FACT BOOK 2009

National Institute on Aging
Intramural Research Program

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Foreword

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

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

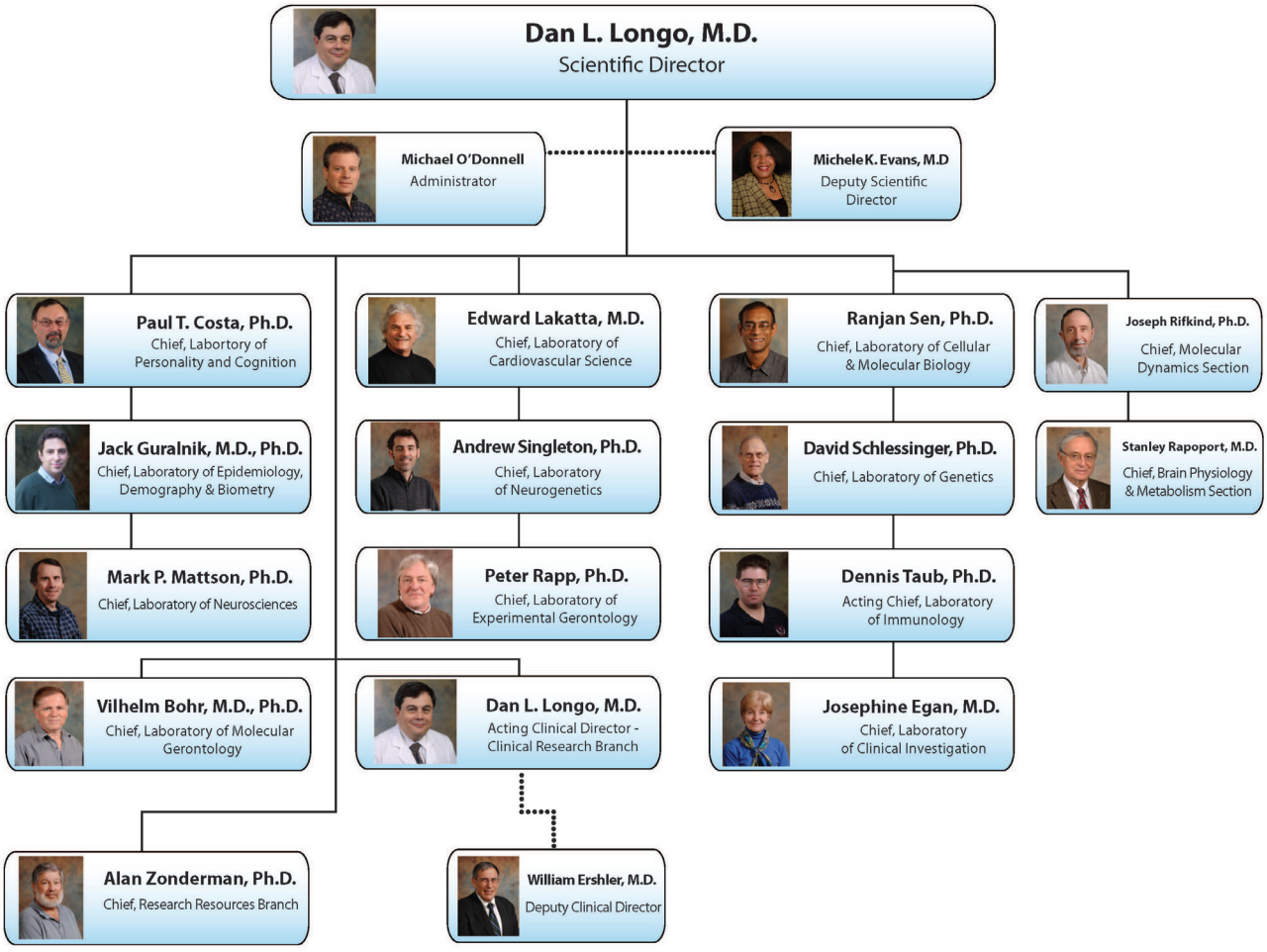
The Intramural Research Program (IRP) in the National Institute on Aging (NIA) comprises 11 scientific laboratories, a clinical research branch, a research resources support branch and 2 sections. The research program includes the scientific disciplines of biochemistry, cell and molecular biology, genetics, physiology, immunology, neuroscience, neurogenetics, behavioral sciences (psychology, cognition, psychophysiology), 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 our 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 a change becomes pathologic. Thus, not only are the common age-related diseases under study (e.g., Alzheimer's Disease, Parkinson's Disease, stroke, atherosclerosis, osteoarthritis, diabetes, cancer), but the determinants of healthy aging are also being defined.

IRP research is conducted in several sites; most of the laboratories are based at the Biomedical Research Center and the Gerontology Research Center on the Johns Hopkins Bayview Campus in Baltimore, Maryland. The Clinical Research Branch's Advanced Studies in Translational Research on Aging (ASTRA) Unit is located at Harbor Hospital, a few miles south of the Bayview Campus in Baltimore, Maryland. The section of *Brain Physiology and Metabolism* and the *Laboratory of Neurogenetics* are located on the NIH main campus in Bethesda, and the *Laboratory of Epidemiology, Demography, and Biometry* is located in the Gateway Building in Bethesda.

The IRP provides a stimulating academic setting for a comprehensive effort to understand aging through multidisciplinary investigator-initiated research. In addition, an effort is made to encourage synergistic interaction through interlaboratory collaboration. 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.

Dan L. Longo, M.D.
Scientific Director
National Institute on Aging

National Institute On Aging
Intramural Research Program



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 four sections and five units, each headed by a senior scientist: Cardiac Function Section, Cardiovascular Biology Unit, Human Cardiovascular Studies Unit, Hypertension Unit, Molecular Cardiology Unit, Cardioprotection Unit, Cellular Biophysics Section, Receptor Signaling Section, and the Translational Cardiovascular Studies Section.

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 determine how aging of the heart and vasculature interacts with chronic disease states to enhance the risk for CV diseases in older persons; (3) to study basic mechanisms in excitation-contraction coupling in cardiac cells and how these are modulated by surface receptor signaling pathways; (4) to elucidate factors that maintain stem cell pluripotentiality, that promote the commitment of stem cells to the cardiac lineage, and that regulate their development as cardiac cells; (5) to elucidate mechanisms that govern cardiac and vascular cell survival; (6) to determine mechanisms that govern neuro-hormonal behavioral aspects of hypertension; and (7) to establish the potentials and limitations of new therapeutic approaches such as changes in lifestyle, novel pharmacologic agents or gene or stem cell transfer techniques in aging or cardiovascular disease states. 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.
To achieve an integrative research program, I have attempted to encourage and foster an LCS environment in which multiple individuals can productively and comfortably interact. Thus, in addition to my continuing efforts to conceptualize the various aspects of the LCS

strategic plan, recasting existing programs, creating and maintaining the research environment, inaugurating new ones, and recruiting qualified individuals to develop these programs, I expend substantial time and energy to create and maintain this interactive research environment. In order to establish links among individuals that capitalize on their strengths and compensate for their shortcomings, I do my best to assess their creative ability, knowledge and motivation. The success of this approach requires an understanding of each person's needs, which vary from outright direction to coaching, support, or complete delegation. (This approach applies not only to the mentoring of junior postdoctoral fellows as they mature, but also to my interactions with tenured scientists, technicians, clerical staff, etc.) Consequently, many of the LCS projects become multi-faceted, spanning a range from humans to molecules due to links among individuals within LCS, and their networking with other institutes within the NIH, academic institutions, and industry. Integration of LCS research efforts, or interdisciplinary research, occurs to a variable extent at multiple interfaces: among different scientific disciplines, e.g., epidemiology, genetics, physiology, pharmacology, biophysics, biochemistry and molecular biology; across species, from humans to rodent models of development to aging; within an organism, e.g., cardiovascular system, heart (H): vascular (V), H-V coupling, tissue, cell, molecule; and among factors that impact on an organism, e.g., age, disease and life style (and soon, genetics). The table on page 4 depicts the resultant LCS Research Program mosaic in schematic form. The left hand column in the scheme lists the various experimental models employed in the Lab's research program (i.e., humans to molecules). The three right hand columns list the general modes of research that may occur within each model system, e.g., intrinsic mechanisms, and acute or chronic modulation of these mechanisms. During any given epoch, each address (horizontal-vertical coordinate) in the scheme may consist of one or several projects, depending upon the personnel constituency and expertise within the Lab at the time. Also, active collaborations have been established within and outside of NIA, including foreign sites.

As Lab Chief, the nature of my specific interactions with individuals within the Lab varies widely. LCS tenured scientists, senior fellows, and tenure track investigators independently choose their specific research projects, within the broad framework of the Lab's mission. These individuals serve as mentors for junior fellows. Occasionally, projects originate at the fellow/investigator level and are coordinated by their mentors. Often, I am invited by tenured or tenure-track scientists, unit heads, or senior fellows, to participate, as a collaborator, in various projects within their programs. In the broad sense, the collective research

output of the LCS can be considered to be a “bottom up” approach. As a result, the LCS environment has, in my opinion, become somewhat unique: it is not strictly akin to a university department, in which each member dictates his/her mission and is required to apply for individual funding in order to implement the proposed program; however, neither is the LCS environment strictly “mission oriented” in the sense that an individual is not mandated to work on specific projects in a “top down” approach.

Laboratory of Cardiovascular Science - Research Program

Experimental Model	Intrinsic Mechanisms	Acute Modulation of Intrinsic Mechanisms	Chronic Modulation of Intrinsic Mechanisms
Humans	Cardiac structure Vascular structure Cardiovascular function at rest	Drugs Postural reflexes Exercise stress	Age, gender, race, socioeconomic status Disease (CAD, hypertension), risk factors, and prevention Genetics
Intact Animals **Heart Failure** **Hypertension** **Aging** **Preconditioning** **Arterial Injury**	Arterial remodeling of aging Cardiac remodeling post myocardial infarction, endogenous na/K ATPase ligands Gene expression VSMC proliferation and migration	Novel drugs	Age Growth factors Diet Thyroid status Local or systemic drug delivery Gene therapy Stem cell therapy
Isolated Heart or Cardiac Muscle	Myocardial contractile properties, excitation-contraction coupling, Ca^{2+}signals, action potentials	Ischemia Anoxia, hypoxia Free radicals Neuropeptides Novel drugs Stretch	Age Diet Exercise Hyperthyroid state Cardiomyopathy Heart failure
Cardiac Cells **Myocytes** **Fibroblasts**	Membrane ionic channel currents Cardiac cell contraction Cystolic Ca^{2+} signals Mitochondrial Ca^{2+} signals Sarcolemmal ion transport Sarcoplasmic Reticulum function Apoptosis	Receptor stimulated second messengers Neuropeptides Stretch Anoxia, hypoxia Free radicals Novel drugs Anesthetics Growth factors Novel endocardial factors Novel endothelial factors	Development Age Disease Heart failure Hypertension Diet Growth factors Hypoxia
Vascular Smooth Muscle and Endothelial Cells	Cystolic Ca^{2+} and pH regulation Proliferation and secretion Chemotaxis and invasion Matrix regulation Tubulin/microtuble dynamics Differentation regulation Angiogenesis	Shear stress Receptor agonists/antagonists Growth factors Anoxia, hypoxia Stretch Anti-microtubule agents Matrix degradation Antisense inhibition and gene overexpression	Atherosclerosis Arterial injury Aging Dedifferentation
Stem Cells	Mechanisms of pluripotency	Homing factors	Differentation into heart and vascular cells
Sub-Cell Organelles	Na/K transport systems Sarcolemmal ion channels Sarcoplasmic reticulum Ca^{2+} cycling Mitochondrial membrane potential regulation, ATP K+ channels	Ionic composition Adenine nucleotides Neuropeptides Ischemia, anoxia Drugs Reactive oxygen species	Age Heart failure Hypertension
Molecules	Genomics-SAGE cDNA assays Control mechanism of gene expression in heart and vascular cells, ryanodine receptors, IP 3 receptors, G proteins Expression of (1) isozymes: e.g. myosin heavy chain, Na-K ATPase, (2) proteins HSP oncogenes, ANF, pump or channels proteins (e.g. SR Ca ATPase, sarcolemmal Ca^{2+} and K^{+} channels	Ionic transportation mechanisms Stretch mechanisms Growth factors Neuropeptides Nitric Oxide Reactive oxygen species	Age Hormones Hypertension Heart failure Genetic manipulation

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Shannon Marshall	Biologist
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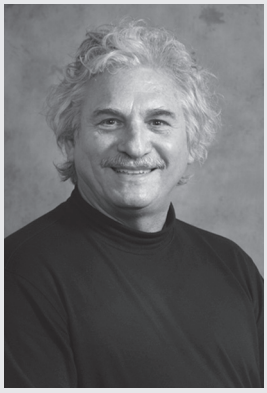
Xiaokun Zeng	Research Scientist (Contractor)



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Biography: Dr. Lakatta received his M.D., magna cum laude, from Georgetown University School of Medicine. Following an internship and residency in Medicine at Strong Memorial Hospital, University of Rochester, Rochester, N.Y., he trained in basic research for two years at the NIH. Subsequently, he completed his cardiology fellowship at Georgetown and Johns Hopkins University Schools of Medicine. This was followed by a year of basic research training in the Department of Physiology, University College and the Cardiothoracic Institute, London England. Dr. Lakatta also holds adjunct appointments as Professor, Department of Physiology, University of Maryland School of Medicine, and Professor, Cardiology Division, Johns Hopkins School of Medicine. Dr. Lakatta is recognized nationally and internationally as an expert in cardiovascular research. He has authored over 340 original publications in top peer reviewed cardiovascular journals, written over 210 invited reviews/book chapters and delivered over 420 invited lectures. He is a member of multiple scholarly societies and journal editorial boards. He has received several awards, among which has been election into the American Society for Clinical Investigation, and the Association of American Physicians. He is the recipient of the Eli Lilly Award in Medical Science, the Paul Dudley White Award in Cardiology, the Allied Signal Achievement Award in Aging, the Novartis Prize in Gerontology, the Irving Wright Award of Distinction of the American Federation for Aging Research (AFAR), a Distinguished Service Medal, Public Health Service, National Institutes of Health, National Institute on Aging and an Honorary Degree from the Universite D'Auverge in Clermont, France. Dr. Lakatta has also been elected as a fellow in the APS Cardiovascular Section, a fellow of the American Heart Association (F.A.H.A.) and is an Inaugural Fellow of the Council on Basic Cardiovascular Sciences of the American Heart Association.

Keywords:

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

Recent Publications:

Vinogradova TM, et al.Circ Res 2006; 98(4): 505-514.

Wang M, et al. Arterioscler Thromb Vasc Biol 2006; 26(7): 1503-1509.

Younes A, et al. Am J Physiol Heart Circ Physiol 2005; 289(4): H1652-H1661.

Fleg JL, et al. Circulation 2005; 112(5): 674-682.

Dr. Lakatta directs the Cardiac Function Section (CFS), which has a broad-based research program ranging from studies in humans to molecules. 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.

Collaborators: Rui-Ping Xiao, M.D., Ph.D., Kenneth R. Boheler, Ph.D.,

Collaborators-Continued:

Steven Sollott, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Michael Crow, Ph.D., Johns Hopkins University; Jerome L. Fleg, M.D., National Heart, Lung and Blood Institute, NIH; George Krause, Ph.D., Max Delbruck Centre for Molecular Medicine; Steven Houser, Ph.D., Temple University School of Medicine; Brian Kobilka, M.D., Stanford University; Robert Lefkowitz, M.D., and Walter Koch, Ph.D., Duke University Medical Center; Remesh Gopal, MBBS, Northwestern University; Ajay Shah, M.D., University of Wales College of Medicine; Konstantin Bogdanov, M.D., Russian Academy of Medical Sciences; Gary Gerstenblith, M.D., Edward Shapiro, M.D., Frank Yin, M.D., and Peter Vaitkevicius, M.D., Johns Hopkins Medical School; Ruth Altschuld, Ph.D., Ohio State University; W. Jonathan Lederer, Ph.D., University of Maryland School of Medicine; Maurizio Capogrossi, M.D., Institute of Dermatology, Rome, Italy; Oscar Bing, M.D., Boston VA Medical Center; David Kass, M.D., Johns Hopkins Hospital; Xilin Long, Ph.D., University of Maryland; Lewis Becker, M.D., Johns Hopkins University; Kostja Bogdanov, Ph.D., National Cardiology Research Center, Moscow, Russia; David Dostal, Ph.D., Pennsylvania State University; Marvin Boluyt, Ph.D., University of Michigan; Kenneth Baker, M.D., Pennsylvania State University; George Roth, Ph.D., GeroScience, Inc.; Donald Ingram, Ph.D., Pennington Biomedical Research Center, Louisiana; Kim Sutton-Tyrrell, Ph.D., University of Pittsburgh; Heping (Peace) Cheng, Ph.D., Peking University, Beijing, China.

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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. Presently, he is a Senior Investigator and Head of the Cardioprotection Unit, Laboratory of Cardiovascular Science. His research attempts to bridge interests spanning basic and clinical science to therapeutics.

Of particular note is his invention, together with former NIA Senior Investigator, James Kinsella, of the use of paclitaxel to prevent vascular restenosis after angioplasty. His research led directly to development of the paclitaxel-coated stent. The technique of local arterial drug therapy with drug-eluting coronary stents has had explosive growth in recent years. Paclitaxel is one of only three currently FDA-approved drug stent coatings proven to prevent in-stent restenosis. Stents coated with paclitaxel deliver it locally only to the site where needed, dramatically reducing the incidence of in-stent restenosis by 50-90% vs. bare-metal stents. Since 2003 when the paclitaxel drug-eluting stent was introduced for clinical use in Europe and 2004 in the United States, 4.6 million have been implanted in patients worldwide.

Accomplishments related to the invention and use of paclitaxel to treat vascular disease were featured in the NIH Record, 58(2), January 27, 2006: "NIA Scientists Honored for Stent Development" http://www.nih.gov/nihrecord/01_27_2006/story05.htm.

This innovation was also recently recognized with two prestigious awards:
-Finalist, 2005 National Inventor of the Year Award, Intellectual Property Owners Association (IPO), for invention of the use of paclitaxel to prevent vascular restenosis (implemented in paclitaxel-eluting vascular stents).
-2005 Federal Laboratory Consortium Mid-Atlantic Regional Award for Excellence in Technology Transfer (September 15, 2005): "Taxus® Express2™: Bypassing By-Pass Surgery with Paclitaxel-Coated Stents."

Keywords:
excitation-contraction coupling
calcium
nitric oxide
reactive oxygen species
mitochondria
permeability transition pore
ROS-induced ROS release
ischemia/reperfusion
cardioprotection
neuroprotection

Keywords-continued

We are studying structure and function of cells from the cardiovascular system along two principal and distinct lines: 1) Nature and control of mitochondrial instability and cell death during oxidant stress, and protection of cardiac myoctes (and neurons) during ischemic stress; and 2) Cellular changes and vascular protection 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.

glycogen synthase kinase-3β
chemotaxis
restenosis
paclitaxel

Recent Publications:

Chiara F, et al. *PLoS ONE* 2008; 3: e1852.

Juhaszova M, et al. *Ann N Y Acad Sci* 2008; 1123: 197-212.

Shivakumar K, et al. *Am J Physiol Heart Circ Physiol* 2008; 294: H2653-H2658.

Lyashkov AE, et al. *Circ Res* 2007; 100: 1723-1731.

Zorov DB, et al. *Biochim Biophys Acta* 2006; 1757: 509-517.

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.

Recent work led to the discovery of a novel phenomenon accompanying induction of the mitochondrial permeability transition pore (mPTP) in cardiomyocytes, termed "ROS induced ROS release." This led to the identification of the mechanism by which ischemia/reperfusion injury damages mitochondria, as well as the mechanism of cardioprotection afforded by ischemic preconditioning. Research proved that the mPTP is the end effector in these processes: the threshold for mPTP induction by ROS being significantly reduced after ischemia reperfusion, but beneficially increased by preconditioning. We concluded that GSK-3β(and specifically its inactivation) is a major, required integration point for a multitude of upstream signals acting on an end-effector responsible for cardioprotection (the mPTP). When cell protection signaling pathways are activated, we found that the Bcl-2 family members relay the signal from GSK-3ß onto a target at or in close proximity to the pore. Thus, the effect of the convergence of these signaling pathways via inhibition of GSK-3ß, relayed through Bcl-2 proteins, on the end effector, the permeability transition pore complex, to limit mPTP induction, is the general mechanism of cardiomyocyte protection. Signaling defects underlying the age associated loss of the capacity for ischemic preconditioning are being examined which could lead to testable clinical therapies relevant to the preservation of healthy aging.

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

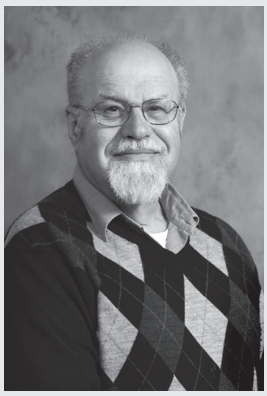
occlusive vascular disorders, including atherosclerosis and post-angioplasty restenosis. We found that a unique intracellular Ca^{2+}-signaling profile is initiated via extracellular cues provided specifically by gradient exposure to PDGF, achieving an apparent threshold for activation of CaM kinase 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. We then went on to prove that paclitaxel prevented restenosis in small and large animal models, and thus could have clinical promise to prevent vascular restenosis which lead to clinical trials worldwide. These trials have demonstrated the safety and efficacy of paclitaxel-eluting stents to prevent restenosis in humans. Indeed, drug eluting stents have become a primary treatment of coronary artery disease, and paclitaxel is one of only three drugs applied to stents that currently have FDA approval. To date, approximately 4.6 million paclitaxel stents have been implanted worldwide, making them the most frequently used drug-eluting stents. My lab is interested in novel strategies for vascular protection.

Collaborators: Salvatore Pepe, Ph.D., Royal Children's Hospital, Melbourne, Australia; Brian O'Rourke, Ph.D., Johns Hopkins University; Nazarino Paolocci, M.D., Ph.D., Johns Hopkins University; Edward G. Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Mark Mattson, Ph.D., Laboratory of Neuroscience, NIA, NIH; Rafael De Cabo, Laboratory of Experimental Gerontology, NIA, NIH; Kirsti Ytrehus, University of Tromsø, Tromsø, Norway; Paolo Bernardi, University of Padova, Italy; Andrea Rasola, University of Padova, Italy; K Shivakumar, PhD, Sree Chitra Tirunal Institute for Medical Sciences and Technology, India.

Collaborators-continued:

Ph.D., University of Louvain Medical School, Brussels, Belgium; Daria Mochly-Rosen, Ph.D., Stanford University School of Medicine; Suhn Hee Kim, M.D., Ph.D., Chonbuk National University Medical School, Chonjen, Korea; Kirsti Ytrehus, University of Tromso, Tromso, Norway.



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

Keywords:

cardiac functions
hemodynamics
microcirculation
heart failure
myocardial infarction

Recent Publications:

Ahmet I, et al. *J Pharmacol Exp Ther* 2008; 325: 491-499.

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

The experimental model of coronary ligation in rats expressed all facets of early and late, structural and functional remodeling described in the literature: increase of earlier and later apoptosis, dilatation of the ventricular chamber, compensatory myocyte hypertrophy, reduction of systolic function, myocardial stiffness, and diastolic dysfunction. For instance, early remodeling was characterized by the fall of ejection fraction (EF) from 60% to less than 40% (echocardiography), and, 24 hrs after coronary ligation, the 35% of cardiomyocyte nuclei across the area at risk were stained positively for apoptosis. During the next seven weeks the EF fell further, by 15% comparing with the value at week 1, and 3 times more of cardiomyocyte nuclei succumbed to apoptosis than in sham operated hearts. The most interesting functional characteristics of late remodeling were shown through pressure-volume analyses of left ventricular performance. Traditional index of systolic function, dP/dt showed a significant, 45% decline in coronary ligated rats. The more sophisticated, load-independent index

of systolic performance, Preload Recruitable Stroke Work showed even larger, more than 50% decline. The end-diastolic stiffness, Eed, doubled in MI rats indicating a diastolic dysfunction. The Ees, end-systolic elastance, one of components of myocardial contractility significantly fell in MI rats, while arterial elastance, the measure of after-load, increased, reflecting the very unfavorable relation (uncoupling) between LV and vascular system from the perspective of energy transfer - Ea/Ees ratio more than doubled in MI animals, i.e., weakened LV was pumping blood against increased vascular load.

Similar characteristics of left ventricular remodeling had been shown in the mouse model of coronary ligation. Moreover, in mice we not only mustered the technique for reliable induction of large myocardial infarctions by ligation of main left descending coronary artery, we delineated a technique for blind ligation of small left ventricular branches which reliably induced small, but transmural MI of predictable location and uniform size.

II. Prophylactic cardioprotective interventions:
Dietary restrictions, either reduced-energy-intake (RI) or Intermittent Fasting (IF), a dietary regimen in which food is available ad-libitum but only every other day, have been proven to increase lifespan and to reduce the incidence of age-associated diseases including cancer, diabetes and kidney disease in animal models. However, the effects of dietary restriction on the heart, its cardioprotective potential, and its potential to attenuate the development of post-MI CHF have not been experimentally documented. Thus, we examined the effects of long term IF and RI on acute and chronic cardiac responses to coronary artery ligation in rats.
After 3 months of IF or regular every day feeding (Control) diets, MI was induced in 5-mo old rats by a coronary artery ligation. Twenty four hours after induction, the MI size in the IF group was 2-fold smaller, the number of apoptotic myocytes in the area at risk was 4-fold less, and the inflammatory response was significantly reduced compared to the Control diet group. Serial echocardiography revealed that during 10 weeks following MI (with continuation of the IF regimen), left ventricular (LV) remodeling and MI expansion observed in the Control diet group were absent in the IF group. In a subgroup of animals with similar MI size at one week following MI, further observation revealed less remodeling, better LV function, and no MI expansion in the IF group compared to the Control group. Thus, IF protected the heart from ischemic injury and attenuated post-MI cardiac remodeling, likely via anti-apoptotic and anti-inflammatory mechanisms. On the other hand, the cardioprotectice effects of RI regimen were less expresed.

III. Translational Studies, Targeting Early and Late Left Ventricular Remodeling:

A) Targeting Early Remodeling: Erythropoietin Reduces Myocardial Infarction and Left Ventricular Functional Decline Following Coronary Artery Ligation in Rats: Erythropoietin (EPO), natural stimulant of erythropoiesis, recently emerged as potential antiapoptotic factor. We tested the hypothesis that single treatment with EPO will reduce the cardiac damage induced by coronary ligation and subsequent decline of cardiac function. In experiments in rats, we showed that single intraperitoneal injection of recombinant human EPO (3000 IU/kg) immediately after ligation of the coronary artery, results in 75% reduction of the size of myocardial infarction eight weeks later. During eight weeks after induction of myocardial infarction, left ventricular remodeling and function decline in EPO treated rats were significantly attenuated and statistically not different from that in sham operated animals. Twenty-four hours after ligation of coronary artery, the amount of apoptotic myocytes measured in the myocardial risk area (area immediately adjacent to the infarct site) was reduced in half in the EPO treated rats in comparison to untreated animals. Further experiment with different doses of EPO and treatment delays indicated that cell death, final MI size, myocardial remodeling, and functional decline are significantly reduced in rats by a single injection of rhEPO in a dose as low as 150 IU/kg, if administered during the first 4 hrs after the ischemic event. Higher doses extend the therapeutic window up to 12 hrs. A single i.v. injection of rhEPO immediately following MI in a dose as low as 150 IU/kg was as effective as 3000 IU/kg in causing a 2-fold reduction of the number of apoptotic nuclei in the AAR 24-hrs later, a 2-fold reduction of the MI size measured 4 weeks later, attenuation of progressive LV dilatation and fall in EF. A 3000 IU/kg dose had similar therapeutic effects when delayed by 4, 8, or 12 hrs following MI, but was not effective after a 24-hr delay. A single dose of 150 IU/kg was effective within 4 hrs post-MI, but was without effect if administered after an 8-hr delay.
In experiments comparing single and multiple, daily, EPO injections following coronary ligation we proved that multiple dosing of rhEPO after induction of MI in rats has no added therapeutic benefits over those achieved by a single dose. A single injection of rhEPO elevated Htc by11% ($p<0.05$) one week after coronary ligation, but after multiple rhEPO injections Htc increased by 40%. In untreated rats a 140% and 340% expansion in end-diastolic and end-systolic LV volumes respectively, and 55% decline in ejection fraction (EF) occurred during the four-week period following coronary ligation. A single rhEPO dose attenuated the LV remodeling and EF reduction by 50%. Repeated rhEPO injections did not

elicit any additional benefits in respect to LV remodeling. Moreover, at the end of four weeks, MI size was significantly reduced (by 40%) by a single injection, while after repeated rhEPO injections the reduction of MI size was not statistically significant.

