table compares and contrasts the conceptual differences between conventional drug approaches and the promise of HSC gene therapy:

	Antiviral Drug Rx	Stem Cell Gene Rx
Life-long Administration	**Yes**	**No**
Systemic Drug Toxicity	**Yes**	**No**
Drug Compliance Issues	**Yes**	**No**
Drug Cytotoxicity to Lymphohematopoietic Cells	**Yes**	**No**
Protection all Blood, Lymphoid & Macrophage Cell Linages	**No**	**Yes**
Life-long Cellular Prophylaxis	**No**	**?**
Retrovirus Strains Become Resistant	**Yes**	**?**

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.

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Biography: Dr. Donovan obtained his B.S. in Biological Sciences at Carnegie-Mellon University in 1976, and worked at the Johns Hopkins University as a Research Technician until 1981. He joined the Biological Sciences graduate program at the University of Maryland, Baltimore County, where he was awarded his Ph.D. in 1987 for work in yeast molecular genetics. Dave then accepted a Postdoctoral position in the Laboratory of Developmental and Molecular Biology at the National Eye Institute, NIH, until 1989, when he joined the Laboratory of Molecular Neurobiology, National Institute on Drug Abuse, NIH. In 1993, he was appointed Acting Section Chief in the Molecular Genetics Section where he participated in the construction and characterization of knockout mice for both the dopamine transporter and the mu opioid receptor. He joined the National Institute on Aging in 1996 where his present research uses transgenic and conditional knockout approaches to address aging related questions in neurobiology.

Keywords:

dopamine transporter
mu opioid receptor
microarray
gene expression profiles

Recent Publications:

Donovan DM, et al. *Mol Brain Res* 1999; 73: 37-49.

Mi QS, et al. *Proc Natl Acad Sci USA* 2000; 97(11): 6031-6036.

Carter MG, et al. *Hum Mol Genet* 2000; 9(3): 413-419.

Research Interests: The two main areas of interest are: (1) dopamine cell gene expression during development, drug treatment, and neurotoxicity, and (2) an understanding of the role of the Mu Opioid Receptor (MOR) in peripheral analgesia and immune response.

(1) Embryonic (E13.5) DA cells have been purified, mRNA isolated, and an E13.5 DA neuron cDNA library constructed. Tyrosine hydroxylase - lacZ (THB) transgenic mouse embryos express β-galactosidase in embryonic ventral midbrain DA neurons. These cells were tagged with fluorescent substrates for β-galactosidase and purified via fluorescent activated cell sorting. 960 DA cDNAs were subjected to partial DNA sequence analysis and used to create a DA microarray. Microarray analysis of ventral midbrain RNA and DA cDNAs on both the DA neuron microarray and the NIA neuro-array (composed of 1100 human cDNAs expressed in the nervous system), provides an initial DA neuron gene expression profile with over 100 new ESTs and numerous embryonic enhanced ventral midbrain cDNAs. Microarray analysis of methamphetamine neurotoxicity reveals greater than 2 fold enhancement of cytochrome c oxidase I (COXI) expression in ventral midbrain at 12 hours post treatment. In a parallel study to search for DA cell-specific gene expression elements, the dopamine transporter gene (DAT) proximal promoter sequences (up to -2.8Kb) were fused to lac Z and introduced into transgenic mice. In situ beta-gal staining has yielded unexpected expression in the locus coeruleus for multiple founder lines from each of 4 x DAT - lacZ constructs, with no DA cell-specific expression identified.

(2) A Cre-loxP strategy to conditionally knockout the MOR in peripheral neurons and T lymphocytes is employed to create a new mouse model of inflammation to study pain and the immune response. This model includes loxP site insertions flanking exon 3 of the MOR gene, Cre expression in the Dorsal Root Ganglion (DRG) via a Peripherin-Cre (PCRE) transgene, and T-cell specific Cre expression in the previously characterized LCK-CRE mouse (from Jamey Marth). Five founder lines of PCRE mice have been analyzed with the ROSA Cre reporter strain that reveal Cre expression in the peripheral nervous system with high expression in the DRG. The resulting knockout mouse should help clarify the role of the MOR vs the other opioid receptors (kappa and delta) in peripheral analgesia and inflammation. The loxP inserted MOR gene mouse is still under construction, but a comparison of the inter-strain differences between the C57BL/6 and 129/Sv MOR genomic sequences identified in this work reveals many polymorphisms that might contribute to strain-specific responses to painful stimuli and opiate drugs. These findings suggest potential genetic sources of inter-individual human variability in these responses.

Collaborators: Dr. George A. Ricaurte, Department of Neurology, Johns Hopkins Medical Institutions; Dr. William J. Freed, National Institute on Drug Abuse, NIH; Dr. Brigitte Kieffer, CNRS, Illkirch, France; Dr. Michel Simonneau, Neurogenetics, Robert Debre Hospital, Paris, France.

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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:

Horska A, et al. *Am J Physiol* 1999; 276: E766-E773.

Morrell CH, et al. *The American Statistician* 2000; 54: 1-4.

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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