B) Targeting Late Remodeling: Effects of Chronic Pharmacological Manipulations of ß-Adrenergic Receptor Subtypes Signaling in an Experimental Model of Dilated Ischemic Cardiomyopathy in Rats: The role of ß-adrenergic receptors (AR) subtype signaling in development of CHF is clearly important but purely understood. It is widely accepted now that ß-1AR activation is associated with development of CHF, thus, the use of ß-1 AR antagonists became a recommended therapy for HF. The possible role of ß-2 AR agonists remains debatable; however, it appears that similarly to ß-1 AR, activation of ß-2 AR during CHF is harmful. Recent research in the Laboratory of Cardiovascular Science using single myocytes indicated that ß-2 AR agonist, fenoterol, possesses a unique ability to activate Gs, but not Gi pathways. Capitalizing on this finding, we studied the effects of chronic treatment with ß-2 AR agonist, fenoterol, and ß-1 AR blocker, metaprolol, in rats starting 2 weeks after ligation of a coronary artery. Our results indicated that both, ß-2 AR agonist and ß-1 AR blocker reduced the apoptosis in myocardium and attenuated the development of CHF, i.e. left ventricular remodeling and functional decline. However, they affected different aspects of cardiac function: metaprolol improved systolic cardiac performance by increasing left ventricular elastance, while fenoterol achieved the same result by reducing the arterial elastance (after-load). Metaprolol did not improve diastolic function, while fenoterol normalized it. Only fenoterol treatment arrested the infarct expansion, resulting in actual decrease of the infarct relative size. The addition of chronic pharmacological ß2AR stimulation to a therapy with ß1AR blockade enhanced the therapeutic effects of ß1AR blockade on cardiac remodeling, but did not exceeded the effect of ß2AR stimulation alone. The ultimate experimental, preclinical evidence of therapeutic benefit of ∃2 AR agonists or combination of ∃2 AR agonists and∃1 AR blockers for CHF requires a long-term experiment with survival as a primary outcome. Thus, at the same experimental model, we conducted a year-long experiment, in which we compared the survival benefits and effects on remodeling in rats subjected to mono-therapy with ß1AR blocker, metoprolol or ß2AR agonist, fenoterol, or treated with combination of both. The results of this experiment clearly demonstrated the superior effect of combined therapy compared to either mono-therapy. Survival analyses showed that 67% mortality observed in untreated MI rats was reduced to 33% in combine treatment group ($p<0.01$). Progressive cardiac remodeling observed in untreated group and group treated with ∃1AR blocker was significantly

attenuated in combined therapy group during the first six months of treatment. Only in the combined therapy group was MI expansion completely prevented, and functional decline significantly attenuated during the entire year. Myocardial apoptosis was significantly reduced in both combined and ∃1AR blocker groups. A reduction of cardiac ∃1AR density and decreases in chronotropic and contractile responses to ∃2 AR specific stimulation in the absence of a reduction of ∃2 AR density in untreated MI rats were precluded in rats receiving combined therapy. Interestingly, during the first three months of therapy, the effects were similar to those previously described, i.e., combined treatment and mono-therapy with ∃2 AR agonist were equally effective. However, after three months the effectiveness of mono-therapy with ∃2 AR agonist started to wane. Therefore, the results demonstrate the cardioprotective and survival benefit of long-term combination therapy of ∃2AR agonists and ∃1AR blockers in the model of DCM.

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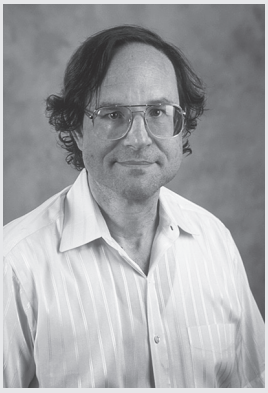

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Biography: Dr. Stern studied theoretical physics at Princeton and received an M.D. degree from University of Pennsylvania. Following internship, he was a Staff Fellow in the Laboratory of Technical Development of the NHLBI, where he invented a method 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, which 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
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Recent Publications:

Tetievsky A, et al. *Physiol Genomics* 2008; 34: 78-87.

Ríos E, et al. J *Gen Physiol* 2008; 131: 335-348.

Cohen O, et al. *J Appl Physiol* 2007; 103: 266-275.

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 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.

More recently, it has been found in our laboratory that "spontaneous" local calcium releases from ryanodine receptors, propagated among several regional couplons, play a critical role in the initiation of the heartbeat in pacemaker cells. We have constructed schematic mathematical models of this process that appear to be capable of explaining many of the pathways of hormonal and neural regulation of the heart rate. To fully understand how this release events are generated and propagated will require extending our previous stochastic couplon model to three dimensions. This computationally daunting task is now within range thanks to improvements in computer hardware. Software to perform this 3D modeling on computational clusters is now under development in our lab.

Mathematical Modeling of Evolutionary Dynamics. As a spin-off from our stochastic modeling efforts, we have developed simulations of the Darwinian evolution of populations of virtual organisms, in order to study how fundamental properties, like robustness to external noise, arise from blind selection. Early versions of this model, which is based on organisms which are Boolean networks, demonstrated a variety of surprising phenomena, including "noise imprinting" in which entirely extraneous data from the environment becomes incorporated into the functioning of organisms as they evolve, so that the advanced organisms are completely dependent on these meaningless elements. With more advanced computer power, we are now examining a model in which organisms exchange "programs", analogous to human cultural evolution, in the hopes of understanding how irrational elements are incorporated into cultural systems, and whether they serve a "survival" function or are parasitic.

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Keywords:

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Recent Publications:

Bagrov AY, et al. *Nat Clin Pract Nephrol* 2008; 4: 378-392.

Anderson DE, et al. *Am J Physiol Regul Integr Comp Physiol* 2008; 294: R1248-R1254.

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Elkareh J, et al. *Hypertension* 2007; 49: 215-224.

Our main objective is to understand the role of Na/K-ATPase (NKA) and its endogenous digitalis-like inhibitors in salt-sensitive hypertension. Endogenous digitalis-like cardiotonic steroids (CTS), endogenous ouabain and marinobufagenin (MBG), coexist in mammalian tissues. MBG acts as a selective inhibitor of alpha-1 isoform of NKA, the main isoform in the kidney and vascular smooth muscle. Salt-sensitive hypertension accounts for 40% of the hypertensives worldwide. The molecular mechanisms of salt-sensitivity are not well understood, and one theory attributes CTS a central role in the pathogenesis of salt-sensitive hypertension. MBG, a natriuretic, inhibits NKA in renal epithelial cells and reduces the sodium ions reabsosrption. An excessive production of MBG induces inhibition of vascular NKA, which raises the blood pressure (BP). In salt-sensitive hypertension, brain endogenous ouabain triggers peripheral MBG though the angiotensin II-sensitive pathway. Our goals are: (i) To perform pharmacological analyses of central prohypertensive effect of a very low (physiologically relevant) concentrations of ouabain in salt-sensitive Dahl rats (DS); (ii) To study age- and gender-associated differences in MBG production after prolonged moderate NaCl-loading of human subjects; (iii) To develop a therapeutic antibody for immunoneutralization of CTS.

In DS, intra-hippocampal administration of 60 pg of ouabain induces activation of renin-angiotensin system (RAS) in the supraoptical nucleus of hypothalamus, and in the pituitary. This activation causes sympathoactivation, which triggers adrenocortical renin-angiotensin system, followed by increase in MBG production. This sequence of events has been analyzed via pharmacological interventions on several levels via administration of anti-ouabain antibody in the supraoptical nucleus of hypothalamus, central and systemic administration of

losartan, and via peripheral adrenoceptor blockade. The results permit to establish a hierarchy in the complex interactions between brain ouabain, central and peripheral RAS, sympathetic nervous system, and peripheral CTS, which underlie the onset of salt-sensitive hypertension.

The impact of gender and age on CTS response to NaCl-loading has been studied in normotensive humans in collaboration with Lund University, Malmo (Sweden). In this study, a moderate (150 mmol/day) NaCl-loading resulted in an increase in MBG levels, and a moderate elevation of BP. In men, plasma and urine MBG levels correlated with BP, and baseline MBG levels predicted salt-sensitivity of the BP. In aged women, however, this relationship was the opposite. Notably, in healthy subjects of both sexes, levels of MBG declined with age, whereas salt-sensitivity of BP is increased with age. Thus, not only excessive production of MBG, but also a failure in the response of this hormone to NaCl-loading may underlie age-associated increase in salt-sensitivity of BP.

MBG promotes natriuresis via inhibition of renotubular NKA, but may cause vasoconstriction via inhibition of the NKA in the vasculature. ANP, via cGMP/PKG-2-dependent phosphorylation of renal alpha-1 NKA, sensitizes renal tubuli to MBG and potentiates natriuretic action of MBG. In the vasculature, on the opposite, ANP, via PKG-1 dependent mechanism, reduces NKA phosphorylation and may offset the excessive vasoconstriction induced by MBG. Since aging is associated with a down-regulation of cGMP/PKG signaling, we hypothesized that in aged rats, ANP would not potentiate renal effects of MBG, and would not oppose vascular effects of MBG. In young (3 month old) and aged (24 months old) Sprague-Dawley rats, we compared systolic blood pressure (BP), natriuresis, NKA activity in renal medulla and in vascular sarcolemma, and levels of MBG and alpha-ANP following acute NaCl loading, and the in vitro interactions of MBG and alpha-ANP on the NKA. As compared to young rats, NaCl-loaded aged rats exhibit greater MBG response, greater BP elevation and greater inhibition of NKA in aortae, less natriuresis and less inhibition of NKA in renal medulla in the presence of comparable changes in alpha-ANP and cGMP levels. Levels of PKG-1 in aorta and PKG-2 in the kidney in aged rats were markedly reduced, while levels of PDE-V in the kidney were increased. In aged rats, ANP did not affect level of NKA phosphorylation in aortic sarcolemma and renal medulla, did not potentiate MBG-induced inhibition of renal NKA, and did not reduce MBG-induced inhibition of vascular NKA. Thus, in aged rats, down-regulation of cGMP/PKG

dependent signaling underlies a shift in ANP modulation of the effect of MBG on renal and vascular sodium pump, which promotes salt-sensitivity.

Although preeclampsia (PE) is a major cause of maternal and fetal mortality, its pathogenesis is not fully understood. CTS are implicated in the pathophysiology of PE, as illustrated by clinical observations that Digibind, a digoxin antibody, which binds CTS, lowers BP in patients with PE. Plasma levels of MBG are increased four-fold in patients with severe PE. We compared levels of MBG in normal and preeclamptic placentae, and tested the antibodies against MBG and against ouabain for their interaction with the material purified from preeclamptic placentae via high-performance liquid chromatography (HPLC). Levels of MBG, but not that of endogenous ouabain, exhibited a four-fold elevation in preeclamptic placentae. The elution time of endogenous placental MBG-like immunoreactive material from reverse-phase HPLC column was identical to that of authentic MBG. Immunoassay, based on Digibind, did not detect cross-reactivity with HPLC fractions, containing ouabain-like immunoreactive material, but cross-reacted with HPLC fractions, having retention time similar to that of MBG and other bufadienolides. This observation indicated that MBG and related bufadienolides are the targets for Digibind. Together with our previous data, showing that anti-MBG monoclonal antibody reduces BP in experimental PE, our results suggest that MBG is a potential target for immunoneutralization in patients with PE.

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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 postdoctoral 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.

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Recent Publications:

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Gundry RL, et al. *Proteomics Clin Appl* 2008; 2: 892-903.

Tarasov KV, et al. *Cells Tissues Organs* 2008; 188: 31-45.

Li J, et al. *J Biol Chem* 2007; 282: 34984-34993.

The primary focus of our research program is two-fold: 1) the analysis of undifferentiated stem cells and early signals of differentiation, and 2) the use of an *in vitro* differentiation model of mouse and human embryonic stem (ES) cells to understand processes associated with cardiomyocyte differentiation. The Unit actively participates with other groups in the Laboratory of Cardiovascular Science to study aging in rodents and humans, human heart failure and questions associated with apoptosis.We employ a number of molecular, cellular and functional techniques to address these questions; and specifically, we examine the consequences of development and of altered gene expression on the function of specific proteins or lineage commitment. The long-term aim of the research in the laboratory is on the eventual use of these cells and other stem cells both as models for cardiomyocyte differentiation and for therapeutic applications in aging and disease. We actively exploit functional genomics and proteomics to examine the molecular basis of differentiation, development, aging and disease.

Mouse Embryonic Stem Cells, Myocardial Development and Therapeutics: Knowledge of the transcriptional circuitry responsible for pluripotentiality and self-renewal in embryonic stem (ES) cells is tantamount to understanding early mammalian development; however the use of ES cells is currently restricted by our limited knowledge of the mechanisms controlling their differentiation. We have therefore employed genomic analyses to identify novel cis-element frameworks that might be implicated in the control of ES cell-restricted gene promoters. Specfically, we exploited the techniques of serial analysis of gene expression (SAGE) to generate a molecular profile of undifferentiated cells and used bioinformatics to model frameworks of cis-binding elements in the promoter regions of ES predominant genes. This has led to the identification and characterization

of novel transcription factors implicated in ES cell regulation. One transcription factor identified from these analyses was myeloblastosis viral oncogene homolog-like 2 (Myblw), which had previously been shown to be critical to the formation of inner cell mass (Tanaka et al, JBC, 1999). Although this transcription factor regulates cell cycle progression and gene transcription in non-ES cells, its role in ES cells has remained enigmatic. We have therefore examined this factor extensively. RNA and immunostaining indicate that Mybl2 is prominently expressed in mouse and human ES cells that that it is dynamically regulated with differentiation. Chromosomal immuno-precipitations (ChIP) show that MYBL2 binds to the promoters of several critical stem cell factors, including oct3/4, sox2, myc and nanog, and that knockdown of Mybl2 with shRNAs in mouse ES cells leads to a decrease in Oct3/4, Sox2 and Nanog transcripts, concomitant with a loss of the undifferentiated phenotype and a decrease in proliferation. The latter is accompanied by a delay or block in the cell cycle phase of G2/M, suggesting that this factor is critical for the regulation of other factors (e.g., Cyclin B1) implicated in cell cycle control. Promoter analyses furthermore indicate that MYBL2 actively regulates oct4 transcriptional activity *in vitro*. Consistent with this observation is the finding that transient transfections to over-express Mybl2 led to elevated levels of Oct3/4 and Sox2, and that mutation of a mybl2 binding site in the human oct4 promoter alters its activity. In stably transfected ES cells lines, Sox2 but not Oct3/4 transcripts were significantly elevated. Mybl2 over-expressing ES cell clones did not differ phenotypically from control ES cells lines, and over-expression of Mybl2 was unable to prevent differentiation following LIF or serum and LIF withdrawal. The over-expressing cell lines, however, demonstrated signficantly higher levels of FGF5 (a marker of primitive ectoderm) upon serum and LIF withdrawal, and the cells had a preferential differentiation to endodermal and mesodermal lineages (including the generation of cardiomyocytes).

Because stably transfected lines acted differently than transiently transfected ES cells, we have gone on to evaluate whether Mybl2 might be regulated post-transcriptional. Our analyses indicate that the protein transiently decreases within 2-4 hours before returning to normal levels 24 hours after serum and LIF withdrawal. Accompanying this was a dramatic change in the phosphorylation status of MYBL2. These studies highlighted changes in the proteome that occur following a stimulus to differentiation. To follow up on these changes, together with Dr. Van Eyk (Johns Hopkins University), we published the first large-scale proteomic analysis of R1 ES cells. The proteomic analysis of undifferentiated mouse R1 ES cell lines using pH 3-10 2-dimensional electrophoresis (2-DE) gels, matrix-assisted laser desorption/ionization & tandem mass spectrometry identified 260 gel spots that were analyzed. Of these, 123 protein species

were identified, which corresponded to 111 unique proteins. A majority of these were functionally implicated in protein expression. This original study has been expanded to compare the proteomes of several ES cell lines and the changes that occur upon differentiation and after manipulation of the transcription factor(s) identified from our SAGE analyses.

With regards to the *in vitro* differentiation of cardiomyocytes, we employ wild type (R1, D3 cell lines) and ES cell clones that are genetically modified. The research is aimed at understanding the developmental processes involved in cardiac myocyte differentiation and development. Of particular interest are signals that promote differentiation, proliferation and survival. To identify, cardiac cells from the heterogeneous population of cells that arise from differentiating ES cells, expression vector constructs have been made that link cardiac restricted promoters to the green or red fluorescence protein (GFP or RFP) and other selection markers. As a tissue-restricted promoter, we employed the Ncx1 promoter (a distal upstream portion of the promoter) to identify and characterize cardiac myocytes derived from mouse ES cells. From the stable clones containing these constructs, we have begun growing and isolating purified cardiomyocytes at different stages of differentiation for molecular, cellular and functional analyses. Our early studies, which relied on an antibiotic-selection cassette, involved the selection and characterization of relatively mature differentiated cells. Cardiac specific resistance to G418 resulted in an apparent homogenous cardiomyocyte population. Purified and non-selected cardiomyocyte populations were characterised electrophysiologically using patch clamp. Based on the cardiospecific expression of cardiac troponin I mRNA we observed that 31±3.7% of the volume of a culture of differentiating ES cells consists of cardiomyocytes. We also found that transcription factors involved in cardiomyogenesis are expressed at levels similar to embryonic, neonatal and adult hearts. Based on these earlier studies, we have now established new clonal lines that contain fluorescent markers to identify very early heart cells that maintain the ability to divide. Our unpublished data indicate that early cells rapidly incorporate BrdU and the cardiac myocyte numbers increase rapidly. Some of these cells, may include cardiac progenitors, and we are now trying to isolate and expand this population to test therapeutic viability in rodent models of heart failure. Interestingly, the differentiated cardiac myocytes appear to survive longer-term when isolated from the heterogeneous embryoid body following antibiotic selection. We also have data with lines over-expressing Mybl2 that suggest an improved differentiation to cardiac myocytes. These latter two findings are the the focus of on-going research.

Finally, in our on-going analyses of excitation-contraction coupling of ES cells, we used wild-type ryanodine receptor ($RyR2^{+/+}$) and RyR2 null ($RyR2^{-/-}$) ES cells-derived cardiomyocytes (ESCMs) as an *in vitro* model of cardiomyogenesis, together with pharmacological approaches and expression profiles of genes relevant for SR function, to elucidate the functional importance of RyR2 and SR on the regulation of Ca^{2+} transients and contraction during early cardiomyocyte development. During differentiation of $RyR2^{+/+}$ ESCMs, SR-function developed progressively with increased basal cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$), enhanced frequency and amplitude and decreased duration of Ca^{2+} transients that were inhibited by ryanodine and thapsigargin. These functional traits correlated with SR Ca^{2+} load and the expression of RyR2, SERCA2a and phospholamban. $RyR2^{-/-}$ ESCMs, comparatively, demonstrated a significantly prolonged time-to-peak and reduced frequency of Ca^{2+} transients and contractions. ß-adrenergic stimulation of $RyR2^{+/+}$ ESCMs increased the frequency and amplitude of Ca^{2+} transients with differentiation, but was much weaker in $RyR2^{-/-}$ ESCMs. We concluded that the function of the RyR and the development of the sarcoplasmic reticulum are crucial to the ability of the cells to respond to ß-adrenergic stimulation, and that these cells progressively mature as a function of *in vitro* cultivation time.

Human Embryonic Stem Cells: Human ESCs are now the subject of intensive investigation in the laboratory for potential applications in developmental biology and medicine. Much of the work is an extension of what we have learned from mouse ES cells, and our focus has been on 1) the characterization of undifferentiated hESCs and early signals for differentiation and 2) the differentiation of these cells to form cardiomycoytes. A promising aspect of hESCs is their ability to differentiate into cardiomyocytes, which generally lack the capacity to regenerate, and therefore their potential for cell-replacement heart therapies. Molecular, cellular and physiological analyses demonstrate that hESC-derived CMs are functionally viable and that they exhibit characteristics typical of heart cells in the early stages of cardiac development.

Adult Stem Cells and Progenitor Cells: Adult stem cells hold some promise for the generation of cardiomyocytes and other adult lineages. To this end, we have examined the potential of selected types of stem or progenitor cells to form cardiomyocytes i.e., mesenchymal stem cells and intestinal derived progenitor cells. In neither case, did these cells readily form cardiomyocytes suggesting that the cells had a limited potential to form cardiomyocytes *in vitro*. These studies did however lead to a greater understanding of the molecular mechanisms underlying stem cell fate. To-

gether with our colleagues in Germany (Anna Wobus), we have described the derivation of nestin-positive cells from adult mouse and human intestinal epithelium (INPs, intestinal epithelium-derived nestin-positive progenitors). The formation of these cells required cultivation on inactivated mouse embryonic fibroblasts (MEFs), which are typically used for cultivation of mouse and human ES cells. A SAGE and Q-RT-PCR analysis of MEFs revealed Wnt/BMP-signaling molecules as potential factors that contributed to the formation of mouse INPs *in vitro*, and we demonstrate that an increase in Lef1, Wnt4, Wnt5a and Wnt/BMP-responsive factors, but a decrease of BMP4/mash-1 transcript abundance was associated with mouse INP formation. Early passage INPs demonstrated a high proliferation capacity, which was lost with continued cultivation. *In vitro*, mouse INPs differentiate into cells expressing neural, pancreatic and hepatic, but not cardiac transcripts and proteins with functional characteristics typical of these immature cell types. We conclude that nestin expression is a functional marker of reprogrammed INPs, and that this intermediate filament will be useful to identify other stem/ progenitor cells, which are amenable to differentiation and regeneration in heterotypic tissues.

Collaborators: Michael Crow, Johns Hopkins University; Jennifer Van Eyk, Johns Hopkins University; Professor Brenda Russell, University of Illinois, Chicago; Professor Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany; Edward G. Lakatta, Laboratory of Cardiovascular Science, NIA, NIH.

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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 physician and pharmacologist at the Tong-Ji Medical University in China and the University of Maryland in the USA, where she received her M.D. and Ph.D., respectively. In 1996, she became an Investigator and the Head of the Receptor Signaling Unit at the Laboratory of Cardiovascular Science (LCS), where she has been a Senior Investigator (tenured) since 2003. Currently, she serves as a Council Member for the International Society of Heart Research (ISHR) and the International Academy of Cardiovascular Science (IACS), and a member of the American Society for Clinical Investigation (ASCI). In addition, she serves as an Editorial Board Member for 6 scientific journals, including *Circulation Research* and *Journal Molecular and Cellular Cardiology*.

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Recent Publications:

Woo AYH, et al. *Mol Pharmacol* 2008; 75: 158-165.

Ahmet I, et al. *J Pharmacol Exp Ther* 2008; 325: 491-499.

Luo D, et al. *Cell Calcium* 2008; 43: 165-174.

Guo X, et al. *Circ Res* 2007; 101: 1113-1122.

Shen T, et al. *J Biol Chem 2007*; 282: 23354-23361.

Jozwiak K, et al. *J Med Chem* 2007; 50: 2903-2915.

Her main scientific focus has been on G-protein coupled receptor (GPCR) signaling in the cardiovascular system. The scope of her scientific work covers three intertwined programs: (**I**) ß-adrenergic receptor (ßAR) subtype signaling in cardiovascular system: from bench to the bedside; (**II**) Regulation of cardiac myocyte viability and excitation-contraction coupling by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) in normal and failing hearts; and (**III**) Identification and characterization of cardiovascular disease-related genes. These studies integrate information gleaned from multidisciplinary approaches at different levels (molecule, pathways, transcriptome, proteome, cell, organ, and whole animal) in conjunction with adenoviral gene transfer, RNA interference, bioinformatics, and gene-targeted mouse models or disease-related animal models.

ß-Adrenergic Receptor Subtype Signaling in the Heart: From Bench To the Bedside

ßAR stimulation by the sympathetic nervous system or circulating catecholamines is broadly involved in metabolic regulation, muscle contraction, and growth control. In the heart, acute ßAR stimulation serves as the most powerful means to regulate cardiac output in response to a fight-or-flight situation, whereas chronic ßAR stimulation plays an important role in physiological and pathological cardiac remodeling.

There are at least two ßAR subtypes, $ß_1$AR and $ß_2$AR, present in cardiac myocytes. Over the past decade, we have vigorously pursued the molecular and cellular mechanisms underlying the distinctly different even

Publications-continued:

Zhu W, et al. *J Biol Chem* 2007; 282: 10833-10839.

Yang D, et al. *Circ Res* 2007; 100: 399-407.

opposite functional roles of these ßAR subtypes in regulating cardiac structure and function, with keen interest in the development of novel therapies based on our bench discoveries. Three discoveries have marked our research line with respect to ßAR subtype-specific signal transduction. These include: (**1**) dual coupling of $ß_2$AR to both G_s and G_i proteins in cardiomyocytes; (**2**) cardiac protective role of $ß_2$AR signaling in improving cardiac function and myocyte viability; (**3**) PKA-independent, CaMKII-mediated $ß_1$AR apoptotic and maladaptive remodeling signaling in the heart. These studies demonstrate that $ß_2$AR might be a "friend" in need due to its concurrent anti-apoptotic effect and contractile support, whereas $ß_1$AR is recognized as a "foe" in the context of heart failure.

Congestive heart failure (CHF) is a complex clinical syndrome characterized by extensive abnormalities in the ßAR system, including ßAR down-regulation, reduced receptor signaling efficiency, increased G_i signaling, and elevated circulating catecholamine levels. However, the heart failure-associated loss of ßAR is selective for $ß_1$AR, with little change in $ß_2$AR subtype. Previous studies have demonstrated that $ß_1$AR and $ß_2$AR stimulation oppositely regulate cardiomyocyte viability and myocardial remodeling with $ß_1$AR detrimental and $ß_2$AR protective and that $ß_1$AR blockade exhibits salutary effects on patients with CHF, becoming a major therapy in the treatment of CHF. Thus, we envisioned and advocated that activation of $ß_2$AR in combination with clinically used $ß_1$AR blockade should provide a potential novel therapy with greater efficacy and fewer side effects for the treatment of CHF. Recently, we have focused on further delineating the molecular and cellular mechanism underlying the unique $ß_2$AR coupling to G_i proteins and translating the newly appreciated ßAR subtype signaling principles into drug development and novel therapies that improve the function and structure of the failing heart.

I. *Dual Coupling of Cardiac $ß_2$-Adrenergic Receptor to G_s and G_i Proteins*

GPCRs constitute the largest class of cell surface signaling molecules and serve as targets for >65% currently available pharmacuetic drugs. By activating their cognate heterotrimeric guanosine triphosphate (GTP) binding proteins (G proteins), GPCRs transduce stimulatory or inhibitory signals for a wide array of endogenous hormones and neurotransmitters, and ambient physical and chemical stimuli, as well as exogenous therapeutic reagents. ß-adrenergic receptors (ßARs) are archetypical members of the GPCR superfamily. There are, at least, both $ß_1$AR and $ß_2$AR present in heart muscle cells. Whereas both ßAR subtypes stimulate the classic G_s-adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade, $ß_2$AR

can activate bifurcated signaling pathways through G_s and G_i proteins. As a consequence, $ß_1$AR and $ß_2$AR exhibit opposing effects on heart cell survival: $ß_1$AR activation can promote programmed heart cell death (apoptosis); in sharp contrast, $ß_2$AR activation can protect heart cells from a wide range of assaulting factors, including enhanced $ß_1$AR stimulation, hypoxia, and reactive oxygen species. Furthermore, we have shown that sustained $ß_1$AR stimulation promotes cardiac myocyte apoptosis by activation of Ca^{2+}/calmodulin kinase II (CaMKII), independently of PKA signaling. Taken together, the differential G protein coupling, to a large extent, accounts for the distinctly different physiological and pathological roles in the heart for $ß_2$AR versus those of $ß_1$AR. The opposite effects of $ß_1$AR and $ß_2$AR on the fate of cardiomyocytes also reveal the rationale for selective $ß_1$AR blockade with concurrent $ß_2$AR activation as a novel therapy to treat chronic heart failure.

II. *Enantiomer-Specific $ß_2$AR-G protein Coupling in Cardiac Myocytes*

It is now well established that any given ligand for a G-protein-coupled receptor (GPCR) does not simply possess a single defined efficacy. Rather, a ligand possesses multiple efficacies, depending on the specific down-stream signal transduction pathway analysed. This diversity reflects ligand-specific GPCR conformations and is often referred to as "Functional Selectivity". It has been known for a century that stereoisomers of catecholamines differ in their potency, and in some systems, differences in catecholamine efficacy were observed, too. However, the molecular basis for differences in efficacy of GPCR ligand stereoisomers has remained poorly defined till now. Specifically, fenoterol, a selective $ß_2$AR full agonist, contains two chiral centers and may exist as four stereoisomers. We have synthesized a series of stereoisomers of fenoterol and its derivatives and characterized their receptor binding and pharmacological properties. We have found that the R,R-isomers of fenoterol and methoxyfenoterol exhibited more potent effects to increase cardiomyocyte contraction than their S,R-isomers. Importantly, while R,R-fenoterol and R,R methoxyfenoterol preferentially activate Gs signaling, their S,R-isomers are able to activate both G_s and G_i proteins as evidenced by the robust pertussis toxin-sensitivities of their effects on cardiomyocyte contraction and on phosphorylation of extracellular signal-regulated kinase 1/2. The differential G protein selectivities of the fenoterol stereoisomers are further confirmed by photoaffinity labeling studies on G_s, G_{i2} and G_{i3} proteins. The inefficient Gi signaling with the R,R-isomers is not caused by the inability of the R,R-isomers to trigger the PKA-mediated phosphorylation of the $ß_2$-adrenoceptor, since the R,R-isomers also markedly increases phosphorylation of the receptor at serine262 by PKA. We conclude that in addition to receptor subtype and phosphorylation status, the stereo-

chemistry of a given agonist plays an important role in determining receptor-G protein selectivity and downstream signaling events.

This study is important, because it is the first account to show that even the subtle chemical differences within a ligand stereoisomer pair are sufficient to stabilize GPCR conformations with distinct G-protein coupling properties. The study highlights of how important it is to carefully examine both the "active" and the "inactive" stereoisomer to understand the exact mechanism of action and cellular effects of a GPCR ligand. This study may "ignite a renaissance of the analysis of ligand stereoisomers, using sensitive pharmacological and biophysical assays", as commented by the associated Perspective published in Molecular Pharmacology 2008. Furthermore, the Perspective pointed out that "meticulous analysis of ligand stereoisomers is a goldmine for understanding mechanisms of GPCR activation, analysis of signal transduction pathways, development of new therapies for important diseases and drug safety".

III. ***Development ß$_2$AR Agonists Into New Drugs for The Treatment of Congestive Heart Failure***

A hallmark of congestive heart failure (CHF) is the desensitization of ßAR signaling, characterized by downregulation of ßAR, reduced signaling efficient of remaining receptors, increased G_i signaling, and elevated circulating catecholamine levels. However, CHF-associated loss of βAR is selective for ß$_1$AR, with little change in ß$_2$AR. Previous studies have demonstrated that (**a**) ß$_2$AR dually couples to the G_i and the G_s signaling pathways in the heart with the G_i coupling negating the G_s-mediated contractile response, whereas ß$_1$AR couples solely to the G_s signaling cascade; (**b**) ß$_1$AR and ß$_2$AR stimulation oppositely regulate cardiomyocyte viability and myocardial remodeling with ß$_1$AR detrimental and ß$_2$AR protective; (**c**) ß$_1$AR blockade exhibits salutary effects on patients with CHF, thus becoming a major therapy in the treatment of CHF. Over the past several years, a joint research program involving efforts from multiple laboratories is to translate ßAR subtype signaling principles into drug development and novel therapies to improve the structure and function of the failing heart. Specifically, we tested the hypothesis that G_s-selective activation of ß$_2$AR alone or in combination with clinically used ß$_1$AR blockade should provide a potential novel therapy with greater efficacy and fewer side effects for the treatment of CHF.

(1) Signaling-Selective β$_2$AR Agonists for the Treatment of Congestive Heart Failure: In CHF, impaired βAR response is often associated with increased Gi signaling and selective downregulation of β_1AR (higher β_2/β_1 ratio). We have demonstrated that inhibition of G_i with PTX restores the markedly depressed β_2AR contractile response in myocytes from two rat

heart failure models. Further, we have identified a unique ß$_2$AR agonist, fenoterol, which selectively activates ß$_2$AR-Gs signaling, bypassing the Gi pathway, fully reverses the diminished ß$_2$AR inotropic effect in myocytes from failing spontaneously hypertensive rat (SHR) hearts in the absence of PTX. This suggests that selective activation of the β_2AR-G_s signaling may provide a useful therapeutic target for the treatment of CHF.

Follow-up *in vivo* studies by colleagues at the LCS have further demonstrated that prolonged use of fenoterol not only improves cardiac function, but also retards cardiac maladaptive remodeling, and that the overall beneficial effects of fenoterol are greater than the salutary effects of ß$_1$AR blockade in a myocardial infarction (MI) induced rat CHF model. Specifically, the effectiveness of the ß$_2$AR agonist in attenuating left ventricle (LV) dilatation, functional decline, and myocyte apoptosis significantly exceeds that of the clinically sued ß$_1$AR blocker, metoprolol. The therapeutic effect of ß$_2$AR stimulation has been recently confirmed in a rat model of autoimmune myocarditis.

(2) *A Combination of ß$_2$AR Activation With ß$_1$AR Blockade Provides A More Effective Therapy For The Treatment of CHF:* In collaboration with Dr. Mark Talan's lab, our long-term (12-mon) in vivo studies have revealed that in the rat CHF model, ß$_2$AR activation alone or a combination of ß$_1$AR blockade with ß$_2$AR activation are superior to β_1AR blockade alone in preventing MI expansion, improving animal survival, and attenuating LV maladaptive remodeling, functional decline, myocyte apoptosis, and arrhythmia. These in vivo studies suggest that a combination of β_2AR activation with the clinically used ß$_1$AR blockade may provide a more effective therapy for the treatment of CHF.

(3) *Characterization of Fenoterol Enantiomers And Derivatives: Developing Novel Therapies With Greater Selectivity and Efficacy and Fewer Side Effects:* To develop this potential therapeutic target and move it toward clinical trial, we have collaborated with colleagues at the LCI of NIA, and initiated this translational approach. This approach is based upon the recognition that fenoterol has two chiral centers, and is, therefore, a mixture of 4 stereoisomers. These stereoisomers can be separated and individually studied to determine which one(s) confer pharmacological activity in this system. We have made following progresses:

- We synthesized these fenoterol enantiomers and a cohort of fenoterol derivatives and filed the necessary patent for the synthesis of R,R-fenoterol and its derivatives to pave the way for the development of ß$_2$AR

agonists with greater selectivity, efficacy and fewer side effects.

- We have demonstrated that R,R-fenoterol is the most potent among the fenoterol enantiomers as evidenced by receptor binding affinity and cardiomyocyte contraction assay. Moreover, R,R-fenoterol could protect myocytes against various insults, including excessive catecholamine (ISO) stimulation and oxidative stress with H_2O_2, while S,S-fenoterol is inactive in either assay.
- We have identified the single isomer, R,R'-fenoterol, with selective activity at the β_2AR signaling provides a unique opportunity for further new drug discovery. Using computer modeling, we have designed and synthesized 25 compounds. Among them, sub-micromolar binding affinities for the β_2AR and positive inotropic effects on adult rat cardiomyocytes are observed for R,R-fenoterol, the R,R-isomer of the methoxy, and R,R- and R,S-isomers of the naphthyl, derivatives. In addition, the $K_i\beta_1$AR/ $K_i\beta_2$AR ratios are >40 for R,R-fenoterol, and the R,R-methoxy and R,S-naphthyl derivatives and 14 for the R,R-napthyl derivative.
- In order to achieve our ultimate goal, a Phase 1 Study of R,R-Fenoterol: "Initial Clinical Evaluation for Pharmacokinetics, Safety and Efficacy Biomarkers" has been initiated. In our ongoing studies, we are systematically characterizing fenoterol derivatives, R,R-methoxy-fenoterol, R,R-naphthyl-fenoterol and R,S-naphthyl-fenoterol in our cell-based experimental system and *in vivo* in genetic or surgical cardiovascular disease models. Initial pharmacokinetic studies in rats have demonstrated that the new fenoterol derivatives have better plasma distribution profiles than R,R-fenoterol. Based upon the results of the Phase 1 study with R,R-fenoterol and *in vivo* animal studies, some of the promising new ß$_2$AR agonists will be moved to clinical trials and developed as new drugs. The development of R,R'-fenoterol and its derivatives into new drugs for the treatment of CHF is currently an institute-wide program at NIA, involving the joint efforts of the LCS, LCI, and ASTRA Unit. Continued efforts on this research line may lead to the development of potential novel therapies with greater selectivity, efficacy and fewer side effects for the treatment of human CHF.

Regulation of cardiac myocyte viability and excitation-contraction coupling by CaMKII in normal and failing hearts

I. *Opposing Functional Roles of CaMKII-δ_B and CaMKIIδ_C in Regulating the Fate of Cardiac Myocytes.*

Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a multifunctional protein kinase activated by the complex of Ca^{2+} and calmodulin. CaMKII

mediates phosphorylation of a wild range of target proteins involved in a multitude of cellular processes such as Ca^{2+} handling, cell growth, and cell death. The δ isoform of CaMKII family is predominantly expressing in cardiac myocytes. There are, at least, two splicing variants of CaMKII-δ, δ_B and δ_C, located in unclear and cytosol compartments, respectively. Our previous studies have shown that enhanced CaMKII-δ_C activation is both necessary and sufficient for β_1AR- and other death stimuli-induced cardiomyocyte apoptosis, in addition to its well-established functions in regulating phosphorylation of cardiac Ca^{2+} handling proteins and thus modulating cardiac excitation-contraction coupling. However, the functional role of CaMKII-δ_B remains elusive. The aim of the present study is to investigate the potential physiological and pathological functional roles of CaMKII-δ_B in the heart and explore its clinical implications.

Here we report a novel function of CaMKII-δ_B as a potent suppressor of oxidative stressed-induced cardiomyocyte apoptosis. First, CaMKII-δ_B expression is remarkably attenuated at both mRNA and protein levels in rat ischemia/reperfusion (I/R) and myocardium infarction (MI) models and in cultured cardiac myocytes in response to oxidative stress with H_2O_2. The reduction of CaMKII-δ_B expression was mirrored by increased CaMKII-δ_C abundance. The inhibitory effects of MI and H2O2 on CaMKII-δ_B are fully prevented by ROS scavengers, indicating ROS constitutes a negative regulator of CaMKII-δ_B gene expression. Concurrently, MI and H_2O_2 markedly increase myocyte apoptosis *in vivo* and in culture, respectively, assayed by DNA laddering, Hoechst or TUNEL staining, and caspase activation. Importantly, overexpression of CaMKII-δ_B using adenoviral gene transfer substantially protects heart cells against ischemia- or oxidative stress-induced apoptosis, whereas overexpression of the cytosolic counterpart promoted myocyte apoptosis. Using cDNA microarray gene expression profiling, real time-PCR and Western blotting, we demonstrated that the protective effect of CaMKII-δ_B was accompanied by elevated expression of heat shock protein 70 (HSP70) family members, including a highly inducible HSP70 (iHSP70, also known as HSP72) and its homologous (Hst70, also called HSPt70). Gene silencing of iHSP70, but not Hst70, abolished CaMKII-δ_B mediated protective effect, indicating that only iHSP70 is required for nuclear CaMKII-δ_B elicited anti-apoptotic signaling. We conclude that, in contrast to cytosolic CaMKII-δ_C, nuclear CaMKII-δ_B serves as a potent suppressor of oxidative stress-induced cardiomyocyte apoptosis via an iHSP70-dependent mechanism, marking CaMKII-δ_B as a promising novel therapeutic target for the treatment of myocardial ischemic disease.

II. ***Roles of Ca^{2+}/Calmodulin-Dependent Protein Kinase II (CaMKII) in Regulating Cardiac Pacemaker Activity and Excitation-Contraction Coupling.***

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 CaMKII-δ_C-mediated positive feedback regulation of the L-type Ca^{2+} current (ICa,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 via modulating properties of $I_{Ca,L}$ inactivation and local Ca^{2+} is critically involved in this process.

In addition to its regulatory effect on cardiac pacemaker activity, CaMKII plays an essential role in regulating cardiac EC coupling and heart rate- or pacing frequency-dependent augmentation of cardiac contractility and acceleration of relaxation. Specifically, we have shown that CaMKII-mediated phosphorylation of PLB at Thr^{17} is augmented in response to increasing pacing frequency in the absence of increase in PKA-dependent phosphorylation of PLB at Ser^{16} or phosphorylation of SR Ca^{2+}-ATPase (SECAR2a). Our results challenged the well-established sequential model for PLB phosphorylation at Ser^{16} and Thr^{17}, and led to a new model in which dual site PLB phosphorylation occurs independently with a synergistic effect of PKA and CaMKII signaling on Thr^{17} phosphorylation. Moreover, CaMKII-mediated phosphorylation of PLB-Thr^{17} plays a crucial role in the positive cardiac contraction/relaxation-frequency relationship. The frequency-encoded PLB-Thr^{17} phosphorylation may represent a previously unrecognized feedback mechanism: elevated intracellular Ca^{2+} regulates its own reuptake into SR, whereas PKA-mediated Ser^{16} phosphorylation is subjected to tight sympathetic regulation. Interplay between ßAR stimulation and heart rate in inducing dual site PLB phosphorylation ensures proper cardiac contractility and relaxation, particularly during stress or exercise.

With respect to cardiac EC coupling, our most recent studies have demonstrated that while CaMKII-δ_C increases sarcolemmal L-type Ca channel activity, it inhibits SR Ca^{2+} release channels, ryanodine receptors (RyR). Using adult rat cardiomyocyte culture and adenoviral gene transfer techniques, we expressed wild type (WT), constitutively active (CA), or domi-

nant negative (DN) CaMKII-δ_C. CaMKII activity was examined by determining the kinase activity and the level of CaMKII-specific phosphorylation of phospholamban at Thr-[17]. Compared with δ-gal adenovirus control, CaMKII-mediated phosphorylation of RyR, assessed by back phosphorylation, was reduced by DN-CaMKII-δ_C, enhanced by CA-CaMKII-δ_C, or unaltered by WT-CaMKII-δ_C expression. Concomitantly, spontaneous Ca2+ sparks at 1mM Ca2+ was hypoactive, hyperactive, or unchanged in CA-, DN- or WT-CaMKII-δ_C groups, respectively; Ca^{2+} transients elicited by action potentials displayed accelerated, slowed or unchanged rate of relaxation in CA-, DN- or WT-CaMKII-δ_C groups, respectively, without affecting the amplitudes. Both WT- and CA-CaMKII-δ_C protected the cells from Ca^{2+} instability as manifested by ~60% attenuation of the frequency of Ca^{2+} waves induced by elevating extracellular Ca^{2+} over a wide range (2-20 mmol/L), whereas DN-CaMKII-δ_C increased Ca^{2+} wave frequency at 20 mmol/L Ca^{2+}. Furthermore, activation of endogenous CaMKII during sustained β_1-adrenergic receptor stimulation (norepinephrine 100 nmol/L, 24h in the presence of α-adrenergic blocker) did not alter Ca^{2+} spark frequency in spite of elevated caffeine-liable Ca^{2+} store at 1 mmol/L Ca^{2+}, and reduced Ca^{2+} wave frequency at high Ca^{2+}concentrations. Taken together, our data support the notion that CaMKII-δ_C negatively regulates the RyR channel activity in intact cells (particularly under Ca^{2+} overload conditions), which counteracts the inherent positive feedback of Ca^{2+}-induced Ca^{2+} release, enhancing the stability of Ca^{2+} signaling in the heart.

Identification and Characterization of Cardiovascular Disease-related Genes

I. HSG Suppresses Cell Growth and Proliferation.

Vascular proliferative disorders, including atherosclerosis, restenosis after balloon angioplasty, and coronary arteriosclerosis, are the most common causes of severe cardiovascular diseases such as myocardial infarction, ischemic heart failure, and strokes. Neointimal VSMC proliferation constitutes an important etiological factor in vascular proliferative disorders. However, the molecular mechanisms governing VSMC proliferation are largely unknown. Thus, identifying genetic modifiers of VSMC proliferation remains as a major focus in cardiovascular biology and medicine.

In order to identify genes involved in VSMC proliferation, we analyzed the gene expression profile of spontaneously hypertensive rat (SHR) VSMCs versus that of Wistar Kyoto rats (WKY) VSMCs using a differential display technique and identified a novel gene. We referred to the cDNA fragment highly expressed in WKY but weakly in SHR as hyperplasia suppressor gene (HSG or mitofusion 2) (accession number:

U41803). The partial (~ 0.35 kb) cDNA identified from differential display was cloned into pGEM-T plasmid vector and sequenced. Using cDNA library screening and 5' RACE reaction, we then cloned the full-length cDNA, consisting of 4151 bp before a poly(A) tail. Sequence analysis revealed an open reading frame encoding a protein of 757 amino acids.

We have demonstrated that the expression of rat HSG (rHSG) is markedly downregulated in hyper-proliferative SHR VSMCs and growth factor-stimulated WKY VSMCs. Overexpression of rHSG overtly suppresses serum-stimulated VSMC proliferation, and attenuates balloon injury-induced neointimal formation by 90%, thereby preventing balloon angioplasty-associated restenosis in rat carotid arteries. The rHSG-induced growth suppression is mediated by cell cycle arrest in G0/G1 phases due to inhibition of the extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling cascade.
Additionally, our preliminary studies have shown that adenoviral gene transfer of the human homolog (hHSG) has a potent anti-proliferative effect in a variety of cancer cell lines, including breast cancer cell lines MCF-7 and BM-1, a leukemia cancer cell line U937, a colon cancer cell line LoVo, and a hepatoma cell line Bel 7402, and that the anti-proliferative effect of hHSG is even more potent than that induced by overexpression of p53 (a well established cancer suppressor). Thus, rHSH functions as a powerful cell proliferation suppressor, and that downregulation or inactivation of rHSG leads to vascular proliferative disorders and might also be involved in the pathogenesis of a variety of cancers.

II. HSG Is The Major Determinant for Oxidative Stress-Induced Cardiac Myocyte Apoptosis.

Among all cell types and tissues, HSG is predominantly expressed in the heart, but its functional role in cardiac myocytes remains elusive. Thus, we have recently explored the potential role of HSG in regulating the fate of heart muscle cells and its potential physiological and pathological relevance. We have demonstrated that HSG, a mitochondria protein, is a major determinant of heart muscle cell apoptosis. Myocardial infarction or ischemia reperfusion injury profoundly elevates endogenous HSG expression and myocyte apoptosis in vivo via a reactive oxygen species (ROS)-dependent mechanism. Similarly, oxidative stress with H_2O_2 leads to concurrent increases in HSG expression and apoptosis in cultured rat cardiomyocytes. Furthermore, overexpression of HSG using adenoviral gene transfer is sufficient to trigger robust cardiomyocyte apoptosis, as manifested by increased mitochondrial cytochrome c release, activation of caspase-9 and caspase-3 and profound DNA fragmentation, and pro-

foundly suppress basal and serum-stimulated Akt activation. HSG-induced apoptosis is fully prevented by overexpression of PI3K or a mitochondrial antiapoptotic protein, Bcl-xL, indicating that HSG promotes cardiomyocytes apoptosis via inhibition of the Ras-PI3K-Akt cell survival signaling and subsequent activation of the primary mitochondrial apoptotic pathway. Importantly, RNAi-mediated gene knockdown of HSG effectively protects cells against oxidative stress-induced apoptosis. These revelations mark HSG as a major determinant of clinically important ischemia/oxidative stress-induced heart disease, and suggest inhibition of HSG function might provide a highly effective novel therapy in treating the devastating disease, heart failure, that currently lacks effective treatments.

Collaborators: Dr. Robert J. Lefkowitz, Howard Hughes Medical Institute; Dr. Walter J. Koch, Duke University Medical Center; Dr. Yibin Wang, University of California, Los Angeles; Dr. Brian Kobilka, Howard Hughes Medical Institutes, Stanford University Medical Center; Heping Cheng, Peking University, China; Drs. Michael Crow and David Kass, Johns Hopkins University; Drs. Edward G. Lakatta and Mark Talan, Laboratory of Cardiovascular Science, NIA, NIH.

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The Laboratory of Cellular and Molecular Biology (LCMB) currently comprises six independent research programs headed by either a tenure-track investigator or a senior investigator. These programs include the Gene Regulation Section, RNA Regulation Section, Cancer Genomics and Signaling Section, DNA Repair Unit, Chromatin Structure and Function Unit and the Molecular Immunology Unit.

Members of LCMB investigate basic mechanisms of gene regulation, DNA repair and recombination and the dysregulation of these processes that lead to the development of age-related deficits, or disorders, such as cancer and Alzheimer's disease. Interest in gene regulation encompasses a broad-based approach that includes epigenetic and genetic modes of transcription control, post-transcriptional mechanisms that affect mRNA stability and protein translational control. These processes are investigated in the biological context of stress-response, lymphocyte development and effector T cell function. Studies of DNA repair are aimed at understanding the mechanisms by which antigen receptor gene diversity is achieved in lymphocytes and the mechanisms that contribute to age-associated loss of genomic integrity. Breakdown of cellular homeostasis and cell cycle control are studied as they pertain to cellular senescence and the development of human cancer. The long-term goal of these programs is to generate new insights that can be applied to prevent or delay the onset of age-related disabilities, and/or to provide new strategies for their diagnosis or treatment.

While the individual research programs within the LCMB function as independent groups, they are highly interactive, conduct weekly 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 and manipulate gene expression is also available within the LCMB.

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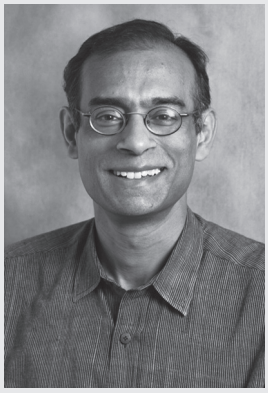

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Biography: Dr. Sen received a Ph.D., degree in chemistry from Columbia University in 1982. He made the transition to molecular biology as a postdoctoral fellow in David Baltimore's laboratory at M.I.T. and the Whitehead Institute. During this stage he developed his current interests in gene regulation. In 1987 Dr. Sen was appointed Assistant Professor in the Department of Biology and Rosenstiel Research Center at Brandeis University. He earned tenure in 1991 and was promoted to Professor of Biology in 1998. He moved to his present position as Chief, Laboratory of Cellular and Molecular Biology, National Institute on Aging in 2003.

Keywords:

immune response
gene regulation
chromatin structure
V(D)J recombination
NF-κB
inflammation
cell death

Recent Publications:

Du H, et al. *Mol Cell* 2008; 31: 641-649.

Gearhart PJ, et al. *Mol Cell* 2008; 31: 615-616.

Standanlick JE, et al. *Nat Immunol* 2008; in press.

Chakraborty TD, et al. *Mol Cell* 2007; 27: 842-850.

Liu Y, et al. *Immunity* 2007; 27: 561-571.

Research Summary: B and T cell differentiation share several common features. B lymphopoiesis takes place in the bone marrow where environmental cues commit multipotent cells to the B lineage. Close to the point of lineage commitment gene rearrangements are initiated at the immunoglobulin heavy chain (IgH) gene locus. Activation of the locus and subsequent V(D)J recombination is regulated in complex ways, and one of our objectives is to understand the molecular mechanisms that underlie this complexity. A parallel pathway operates in the thymus where multipotent cells commit to the T lineage. One important consequence is the activation and recombination of T cell receptor (TCR) β chain genes. The TCRβ gene enhancer has been shown to be essential in this process and we have used it to probe this differentiation step.

1) Regulatory Mechanisms in Pro-B Cells: The immunoglobulin heavy chain gene locus is spread over several megabases. Functional IgH genes are assembled in pro-B cells by gene recombination events that bring together V_H, D_H and J_H gene segments. We have recently found that this locus is activated in discrete, independently regulated steps. An approximately 90 kb domain is activated first prior to the initiation of V(D)J recombination. This domain includes all the D_H gene segments and extends to Cμ. V_H genes are inactive at this stage, which ensures that D_H to J_H recombination takes place first. Our analysis suggests that DJ_H recombination activates V_H genes that lie closest to the D_H/Cμ regions. Other parts of the V_H locus are activated independently: the 5'V_H J558 family requiring IL-7 and the intermediate V_H10 genes responding to tyrosine kinase signals.

The problem of IgH locus activation can therefore be broadly divided into two parts. First, regulation of the 90 kb D_H/Cμ domain and second, the regulation of V_H genes. Our objective is to understand the molecular basis for these regulatory events.

1A. The D_H/Cμ Locus: Within this 90 kb lies the first tissue-specific transcriptional enhancer identified, the μ enhancer (Eμ). This regulatory element was subsequently shown to be a recombinational enhancer in artificial recombination substrates, further strengthening its importance as a regulator of IgH gene expression in pro-B cells. The presence of other recombinational enhancers in the locus was inferred from the observation that deletion of Eμ from the endogenous locus had little effect on D_H to J_H recombination. We have examined approximately 60kb of the 90kb region and found evidence for only one other regulatory sequence, which is close to Dq52. Multiple approaches to study this domain are currently ongoing in our laboratory.

Does RNA interference determine the chromatin structure of the 90kb D_H-Cμ domain? We have recently found that the majority of D_H gene segments are in an epigenetic state that corresponds to inactive chromatin in B cell precursors that are poised to initiate D_H to J_H recombination. This is counter-intuitive and we are trying to understand why this is so. One hypothesis is that these gene segments are suppressed via repeat-induced gene silencing, a phenomenon best characterized in lower organisms, that utilizes the RNA interference machinery.

How do known cis-regulatory sequences set the epigenetic landscape of the germline IgH locus? In collaboration with Drs. F. Alt and E. Oltz, we are analyzing the chromatin structure, and recombination efficiencies, of IgH alleles that lack Eμ, or the Dq52 regulatory sequence or both. These studies are expected to provide insights into the functions of these sequences at the endogenous loci. Coupled with the in vitro analysis of the Eμ (described below), our goal is to understand how DNA binding proteins that interact with these sequences lead to recombination and transcription activation.

Recombination-dependent alteration in IgH chromatin structure. We find evidence for significant changes in the epigenetic status of the locus as recombination proceeds. Characterization of these changes will allow a perspective on how the first recombination event (D_H to J_H) sets the stage for the second (V_H to DJ_H) recombination event. Parallel studies in Eμ-deficient pro-B cells will indicate which of these changes are caused by Eμ.

Chromosomal dynamics in developing B lymphocytes. We are interested in characterizing chromosomal movements that accompany IgH expression during B cell development. Two broad categories of movements have been identified. First, the IgH locus moves away from the nuclear periphery to the interior of the nucleus. We plan to identify proteins that interact with the IgH locus when it is at the periphery, the precise developmental stage when it moves to the interior, and the cis-sequences that are necessary for this movement. Second, the IgH locus undergoes compaction, presumably in order to bring distal gene segments into proximity to undergo recombination. The Pax5 protein has been implicated in this process, but molecular mechanisms are not known. We are undertaking a high resolution genome proximity analysis to determine which parts of the locus come together at specific developmental stages, with the goal of understanding mechanisms that regulate chromosomal conformation.

Biochemical studies of Eμ function. We have studied this enhancer for several years from the perspective of transcriptional activation. We know the proteins that bind, the functional consequences of disrupting protein binding, proteins that interact with other Eμ binding proteins, and the biochemical consequences of some of these interactions. Yet, a deep understanding of the basis of enhancer function is still lacking. For example, we do not understand why certain protein binding sites need to be next to each other, or why they are spaced the way they are, or even the function of individual, or combinations of, proteins. Ongoing biochemical studies in the laboratory are aimed at reconstituting recombination and transcription activation by Eμ in the context of nucleosome-assembled plasmids, recombinant transcription factors and chromatin remodeling/modifying activities.

1B. The V_H locus: We have evidence for three independently regulated domains of V_H genes: the 5' V_H J J558 genes are IL-7 responsive, the 3' V_H 7183 and SM7 genes are activated by DJ_H recombination, and the intermediate V_H10 genes are activated by the v-abl tyrosine kinase. Our immediate objectives are to i) confirm the model that DH-proximal V_H genes are activated in response to DJ_H recombination, ii) to identify the normal signals that activate V_H10 and co-regulated genes, and iii) to study the mechanism of V_H allelic exclusion. An example of ongoing studies is described below.

Allelic exclusion refers to the phenomena that B and T lymphocytes express only one antigen receptor. Though this could result from low probability of generating two functional rearrangements, it has been convincingly demonstrated that allelic exclusion at IgH (and TCRβ) is actively regulated by a feedback mechanism. Cells sense IgH protein via the pre-B cell receptor and terminate further V_H to DJ_H recombination. Based on our recent insights into the activation of V_H genes, we proposed the simple hypothesis that allelic exclusion is the opposite of V_H gene activation. For example, since IL-7 activates V_HJ558 genes, according to our model loss of IL-7 signals results in allelic exclusion of this family. We are currently testing several predictions of this model as well as investigating the mechanism of V_H gene inactivation.

2) Regulatory Mechanisms in Pro-T Cells: TCRβ chain gene recombination and expression requires an enhancer located several kilobases 3' of the Cβ2 exons. We have initiated a systematic analysis of this enhancer with the goal of identifying critical motifs (and associated DNA binding proteins) that are responsible for activating it at the earliest stages of T cell development. The working hypothesis is that thymic environmental signals that commit a multipotent cell to the T cell lineage also activate the TCRβ enhancer. Thus, working back from the enhancer provides one route to identifying the signaling pathways that operate in the earliest thymocytes. We identified two novel sequence motifs in the TCRβ enhancer that lie between two composite ETS/CBF elements. We plan to identify these proteins biochemically and/or genetically. Their role in early thymocytes will be further addressed once we have antibodies and gene sequences.

Allelic exclusion of Vβ gene segments. Like V_H genes, Vβ gene segments are also targeted for feedback inhibition of recombination. Vβ allelic exclusion is accompanied by reduced levels of histone acetylation, but the mechanisms that up- or down-regulate Vβ gene recombination are not known. Extrapolating from our model of VH allelic exclusion, we have suggested that Vβs may also be activated by transient signals, whose down-regulation as differentiation proceeds reverts these gene segments to a hypo-recombinogenic state. To test this hypothesis, we are 1) identifying the chromatin changes that occur in developing thymocytes as Vβ gene segments are activated for recombination, 2) testing various genetic mutations that may affect the transition to active Vβs, and 3) re-activating silenced Vβ gene segments in allelically excluded cells based on inferences derived from parts 1 and 2.

3) Function of NF-κB Proteins: NF-κB proteins are a family of inducible transcription factors that allow cells to respond to extracellular stimuli. The diverse stimuli that activate NF-κB and the distinct cellular responses that ensue raise the question as to how specificity of the response is regulated. This complexity is most likely a reflection of the several different Rel proteins that constitute the NF-κB family and the several different IκB proteins that inactivate them. For example, there may be differences in the way Rel proteins are sequestered in the cytoplasm, different signals may target different IκBs, and different family members may activate different genes. However, there are very few well characterized examples of such differences and even fewer molecular mechanisms to explain them. Our long-term interest is to attempt to unravel some of this complexity, particularly in cells of the immune system. Current research interests are summarized below.

Innate/adaptive immune cross-talk via NF-κB proteins. We have found that pro-inflammatory cytokines produced by cells of the innate immune system prime naïve $CD4^+$ T cells for heightened response to T cell receptor signals. The mechanism we proposed is via generation of c-Rel/IκBα complexes which are rapidly activated in response to TCR cross-linking. Current experiments extend these observations in several directions. First, we are interested in determining whether NF-κB-dependent priming is a feature of $CD8^+$ T cells and B cells. Second, we are exploring the roles of c-Rel and IκBα in the maintenance of short or long-term immunological memory. Third, we would like to understand the mechanism by which c-Rel-dependent priming is lost in effector cells. Our studies involve a combination of in vitro and in vivo manipulation of cells from genetic deficiencies in NF-κB components, and biochemical analyses of cytosolic signaling pathways that activate NF-κB/IκB complexes.

NF-κB regulation of the timing of activation-induced cell death of $CD4^+$ T effector cells. Many forms of cell stimulation that activate NF-κB also trigger cell death. Because anti-apoptotic genes are an important class of NF-κB targets, it is interesting to consider how NF-κB-dependent anti-apoptosis is balanced by stimulus-induced programmed cell death. In activated $CD4^+$ T cells, that are programmed to die via death receptor-initiated signals, we found that onset of cell death closely parallels p65/Rel A down-regulation from the nucleus. Based on expression analysis of putative NF-κB-dependent anti-apoptotic genes, and ectopic expression of

these genes, we proposed that the balance between cellular life and death is determined by the timing of NF-κB down-regulation. Current studies are focused on 1) mechanisms that dictate the timing of NF-κB down-regulation in T cells, 2) mechanism by which the duration of nuclear NF-κB may be changed, and the consequences thereof to T cell effector function, 3) mechanisms that turn down expression of NF-κB-dependent genes after loss of nuclear NF-κB, and 4) the analysis of transient NF-κB-dependent gene expression to other cell types involved in the immune response. The central hypothesis underlying our efforts is that timing of cell death, and thus the duration of effector phase, directly affects inflammatory responses and may thereby play a role in the pathophysiology of inflammatory disease.

Collaborators: Eugene Oltz, Ph.D., Vanderbilt University; David Schatz, Ph.D., Yale University; Michael Pazin, Ph.D., Chromatin Structure and Function Unit, Laboratory of Cellular and Molecular Biology, NIA, NIH; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Stephen Smale, Ph.D., University of California, Los Angeles; Satyajit Rath, M.D., Ph.D., N.I.I., India; Joan Press, Ph.D., Brandeis University; Robert Woodland, Ph.D., University of Massachusetts; Rachel Gerstein, Ph.D., University of Massachusetts; Janet Stavnezer, Ph.D., University of Massachusetts; Fred Alt, Ph.D., Harvard Medical School; Carl Schildkraut, Ph.D., Albert Einstein College of Medicine; Juan-Carlos Zuniga-Pflucker, Ph.D., University of Toronto; Klaus Rajewskly, Ph.D., Harvard Medical School; Luigi Ferrucci, M.D., Longitudinal Studies Section, NIA, NIH; Antony Rosen, M.D., MB ChB, Johns Hopkins University School of Medicine; Myriam Gorospe, Ph.D., RNA Regulation Section, Laboratory of Cellular and Molecular Biology, NIA, NIH; Kevin Becker, Ph.D., Gene Expression and Genomics Unit, NIA, NIH; Mitsuo Oshimura, D.Sc., Tottori University, Japan.
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Biography: Dr. Gorospe received her Ph.D. from the State University of New York at Albany (New York) in 1993. She completed her postdoctoral training at the Section on Gene Expression and Aging (renamed Cell Stress and Aging in 2000), National Institute on Aging, and assumed the position of Investigator in 1998 and Senior Investigator in 2003. Her research program focuses on posttranscriptional mechanisms serving to modulate gene expression, with a particular interest in studying proliferative, stress-response, and cell cycle regulatory genes.

Keywords:

posttranscriptional regulation
mRNA turnover
translation
RNA-binding proteins
microRNAs
cell cycle control
stress response
senescence
microarray

Recent Publications:

Mazan-Mamczarz K, et al., *Oncogene* 2008; 27: 6151-6163.

van der Brug MP, et al. *Proc Natl Acad Sci* U S A 2008; 105: 10244-12049.

Hucl T, et al. *Cancer Res* 2008; 68: 5023-5030.

Kim HH, et al. *Genes Dev* 2008; 22: 1804-1815.

Chen J, et al., *Mol Biol Cell* 2008; 19: 3701-3712.

Ishmael FT et al. *J Immunol* 2008; 180: 8342-8353.

Kuwano Y, et al. *Mol Cell Biol* 2008; 28: 4562-4575.

Research Summary: Aging is characterized by a general decline in the ability of individuals to respond adequately to different stimuli from either environmental or endogenous sources. Changes in the expression of stress-response and proliferative genes are widely believed to play an important role in determining cell fate. While the transcriptional control of gene expression has been extensively studied, posttranscriptional regulatory mechanisms are increasingly recognized to regulate gene expression potently. Posttranscriptional gene regulation comprises changes in the stability and translation rate of mRNAs, as well as splicing, nuclear export of mRNA, and intracellular storage. Broadly speaking, our long-term aims are: 1) to study the ribonucleoprotein complexes (RNPs) [RNA-binding proteins (RBPs), microRNAs (miRNAs) and target mRNAs] that influence the stability and translation of specific mRNA subsets; 2) to elucidate the signaling events that regulate RNP associations; and 3) to study the implications of RNPs on physiological and pathological processes. These aims are pursued in a variety of research efforts, as described below.

Initiatives to investigate the turnover and translation of specific mRNAs: We hypothesize that the net influence of the RNA-binding proteins (RBPs) and microRNAs (miRNAs) that associate dynamically with a given mRNA determine its stability and translation rate. To test this hypothesis, we use human diploid fibroblasts (HDFs) and immortal human cell lines to study specific mRNAs of interest. The mRNAs examined are primarily those that encode proteins involved in the response to cell damage and division (e.g., cdks, cyclins, cdk inhibitors,

Publications-continued
Lal A et al. *PLoS ONE* 2008; 3: e1864.

Robinson VL, et al. *Mol Cancer Res* 2008; 6: 501-508.

Casolaro V, et al. *J Allergy Clin Immunol* 2008; 121: 853-859.e4.

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Pullmann R Jr, et al., *Mol Cell Biol.* 2007; 27(18):6265-78.

Zahn JM, et al. *PLoS Genet* 2007; 3: e201.

Xiao L, et al., *Mol Biol Cell*. 2007; 18(11):4579-90.

apoptosis-related proteins, oncoproteins, and tumor suppressors). We study the 5'-untranslated region (UTR), the coding region (CR) and the 3'UTR to identify sequences that affect mRNA half-life and translation, such as regions of association with RBPs, sequences of interaction with microRNAs, and internal ribosome entry sites (IRES).

Efforts to study the mRNA-binding factors involved in mRNA turnover and translation: We hypothesize that RBPs and miRNAs are essential regulators of broad subsets of mRNAs in cells responding to injury and mitogens. By performing these functions, we propose that RBPs/miRNAs orchestrate gene expression programs associated with senescence and aging. To test this notion, se have several projects that seek to identify collections of RBP/miRNA target mRNAs using microarrays. In RBP studies, we also aim to uncover shared RNA motifs and to investigate the signaling pathways that regulate RBP levels, localization, and activity. Our approaches also include the analysis of endogenous mRNAs and ectopic reporter transcripts to test the influence of RBPs/miRNAs upon the stability and translation of target mRNAs.

Research projects to investigate the influence of mRNA turnover and translation on biological processes: We hypothesize that RBPs/miRNAs influencing cytoplasmic mRNA metabolism can coordinate gene expression programs that influence specific biological processes. We are particularly interested in studying the impact of ribonucleoprotein associations on the cell division cycle, carcinogenesis, apoptosis, replicative senescence, and the stress response. Thus, a major initiative in our laboratory is to investigate biological processes that are orchestrated through joint alterations in mRNA half-life and translation rates. Interventions include increasing or decreasing specific RBPs or miRNAs in different model systems to test the consequences of altering RNP function.

Collaborators: Kevin Becker, Research Resources Branch, NIA, NIH; Mark R. Cookson, Laboratory of Neurogenetics, NIA, NIH; Rafael De Cabo, Laboratory of Experimental Gerontology, NIA, NIH; Fred E. Indig, Research Resources Branch, NIA, NIH; Marcel van der Brug, Laboratory of Neurogenetics, NIA, NIH; Dongmei Yang, Laboratory of Cardiovascular Science, NIA, NIH; Ming Zhan, Research Resources Branch, NIA, NIH; Henry Furneaux, University of Connecticut Health Center, CT; Gary Brewer, University of Medicine and Dentistry of New Jersey, NJ;

Collaborators-continued: Imed Gallouzi, McGill University, Canada; Cristiana Stellato, Johns Hopkins Medical Institutions, MD; Ulus Atasoy, University of Missouri, MO; Paul Anderson, Harvard University, MA; Justin Blethrow, University of California, San Francisco, CA; Ching-Yi Chen, University of Alabama, AL; Jinshui Fan, Johns Hopkins University, MD; Angelica Figueroa, Conseji Superior de Investigaciones, Spain; Ronald Gartenhaus, University of Maryland School of Medicine, MD; Bret A Hassel, University of Maryland School of Medicine, MD; Martin Holcik, McGill University, Canada; Nancy Kedersha, Harvard University, MA; Jack Keene, Duke University Medical Center, NC; A. Lal, Harvard University, MA, J. Lieberman, Harvard University, MA; Jun Liu, Johns Hopkins University, MD; Isabel Lopez de Silanes, Centro Nacional de Investigaciones Oncologicas, Spain; Maria Luz Martinez-Chantar, Biogune, Spain; Carrie Rinker-Schaeffer, University of Chicago, IL; Victoria Robinson, University of Chicago, IL; Kevan M. Shokat, University of California, San Francisco, CA; David Sinclair, Harvard University, MA; Mercedes Vazquez, Biogune, Spain; Jian-Ying Wang, University of Maryland, MD; Jackie Wilce, University of Queensland, Australia; Matthew Wilce, University of Queensland, Australia; Gerald Wilson, University of Maryland, MD.

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Keywords:

DNA damage
DNA repair
oxoguanine-DNA glycosylase
base excision repair
genetic polymorphisms
health disparities
translational research

Recent Publications:

Trzeciak AR, et al. *Free Radic Biol Med* 2008; 45 :1631-1641.

Nyaga SG, et al. *Biochem Biophys Res Commun* 2008; 376: 336-340.

Dotson VM, et al. *Clin Neuropsychol* 2008; 21: 1-29.

Hill JW, et al. *DNA Repair (Amst)* 2008; 7: 648-654.

Trzeciak AR, et al. *Radiat Res* 2008; 169: 110-121.

Research Summary: The DNA Repair Unit of the Laboratory of Cellular and Molecular Biology conducts research focused on understanding the role of oxidative damage, oxidative stress and oxidative DNA repair in the development of age-related diseases and health disparities. This unit approaches these questions within the framework of translational research. We have attempted over the last 4 years to build a two-way bridge between basic science laboratory studies and clinical research that spans from the targeted population to the bench. The ultimate goal of this approach is to transform scientific discoveries arising from laboratory, clinical, or population studies into clinical applications to reduce incidence, morbidity, and mortality of age-associated diseases and health disparities. This is accomplished by the development and implementation of a clinical component and a basic science component that are interdependent and pursuing related hypotheses.

The hypotheses pursued in this work include the following: Project 1-Race and socioeconomic status influence health status and age-related health disparities separately or synergistically as co-factors of biological, behavioral, psychosocial, and environmental conditions, Project 2-Health disparities promote the development of an Accelerated Aging Phenotype in vulnerable population cohorts characterized by accumulation of oxidative

Hill JW, et al. *Cancer Detect Prev* 2007; 31: 237-243.

Nguyen HT, et al. *African Americans Arch Clin Neuropsychol* 2007; 22: 689-698.

Nyaga SG, et al. **Cell Cycle** 2007; 6: 1472-1478.

Rodriguez H, et al. *Biochemistry* 2007; 46: 2488-2496.

Evans MK, et al. Oncogene 2007; 26: 1941-1948.

stress and damage to DNA and other cellular components, possible defects in repair of oxidative DNA damage and the augmentation of inflammatory processes that ultimately result in the disparities noted in national statistics of overall longevity and other health outcomes, and Project 3-Oxidative DNA damage and repair mechanisms are required for the maintenance of genomic stability and genetic fidelity. Alterations in oxidative DNA repair capacity play a role in cancer susceptibility. This project examines the functional consequences of human genetic polymorphisms of hOGG1, a base excision repair gene that has been implicated in commonly occurring neoplasms.

Clinical Component: Epidemiologic Population-based Research focused on Health Disparities The Healthy Aging in Neighborhoods of Diversity across the Life Span study (HANDLS) is a multidisciplinary, community-based, prospective longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities among socioeconomically diverse African Americans and whites in Baltimore. This study investigates whether health disparities develop or persist due to differences in SES, differences in race, or their interaction. This study is unique because it will assess over a 20-year period physical parameters as well as evaluate genetic, biologic, demographic, psychosocial, and psychophysiological parameters of African American and white participants in higher and lower SES. It also employs novel research tools, mobile medical research vehicles, in hopes of improving participation rates and retention among non-traditional research participants. The domains of the HANDLS study include: nutrition, cognition, biologic biomarkers, body composition and bone quality, psychophysiology, physical function and performance, sociodemographics, psychosocial, neighborhood environment and cardiovascular disease. Utilizing data from these study domains will facilitate understanding of the driving factors behind persistent black-white health disparities in overall longevity, cardiovascular disease, and cognitive decline.

HANDLS as a translational research study permits investigation of health disparities in terms of socioeconomic, socio-cultural, and psychosocial parameters but also allows us to define a medical/biologic phenotype that may be amenable to dissection by bench scientists examining the molecular aspects of aging, disease and disability. The early appearance and increased severity of age-associated disease among African Americans and low SES individuals suggests that the factors contributing to the

emergence of health disparities produce a phenotype of 'accelerated aging' while others have attributed this to racism and other socio-cultural factors, we seek to understand the underlying biologic, genetic, and environmental factors that may result in this phenotype that ultimately contribute to the disparate life expectancies seen for low-SES and minority sub-populations.

We hypothesize that in low SES populations and in minority populations with high rates of early onset, age-associated disease the interaction of biologic, psychosocial, socioeconomic and environmental and genetics factors may result in a phenotype of premature or accelerated aging. The underlying biologic processes maybe the same as those postulated in theories of normal human aging. The Harman Free Radical Theory of Aging states that the accumulation of oxidative stress and damage to DNA and other cellular components and tissues over the lifespan leads to aging, disease and death. The health disparities induced phenotype of accelerated aging may be biologically similar to heritable 'progeroid' syndromes whose manifestations include increased susceptibility to oxidative stress, premature accumulation of oxidative DNA damage, defects in DNA repair and higher levels of biomarkers of oxidative stress and inflammation. While genetic background, environmental and behavioral factors influence health outcomes in all populations over the lifespan, health disparities may be the end product of an accelerated trajectory of dysfunctional interactions of these factors in populations at high risk or with high levels of risk exposure. We are pursuing the following aims: to determine whether defects in oxidative DNA repair pathways can be accurately measured in an epidemiologic population sample, to determine whether of oxidative DNA damage can be accurately and reproducibly assessed in an epidemiologic population sample and to determine whether there are differences in measured DNA repair capacity and DNA damage levels that correlate with race, sex, health outcomes or other demographic or biologic correlates.

We have chosen to pursue these endpoints because both oxidative DNA repair capacity and levels of oxidative DNA damage have been implicated cancer, cerebral ischemia, atherosclerosis and other age-associated diseases that have incidence and prevalence rates that are disproportionate in poor and minority populations. The alkaline comet assay is a sensitive and relatively inexpensive technique used for the detection of DNA damage and DNA repair. One of the major advantages of the alkaline comet assay is the possibility of analysis of DNA damage and repair in

individual cells. Furthermore, small numbers of cells are required for this assay which is particularly advantageous for analyses performed in samples from human populations.

Though, the Comet assay does not specifically probe the fidelity of the process or the activity of the specific enzymatic repair components that participate in the biologic pathway of SSB repair, defects in SSB repair identified through the Comet assay may provide targets for further investigation of possible DNA repair genes or gene products related to disease states identified among human cohort. We have recently published our work on the refinement of an accurate, reproducible and efficient comet assay methodology for evaluating DNA repair capacity in cryopreserved PBMCs and our first set of single strand break repair capacities in the HANDLS cohort. This protocol includes estimation of the initial rate of DNA repair, a newly introduced DNA repair parameter showing the rate of DNA repair immediately after a genotoxic exposure. While no single test can reveal all the complexities of DNA repair capacity in individuals, the comet assay when performed using reference standardization procedures can serve as a general screening method assessing capacity of intact cells to repair AP sites, SSB and DSB.

Basic Science Component: Oxidative DNA Repair and Human Disease Although numerous genetic association studies have established links between human genetic polymorphisms of DNA repair genes and several cancers, there has been little work done examining the functional biochemical consequences of these polymorphisms and how they increase cancer susceptibility. Since our interest lies in oxidative DNA base damage and base excision repair pathways in human disease, we have examined biochemical characteristics of the gene products that result from hOGG1 polymorphisms. A major base damage produced by reactive oxygen species is 7,8-dihydro-8-oxoguanine (8-oxoG). Unlike normal guanine, 8-oxoG has the propensity to mispair with adenine during DNA replication, resulting in the fixation of G:C to T:A transversion mutations. Oxidatively modified bases, such as 8-oxoG, are repaired primarily by the base excision repair pathway (BER), the first steps of which are the recognition and excision of the damaged base by a specific DNA glycosylase. The major mammalian enzyme for removing 8-oxoG from DNA is 8-oxoguanine-DNA glycosylase (OGG1). OGG1 is a bifunctional enzyme, having both 8-oxoG excision activity and a weak AP-lyase strand incision activity at abasic sites. Several OGG1 polymorphisms have been reported and positively correlate with a variety of cancers. A

frequently occurring polymorphism results in the substitution of serine for cysteine at position 326 in the C-terminus of OGG1. The allele frequency of S326C OGG1 measured in human populations ranges from 0.13 to as high as 0.62 and varies significantly with ethnicity. Association studies have identified that individuals homozygous for the S326C OGG1 allele have increased incidence of lung, prostate and orolaryngeal cancers. A previous study found decreased catalytic efficiency (kcat/Km) of purified polymorphic S326C OGG1, while another study implicated the S326C genotype with decreased 8-oxoguanine repair capacity in vivo. We thought it was critical to investigate the functional biochemical consequences of this polymorphism that is associated with malignancies that have disproportionate incidence, morbidity and mortality among African Americans. We have characterized the glycosylase and AP-lyase activities and DNA damage binding affinity of purified S326C and found novel functional defects in the polymorphic OGG1 and a distinct dimeric DNA binding conformation compared to the wild-type enzyme. Our results confirm that S326C has decreased repair activity towards 8-oxoG paired with C and further show that S326C OGG1 is particularly deficient in 8-oxoguanine excision activity when the lesion is opposite T or G.

Since we were able to show definitively that a single amino acid change in polymorphic S326C OGG1 altered repair activity, substrate specificity, molecular stoichiometry, and stimulation of the enzyme by AP-endonuclease 1, we proceeded to examine the effect of another documented human genetic polymorphism of OGG1 on 8-oxoG excision activity. We characterized the enzymatic activity of R229Q. R229Q is a validated OGG1 polymorphism for which approximately 6% of the US population, or 18 million individuals, may be heterozygous. Like the more prevalent cancer-associated S326C OGG1 polymorphism, the frequency of the R229Q OGG1 allele varies significantly with ethnicity, with particularly high incidence in a West African population. Our observations of R229Q thermolability in vitro and in vivo explain previous reports of low 8-oxoguanine excision activity in KG-1 cells. Because of the destabilization of OGG1 resulting from the substitution of a large basic amino acid (arginine) for a small neutral amino acid (glutamine) at position 229, cells harboring the R229Q variant can be anticipated to have a significantly elevated spontaneous mutation frequency due to increased 8-oxoguanine in DNA. Such individuals may have decreased 8-oxoG repair capacity and increased cellular sensitivity to ROS-induced DNA damage that could promote susceptibility to carcinogenesis.

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Keywords:

ovarian cancer
gene expression profiling
drug resistance
SAGE
Claudin

Recent Publications:

Dahiya N, et al. *Plos ONE* 2008; 3: e2436.

Choi JH, et al. *Cancer Res* 2008; 68: 5716-5723.

Honda H, et al. *Cancer Biol* Ther 2008; 6: 1733-1742.

D'Souza T, et al. *Exp Cell Res*. 2007; 313: 3364-3375.

Li J, et al. *Oncogene* 2007; 26: 2860-2872.

Morin PJ, *Disease Markers* 2007; 23: 453-457.

Research Summary: Ovarian cancer is the fifth most common cause of cancer deaths among women in the United States, yet very little is known about the molecular mechanisms involved in the development of this disease. In order to address this problem, we are analyzing gene expression in ovarian cancer and in normal ovarian cells using a variety of techniques such as serial analysis of gene expression (SAGE), microarrays, and various PCR-based approaches. Our focus is directed at two major clinical problems in ovarian cancer: difficulty of detection and drug resistance. A better understanding of the molecular pathways important in ovarian cancer progression and drug resistance development may lead to novel approaches for detection and therapy of this disease.

Expression and roles of claudin-3 and claudin-4 in ovarian cancer. Because we have demonstrated that claudin-3 and claudin-4 are consistently overexpressed in ovarian cancer, we are interested in elucidating the roles of these claudins in ovarian tumorigenesis. We are approaching this problem from three different angles: 1) Characterization of the gene expression patterns for different claudins and investigate whether these claudins may be used as ovarian cancer biomarkers, 2) elucidation of the roles and functions of claudins in ovarian tumorigenesis, and 3) characterization of the mechanisms of transcriptional, post-transcriptional, and post-translational regulation of claudin proteins. Because both CLDN3 and CLDN4 are elevated in a large fraction of ovarian cancer, the mechanisms leading to their deregulation may represent a general pathway in ovarian tumorigenesis. In addition, we are using Illumina oligonucleotide arrays to characterize the roles of claudin-3 and -4 in cancer. We have identified genes that are altered by expression of claudins and are currently characterizing the roles of these genes in ovarian cancer.

Expression and roles of microRNAs in ovarian tumorigenesis: MicroRNAs (miRNAs) represent a class of small non-coding RNAs that control gene expression by targeting mRNAs and triggering either translation repression or RNA degradation. Emerging evidence suggests the potential involvement of altered regulation of miRNA in the pathogenesis of cancers, and these genes are thought to function as both tumor suppressors and oncogenes. Our goal for this project is to identify the roles of various microRNAs in ovarian cancer development. In order to reach this goal, we have used the Exiqon microRNA microarrays to identify several miRNAs aberrantly expressed in human ovarian cancer tissues and cell lines. miR-221 stands out as a highly elevated miRNA in ovarian cancer, while miR-21 and several members of the let-7 family are found downregulated. Public databases were used to reveal potential targets for the highly differentially expressed miRNAs. We are currently investigating the targets of these miRNAs in order to clarify their roles in ovarian cancer. This work involves the validation of the targets by RT-PCR in cell lines and tissues, as well as the expression of these targets in ovarian cells to functionally test their functions.

Analysis of Gene Expression Associated with Drug Resistance: Resistance to chemotherapy is a major problem in the treatment of ovarian cancer, as half of the patients present with cisplatin-resistant tumors. In addition, many ovarian tumors initially responsive to treatment often become refractory to chemotherapy. In order to study this problem, we have generated a series of lines individually resistant to 3 different drugs (cisplatin, doxorubicin and paclitaxel).. Using these models, we have utilized SAGE and microarrays to identify genes whose expression is altered in cisplatin-resistant cells. Among the genes differentially expressed in cisplatin-resistant cells, we have found many genes encoding proteins of the extracellular matrix. In addition, we have identified several genes that are common to several resistance types. For example, ABCB1 and GAGE genes were consistently elevated in resistant cells, while PRSS8 and MSMB1 were consistently downregulated. We are currently validating these and other candidate genes by quantitative RT-PCR in these cell lines. We are also planning to validate these genes in patient tissues using RT-PCR and immunohistochemistry. We are interested in identifying the mechanisms of cell adhesion-mediated drug resistance (CAM-DR) in ovarian cancer.

Collaborators: Kathleen R. Cho, M.D., University of Michigan Medical School; Michael Pazin, Ph.D., Laboratory of Cellular and Molecular Biology, NIA, NIH; James M. Mullin, Ph.D., Lankenau Institute for Medical Research. Ashani Weeraratna, Ph.D., Laboratory of Immunology, NIA, NIH; Richard B. Roden, Ph.D., Johns Hopkin Medical Institutions.

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Biography: Dr. Fugmann received his Ph.D. in human biology from the University of Ulm, Germany, in 1998. He went on to do his postdoctoral training at Yale University in the laboratory of Dr. David. G. Schatz, and joined the NIA as a tenure-track investigator in September of 2004.

Keywords:

V(D)J recombination
gene conversion
somatic hypermutation
RAG 1/2
evolution of adaptive immunity

Recent Publications:

Wilson et al., *Dev Comp Immunol* 2008, 32: 1221-1230.

Kothapalli et al. *J Immunol* 2008, 180: 2019-2023.

Gopal et al. *Mol Immunol* 2008, 45: 2062-2068.

Drejer-Teel et al. *Mol Cell Biol* 2007; 27: 6288-6299.

Yang SY, et al. *J Biol Chem* 2007; 282: 25308-25313.

Research Summary:

Mechanisms of Genome Rearrangements in Lymphocyte Development: B- and T-lymphocytes are unique components of the adaptive immune system in higher vertebrates. They stand out by their abilities to recognize an enormous variety of pathogens and to initiate a highly specific response to fight the infection. In order to do so, each individual lymphocyte displays a unique antigen receptor molecule (named immunoglobulin, Ig, in B-cells, and T-cell receptor, TCR, in T-cells) on its surface. There are four processes that generate such a remarkable diversity within the genes encoding these antigen recognition proteins: V(D)J recombination, somatic hypermutation (SHM), class switch recombination (CSR) and immunoglobulin gene conversion (GCV). They share a common theme in that they involve an active modification of the antigen receptor gene loci in each developing lymphocyte. Thus they also impose a high risk to each cell (and the entire organism) as an error in any of these processes can result in chromosomal aberrations that could ultimately lead to cancer.

V(D)J Recombination: The initial Ig and TCR repertoire is generated by V(D)J recombination. In this tightly regulated cut-and-paste process, functional antigen receptor genes get assembled from individual V, D and J gene segments. The only lymphocyte specific factors are encoded by the recombination activating genes RAG1/2. Together with the ubiquitous DNA-bending protein HMG1/2, they form the recombinase complex that cleaves the DNA in the first phase of the process. In the second phase, joining, this complex acts together with many non-homologous end-joining (NHEJ) family members (Ku70, Ku80, DNA-PKcs, artemis, xrcc4, DNA ligase IV) to religate the broken chromosome.

One focus of our lab is to gain a detailed understanding of the mechanism and regulation of V(D)J recombination starting from the initial recombinase complex formation, its interactions with its cognate DNA substrate to the final resealing of the broken DNA. To address these questions we are performing cell-based assays and biochemical experiments using purified (recombinant) proteins.

Evolution of Adaptive Immunity: We recently identified a gene pair in the genome of the purple sea urchin (Strongylocentrotus purpuratus) with striking similarity to the vertebrate RAG1/2 genes. The function of the encoded sea urchin Rag1/2-like proteins (spRag1L/spRag2L) is unknown, as this organism, to our current knowledge, shows no evidence of rearranging antigen receptor genes. We are currently pursuing *in vitro* and *in vivo* studies to characterize their molecular function, their role in sea urchin development and immunity, and the relationship to the classical vertebrate RAG1/2 proteins.

Somatic hypermutation and Immunoglobulin Gene Conversion: These closely related Ig gene diversification processes are the secondary diversification processes important for the fine-tuning of antibodies towards high-affinity recognition of pathogens. While SHM is common to all jawed vertebrates, GCV has been identified thus far in chicken, rabbits and sheep (but not in humans or mice). Both processes are initiated by the action of activation induced cytidine deaminase (AID), converting C to U in the context of single stranded DNA. The resulting mismatch is resolved by base excision repair and mismatch repair leading to individual point mutations in the case of SHM. In GCV the DNA lesion gets repaired by copying sequence information from upstream pseudogenes is into the exon encoding the antigen binding domain of the Ig molecules.

We use the DT40 chicken B-cell line as a model system to study the role of distinct (candidate) proteins in these processes, and to determine cis-acting DNA elements that target AID-dependent sequence diversification processes (somatic hypermutation and Ig gene conversion) to Ig genes.

Collaborators: Jonathan P. Rast, Ph.D., University of Toronto, Canada; Weidong Wang, Ph.D., Laboratory of Genetics, National Institute on Aging, NIH; David G. Schatz, Ph.D., HHMI , Yale University, New Haven, CT; F. Nina Papavasiliou, The Rockefeller University, NY.

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Biography: Dr. Pazin received his B.S. degree in chemistry from MIT in 1986, and his Ph.D. degree in cell biology from the University of California, San Francisco in 1992, and began working on chromatin biology as a postdoctoral fellow at the University of California, San Diego. Dr. Pazin was an Assistant Biologist/Assistant Professor at MGH/Harvard Medical School, and moved to his present position in the Laboratory of Cellular and Molecular Biology, National Institute on Aging in 2004. The main goal of his research is to understand the role of chromatin remodeling in gene expression, using lymphocytes and neurons as model systems.

Keywords:

chromatin remodeling
gene regulation
epigenetics
lymphocyte activation
neuronal differentiation

Recent Publications:

Du H, et al. *Mol Cell* 2008; 31: 641-649.

Wurster AL, et al. *Mol Cell Biol* 2008; 28: 7274-7285.

Zhang P, et al. *Curr Biol* 2008; 18: 1489-1494.

Research Summary: We are trying to understand how changes in chromatin structure regulate gene expression. Chromatin plays a critical role in regulating access to the information contained in the genome. Chromatin structure can change rapidly following chromatin remodeling enzymes are recruited, yet can also apparently be a stable source of epigenetic memory. We are interested in how remodeling enzymes change chromatin structure, how they find their target sites, and how remodeling causes changes in gene expression. We focus on the ATP-dependent class of remodeling enzymes. We use cell-based and cell-free assays to examine this problem, using lymphocytes, neuronal cells and their genes as model systems.

T Cell Activation and Differentiation: Activated T cells express a number of transcription factors and cytokines, and the gene expression program changes when T cells differentiate. We are using T cell activation and T cell differentiation as model systems to investigate the function of ATP-dependent remodeling enzymes. We are asking what remodeling enzymes are required for gene expression, and measuring how they change the chromatin structure of their target loci. We use primary murine T cells as well as human and mouse T cell lines in these studies. Recently, we found that the remodeling ATPase BRG1 regulates expression of the Th2 cytokine cluster (IL-4, IL-5, and IL-13) by binding to the LCR and other elements, and programming chromatin structure. BRG1 is recruited by STAT6 and NFAT transcription factors. We have identified other genes regulated by BRG1 and other remodeling enzymes.

Neurons, Gene Expression and Differentiation: Neuronal cells undergo changes in gene expression during embryogenesis and differentiation, as well as during pathological processes such as Alzheimer's and Huntington's diseases. We are interested in how remodeling plays a role in regulating these gene expression programs. In collaboration with Mark Mattson, we are examining the chromatin structure of developmentally regulated genes.

Collaborators: Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Patrice Morin, Ph.D, Laboratory of Cellular and Molecular Biology, NIA, NIH.

Laboratory of Clinical Investigation

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The Laboratory of Clinical Investigation (LCI) is comprised of 3 Sections and 1 Unit, with 4 principal investigators in total. These are as follows: Bioanalytical Chemistry and Drug Discovery Section; Diabetes and Genetics Section; Nuclear Magnetic Resonance Section; Metabolic Unit. The common thread among these research groups is the identification and development of new therapeutic targets for the treatment of age-related diseases. The Laboratory serves as an infrastructure to facilitate the creation and development of therapeutic targets within the Laboratory and across the Intramural Research Program. Activities relating to this theme within each Section are as follows:

Bioanalytical Chemistry and Drug Discovery Section (BCDDS): (PI Dr. Irving Wainer) In addition to developing the original science using receptor-immobilized columns and receptor structural modeling, this Section serves as a resource for determination of drug and metabolite structure and quantification, and for assignment of structure to larger proteins. It therefore conducts receptor/target conformational studies that provide the basis for understanding drug and receptor interactions. A goal is the creation and/or modification of drug structure that optimizes ligand (drug) receptor interactions. Use of receptor-immobilized columns for the nicotinic receptor, for example, is leading to better understanding of the on-off kinetics for various ligands that, coupled with animal or clinical pharmacodynamic data, suggest structural modification of known ligands or prediction of structure for ligands to be synthesized to achieve improvement in ligand-receptor binding characteristics. Similar studies are underway with the drug transporter, P-glycoprotein, and are beginning, in collaboration with Dr. Rui-Ping Xiao in the Laboratory of Cardiovascular Science, on the β_2 -adrenergic receptor.

Diabetes and Genetics Section (DGS): (PI Dr. Josephine Egan) Type 2 diabetes mellitus and the identification of new targets for its treatment is one of the focuses of this Section as it relates to drug discovery and development. Dr. Egan identified the GLP-1 receptor as a promising target as an insulinotropic agent. In the past Dr. Egan has shown in preclinical and clinical study that the GLP-1 receptor ligand exendin-4 may provide a new approach for the treatment of Type II diabetes mellitus. This work has provided the scientific basis, both preclinical and clinical, that made this therapeutic target and drug appropriate for clinical development and it is now FDA approved as exenatide (Byetta, Eli Lilly) and in use for the treatment of diabetes mellitus. The extension of this work is the evaluation of protein chimera analogs of GLP-1. These drug candidates appear not to cross the blood brain barrier and may offer an improvement in therapy over exenatide, as frequently nausea and vomiting limit its use. In addition, studies of the relationship of taste receptors and gut peptide release are complete. The present focus is the study of the mechanisms of the release of enteric peptides that modulate insulin release, and study of novel factors that regulate beta cell turnover.
Dr. Bernier focuses on insulin receptor activity. He is investigating the protein-protein interactions that make up the signaling unit of the insulin receptor, any part of which may be disrupted in type 2 diabetes mellitus. Dr. McDonnell studies the clinical genetics of connective tissue disorders such as Ehlers-Danlos Syndrome as well as the underlying metabolic abnormalities in the pathophysiology of these diseases. While the genetic abnormalities may not be amenable to engineering, the metabolic conditions may be treatable.

Nuclear Magnetic Resonance Section (NMRS): (PI Dr. Richard Spencer) The people in this section continue to make important contributions in the field of chrondrocyte biology using tissue bioreactors as cellular models of arthritis. With respect to identification of new therapeutic targets and drug development, the major effort is in characterization (phenotyping) of transgenic mice, studies of tissue bioenergetics in various animal disease models, studies of body composition and organ function, and evaluating the effects of treatments in the disease models. Collaborations with Dr. Egan, evaluating diabetes animal models, and with Dr. Lakatta in the Laboratory of Cardiovascular Science, studying heart failure models, have been quite informative and productive. Studies with Dr. McDonnell to characterize the phenotype of animals that model human skeletal dysplasias such as Ehlers-Danlos-like Syndromes and with Dr Martin on metabolic conditions that occur

as a complication of neurological diseases (Huntingon's and Alzheimer's Disease) are expected to produce animal models in which therapeutic interventions can be implemented and pharmacodynamics sensitively measured using *in vivo* NMR.

Metabolic Unit: (PI Dr. Bronwen Martin) The aim of this new unit it is to use an integrative systems biology approach to understand the multitude of factors that control metabolic function and energy homeostasis. Metabolism and aging are highly complex biological traits that involve entire networks of changes at both a molecular and systemic level, and require a broad systems approach to understanding how these networks change with age or are disrupted during disease. Dr Martin's group will focus on the development of novel therapeutic strategies that enhance metabolic function during the aging process and it aims to uncover effective treatment avenues for complex aging-related disorders that have underlying endocrine pathophysiologies. Additionally, the Metabolic Unit will investigate how cognitive ability or susceptibility to neurodegenerative disorders is affected by general systemic metabolic function, and how cognitive decline during aging could potentially be reduced by enhancing general metabolic health. In collaboration with the other sections in LCI and other laboratories at the NIA, the Metabolic Unit will use genetic, proteomic, metabolomic, NMR, *in vitro* and animal behavioral approaches to investigate alterations in metabolic function and energy homeostasis during aging, and to screen potential drug candidates and targets that can maintain or enhance metabolic control.

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Biography: Dr. Josephine M. Egan M.D. is a Board Certified Endocrinologist since 1989. After completing a medical residency and clinical pharmacology fellowship at Baylor College of Medicine in 1987, she continued on to endocrine training at the University of Virginia, Charlottesville. Since July 1990, she has been with NIA and is currently chief of the Diabetes Section within the Laboratory of Clinical Investigation. Her early work focused on investigating and quantifying insulin secretion from single beta cells of rat islets. This lead to further in-depth investigations of factors that mediate insulin secretion. Because insulin secretion decreases in type 2 diabetes, she specifically studied factors that might be of use to overcome this defect. This lead to a series of studies outlining the mechanisms of action of GLP-1, uncovering its biological effects and reaching the realization that GLP-1 agonists could be used to improve beta cell function in type 2 diabetes and aging. Subsequently, she demonstrated that exendin-4, a GLP-1 mimetic, was a superagonist of the GLP-1 receptor and she performed the first glucose clamp experiments in humans in the presence of exendin-4, showing it to be a powerful insulin secretagogue. Exendin-4 is now a treatment available for type 2 diabetic subjects. She also showed that chronic treatment of obese rodents with exendin-4 led to a slow and progressive weight loss, which is also of great benefit to diabetic subjects. Further work showed that exendin-4 increases beta cell turnover in rodents and this made it a powerful research tool to study beta cell division. Prior to that time, all models of beta cell turnover involved injury to the pancreas. Her research interests include Endocrinology, Clinical Pharmacology and studies of beta cells of the islets of Langerhans.

Keywords:

insulin
beta calls
GLP-1
Notch

Recent Publications:

Chia CW, et al. J Clin Endocrinol Metab 2008; 93: 3703-3716.

Martin B, et al. PLoS One 2008; 3: e2398.

Elahi D, et al. Obesity (Silver Spring) 2008; 16: 1501-1509.

Egan JM, et al. Mol Interv 2008; 8: 78-81.

Shin YK, et al. J Neurochem 2008; 106: 455-463.

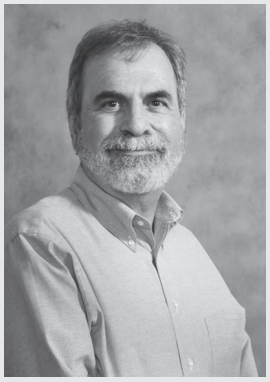
Designing Drugs for Treatment of Type 2 Diabetes: For most people, as they age, glucose tolerance declines progressively, and consequently the prevalence of type 2 diabetes is > 20% in Americans aged 65 years and older. Increasing resistance to insulin's peripheral effects is a near-universal finding in the elderly but insulin secretion in response to insulin secretagogues, especially glucose, also declines with age. Therefore, with increasing age, beta cells of the pancreas are less well able to compensate for increasing insulin resistance. Other substances, besides glucose, also influence insulin secretion, most notably insulinotropic peptides of the gut that are secreted from enteroendocrine cells lining the lumen of the gut. The most studied insulinotropic agents are two peptides called GLP-1 and GIP, sometimes collectively referred to as incretins. Upon food entering the intestine, the incretins are secreted and their plasma concentrations become elevated. They then activate specific incretin receptors on beta cells, and consequently insulin is secreted in a glucose-dependent manner. GLP-1 receptor agonists are under intense study as treatment for type 2 diabetes and one such agonist, exenatide, is indeed a new treatment for type 2 diabetes. It, however, must be injected twice daily in order to maintain increased insulin secretion. We are currently testing an analog

of GLP-1 that has been fused to human transferrin (hTf). hTf protects the GLP-1 from rapid breakdown and from renal excretion. Animal data shows that the half-life of GLP-1-hTf is about 3 days and so the hope is that one weekly injection may be sufficient to control blood glucose. A clinical study to evaluate this will employ state-of-the-art glucose-sensing equipment whereby continuous glucose monitoring before and immediately after drug administration will be carried out for up to 72 hours in free-living subjects. We therefore will have extremely accurate data on the length of the glucose-lowering effects. GIP is not the subject of quite as much research as is GLP-1, the reason being that the GIP receptor, unlike the GLP-1 receptor, is severely down-regulated in type 2 diabetes. Even attaining plasma GIP levels in type 2 diabetic subjects that are 7-10-fold higher than those found in plasma after eating, insulin secretion is still not increased by GIP. Therefore, in addition to on-going work related to GLP-1 and GLP-1 receptor actions, we are testing a superagonist of the GIP receptor in a double-blinded randomized trial of type 2 diabetic subjects. In parallel basic studies, we are attempting to unravel the mechanism whereby incretin-secreting cells sense macronutrients and respond with the appropriate amount of hormone. We have uncovered a taste signaling pathway for hormone-secreting cells homologous to chemosensation in taste receptor cells of the tongue and oropharynx. We are currently attempting to develop non-nutrient incretin secretagogues that we feel may have a place in treating diabetes and obesity.

The Aging Pancreas and its Relationship to Type 2 Diabetes: As the body as a whole, and the pancreas in particular, ages, beta cell turnover rates decline. While the topic of beta cell turnover is difficult to study in humans (one cannot simply biopsy pancreata and longitudinal studies are therefore not possible), in rodents all evidence suggests that beta cell replication is minimal after the age of 12-13 months. If this is also true for humans, it can be easily seen why beta cells cannot compensate for increasing insulin resistance associated with aging--a finite number of beta cells may reach a critical mass of insulin secretion and replication would not be an option to increase insulin secretory amounts. Incretins not only increase insulin secretion, as discussed above, but they also appear to increase beta cell mass through increased replication. Twenty-eight days continuous treatment of mice with exenatide increased beta cell mass about 2-fold and this was true for both young and old (17-month) animals. The IRS2 limb of the insulin/IGF-signaling pathway appears essential for beta cell replication because in IRS2 knockout animals, exenatide treatment did not lead to increased beta cell replication and the animals

eventually developed fatal diabetes. We have recently uncovered a novel pathway that controls beta cell turnover and hope to have made progress on fully elucidating this by next year. Impacting such a pathway would be useful for treatment type 1 and 2 diabetes.

Collaborators: Maire E. Doyle, Assistant Professor, Johns Hopkins, Baltimore, Maryland; Robert F. Margolskee, Department of Neuroscience, Mount Sinai School of Medicine, New York; Daniel Drucker, Department of Medicine, Banting and Best Diabetes Centre, Toronto General Hospital, Canada; Mark P. Mattson, Laboratory of Neurosciences, NIA, NIH.



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

He has published over 325 scientific papers and eight books. He was founding editor of the journal Chirality and Senior Editor of the Journal of Chromatography B: Biomedical Sciences and Applications for 11 years. His awards include: co recipient with Dr. John E. Stambaugh of the "Harry Gold Award" from the American College of Clinical Pharmacologists; "Sigma Xi Science Award", FDA Sigma Xi Club; "A.J.P. Martin Medal" presented by the Chromatographic Society for contributions to the development of chromatographic science; Elected Fellow of the American Academy of Pharmaceutical Sciences; Elected Member United States Pharmacopeial Convention Committee of Revision for 1995-2000. In June 2006 he was awarded an honorary doctorate in medicine (Doctoris Honoris Causa) from the Medical University of Gdansk, Poland. His research interests include Clinical Pharmacology, Bioanalytical Chemistry, proteomics and the development of on-line high throughput screens for new drug discovery with an emphasis in oncology and CNS diseases.

Keywords:

cancer cachexia
drug metabolism
immobilized receptors
high throughput screens
new drug discovery
molecular modeling

Recent Publications:

Jozwiak, K, et al. J Med Chem 2008, 50: 6279-6283.

Moaddel, R. et al. Anal Chem 2008, 80: 48-54.

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

Marszałł MP, et al. Anal Biochem 2008, 373: 313-321.

Moaddel R, et al. Anal Chem 2007; 79: 5414-5417.

Siluk D, et al. J Pharm Biomed Anal 2007; 44: 1001-1007.

Publications-continued

Moaddel R, et al. Br J Pharmacol 2007; 151: 1305-1314.

Since these observations were associated with advanced disease, we have initiated studies in patients suffering from terminal syndromes such as cancer cachexia. In particular, we have developed a direct measure of a proteolysis inducing factor (PIF) associated with cachexia. The PIF is measured in spot urines using capillary electrophoresis (CE). The presence of PIF in urine has been correlated with clinical status and with the identification of PIF in tumor biopsies. We have also correlated the presence of PIF in urine with treatment response and clinical relapse.

Based on these results, we have initiated a study of the effect of cancer cachexia and advanced aging on mitochondrial function. We have developed capillary electrophoresis and liquid chromatography methods coupled with mass spectrometry to assess beta-oxidation and ATP production in mitochondria obtained from control and diseased animals. In addition, techniques to assess the effect of disease status on the function and expression of mitochondrial enzymes, transporters and chaperons are under development and will be applied in cell-based, animal and human studies.

Immobilized Receptors, Transporters and Enzymes: We have developed liquid chromatographic stationary phases containing immobilized receptors, enzymes and transporters as an on-line, flow system for use in new drug discovery and in the characterization of lead drug candidates. These columns can range in size from standard lc columns to micro-columns, can be used to screen complex chemical mixtures, to characterize single compounds and to screen botanical extracts. The columns can be used with characterized targets such as ligand gated ion channels (nicotinic, GABA, NMDA receptors), G-protein receptors (opioid and □-adrenergic receptors), nuclear receptors (estrogen receptor and DNA unwinding binding element) drug transporters (P-glycoprotein, human organic cation and anion transporters), enzymes (cytochrome P450, phenylethanolamine N-methyltransferase, dopamine □-hydroxylase) as well as orphan receptors and other expressed proteins. In addition, the columns can be used to study the thermodynamics and kinetic of ligand-protein interactions and can provide data for molecular modeling studies. The technique can be used to pattern the expression of multiple receptors, ion channels and transporters contained in cellular membranes and we have demonstrated that the technique can identify inter-cell line differences in the expressions of the probed targets. Thus,

the approach can be used to study disease-related differences and to help develop disease-specific drugs. We have also immobilized proteins and receptors on the surface of magnetic beads and demonstrated that the beads can be used to "fish" out small molecules from complex mixtures and proteins from cellular extracts. This approach is being used to discover new drug candidates in herbal extracts and new cellular protein targets.

Bioanalytical Chemistry: We have developed a wide variety of new and unique bioanalytical methods for the quantification of drugs in biological matrices. These methods have been applied to pharmacokinetic and clinical studies. The studies involve the development and validation of the assay, the application of the assay to clinical studies and the pharmacokinetic analysis of the data. *In vivo* and *in vitro* metabolic studies are also associated with the studies and include the identification of the enzymes involved in the metabolic transformations. In addition, the studies have included the examination of the effect of disease status on hepatic and pre-systemic metabolism.

Collaborators: Michel Bernier, Laboratory of Clinical Investigation, NIA, NIH; Robert Clarke, Georgetown University; Alex Macieuk, Paris XI University, France; Jean-Francois Cloix, University of Lyon, France; John Lough, Sunderland University, UK; Carlo Bertucci, University of Bologna, Italy; Vencenza Andriasano, University of Bologna, Italy, Jun Haginaka, Mukogawa University, Japan.

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Biography: Dr. Nazli McDonnell graduated from University College Cork and Towson University with a B.A. degree in philosophy and a B.S. degree in chemistry and biology. She subsequently worked as a research assistant in Dr. Jeff Corden's laboratory at Johns Hopkins Medical School, Department of Molecular Biology and Genetics. She completed the M.D./Ph.D. program in University of Maryland Medical School in 1998. Her Ph.D. supervisor was Dr. Michael Summers in the Department of Biochemistry and Molecular Biology at the University of Maryland, Baltimore County. The focus of Dr. McDonnell's Ph.D. research was the study of protein-drug interactions by Nuclear Magnetic Resonance Spectroscopy. Dr. McDonnell's clinical training consisted of a residency in Internal Medicine at York Hospital, Pennsylvania, and a Medical Genetics Fellowship at the Metropolitan Washington D.C. Genetics Fellowship Training Program at National Human Genome Research Institute at the National Institutes of Health. In 2003, Dr. McDonnell joined Dr. Clair Francomano's laboratory at the National Institute on Aging, Laboratory of Genetics to study hereditary disorders of connective tissue. Upon Dr. Francomano's departure, she moved to the Laboratory of Clinical Investigation. Her professional memberships include Sigma Xi, American Medical Association, American Women's Medical Association, American Association for the Advancement of Science, American Society of Human Genetics, and Phi Kappa Phi.

Keywords:

genetics
connective tissue
aneurysm

Research Interests: Dr. McDonnell's research is focused on clinical and molecular investigations of hereditary disorders of connective tissue (HDCT). The disorders of interest are Ehlers-Danlos syndrome (EDS), Marfan syndrome, Stickler syndrome, hereditary aneurysm syndromes and fibromuscular dysplasia (FMD). Dr. McDonnell is investigating the natural history of these disorders at the NIA-ASTRA Unit, as well as studying genotype/phenotype correlations, molecular and cellular mechanisms and exploring treatment strategies in the laboratory in Laboratory of Clinical Investigation.

Current Laboratory Projects: In collaboration with Dr. Mark Talan's group, we are working with a mouse model of VEDS to discover and assess treatment strategies for VEDS. Other investigations include the role of tenascin X (TNXB) mutations and deletions in Hypermobile EDS and in patients with Congenital Adrenal Hyperplasia (in collaboration with Dr. Debbie Merke, NICHD), the study of genotype/phenotype correlations in Stickler Syndrome (with Dr. Ala-Kokko), discovery of new causative genes for familial aneurysm syndromes and in families with HDCT where no mutation in the known genes such as COL5A1, COL5A2, COL2A1, COL11A1, COL3A1, TGFBR1, TGFBR2 or fibrillin has been identified (with Dr. Andrew Singleton). With help from the NIA Research Resources Branch, we are studying tissue samples from patients with connective

tissue disorders utilizing cDNA microarray experiments and proteomics approach in order to identify treatment targets for the disorders of interest. Projects on the horizon include siRNA or pharmacological treatment strategies for dominantly inherited genetic disorders.

Current Clinical Projects - Natural History of Hereditary Disorders of Connective Tissue: We are investigating the cardiovascular and musculoskeletal complications of hereditary disorders of connective tissue, including autonomic dysfunction observed in patients with EDS, incidence of aneurysms and cardiovascular abnormalities in patients with all forms of HDCT, incidence of spine abnormalities and bone density loss in patients with HDCT, and pain and quality of life issues associated with HDCT. These investigations have uncovered a predisposition to craniocervical junction abnormalities including Chiari I malformation in patients with HDCT. We are also enrolling a group of patients with a diagnosis of FMD in order to define this disorder clinically and discover causative genes.

Principal Investigator, IRB approved protocol, National Institute on Aging: Project # 2003-86: "Clinical and Molecular Manifestations of Heritable Disorders of Connective Tissue." Patients with hereditary disorders of connective tissue have many early manifestations that usually afflict the elderly including osteoarthritis, loss of bone density, spinal disc disease, musculoskeletal weakness, arterial aneurysms, and alterations in vascular remodeling. Through clinical and laboratory evaluations in this group of patients, we expect to elucidate underlying mechanisms contributing to these common conditions associated with aging.

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Biography: Richard Spencer obtained his Master's Degree in Physics from U.C. Berkeley in 1981, and 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. He received his M.D. from Harvard Medical School in 1988, in the Health Sciences and Technology Division. He was a postdoctoral fellow with Professor Robert Griffin at the Francis Bitter National Magnet Laboratory of MIT before joining the National Institute on Aging in 1991. He completed internal medical residency training at Johns Hopkins Bayview Medical Center in Baltimore and is an Associate Professor of Medicine at Johns Hopkins Medical School in Baltimore, Maryland, and a Fellow of the American College of Physicians. Dr. Spencer has published over 95 articles and book chapters and is on the editorial board of several journals. He has been on the Faculty of the International Society of Magnetic Resonance in Medicine, delivering educational lectures on MRI hardware and signal processing. His research interests are primarily in magnetic resonance studies of engineered cartilage tissue, including three-dimensional cartilage grown from chondrocytes in an MRI-compatible hollow-fiber bioreactor, in spectroscopic studies of metabolism in cartilage, muscle, and heart under a variety of pharmacologic and physiologic conditions, phenotypic studies of mice, and methodology development in magnetic resonance imaging and spectroscopy.

Keywords:

magnetic resonance
imaging and spectroscopy
heart
cartilage
muscle
transgenic

Selected Recent Publications:

Ramaswamy S, et al. *J Biomed Mater Res B Appl Biomater* 2008; 86B: 375-380.

Ramaswamy S, et al. *Tissue Eng Part C Methods* 2008; 14: 243-249.

Velan SS, et al. *Am J Physiol Regul Integr Comp Physiol* 2008, 295: R1060-R1065.

A Bioreactor System for Magnetic Resonance Microimaging and Spectroscopy of Chondrocytes and Tissue: Repair of articular cartilage secondary to either traumatic injury or degenerative joint disease represents an important therapeutic challenge. In spite of significant progress in understanding the pathogenesis of this highly prevalent disease, there are no well-accepted disease-modifying interventions available. The development of a flexible and reliable MRI-compatible cartilage hollow fiber bioreactor (HFBR) system for neocartilage growth has the potential to contribute to therapeutic approaches. First, conditions promoting the growth of high-quality cartilage from cells can be studied intensively in such a system, which provides full control over exposure of the developing neocartilage to growth factors, substrate composition, dissolved O2 and CO2 concentrations, temperature, and other environmental factors. While in situ development of cartilage from cells, including both chondrocytes and, potentially, bone marrow stromal cells, in an organism will differ in important ways from the bioreactor conditions, in vitro studies will be able to point the way to appropriate conditions for development of functioning neocartilage from cells. A related strength of this system is that it provides a flexible test-bed for current and future therapeutic agents and interventions. Second, growth

Bi X, et al. *Anal Bioanal Chem* 2007; 387: 1601-1612.

Galbán CJ, et al. *Osteoarthr Cartil* 2007; 15: 550-558.

Galban CJ, et al. *Magn Reson Med* 2007; 58: 8-18.

Fishbein KW, et al. *Magn Reson Med* 2007; 57: 1000-1011.

McConville P, et al. *Am J Physiol Endocrinol Metab* 2007; 293: E1828-E1835.

of high-quality cartilage in the bioreactor may result in a source of tissue for actual transplantation. Finally, and most generally, regardless of the specifics of eventual cartilage repair and regeneration procedures, the ability to monitor tissue quality is of great importance. Our bioreactor system permits such methods to be developed across a wide range of conditions. All MRI studies for this work are performed on our 9.4 Tesla system at the Gerontology Research Center.

We have successfully demonstrated that cartilage grown from chick sternal chondrocytes and bovine articular chondrocytes in the HFBR will develop and maintain the hyaline phenotype, that morphologic measurements with MRI correlate with tissue histology, and that MRI measurements of local T1, T2, diffusion and MT correlate with biochemical assays of collagen, proteoglycans and hydration. Thus, noninvasive MRI measures provide reliable information about cartilage matrix composition. We have further demonstrated that cartilage growth in the HFBR can be modified by introduction of biologically active compounds, and that the correlations between MRI-derived parameters and biochemical results noted above are maintained in spite of the greater dynamic range of tissue characteristics resulting from these interventions. In addition, a major focus of our work has been to demonstrate that MRI measurements of matrix fixed density correlate with measurements of dynamic and equilibrium compressive moduli.

Mouse Phenotyping Studies:

Assessment of skin abnormalities in a mouse model of osteogenesis imperfecta (OI) (with Nancy Pleshko)

We have studied the *oim/oim* mouse, which exhibits a mutation leading to abnormal collagen structure. This results in bone fragility similar to that seen in patients with OI. Because the characteristics of skin are also highly dependent upon collagen integrity and organization, we are using MRI, infrared spectroscopy, and histology to assess the properties of skin in these animals, and have found significant abnormalities. While these modalities are all sensitive to different biophysical properties, the results among them are consistent and together indicate a state of disordered collagen packing in the skin of the *oim/oim* mouse. Key findings include: MR microscopic images correspond well to histologic images for overall skin structure, fat, and collagen. Dermal layer thickness is reduced in *oim/oim* mice, although overall skin thickness is greater. The *oim/oim* mice show a deep dermal layer not seen in wild type. Reduced concentration

and likely impaired structure of collagen in the dermal layer of *oim/oim* mice is suggested by reduced magnetization transfer and long T2 values. Based on these results, we are currently investigating the question of whether MRI will be sensitive to skin abnormalities in OI patients. If this proves to be the case, this approach may lead to rapid and non-invasive diagnostics for OI in patients who do not exhibit multiple fractures. These MRI studies are performed on our 9.4Tesla system at the Gerontology Research Center.

***In vivo* studies of a transgenic mouse with an altered cardiac calcium channel (with Nikolai Soldatov and Darrell Abernethy)**

We are performing anatomic and functional cardiac studies on a mouse that overexpresses a human calcium channel type which appears to be characteristic of atherosclerotic disease. While the mice exhibit no physiological abnormalities at rest, it is frequently the case that transgenic animals or those representing a disease model exhibit a non-normative response only under chronic or acute stressors. Accordingly, we are studying the response of these animals to a 7 day infusion of isoproterenol via an implanted Alzet pump. We have found significant differences in the effects of the isoproterenol infusion in the wild type (WT) in comparison to the transgenic (TG) mouse. Current results indicate that the LV weight increased by 11% in the WT animals, but by 19% in the TG animals. The end-systolic volume increased by 89% in the WT, but by 176% in the TG. The cardiac output increased by 27% in the WT, but by only 7% in the TG. All of these differences were statistically significant. Overall, these findings are consistent with increased intracellular calcium as a signal for cardiac hypertrophy, exaggerated by isoproterenol, and an exaggerated isoproterenol-induced cardiomyopathy.

Cardiac MRI studies are performed on our 7 Tesla system at the Gerontology Research Center.

Collaborators: Edward Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Nancy Pleshko Ph.D., Exponent, Philadelphia; Nikolai Soldatov, Ph.D., Laboratory of Clinical Investigation, NIA, NIH; Al Grodzinsky, Ph.D., MIT, Boston, MA; Thorsten Kirsch, Ph.D., NYU, New York, New York

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Biography: Dr Bronwen Martin received her B.Sc. (Hons) from the University of Kent at Canterbury in Biochemistry, and her Ph.D. in Endocrinology from the University of Edinburgh (College of Medicine and Veterinary Medicine), where she had a Medical Research Council Postgraduate Scholarship. During this time, she worked with Professor Alan McNeilly and Professor Richard Sharpe at the MRC Human Reproductive Sciences Unit, investigating the effects of neonatal endocrine disruption on the function of the hypothalamic-pituitary-gonadal hormonal axis.

In 2004, she joined the Laboratory of Neurosciences at the National Institute on Aging in Baltimore, MD, and conducted postdoctoral work investigating neuro-endocrine alterations during aging and age-related neurodegenerative disorders. During her postdoctoral training, she was funded by the Huntington's Disease Society of America (HDSA) to investigate novel endocrine-related therapeutic targets for the treatment of Huntington's disease. In 2009 she was appointed as a tenure track investigator in the Laboratory of Clinical Investigation, where she now heads the Metabolism Unit.

Keywords:

metabolism
energy homeostasis
endocrinology
systems biology
aging

Recent publications:

Martin B, et al. *Trends Endocrinol Metab* 2009; in press.

Martin B, et al. *J Biol Chem* 2009; 284: 2493-2511.

Martin B, et al. *Diabetes* 2009; 58: 318-328.

Chadwick W, et al. *Trends Neurosci* 2008; 31: 504-511.

Martin B, et al. *J Neurochem* 2008; 106: 455-463.

Martin B, et al. *Histol Histopathol* 2008; 23: 237-**250.**

Research overview: Metabolic function and aging are complex traits that involve entire networks of changes at the molecular level, driven by genetic and environmental perturbations. The aim of the Metabolism Unit is to use an integrative systems biology approach to understand the myriad of factors that control metabolic function and energy homeostasis and how metabolic control is altered/dysregulated during aging and age-related disorders. The Metabolism Unit strives to develop novel therapeutic strategies and compounds that enhance metabolic function and energy homeostasis during the aging process and to uncover effective treatment strategies for complex disorders that have underlying endocrine pathophysiologies.

Aging and age-related disorders are often associated with endocrine axes imbalances. These endocrine axes are multi-level entities that require a systems-level mode of investigation. In the face of disease/pathophysiology, these axes become disrupted and potentially exacerbate the pathology. It is likely that dietary/pharmacological/behavioral re-adjustment of these disrupted endocrine axes may be the best mechanism to create an effective, long-lasting and least deleterious treatment for age-related disorders such as diabetes, infertility, obesity, metabolic syndrome, and various neurological disorders such as Alzheimer's disease,

Publications-continued
Martin B, et al.
Endocrinology 2007; 148: 4318-4333.

Parkinson's disease, and Huntington's disease. With endocrine axis re-adjustment, the beneficial effects of the therapeutic intervention may be more stable and greatly amplified by the axis itself, compared to the traditional one-target therapeutic strategies.

The research focus of the Metabolism Unit is as follows:

1) <u>The energy-behavior 'super-axis'.</u> This series of projects aim to elucidate and characterize - at a systemic level - endocrine regulation and feedback during the aging process, with special attention to euglycemic control axes and how these major energy axes may act as a master integrator for additional endocrine axes such as the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. Therefore, multiple dietary, digestive and reproductive axes can be integrated at the humoral level to form a higher order 'super-axis'. Many of the hormones that create the complex feedback loops in this super-axis provide a multi-level connection between cognitive appreciation of food supply, energy sensation/perception and eventual energy metabolism. Using animal models of aging or metabolic pathophysiology (e.g. type 2 diabetes, metabolic syndrome, Alzheimer's disease) we use various genomic, proteomic, bioinformatic and behavioral approaches to model and predict complex endocrine feedback loops and determine which pharmacological endocrine axes targets could be best used to enhance metabolic health and improve health-span during the aging process.

2) <u>Flavor perception and metabolism.</u> These projects aim to investigate how different forms of dietary energy intake and nutritional input affect neuroendocrine function and general metabolic health during development, puberty and aging. We are investigating how flavor perception – through vision, olfaction and gustation – is linked to general metabolic control and how flavor perception can be altered pharmacologically to enhance peripheral metabolic function and energy homeostasis.

3) <u>Cognitive function and glycemic control.</u> These projects aim to investigate how multiple forms of cognitive ability or susceptibility to neurodegenerative disorders are affected by general systemic metabolic function, and how cognitive decline during aging could potentially be reduced by enhancing glycemic function and maintaining general metabolic health. Previously, we have already pioneered this work by linking the progression of Huntington's disease to a diabetic-like

state. It is clear from several lines of evidence, including our own, that hormones responsible for glycemic regulation also play vital roles in cognitive function. It is likely that through the creation of multiple types of euglycemic therapeutics there will also be the creation of distinct mechanisms by which these strategies affect cognitive function. These projects will aim to first understand how multiple types of glycemic control can affect neuronal function and then to tailor distinct novel therapeutics with enhanced desirable characteristics.

4) Strategies to enhance metabolic function during aging. These projects will use a systems biology approach (using genetic, proteomic, bioinformatic and mathematical methods) to gain a greater insight into how metabolic function changes during life-span. This will involve conducting multiple tissue analyses on organs that are known to play a role in controlling metabolic function, such as: hypothalamus, pituitary gland, olfactory bulb/tongue, gut, pancreas, liver, adipose tissue, muscle, bone, thyroid, and gonads. Once we gain a greater insight into alterations in the metabolic/energy homeostasis 'super-axis' during the aging process, we can then elucidate which pharmacological endocrine axes targets could be best used to enhance metabolic health.

Summary: The rapidly rising numbers of patients suffering from type 2 diabetes, metabolic syndrome, obesity, and obesity-related disorders call for much needed innovative and clinically-oriented research aimed at finding novel strategies for maintaining or enhancing glycemic heath and improving general metabolic well-being in an increasingly elderly population. The goal of the Metabolism Unit is to improve metabolic health in an aging population and to find novel strategies to help patients suffering from disorders that have underlying endocrine pathophysiology.

Collaborators: Jacki Crawley, Ph.D., NIMH, Jean-Lud Cadet, M.D., NIDA, Chris Peers, Ph.D., University of Leeds, Stephan von Hörsten, M.D., Friedrich-Alexander-University of Erlangen-Nürnberg, James Waschek, Ph.D., UCLA, Christopher Ross, M.D., Johns Hopkins University School of Medicine, Bob Cole, Ph.D. Johns Hopkins University School of Medicine, Jennifer Payne, M.D., Johns Hopkins University School of Medicine, Kevin Becker, Ph.D. NIA, Andrew Zimmerman, M.D., , Kennedy Krieger Institute, Johns Hopkins University, Michelle Harvie, Ph.D., University of Manchester, Steven Munger, Ph.D. and Shawn Dotson, Ph.D., University of Maryland, School of Medicine.

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The Laboratory of Epidemiology, Demography, and Biometry (LEDB) conducts research on aging and age-associated diseases and conditions using population-based epidemiologic and biometric methods. Laboratory staff work collaboratively both within and among four groups: the Epidemiology and Demography Section, the Neuroepidemiology Section, the Geriatric Epidemiology Section, and the Biometry Section and with other NIA and outside investigators. The mission of LEDB is to elucidate the etiology of diseases of old age by combining epidemiologic data with information from other disciplines; evaluate the consistency of epidemiologic data with etiologic hypotheses developed either clinically or experimentally; and to provide the basis for developing and evaluating preventive procedures and public health practices. These general principles have guided a research agenda that emphasizes three important and interrelated areas: Physical Function and Disability, Cognitive Function and Dementia, and Age-associated Diseases and Conditions – including successful or effective aging. In each area, studies are influenced by results of analytic efforts of current LEDB-sponsored studies and by opportunities created by advances in biology. Cross-cutting research themes being addressed by more than one LEDB investigator are: Comorbidity/Coimpairment, Genetic Epidemiology, Inflammation, Socioeconomic Status and Health, Diabetes/Metabolism, and Energy Balance-Physical Activity/Obesity.

The Epidemiology and Demography Section plans and conducts studies on chronic diseases, functional status and disability in the older population. The Neuroepidemiology Section conducts interdisciplinary research on the association of genetic, molecular, and behavioral factors in relation to brain disease in old age. The Geriatric Epidemiology Section carries out interdisciplinary studies of the association of molecular and genetic

risk factors with health outcomes in old age, including discrete diseases, disability and mortality. The Biometry Section conducts research in the mathematical, statistical and numerical aspects of aging and health. This Section provides statistical consulting, computing, graphics, and data management services to the other units within LEDB. Senior LEDB staff consult with other components within the Intramural Research Program, NIA, other NIH Institutes, other government agencies, and the private sector. LEDB research interests use data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE); the Women's Health and Aging Study (WHAS); the Honolulu-Asia Aging Study (HAAS); the Health, Aging and Body Composition (Health ABC) Study; Age, Gene/Environment Susceptibility (AGES) Study Reykjavik, Iceland; and the InChianti Study. Senior investigators are leading efforts in two large clinical trials: ACCORD-MIND (Action to Control Cardiovascular Risk in Diabetes), a study to evaluate whether aggressive control of risk factors for atherosclerosis in diabetics reduces cognitive decline, and LIFE (Lifestyle Interventions and Independence for Elders) trial to evaluate if physical activity prevents the onset of disability.

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Biography: Dr. Guralnik received his M.D. from Jefferson Medical College in Philadelphia and his M.P.H. and Ph.D. from the School of Public Health, University of California, Berkeley. He practiced as a primary care and public health physician prior to his Ph.D. training. He is Board Certified in Public Health and General Preventive Medicine. Before coming to NIH he did research on predictors of healthy aging in the Human Population Laboratory Alameda County Study in Berkeley, California. He has been in the Laboratory of Epidemiology, Demography, and Biometry at the National Institute on Aging since 1985, has been the Chief of the Epidemiology and Demography Section since 1991, and has been Chief of the Laboratory since 2004.

Keywords:

epidemiology
chronic diseases
disability
functional status
physical activity

Recent Publications:

Volpato S, et al. *J Am Geriatr Soc* 2008; 56: 621-629.

Guralnik JM. *Arch Int Med* 2008; 168: 131-132.

Ferrucci L, et al. *Br J Haematol* 2007; 136: 849-855.

Shumway-Cook A, et al. *J Am Geriatr Soc* 2007; 55: 58-65.

Giannelli SV, et al. *J Am Geriatr Soc* 2007; 62: 296-300.

The Epidemiology and Demography Section plans and conducts epidemiologic studies of the risk factors for specific chronic diseases important in aging and pursues research on the consequences of disease, especially the effects of chronic disease on functional limitations, disability, and the ability to remain independent in the community. Assessing the roles of behavioral, psychosocial, and demographic risk factors in the development of disease and disability is also an important area of research. Particular attention has been focused on the development of mobility disability and how factors such as strength and balance, exercise, and measures of physical performance predict the loss of walking ability. Research interests have been pursued using data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE), the Women's Health and Aging Study (WHAS), the InChianti Study and the British 1946 Birth Cohort Study.

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

was produced to instruct physicians and investigators in the standardized battery that has been extensively studied in the EPESE study, the Women's Health and Aging Study, and others. This battery, known as the Short Physical Performance Battery (SPPB) has now been evaluated in the outpatient clinical setting and found to be feasible to administer and highly predictive of adverse clinical events. It is being employed in the LIFE clinical trial as a method to screen in older persons who have functional limitations and as an outcome variable. Research into the use of the SPPB in the hospital setting is now underway and application of the battery in the assessment of persons with heart failure has been performed.

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

Physical Activity and Exercise: A major research interest has been in examining the impact of physical activity and exercise on disability and other health outcomes in older people. Past work demonstrated the risk of incident disability related to sedentary lifestyle. Data from the WHAS have shown that many women with difficulty walking continue to walk for exercise while nearly half of the women without difficulty don't walk at all for exercise. The amount of walking for exercise done by older women is strongly influenced by their level of disease and disability, but many psychosocial variables also influence the amount of walking these women do. Recent findings indicate that even very modest amounts of

walking are associated with lower rates of disability onset. Dr. Guralnik has participated in the development of the LIFE Study, a randomized clinical trial evaluating the impact of physical activity in preventing mobility disability in non-disabled but at-risk older persons. A large pilot sutdy, LIFE-P, with over 400 participants showed statistically significant difference between the physical activity group and the successful aging control group in two measures of mobility-related functioning, the SPPB and gait speed in the 400 meter walk.

Anemia: Anemia is common in the older population, but the impact of anemia on older persons, especially mild anemia, had not received much research attention prior to 5 years ago. In 2004 we published a paper using the nationally representative Third National Health and Nutrition Examination Survey (NHANES III) that described the prevalence of anemia across the full age spectrum, showing steep increases in its prevalence at older ages, and demonstrating that, in older persons with anemia, evidence of nutrient deficiency or blood loss was present in one third, anemia of chronic inflammation (formerly called anemia of chronic disease) or anemia of chronic renal disease or both were present in one third, and unexplained anemia was present in one third. Our research on the impact of anemia on change in the SPPB, using data from the EPESE, demonstrated greater functional decline at lower levels of hemoglobin. A further series of papers explored the consequences of anemia in terms of mortality, hospitalization and physical and cognitive functional outcomes, with results showing that hemoglobin levels below the established WHO cutpoints (12 g/dL for women and 13 g/dL for me) and in some instances hemoglobin just over the cutpoints were generally associated with adverse outcomes even after adjusting for multiple measures of baseline health status and disease burden.

Health Disparities: We have had a long-standing interest in the impact of social class on health and have demonstrated that educational status and income are powerful predictors of disability onset and mortality. We have also shown that active, or disability-free, life expectancy is considerably longer in persons with higher levels of education. Race also plays a role in the health of older persons although its influence, after adjustment for education and income, has not been consistently demonstrated. Recent work using data from the British 1946 cohort study has been evaluating the relationship of parental occupation and education in early life relates to functional status in middle age.

Collaborators: Dr. Luigi Ferrucci, Longitudinal Studies Section, Clinical Research Branch, NIA, NIH; Dr. Andrew Singleton, Laboratory of Neurogenetics, NIA; Dr. Linda Fried, Columbia University School of Public Health; Drs. Karen Bandeen-Roche, Richard Semba, Johns Hopkins Medical Institutions; Dr. Helaine Resnick, American Association for Homes and Services for the Aged, Washington, DC; Dr. Mary McDermott, Northwestern University School of Medicine; Dr. Marco Pahor, University of Florida; Drs. Steven Kritchevsky, Mike Miller, Mark Espeland, Wake Forest University School of Medicine; Dr. Ann Shumway-Cook, University of Washington, Seattle; Dr. Stephanie Studenski, University of Pittsburgh; Dr. Suzanne Leveille, Beth Israel Deaconess Medical Center and Harvard Medical School; Dr. Meredith Minkler, University of California, Berkeley; Dr. Diana Kuh, Medical Research Council National Survey of Health and Development, London, England; Dr. Chiara Corti, Regional Health Administration, Padua, Italy; Drs. Howard Bergman and Francois Beland, McGill University, Montreal, Canada; Dr. David Melzer, Penninsula Medical School, Exeter, England; Professor Sir Michael Marmot and Dr. Eric Bruner, University College, London, England; Dr. Sallie Lamb, Oxford University, England; Dr. Marja Jylhä, University of Tampere, Finland; Dr. Taina Rantanen, University of Jyväskylä, Finland.

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

Keywords:

molecular and genetic epidemiology
bioimaging
chronic disease
body composition
inflammation
aging

Recent Publications:

Manini TM, *et al. JAMA 2006; 296(2): 171-179*

Nicklas BJ, *et al. J Am Geriatr Soc 2006; 54(3): 413-420.*

Eiriksdottir G, *et al. Atherosclerosis 2006; 186(1): 222-224.*

Visser M, *et al. J Gerontol A Biol Sci Med Sci 2005; 60(3): 324-333.*

Newman AB, *et al.Am J Clin Nutr 2005; 82(4): 872-878.*

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

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

which multiple conditions contribute to disability. Since little was known about sarcopenia in an unselected population, the Health ABC population was selected as well-functioning and relatively health-stable, but at high risk of health transitions secondary to age, race and gender characteristics. The Health ABC cohort consists of 3,075 black and white men and women aged 70-79 (46 percent of the women and 37 percent of the men enrolled are black) who initially reported no difficulty walking at least 1/4 mile and or up a flight of stairs. The major study outcome is report of new limitation in walking 1/4 mile or up stairs, complemented by assessment of performance on a 400-meter walk, quadriceps strength, and other objective functional tests. Morbidity and mortality are also assessed.

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

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

The Geriatric Interdisciplinary Studies Section also has an ancillary study in the Osteoarthritis Initiative to investigate the relationship of muscle mass in the leg, strength, and the importance of fatty infiltration into muscle in relationship to incidence and progression of knee osteoarthritis. This involves a measure of quadriceps and hamstrings strength as well as a protocol for imaging of the muscles of the leg with a quantitative assessment of muscle lipid.

Causes and Consequences of Inflammation in Diseases of Old Age: The focus of efforts in the Geriatric Interdisciplinary Studies Section has been on the contribution of chronic low-level inflammation to health outcomes apart from cardiovascular disease, and to understanding what conditions and behaviors appear to be linked to low-level inflammation. A number of data sets have been used to explore the relationship of chronic low-level inflammation with health risks in old age. These efforts have involved studies of mortality, disability, cardiovascular disease, diabetes and glucose metabolism, smoking and pulmonary function, cognition, and weight and fat distribution. Visceral fat has been identified as the fat depot most consistently associated with higher levels of cytokines; however, fat infiltrating into muscle also appears to be associated with higher cytokines as well. There is on-going analysis of these data to assess whether the poor health outcomes associated with elevated cytokines is due to direct effects of elevated cytokines or whether the elevated cytokines represent severity of the underlying condition and the condition ultimately is responsible for the increased health risk.

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

The Geriatric Interdisciplinary Studies Section has also carried out studies of selected polymorphisms pertinent to inflammation and body composition measures in nested case-control studies in the Health ABC

Study and in other datasets developed for this purpose. Efforts have been made to broaden the application of emerging techniques for genomic and proteomic studies to populations by development of new methods in collaboration with laboratory-based investigators. These efforts include panels of SNP's in Health ABC including admixture markers and several panels of SNP's in the AGES-Reykjavik Study.

Collaborators: Lenore Launer, Ph.D., Neuroepidemiology Section, NIA, NIH; Dennis Taub, Ph.D., Laboratory of Immunology, NIA, NIH; Eleanor Simonsick, Ph.D., Luigi Ferrucci, M.D., Ph.D., Longitudinal Studies Section, Clinical Research Branch, NIA, NIH; Gayle Lester, Ph.D., Project Director, OAI, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH; Anne Newman, M.D., M.P.H., Lewis Kuller, M.D., Jane Cauley, Ph.D., Bret Goodpaster, Ph.D., University of Pittsburgh; Stephen Kritchevsky, Ph.D., Wake Forest University School of Medicine; Fran Tylavsky, Ph.D., Ron Shorr, M.D., University of Tennessee, Memphis; Steven Cummings, M.D., M.P.H., Michael Nevitt, Ph.D., Susan Rubin, M.S., Susan Averbach, M.S., Emily Kenyon, Ph.D., Thomas Lang, Ph.D., Thomas Fuerst, Ph.D., Charles Peterfy, M.D., University of California, San Francisco; Russell Tracy, Ph.D., University of Vermont; Marjolein Visser, Ph.D., Free University, Amsterdam, Netherlands; Stefania Maggi, M.D., M.P.H., University of Padua, Padua, Italy; Mauro Zamboni, M.D., University of Verona, Verona, Italy; Dennis Taaffe, Ph.D., University of Brisbane, Australia; Dympna Gallagher, Ph.D., Columbia University College of Physicians and Surgeons, New York, New York; Helaine Resnick, Ph.D., Washington Hospital Center, Washington, D.C.; John Robbins, M.D., University of California, Davis; Teresa Seeman, Ph.D., David Reuben, M.D., University of California, Los Angeles; Harvey Cohen, M.D., Duke University; Vilmundur Gudnason, M.D., Ph.D., Palmi Jonsson, M.D., Gudmundur Thorgeirsson, M.D., Ph.D., Gunnar Sigurdsson, M.D., Ph.D., University of Iceland and Icelandic Heart Association.

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

Keywords:

epidemiology
neurologic diseases
genetic and environmental risk factors

Recent Publications:

Saczynski J, et al. *Am J Epidemiol* 2008, 168: 1132-1139.

Qiu C, et al. *Diabetes* 2008; 57: 1645-1650.

Laurin D, et al. *Circulation* 2007; 116: 2269 -2274.

Harris T, et al. *Am J Epidemiol* 2007; 165: 1076-1087.

The paradigm of Alzheimer's disease (AD) has shifted. Whereas previously co-morbid cardiovascular disease was an exclusion criterion for a diagnosis of AD, many recent epidemiologic studies, including those from the **Neuroepidemiology Section** show that AD often co-occurs with sub-clinical and clinical cardiovascular disease, and that this co-morbidity may contribute to the pathogenesis of the disease or its clinical presentation. Further, neuropathologic data show that there are multiple pathologies in the brains of older persons, all of which may contribute to dementia.

Within this paradigm, studies in the Neuroepidemiology Section focus on understanding the contribution of genetic, life style, inflammatory, metabolic, vascular, and hormonal factors to sub-clinical and clinical outcomes in brain disease; investigating the links between different markers of brain disease; and identifying the associations of brain markers to other common diseases of old age. Also of interest is the investigation of the functional consequences of different markers of brain disease. Research is largely based on population-based epidemiologic studies, which allow us to test in the general population, hypotheses on risk/protective factors and mechanisms of brain aging. Investigations into novel biomarkers and methodologic studies support this research.

Studies conducted by the Neuroepidemiology Section include a broad range of brain structure and function measures, including memory and executive domains of cognitive function; MRI measures of white matter lesions, (sub)-clinical stroke, regional brain atrophy; brain tissue integrity; and vascular function; clinical dementia and sub-types (AD and vascular

dementia); and neuropathologic markers of neurodegeneration and vascular disease.

Research Resources

Much of the research in the Section is based on two large prospective community-based cohorts: the AGES-Reykjavik (Age Gene-Environment Susceptibility – Reykjavik Study), and the HAAS (Honolulu Asia Aging Study). Both studies were established in the mid-1960s to answer questions about the heart disease epidemic that became a public health priority during that decade. Both have similar measures of cardiovascular risk factors in middle-age. The studies also have complementary measures of brain aging; whereas the HAAS includes a rich autopsy sub-study, AGES-Reykjavik has a wealth of cognitive and MRI data.

The HAAS is based on a prospective population-based cohort of Japanese American men born 1900-1919 that was initiated in 1965 as a part of the Honolulu Heart Program (HHP). To better understand the factors associated with diseases of old age, the HHP was extended to the HAAS in 1991 (n=3734); the study is currently on-going. Starting in 1991, global cognitive function was measured in the total sample and cases of dementia ascertained. Clinical measurements, demographic medical information, and biospecimens are collected at the exams. An autopsy study nested within the cohort was also started in 1991; a MRI sub-study of 575 men was performed in 1995-1996. The HAAS has contributed importantly to understanding the role of risk factor levels in middle age, measured in the HHP, to late life brain disease measured in the HAAS. Research in HAAS is done in close collaboration with Dr. Lon White [PI of the HAAS] and the other members of the HAAS team.

AGES-Reykjavik is a prospective population-based cohort of men and women (n=5764) born 1907-1934. The cohort was established in 1967 as the Reykjavik Study, by the Icelandic Heart Association [IHA]; participants were followed up to six times. In 2002 this study was extended to the AGES-Reykjavik Study to advance our understanding of genetic and non-genetic risk factors for diseases in old age. The AGES-Reykjavik Study focuses on multiple physiological systems, including neurocognitive, vascular, musculoskeletal, and body composition, and metabolic measures. The phenotypes are measured with high quality quantitative measures of phenotypes, including bio-images and bio-specimens. Owing to the excellent archival system in Iceland, birth and school records on this cohort are available. In addition, a registry on

coronary disease and interventions, fractures, and mortality is maintained, and a registry on stroke and congestive heart failure is under development. The second exam in the AGES-Reykjavik Study is currently on-going. Research in AGES-RS is a collaborative effort with Dr. Tamara Harris [NIA] and the team at the IHA [Dr. Gudnason, PI].

Translation of the vascular hypothesis of dementing diseases is studied in the context of a large NHLBI clinical trial - ACCORD (Action to Control Cardiovascular Risk in Diabetes) [http://www.accordtrial.org]. This trial includes a large sample of persons with type 2 diabetes over 55 years of age and persons 45 years of age and older with a history of cardiovascular disease. It is designed to compare the effects on cardiovascular disease of standard versus intensive treatment of major risk factors in diabetes: hyperglycemia, hypertension, and dyslipidemia. In 2002 the MIND (Memory in Diabetes) sub-study was initiated to measure the impact of the intensive treatment strategy on brain function as measured by cognitive tests (n=2970) and brain structure as measured by magnetic resonance imaging (n=620). Research is conducted in collaboration with Wake Forest University [Dr. J. Williamson, PI Coordinating Center] and participating network PIs.

To better understand the genetic components of normal and pathologic brain morphology and function, several projects have been established within the major research resources. Studies are in progress to identify accurate phenotypes to better identify genes that regulate pathology in the pathways leading to such diseases as Alzheimer's disease, MRI outcomes such as ischemic lesions, and neuropathologic markers such as neuritic plaques. We have completed a genome wide scan on 3200 individuals participating in the AGES-Reykjavik Study, and have completed, or are planning to follow-up, investigations into more targeted genes. This research requires an extensive network of collaborators from all over the world.

Ongoing research in the Neuroepidemiology Section also includes smaller studies to investigate novel markers of disease and studies to specifically address the public health impact of cardiovascular risk factors on brain disease in old age.

Collaborators: TB Harris, M.D., M.S., J. Guralnik, M.D., Ph.D., Laboratory of Epidemiology, Demography, and Biometry, NIA, NIH; L.R. White, M.D., G.W. Ross, H Petrovitch, K. Masaki Pacific Health

Research Institute, Hawaii; M.M.B. Breteler, M.D., Ph.D., Erasmus University Medical Centre, Netherlands; M.F. Ferrari, M.D., Ph.D., M.A. van Buchem, M.D., Ph.D., Leiden University Medical Centre, Netherlands; A. Zijdenbos, Montreal Neurologic Institute, Montreal Canada, A; O. Lopez, University of Pittsburgh; V. Gudnason, M.D. PhD; G. Eiriksdottir, M.S., P. Jonsson, M.D., G.Thorgeirsson, M.D., Ph.D., G. Sigurdsson, M.D., Ph.D., Icelandic Heart Association, Iceland; P. Scheltens, M.D., Ph.D., Free University, Netherlands; A. Singleton, PhD, Laboratory of Neurogenetics, NIA, NIH; R. Tracey PhD, N. Jenny PhD., University of Vermont; J. Williamson, M.D., Wake Forest University School of Medicine; R. Lazar, Ph.D., Columbia University; A. Murray, M.D., University of Minnesota; M. Sullivan, M.D., Ph.D., University of Washington; N.R. Bryan, University of Pennsylvania. Dr. D. Fallin, Johns Hopkins University, Maryland.

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Biography: Dr. Yu received his B.S. in Probability and Statistics from Peking University, Beijing and Ph.D. in Statistics from the George Washington University.

Keywords:

Bayesian method
biomarkers
diagnostic test
longitudinal studies
missing data

Recent Publications:

Yu B, et al. *J R Stat Soc Ser A* 2008; in press.

Yu B. *J Appl Stat* 2008; in press.

Yu B, et al. *Philos Transact A Math Phys Eng Sci* 2008; 366: 2377-2388.

Yu B. *Biom J* 2008; 50: 386-394.

Yu B, et al. *Comput Stat Data Anal* 2008; 52: 1524-1532.

Kim HJ, et al. *Journal of Statistical Computation and Simulations* 2008; 78: 1087-1103.

Kim HJ, et al. *Statistics Sinica* 2008; in press.

Overview: The Biometry Section conducts research in mathematical, statistical and numerical aspects of aging and health. The Section is responsible for methodologies used in research programs related to aging and studies factors which affect health, disease and longevity. The Section performs a variety of functions to develop and apply mathematical and statistical methods from epidemiology to LEDB data.

As a group, we live by John Tukey's saying "We need to find approximate solutions for the right problem, which may be vaguely stated, rather than exact solutions for the wrong problem, which can always be made precise." My view is that development of statistical methods should be driven by problem solving rather than mathematical elegance. In the role of statistical scientists, we conduct independent research on statistical methods as well as collaborate with medical scientists on research related to diseases, treatments, patient outcomes and biology. In the role of consultants and data managers, we provide computational and programming services and apply modern statistical methods to scientific problems in study design, data collection, analysis and interpretation.

Research Interest: Research interests of the Biometry Section span a wide variety of both methodological and substantive topics, chosen to represent individual interests, Institute priorities, and to take advantage of scientific opportunities. The research projects of the Biometry Section include: estimating the age-specific risk of dementia onset and its impact on survival; assessing the accuracy of cognitive screening tests in various settings; examining the effects of non-ignorable missing data and unobserved confounders in observational studies and clinical trials;

Collaborators: Dr. Chuan Zhou, University of Washington; Dr. Ram Tiwari, CDER/FDA; Dr. Pulak Ghosh, Georgia State University; Dr. Lan Hang, Dr. Eric Feuer National Cancer Institute/NIH

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The **Laboratory of Experimental Gerontology (LEG)** conducts basic research in experimental models aimed at defining the basis of age-related functional impairment, and the identification of strategies to promote optimally healthy aging. A major line of investigation involves a longitudinal study of the potential benefits of diet restriction on aging in nonhuman primates. In this long-standing program of over two decades, various parameters of aging have been assessed to determine the effects of 30% calorie restriction on multiple indices of aging. Areas of particular interest include studies of learning and memory; motor function and general locomotor activity; immune function—both local response and T-cell populations; osteoarthritis; reproductive function, including the onset of menopause; cardiovascular function---blood pressure changes, arterial stiffening; ocular function and disease. Other research in the LEG nonhuman primate program is assessing the effects of a noradrenergic neurotoxin (DSP-4) on the development of Alzheimer's plaque pathology and neuroinflammation, and the potential protective influence of resveratrol on biomarkers of aging. The component divisions of LEG manage a wide variety of related research initiatives on aging, as described below.

The **Aging, Metabolism, and Nutrition Unit (AMNU)** (PI Dr. Rafael de Cabo), applies whole body physiological analysis coupled with tissue-specific molecular approaches to investigate the effects of nutritional interventions on basic mechanisms of aging and age-related disease. Caloric restriction (CR), without malnutrition, extends lifespan and retards a wide variety of aging processes in several short-lived species, and is the primary paradigm employed by AMNU scientists. Research within this unit uses both rodent and *in vitro* models of CR. This manipulation affects metabolic regulation to induce an overall phenotypic change leading to a decrease in cellular proliferation and growth rates. CR also induces

measurable change in circulating levels of several hormones and growth factors that regulate cell growth and proliferation. Serum obtained from CR animals alters growth, proliferation and stress responses of cells in culture. We have demonstrated that it is possible to investigate certain aspects of CR using this *in vitro* approach. This approach lends itself to the rapid identification of possible mechanisms and, perhaps more important, the development of interventions that induce or promote a CR phenotype, essentially a CR mimetic. We are currently testing several such CR mimetics identified by our laboratory.

CR extends lifespan in a variety of animal model systems and reduces oxidative stress during aging. The reduction in oxidative stress may be explained, at least in part, by the fact that animals on CR reach a new bioenergetic equilibrium. Two major components in the bioenergetic pathway are the mitochondria electron transport chain and the plasma membrane (PM) redox system (PMRS). Ubiquinone is the central molecule of the PMRS and protects the membrane under different stress conditions. Aging induces general macromolecular damage that can be prevented and reversed by CR. Preliminary data suggest that several components of the PMRS are altered during aging and that several of these changes are modified by CR in rats and mice. Analysis of the bioenergetic balance between mitochondria and PM in rats and mice on CR yields information that might explain the enhanced resistance to oxidative stress that CR affords during aging. The role of the PMRS in the prevention of age-related increases oxidative stress by CR can also establish a basis for designing potential CR mimetics and nutritional interventions. We have recently generated two transgenic animals that will allow us to further study the role of the PMRS during CR and normal aging.

The mission of the **Functional Genomics Unit (FGU)** (PI Dr. Sige Zou, PI) is to identify the mechanisms of aging at molecular, cellular and tissue levels, and develop prolongevity interventions that modulate aging processes. To achieve these aims, we have initiated three research projects. The first addresses how different tissues age and how a particular tissue contributes to organismal aging. We have measured global transcriptional profiles for seven tissues in the fly Drosophila *melanogaster* and identified hundreds of genes that display tissue-specific changes with age, including brain, muscle and the digestive and reproductive systems. We are currently characterizing the function of these tissue-specific, age-associated genes in modulating lifespan. The second project investigates the molecular and cellular mechanisms of lifespan modulation by dietary restriction (DR) using the invertebrate models, D. *melanogaster* and the worm, C. *elegans*.

DR is one of the most effective ways to extend lifespan and delay age-related physiological decline. In D. *melanogaster*, we are conducting a genetic screen to identify genes that are required for lifespan extension by DR. We are also assessing the role of several tissues, including the fat body, reproductive system and brain, in regulating DR effects. In *C. elegans*, we have previously published a new DR paradigm, dietary deprivation (DD), that significantly extends lifespan in adult sterile worms. Building on this background we are now using genomic and genetic approaches to identify which genes and biological pathways are critical for lifespan extension by DD. The third project aims to develop effective aging interventions by non-genetic approaches using various invertebrate and rodent models, including D. *melanogaster*, Mexican fruit flies, mice and rats. We are assessing a number of chemical compounds and fruit extracts to evaluate their prolongevity and anti-aging effects. Ultimately, results of this research will provide insight into the mechanisms of aging at the molecular and cellular levels and fuel the development of effective strategies for aging interventions in humans.

Research in the recently established **Neurocognitive Aging Section (NAS)** (PI Dr. Peter Rapp, and Dr. Bonnie Fletcher, Staff Scientist) aims to understand the mechanisms of normal cognitive aging as a basis for developing effective therapeutic interventions. Our early studies in nonhuman primates succeeded in establishing a basic neuropsychological profile of aging, and we have now turned attention to the specific nature of decline, with the aim of defining effects on the component processes of declarative/episodic memory. An important goal is to develop a detailed and sensitive framework for testing the working hypothesis that age-related decline results from large-scale restructuring of the neural networks that support normal memory. Toward this end, young and aged monkeys receive periodic high resolution, structural MRI and corresponding fluorodeoxyglucose PET scans over the course of neuropsychological testing. Metabolic activity in the prefrontal cortex and medial temporal lobe system is then evaluated in relation to individual variability in the cognitive outcome of aging. The incidence of menstruation and urinary hormone profiles are also tracked, enabling analysis of the behavioral and imaging results in the context of naturally occurring ovarian failure.

Additional collaborative studies in nonhuman primates take advantage of the uniquely valuable translational potential of this animal model. Although available evidence indicates that aging modulates the cognitive and neurobiological effects of ovarian hormone manipulation, this

proposal has proved difficult to test in women. Studies currently underway in young and aged monkeys are therefore designed to compare the cognitive effects of multiple hormone replacement strategies, modeled on regimens available for clinical use in women. These investigations establish a unique framework of behavioral data for related initiatives focusing on the neurobiological effects of ovarian hormone manipulation.

Age-associated cognitive decline in humans prominently involves disrupted interactions between multiple memory-related brain systems. Ongoing collaborative studies in NAS are among the first explore this issue in an aged rat model, using a plus-maze procedure and quantitative *in situ* hybridization for the plasticity-related gene Arc to test the possibility that deficits in cognitive flexibility are coupled with functional network reorganization across the prefrontal cortex, dorsal striatum and hippocampus. Current perspectives implicate alterations in plasticity mechanisms as a basis for cognitive aging. Related evidence indicates that promoting chromatin rearrangement permissive for gene transcription by pharmacological means enhances hippocampal long-term potentiation, and benefits memory. These results predict that treatments targeting epigenetic transcriptional control may improve the neurocognitive outcome of aging. NAS is testing this proposal in both rats and nonhuman primates, coordinating behavioral assessment with the analysis of relevant molecular signatures of successful aging. Other studies are examining the resting basal status of epigenetic transcriptional control, and the dynamic regulation of these mechanisms under learning activated conditions.

Progress in research on neurocognitive aging is critically supported by advances in understanding the fundamental structure and organization of memory in brain. Based on this perspective, and guided by the consensus that the medial temporal lobe system is critical for normal episodic memory, an additional line of investigation in NAS aims to identify the information processing functions of the primate hippocampus that mediate this capacity. In these studies, monkeys are tested across a battery of both standard and novel tasks, manipulating demands on candidate properties of episodic memory: 1) the temporal organization of memory, 2) memory for spatial and nonspatial context, 3) “autobiographical” memory, and 4) the relational organization of memory. Taken together, these investigations are expected to substantially advance our understanding of the structure and organization of medial temporal lobe memory in primates and, ultimately, fuel research on a variety of conditions in which memory is prominently affected.

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Biography: Peter R. Rapp, Ph.D., joined the NIA from the Mount Sinai School of Medicine, New York, NY in 2008 he where held joint primary appointments in the Fishberg Department of Neuroscience and the Department of Geriatrics and Adult Development. He served in various capacities at Mount Sinai including Interim Chair of the Department of Neuroscience (2006-2008), the Mount Sinai Endowed Chair in Neuroscience, and Co-Director of the Graduate Training Program in Neuroscience. His extramural research on the cognitive and neurobiological consequences of normal aging has been continuously funded since 1989, including a recent M.E.R.I.T award from the NIA. Dr. Rapp earned his Ph.D. in Biopsychology from the University of North Carolina at Chapel Hill in 1986, and after training at the Salk Institute for Biological Studies, San Diego, CA, he joined the faculty of the Center for Behavioral Neuroscience at the State University of New York, Stony Brook. He serves on several editorial boards, including the position of Section Editor for Neurobiology of Aging.

Keywords:

aging
epigenetics
hippocampus
macaque
memory
prefrontal cortex
quantitative morphometry

Recent Publications:

Rapp PR. In: Encyclopedia of Neuroscience, Squire LR, et al., eds. In press.

Alexander GE, etal. *J Neurosci* 2008; 28: 2710-2718.

Calhoun ME, etal. *Neurobiol Aging* 2008; 29: 1256-1264.

Shamy JL, etal. *J Comp Neurol* 2007; 502: 192-201.

Research Overview

Models of Neurocognitive Aging. Age-related deficits in cognitive function compromise the quality of life and are among the most troubling consequences of growing older. A major line of research in NAS is aimed at establishing a nonhuman primate model for defining the basis of normal cognitive aging, and for developing effective interventions. Our early studies helped reveal the basic neuropsychological profile of aging, and we have now turned attention to the specific nature of decline, with the aim of defining the effects of aging on the component processes of declarative/episodic memory. Young and aged monkeys are tested across a battery of novel assessments that manipulate demands on key operating characteristics of memory: 1) the contributions of recollection and familiarity to visual recognition, 2) memory for spatial and non-spatial context, 3) the temporal structure of experience, and 4) the relational organization of memory. An important goal of these studies is to develop a detailed and sensitive framework for testing the working hypothesis that age-related decline results from large-scale restructuring of the neural networks that support normal memory. Toward this end, subjects receive periodic high resolution, structural MRI and corresponding [^{18}F] fluorodeoxyglucose PET scans over the

Fletcher BR, etal. *Hippocampus* 2007; 17: 227-234.

Hao J, etal. *Proc Natl Acad Sci U S A* 2007; 104: 11465-11470.

course of neuropsychological testing. Metabolic activity in the prefrontal cortex and medial temporal lobe system is then evaluated in relation to individual variability in the cognitive outcome of aging. The incidence of menstruation and urinary hormone profiles are also tracked, enabling evaluation of the behavioral and imaging results in relation to naturally occurring ovarian failure.

Enabled by collaborations with Dr. Michela Gallagher at Johns Hopkins University, John Morrison at the Mount Sinai School of Medicine, Howard Eichenbaum at Boston University, and others, NAS has configured additional basic research efforts around several integrated themes. Age-associated cognitive decline in humans prominently involves disrupted interactions between multiple memory-related brain systems. Ongoing studies in NAS are among the first to address this issue in an aged rat model, using a plus-maze procedure and subsequent *in situ* hybridization for the plasticity-related gene Arc to test the possibility that deficits in cognitive flexibility are coupled with functional network reorganization across the prefrontal cortex, dorsal striatum and hippocampus.

Current perspectives implicate alterations in plasticity mechanisms as a basis for cognitive aging, and accordingly, our effort toward the development of effective interventions focus on this target from multiple levels of analysis. It is noteworthy in this context that histone deacetylase inhibitor administration in young subjects promotes chromatin rearrangement permissive for normal gene transcription, enhances hippocampal long-term potentiation, and benefits memory. These results predict that, even in the absence of underlying defects in chromatin remodeling, treatments targeting epigenetic transcriptional control may improve the neurocognitive outcome of aging. NAS is testing this proposal in both rats and nonhuman primates, coordinating behavioral assessment with the analysis of pharmacological effects on relevant molecular signatures of successful aging. We will also pursue a systematic examination of chromatin remodeling contributions to normal cognitive aging, focusing on both the resting basal status of epigenetic transcriptional control, and the dynamic regulation of these mechanisms under learning activated conditions.

Ovarian Hormone Influences on Neurocognitive Health. Additional collaborative studies in nonhuman primates take advantage of the uniquely valuable translational potential of this animal model. Although available evidence indicates that aging modulates the cognitive and

neurobiological effects of ovarian hormone manipulation, this proposal has proved difficult to test in women. Studies currently underway in young and aged monkeys are therefore designed to compare the cognitive effects of multiple hormone replacement strategies, modeled on regimens available for clinical use in women. Our strategy for neuropsychological assessment takes advantage of a battery of extensively standardized tasks with sensitivity to both aging and ovarian hormone manipulation, and that facilitates the development of testable predictions about the neural systems that might mediate the effects of hormone administration. Behavioral testing also includes systematic manipulations of interference and distraction, aimed at illuminating the specific information processing capacities responsible for the cognitive benefits of treatment. These studies will establish a unique framework of behavioral data for related collaborative initiatives focusing on the neurobiological effects of ovarian hormone manipulation. Taken together, the results can be expected to inform a number of pressing issues in the clinical use of hormone replacement, and to substantially advance research on women's neurocognitive health.

Structure and Organization of Memory. Progress in research on neurocognitive aging is critically supported by advances in understanding the fundamental structure and organization of memory in brain. Based on this perspective, another line of investigation in NAS brings into convergence research on the neurology and cognitive psychology of memory. Guided by the consensus that the medial temporal lobe system is critical for normal episodic memory, a key aim is to identify the information processing functions of the primate hippocampus that mediate this capacity. In these studies, monkeys are tested across a battery of both standard and novel tasks, manipulating demands on candidate properties of episodic memory. Although a variety of tasks are used, the underlying strategy throughout is to incorporate "probe" tests that go beyond task accuracy to reveal the nature of representations established during training. Taken together, these investigations are expected to advance our understanding of the structure and organization of medial temporal lobe memory in primates and, ultimately, fuel research on a variety of age-related conditions in which memory is prominently affected.

Collaborators: David Amaral, Ph.D., UC Davis; Carol Barnes, Ph.D., University of Arizona; Howard Eichenbaum, Ph.D., Boston University; Bryan Devan, Ph.D., Towson University; Michela Gallagher, Ph.D., Johns Hopkins University; Patrick Hof, M.D., Mount Sinai School of Medicine; Don Ingram, Ph.D., Pennington Biomedical Research Center; Sally Mendoza, Ph.D., UC Davis; John Morrison, Ph.D., Mount Sinai School of Medicine; Peter Mouton, Ph.D., Stereology Resource Center; Elisabeth Murray, Ph.D., Laboratory of Neuropsychology, NIHM; Jul Lea Shamy, Ph.D., Mount Sinai School of Medicine; Matthew Shapiro, Ph.D., Mount Sinai School of Medicine; Yaakov Stern, Ph.D., Columbia University.

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Biography: After receiving his BS/MS from the University of Cordoba, Spain, Dr. de Cabo earned his Ph.D. in 2000 from the Department of Foods and Nutrition at Purdue University. Upon completion of his graduate education, he received a postdoctoral position in the Laboratory of Neurosciences at the National Institute on Aging in Baltimore, Maryland. In 2004, he was appointed as a tenure track investigator in the Laboratory of Experimental Gerontology, where he now heads the Aging, Metabolism, and Nutrition Unit (AMNU). The AMNU applies both physiological and tissue-specific molecular approaches to investigate effects of nutritional interventions on basic mechanisms of aging and age-related diseases. Research within his unit strives to identify protective mechanisms invoked by caloric restriction and to evaluate the consequences of dietary interventions on lifespan, pathology, and behavioral function. The AMNU balances the exploration of *in vivo* rodent, as well as *in vitro*, paradigms of caloric restriction. Dr. de Cabo is an active member of the Board of the American Aging Association.

Keywords:

aging
calorie restriction
NRF2
calorie restiction mimetics
nutraceuticals
oxidative stress

Recent Publications:

Pearson KJ, etal. *Proc Nal Acad Sci U S A* 2008; 105: 2325-2330.

Minor RK, etal. *Behav Brain Res* 2008; 189: 202-211.

Pearson KJ, etal. *Cell Metabolism* 2008; 8: 157-168.

Gorospe M, etal. *Trends Cell Biol* 2008; 18: 77-83.

Ungvari Z, etal. *Circ Res* 2008; 102: 519-528.

Program Overview:

The Aging, Metabolism, and Nutrition Unit (AMNU), applies whole body physiological coupled with tissue-specific molecular approaches to investigate effects of nutritional interventions on basic mechanisms of aging and age-related diseases. Caloric restriction (CR), without malnutrition, is widely known to extend lifespan and retard a wide variety of aging processes in several short-lived species and is the primary paradigm employed by AMNU scientists. Research within this unit uses both rodent models of CR as well as an *in vitro* model for CR. CR affects metabolic regulation to induce an overall phenotypic change leading to a decrease in cellular proliferation and growth rates. CR induces measurable changes on circulating levels of several hormones and growth factors that regulate cell growth and proliferation. Serum obtained from CR animals alters growth, proliferation and stress responses of cells in culture. We have demonstrated that it is possible to investigate certain aspects of CR using this *in vitro* approach. This approach lends itself to a more rapid investigation of possible mechanisms and, perhaps more importantly to the research, development and rapid evaluation of interventions that would be able to induce or promote a phenotype similar to that seen with CR, essentially a CR mimetic. In this regard, we are studying a number of compounds that mimic calorie restriction, the first one was resveratrol. We knew that extra copies of Sir2-1 gene or small molecules,

Publicaitons-continued

Allard JS, etal. *PLoS ONE* 2008; 3: e3211.

Navas P, etal. *Mitochondrion* 2007; Suppl 1: S34-S40.

Hyun DH, etal. *J Neurochem* 2007; 100: 1364-1374.

Yang H, etal. *Cell* 2007; 130: 1095-1107.

such as resveratrol, directly activate the enzyme and extend lifespan. Mammalian Sir2 gene, SIRT1, is induced in tissues from CR rats and in cells treated with their serum. CR and genetic manipulations that extend lifespan have been shown to involve the insulin signaling pathway and can also attenuate stress-induced apoptosis. *In vitro* induction of SIRT1 by resveratrol attenuates stress-induced apoptosis. We assessed the effects of *in vivo* induction of SIRT1 by resveratrol and its effects on insulin signaling, stress-induced apoptosis, and lifespan. These results are now published and we are continuing our research on sirtuin activators through a CRADA with SIRTRIS.

CR extends lifespan in a variety of animal model systems and reduces oxidative stress during aging. At least in part, the reduction in oxidative stress may be explained by the fact that animals on CR reach a new bioenergetic equilibrium. Two major components in the bioenergetic pathway are the mitochondria electron transport chain and the plasma membrane (PM) redox system (PMRS). Ubiquinone is the central molecule of the PMRS and protects the membrane under different stress conditions. Aging induces general macromolecular damage that can be prevented and reversed by CR. Preliminary data suggest that several components of the PMRS are altered during aging and that several of these changes are modified by CR in rats and mice. Analysis of the bioenergetic balance between mitochondria and PM in rats and mice on CR can provide the information that might explain the enhanced resistance to oxidative stress that CR affords during aging. The role of the PMRS in the prevention of oxidative stress by CR during aging can provide the basis for the design of potential CR mimetics and nutritional interventions.

Collaborators:

U.S. Federal Government:

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Universities and Institutes:

Albert Einstein College of Medicine: A.M. Cuervo,E. Snapp, Harvard University: D. Sinclair, J. Clardy, Johns Hopkins University: P. Puigserver, C. Lerin, C. Holcroft, A.Burke, Louisiana State University, Pennington: E. Ravussin, D. Ingram, J. Keller, V. Deep-Dixit, Massachusets Institute of Technology: L. Bordone, Oregon Health Sciences University: G. Kisby, S.

Kohama, M. Zelinski-Wooten, Purdue University: J.R. Burgess, Standford University: A. Brunet, University of California Davis: J.J. Ramsey University of California San Diego: A. De Maio, University of California Los Angeles: V. Longo, University of Kentucky School of Medicine: P. Sullivan, University of Maryland: M.A. Ottinger, University of Medicine and Dentistry of New Jersey: A. Vaquero, University of Washington School of Medicine: W. Pendergrass, N. Wolf, University of Wisconsin: R. Anderson

International:

Universidad Pablo de Olavide, Sevilla, Spain: P. Navas, G. Lopez, E. Siendones Universidad de Cordoba,Cordoba, Spain: J.M. Villalba, J.A. Gonzalez, Flanders Interuniversity Institute for Biotechnology: C. Chen, University of Sydney, Australia: D. Le Couteur, S. Hilmer, Universidad de Barcelona: A.M. Gomez-Foix , Bar-Ilam University, Ramat-Gan, Israel: H. Cohen, Institute for Biomedical Aging Research, Innsbruck, Austria: W. Zwerschke

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Biography: Dr. Sige Zou received his B.S. in Genetics and Genetic Engineering in 1990 from Fudan University, Shanghai, China and his Ph.D. in 1996 from Iowa State University, Ames, IA. Dr. Zou went to the University of California San Francisco in 1997 as a postdoctoral fellow working on "Aging in *Drosophila melanogaster*". He became a Senior Scientist with the Bio-Rad Laboratories Inc. in 2003. Dr. Zou then came to the Laboratory of Experimental Gerontology at NIA in 2004 as a Tenure-Track Investigator in the Functional Genomics Unit.

Keywords:

aging
microarray
tissue-specific
dietary restriction
dietary deprivation
C. *elegans*
Drosophila melanogaster

Recent Publications:

Zhang W, etal. *BMC Bioinformatics* 2008; 9: 129.

Zou S and Sinclair J (Co-first author), etal. *Mech Ageing Dev* 2007; 128: 222-226.

Norgate M, etal. *Biometals* 2007; 20: 683-697.

He HJ, etal. *Biotechniques* 2007; 43: 93-98.

Zhu M, etal. *Exp Gerontol* 2007; 42: 733-744.

Zhan M, etal. *Genome Res* 2007; 17: 1236-1243.

Program Overview:

The mission of the Function Genomics Unit is to investigate the mechanisms of aging at molecular, cellular and tissue levels, and develop prolongevity interventions that modulate aging processes. To achieve these aims, we have initiated and would like to fully support three research projects. The first project is to address how different tissues age and how a particular tissue contributes to the organismal aging. We have previously published the identification of hundreds of genes showing tissue-specific changes with age from measuring global transcription profiles of aging for seven tissues in the fly *Drosophila melanogaster*, including brain, muscle and tissues in the digestive and reproductive systems. We are currently characterizing functions of these tissue-specific age-associated genes in modulating lifespan. The second project is to investigate the molecular and cellular mechanisms of lifespan modulation by dietary restriction (DR) using the invertebrate models, *D. melanogaster* and the worm, *C. elegans*. DR is one of the most effective ways to extend lifespan and delay the gradual decline of physiological functions with increasing age. In *D. melanogaster*, we have started conducting a genetic screen to identify genes that are required for lifespan extension by DR. We are also assessing the role of several tissues, including the fat body, reproductive system and brain, in regulating DR effects. In *C. elegans*, we have previously published a new DR paradigm, dietary deprivation (DD), which can effectively extend lifespan of adult sterile worms. We are using genomic and genetic approaches to identify which genes and biological pathways are critical for lifespan extension by DD. The third project is to develop effective aging interventions by non-genetic approaches using various invertebrate and rodent models, including *D. melanogaster*,

the Mexican fruit flies, mice and rats. We are assessing a number of chemical compounds and fruit extracts to evaluate their prolongevity and anti-aging effects. Accomplishing these aims will provide insight into the mechanisms of aging at molecular and cellular levels and develop effective strategies for aging interventions in humans.

Translational Efforts

The Effects of Cranberry on Delaying Pathogenesis of a Mouse Model of Alzheimer's Disease.

We have recently demonstrated that cranberry extracts can extend lifespan in *D. melanogaster* and delay age-related functional decline in rats. To further assess the beneficial effects of cranberry consumption, we have started to investigate the effects of cranberry supplementation on delaying the pathogenesis of a common aging-associated neurodegenerative disease, Alzheimer's disease (AD), using a transgenic mouse model. Specifically, we will assess the effect of cranberry on cognitive function, formation of the senile plaque, a hallmark of AD, and inflammatory response in the AD mice. Accomplishing of these aims will provide scientific information on the anti-aging effects of cranberry. This project is partly funded by the Wisconsin Cranberry Board.

Collaborators:

Ming Zhan, Ph.D., NIA; Catherine A. Wolkow, Ph.D., NIA; James Carey, Ph.D., University of California, Davis; Pablo Liedo, Ph.D., ECOSUR, Mexico; Donald K. Ingram, Ph.D., Louisiana State University; Pablo M. Irusta, Ph.D., Georgetown University; Chaoyang Zeng, Ph.D., University of Wisconsin-Milwaukee; Lili Wang, Ph.D., National Institute of Standard Technology.

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NIA Primate Aging Study

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Biography: Dr. Julie Mattison received a B.S. in Biology from UCSD, a M.S. in Exercise Physiology from Central Washington University and completed her education at Southern Illinois University with a Ph.D. in Physiology. Dr. Mattison came to NIA in 2000 as a post-doctoral fellow with a cross appointment in the Laboratories of Neurosciences and Cardiovascular Science to manage the ongoing study of calorie restriction in nonhuman primates and begin new studies of nutrition and vascular aging. In 2004, she became a contract Facility Head of the nonhuman primate program and was appointed as a Staff Scientist/ Facility Head in 2006.

Keywords:

calorie restriction
rhesus monkey
behavior
immune function
resveratrol
Alzheimer's Disease
aging
obesity

Recent Publications:

Reynolds MA, etal. *Nutrition* 2009; 25: 88-97.

Downs JL, etal. *Neurobiol Aging* 2008; 29: 1412-1422.

Branch-Mays G, etal. *J Periodontol* 2008; 79: 1184-1191.

Ebersole JL, etal. *J Periodont Res* 2008; 43: 500-507.

Gouras P, etal. *Graefes Arch Clin Exp Ophthalmol* 2008; 246: 1395-1402.

Gouras P, etal. *Graefes Arch Clin Exp Ophthalmol* 2008; 246: 1403-1411.

Primate Aging Studies: The NIH Animal Center in Poolesville, Maryland is home to the NIA Primate Aging Study. Although the primary focus has been a long-term study of calorie restriction, the Laboratory of Experimental Gerontology (LEG) has conducted additional studies of dietary interventions that more specifically affect cardiovascular aging and a model of Parkinson's Disease. Currently, our lab is assessing a primate model of Alzheimer's Disease and evaluating the effects of resveratrol treatment in monkeys fed a high fat and sugar diet.

Dietary calorie restriction (CR) has been shown to benefit health and longevity in a wide variety of species, although most have maximal lifespans of only a few years. In 1987, the National Institute on Aging Intramural Research Program began the first well controlled long-term study in a species with a considerably longer lifespan and a closer physiology to humans. Using rhesus monkeys (Macaca mulatta), an extensive array of physiological measures have been conducted in both male and females to evaluate the effects of CR. A smaller group of squirrel monkeys (Saimiri sciureus) has also been studied. Although it is not yet known if CR extends maximal lifespan in these long-lived primate species, our findings indicate that physiological responses are in general agreement with the extensive literature in rodents and that nonhuman primates on CR are likely to experience fewer incidences and less severe effects of age-related disease, in particular, cardiovascular disease and diabetes.

With an average lifespan of 25 years and a maximum of 40 years, studies of longevity in rhesus monkeys are challenging to conduct. Effective anti-aging interventions should result in decreasing the incidence and delaying

Collaborators-continued

Moore CM, etal. *AGE: J Am Aging Assoc* 2007; 29: 15-28.

Mattison JA, etal. *J Med Primatol* 2007; 36: 391-398.

the age of onset of characteristic age-related diseases and pathology. In addition, there must be maintenance of cellular, organ, physiologic, and behavioral function into old age. By using criteria in the three main categories of mortality, morbidity, and function, the NIA hopes to clearly establish whether CR retards the rate of aging in rhesus monkeys.

A second study is underway to establish a primate model of Alzheimer's Disease. The primary animal models of Alzheimer's Disease (AD) consist of mice that are made to express select aberrant human genes and thus produce AD-like pathology. However, the majority of human AD cases do not contain these mutant genes, and thus there exists no animal model of idiopathic AD, which accounts for greater than 90% of all AD cases. Nonhuman primates (NHPs) naturally express AD-like pathology and hence may prove to be an invaluable animal model for the study of AD. However, the AD-like pathology in NHPs does not occur until very advanced ages; thus, this study examines a mechanism for inducing AD pathology which has been effective in rodents. We will determine if a chemical lesion (N-(2-chloroethyl)-N-ethyl-2 bromobenzylamine (DSP-4)) of the locus coeruleus in rhesus and squirrel monkeys will accelerate the deposition of amyloid-beta and corresponding neuroinflammation.

A third study assesses the health benefits of resveratrol, a naturally occurring plant compound with strong anti-oxidant properties, in conjunction with a high fat and sugar diet (typical in western cultures) in rhesus monkeys. In a previous study, mice fed resveratrol with an obesity-inducing diet maintained better health than those fed a high fat diet alone as indicated by overall lifespan, and measures of motor function, insulin sensitivity, organ pathology, PGC-1a activity, and mitochondrial number. In the present study, we will analyze several indicators of health and aging including serum markers, insulin sensitivity, cardiovascular health, activity level, learning and memory, and tissue pathology.

Collaborators: M.J. Novak, J.L. Ebersole, D. Dawson, University of Kentucky; M. Reynolds, G. Branch-Mays, University of Maryland Dental School, J. Nikolich-Zugich, M. Zelinski, H. Urbanski, M. Neuringer, S. Kohama, Oregon National Primate Research Center; P. Kramer, University of Washington; P. Gouras, Columbia University; L. Ivert, St. Erik's Eye Hospital, Sweden; B. Wasserman, Johns Hopkins University; M.A. Ottinger, University of Maryland; C. Moore, Y. Ikeno, G. Hubbard, University of Texas Health Sciences Center, San Antonio; D. Allison, M. Beasley, University of Alabama, Birmingham.

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The **Laboratory of Genetics (LG)** includes the Human Genetics Section, directed by David Schlessinger, the Genome Instability and Chromatin Remodeling Section directed by Weidong Wang, the Developmental Genomics and Aging Section under the direction of Minoru S.H. Ko, the Image Informatics and Computational Biology Unit led by Ilya Goldberg, and the 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*. Major studies include the following.

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 mouse models, by differential assays of gene expression in oocytes, preimplantation embryos, placenta, and stem cells differentiating along selected lineages (in the Developmental Genomics and Aging Section). The studies have inferred a set of molecular markers of pluripotentiality and defined expression profiles of stem cells along trajectories to neural, endodermal, and placental lineages. They are continuing with systematic evaluation of the contribution of 300 sleected transcription factors to maintenance of pluripotency or differentiation in stem cells and incipient embryos.

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 studied. Studies start from human or mouse hereditary defects that have been attributed to single genes, such as the

ectodysplasin-A (EDA) gene involved in X-linked ectodermal dysplasia and the mitochondrial ribosomal RNA processing (MRP) gene mutated in Cartilage-hair hypoplasia.

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 and DNA repair. The Genome Instability and Chromatin Remodeling Section is using a combination of approaches to isolate and characterize critical complexes, including the ones that are modified to cause the Werner, ATRX, and Bloom Syndromes, and Fanconi Anemia (FA). For FA, the studies have uncovered 3 new proteins that define the enzymatic function of the corresponding complex, including a specific ubiquitin ligase and a motor that moves the complex along damaged DNA.

4. Genes involved in embryonic events that prefigure aging-related phenomena. For example, the Human Genetics Unit is involved in studies of premature ovarian failure, in which the aging phenomenon of early menopause is determined by the balance of follicule formation and atresia during fetal life. Comparable studies are being carried out characterizing skin appendage and cartilage formation and regeneration. For the ovary, for example, the studies have revealed the pivotal role of FOXL2, a forkhead transcription factor, in the maturation of the ovary, the stabilization of female sex fate, and the regulation of menopause.

5. The genetics of aging-related complex conditions is being approached by interactive studies with the "founder" population in Sardinia. Initial phenotypes that have been studied along with epidemiological factors include arterial stiffness and selected psychiatric/psychological traits. For this project investigators from the Laboratory of Cardiovascular Science (Edward Lakatta, Samer Najjar, and Angelo Scuteri), the Laboratory of Personality and Cognition (Paul Costa, Antonio Terracciano, and Alan Zonderman), and the Laboratory of Genetics are working with Antonio Cao, Manuela Uda, and Serena Sanna, human geneticists at the University of Cagliari, Sardinia, and Goncalo Abecasis, a statistical geneticist at the University of Michigan. Genes have been detected in which variants contribute significantly to the levels of many quantitative traits and risk factors (for example, obesity, fasting glucose levels, blood pressure, cholesterol and lipids, etc.).

6. The Image Informatics and Computational Biology Unit is helping to

develop quantitative visual assays. The unit is principal developer and co-founder of the Open Microscopy Environment (OME) project. OME is a software package and a set of standards for the collection, maintenance, and analysis of biological images. The analysis package developed by the group includes a set of algorithms that recognize texture, repetitive features, and other characteristics of images. The set has been successfully applied to determine the spatial distribution of differentially expressed gene products in pre-implantation mouse embryos, to identify the intracellular localization of antibodies, and to score the age of nematode worms and screen for morphological mutants. It is currently being applied to computer-aided diagnosis of lymphoma types and osteoarthritis.

7. The Gene Recovery and Analysis Unit equips the Laboratory with other state-of-the-art resources for genomic approaches, including large-insert clones and recovery methods, Solexa sequencing, chromatin analysis techniques, and site-specific modification of large-insert clones by recombineering techniques. Among the projects are the protein profiling of ES cells and selected tissues by mass spectrometry and the study of gene regulatory elements that confer highly restricted expression patterns. The current focus is on PLAC1, a gene normally expressed only in placenta in mice and humans, but also turned on in many cancers. In addition, high-throughput sequencing is being applied to genes inferred in the SardiNIA project (see 3. above) as associated with variation in the levels of quantitative traits; the sequencing aims to identify the causal variants involved.

In other technology-related activities, the Laboratory has made high-quality cDNA libraries from very few cells from embryos (in the Developmental Genomics and Aging Section), and in collaboration with Agilent Technologies, has developed gene expression profiling with microarrays bearing 44,000 oligonucleotide features based on the cDNAs. The laboratory also benefits from joint efforts with other groups and resource providers both within NIA and at a number of extramural sites in the United States and abroad.

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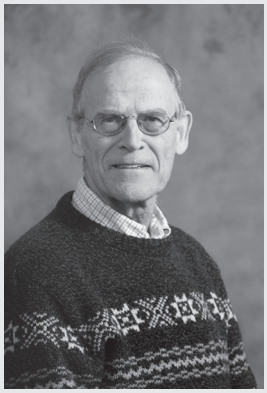

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Biography: Dr. Schlessinger received his Ph.D. from Harvard University in 1960. Following postdoctoral training at the Pasteur Institute in Paris, he joined Washington University in St. Louis, where he served as Professor of Molecular Microbiology, Genetics, and Microbiology in Medicine until his move to NIA in September 1997. He has contributed both to microbial and human genome studies. He 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. His genome-related activities have included serving as a councilor of the International Human Genome Organization (HUGO), and as President, HUGO Americas.

Keywords:

ectodermal dysplasia
skin appendages
premature ovarian failure
ovary development
open microscopy environment (OME)
sex determination
genome-wide association scan
quantitative traits

Recent Publications:

Arnaud-Lopez L, et al. *Am J Hum Genet* 2008; 82: 1270-1280.

Chen WM, et al. *J Clin Invest* 2008; 118: 2620-2628.

Lettre G, et al. *Nat Genet* 2008; 40: 584-591.

Lettre G, et al. *Proc Natl Acad Sci U S A* 2008; 105: 11869-11874.

Loos RJ, etal. *Nat Genet* 2008; 40: 768-775.

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

1. Technologies: We aim to understand tissue- and developmentally-restricted expression of selected genes at the level of RNA expression, gene regulation in chromatin, and protein diversity (proteomics), and to use mouse models to determine the physiological roles of the genes. Technologies being adapted include the generation of constructs for knock-out mice and the definition of regulatory element functions, using recombineering-based approaches in the Gene Recovery and Analysis Unit, headed by Ramaiah Nagaraja (q.v.).

2. Areas of Research: Projects are designed to identify and characterize 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 assessed, and knockout technologies are available) and attempts to distinguish the factors responsible for the initiation and maintenance of the processes of interest.

Publications-continued
Sanna S, etal. *Nat Genet* 2008; 40: 198-203.

Uda M, et al. *Proc Natl Acad Sci U S A* 2008; 105: 1620-1625.

Willer CJ, et al. *Nat Genet* 2008; 40: 161-169.

Hashimoto T, et al. 2007; *Cell Cycle* 7: 106-111.

Li S, et al. *PLoS Genet* 2007; 3: e194.

Nakashima E, et al. *Am J Med Genet A* 2007; 143A: 2675-2681.

Ottolenghi C, et al. *Hum Mol Genet* 2007; 16: 2795-2804.

Examples of model systems under study in the Human Genetics Section include:

Premature ovarian failure. The progressive depletion of oocytes leads to the aging-related phenomenon of menopause. Its acceleration or anticipation define premature ovarian failure (POF), which occurs in up to 5% of women. Current work in the laboratory has identified part of a mechanism that may sustain the reproductive competence of the ovary based on the maintenance of gene activities that are initiated during embryo-fetal development. A subset of women with POF have a defect that is also associated with eyelid dysplasia (BPES, the blepharophimosis-ptosis-epicanthus inversus syndrome). We identified a "winged helix" transcription factor, FOXL2, that is mutated to cause both the eyelid and ovarian follicle defects. In correlated developmental work, a mouse knockout model has been developed that recapitulates features of BPES. Systematic studies have defined gene cohorts specifically expressed during the development of ovarian follicles, including the target genes controlled by FOXL2. In the absence of FOXL2, all follicle formation and ovary maturation fails, and partial sex reversal ensues. Thus, FOXL2 is involved in ovary formation, in the regulation of female reproductive life span, and in the maintenance of female sex determination - thereby providing a mechanism for the continued action of developmental processes in female reproductive competence.

Skin appendage formation. Teeth, hair follicles and sebaceous and sweat glands, the latter being essential for regulation of the body temperature, are defective or lacking in patients with X-linked anhidrotic ectodermal dysplasia (EDA). We identified the gene mutated in most of these patients and characterized the developmental course of the anomalies affecting the Tabby mouse, an experimental model for the human condition. We showed that EDA is required transiently during development to initiate skin appendage formation, yet maintains a trophic effect throughout life. Transgenic experiments found that in mice, one EDA isoform can differentially affect distinct hair types, rescue sweat glands, and also prevent ocular surface disease that is otherwise seen in the mice (and in EDA-deficient patients). Further study is aimed at understanding the aging-related defects in skin appendages, which are extensive and highly diverse among individuals. Expression profiling has revealed downstream NF-κB-dependent pathways, including the dependence of hair type on the non-canonical lymphotoxin-beta pathway. EDA thereby provides an entree to an embryonic branch point that leads to the formation of the whole range of skin appendages and functions.

Population-based study of genetic risk factors. More proximal to complex human diseases, an extensive collaborative project is studying a favorably inter-related population in Sardinia to determine critical genes involved in aging-related traits, with the long-term aim of promoting patient benefit. To date, 98 quantitative traits, including personality traits and risk factors for cardiovascular disease, have been assessed on 6,162 participants ages 14-102, comprising over 60% of the population of a cluster of 4 towns. Genome-wide association studies of the population have led to the inference of genes and gene variants involved in determining the levels of a variety of traits, including obesity, lipids, and blood components. In addition, second visits have been carried out and third visits will be done for the study cohort to permit the assessment of longitudinal trends and outcomes, as well as the assessment of additional phenotypes related to bone density and frailty as a function of age. Targeted data analysis has also been initiated to replicate studies and look for correlations in other large population cohort studies, including the Baltimore Longitudinal Study of Aging and the InCHIANTI study supported by the NIA.

Collaborators: Dr. Goncalo Abecasis, University of Michigan, Ann Arbor; Dr. Antonio Cao, Institute of Neurogenetics and Neuropharmacology, Cagliari, Italy; Dr. Michael Fant, University of Texas, Houston; Dr. Antonino Forabosco, University of Modena, Italy; Dr. Jose Elias Garcia, University of Guadalajara Medical School, Mexico; Dr. Juha Kere, Karolinska Institute, Sweden; Dr. Anand Srivastava, Greenwood Genetics Center, South Carolina; Dr. Raj Thakker, Oxford University, United Kingdom; Dr. Valeria Ursini, Institute of Genetics and Biophysics, Naples, Italy; GIANT and MAGIC International Consortia for the finding of genes associated with anthropometric and glucose/insulin-related quantitative traits and diseases.

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Biography: Dr. Wang was trained as a biochemist and molecular biologist at UCLA, where he obtained his Ph.D., and Stanford University, where he worked as a postdoctoral fellow. He joined the Laboratory of Genetics at the National Institute on Aging as a tenure-track Investigator in 1997, and was awarded tenure in 2004. He is currently the chief of the Genome Instability and Chromatin-Remodeling Section. Dr. Wang has received several awards, including the Presidential Early Career Award for Scientists and Engineers, the Merit Award from the Fanconi Anemia Research Foundation, and the Award of Merit from NIH. He serves on the Editorial Board of Molecular and Cellular Biology.

Keywords:

chromatin-remodeling
SWI/SNF
helicase
genome instability
cancer
Fanconi anemia
Bloom syndrome
Rothmund-Thompson Syndrome
ATR-X syndrome
aging
DNA repair
BRCA1
BRCA2
ubiquitin

Recent Publications:

Ciccia A, etal. *Mol Cell* 2007; 25: 331-343.

Xu D, etal. *Genes Dev* 2008; in press.

Research Description: Recently, multiprotein complexes have been implicated in the regulation or modulation of many cellular processes. Often, one protein can be discovered in several complexes, with each complex performing its unique function. Thus, the biological functions of a given protein can be understood only when the consequences of its association in complexes are defined. The Genome Instability and Chromatin Remodeling Section studies selected nuclear regulatory complexes. Through purification and characterization of these complexes, we aim to identify new genes that prevent premature aging and guard genome integrity, and discover new mechanisms for gene regulation and genome maintenance.

In the eucaryotic nucleus, the chromatin structures that allow efficient storage of genetic information also tend to render the DNA inaccessible to metabolizing enzymes. The repressive chromatin structure must be remodeled to allow transcription and other metabolic reactions to occur. Chromatin-remodeling multiprotein complexes are critically involved in processes that include transcription, replication, repair, chromatin assembly, and chromosome condensation. Furthermore, multiple human diseases, including several types of cancer, are caused by mutations in remodeling complexes; and aging in several lower species (and in several human disorders with features of premature aging) can be modulated by alterations in remodeling enzymes. Our Section aims to uncover novel chromatin-remodeling molecules and investigate their composition and mechanism of action. We have taken a biochemical approach to defining

targeted complexes, starting with the development of a highly efficient immunopurification protocol to isolate the endogenous complexes from mammalian nuclear extracts in highly purified form. We have focused on studies of two families of multiprotein complexes involved in DNA expression and genome stability, in two corresponding projects:

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

1. Purification of a Complex Containing BLM, the Helicase Involved in Bloom Syndrome: Bloom syndrome features genomic instability and cancer predisposition. The gene defective in this disease belongs to the family of RecQ helicases. We have purified three distinct BLM-containing complexes from HeLa cells. Interestingly, one of the complexes, termed BRAFT, contains five of the Fanconi anemia (FA) complementation group proteins (see below). The complex also contains topoisomerase IIIa and replication protein A, proteins that are known to interact with BLM and could facilitate unwinding of DNA. Importantly, we identified a protein, termed RMI1 or BLAP75, which is present in all BLM complexes. RMI1/BLAP75 is essential for the stability of the BLM complex, and its depletion results in genomic instability similar to that observed in BLM-depleted cells. After our work was published (Yin et al. EMBO J. 2005), two other labs have shown that the homolog of BLAP75 in yeast is involved in a similar complex with yeast BLM homolog and topoisomerase IIIa, and is essential for maintaining genome stability. Most recently, we identified a new component of the BLM complex, named RMI2 (Xu et al. Genes & Dev. In press). We demonstrate that RMI1 and RMI2 form a novel complex that plays a critical role in mediating BLM function.

2. Identify New Fanconi Anemia Genes and Understand the Disease Mechanism: Fanconi anemia (FA) is a genome instability disease and the patients have higher risks to develop cancer. Recently, this rare disease has attracted wide-spread attention, because FA gene products have been shown to function in the same DNA damage response network as the breast cancer susceptibility proteins BRCA1 and BRCA2. The cells derived from FA patients exhibit hypersensitivity to DNA crosslinking

drugs, making FA a disease model for studies of repair of crosslinked DNA damage. We have purified an FA core complex, and shown that this complex has five known FA proteins and five new components (they are named FAAPs for FA-Associated Proteins). We demonstrate that three of the four FAAPs are defective in FA patients, and thus are encoded by three new FA genes. They are named FANCL, FANCB, and FANCM (Meetei et al. Nature Genetics. 2003, 2004 and 2005). Importantly, FANCL and FANCM proteins have defined enzymatic domains and activities. FANCL contains a ubiquitin ligase motif as well as the corresponding activity, and is required for FANCD2 monoubiquitylation *in vivo*, a critical step in DNA repair. It likely plays a crucial role in the FA/BRCA pathway as the catalytic subunit required for FANCD2 monoubiquitylation. FANCM has a DNA-translocase activity, and can process DNA intermediates generated during repair. FANCM is also hyperphosphorylated in response to DNA damage, and may serve as a signal transducer through which the complex is regulated by DNA damage signals. We are currently identifying other components of the FA core complex to better understand its role in genome maintenance.

3. Purification of a Complex Involved in Rothmund-Thompson Syndrome: This disease is characterized by genome instability and higher risk of cancer. The gene mutated in the disease, RECQL4, belongs to the same RecQ helicase family as WRN and BLM. We have now purified the RECQL4 complex from HeLa cells, and shown that this complex contains not only RECQL4, but also UBR1 and UBR2, which are two homologous ubiquitin ligases involved in the "N-end-rule" pathway that regulates protein degradation. Unlike the WRN and BLM complexes, which contain DNA binding proteins and helicase activities, RECQL4 complex does not have any other subunits that can bind DNA and it lacks detectable helicase activity. Our data suggest that RECQL4 may use a different mechanism to maintain genome stability, possibly dependent on ubiquitination and the N-end-rule pathway.

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

1. Mammalian SWI/SNF-related Chromatin-Remodeling Complexes: The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. In mammals, the SWI/SNF-related complexes are involved not only in gene regulation, but also in targeting

of HIV integration, cell cycle regulation, and in tumor suppression by interacting with Rb protein. Mutation of the hSNF5 subunit has been shown to be a cause for pediatric rhabdomyosarcoma. We have completely purified several distinct mammalian SWI/SNF-related complexes. By microsequencing, we have cloned all subunits from two major complexes of human HeLa cells, BAF and PBAF. We demonstrated that these two complexes have selectivity in regulating gene expression *in vivo*, and have identified the subunits that provide such selectivity (Yan et al. Genes & Dev. 2005). We have also shown that one of the subunits is required for embryonic stem cells to maintain its pluripotency (Yan et al. Stem Cells, 2008).

2. Chromatin Remodeling in ATRX Syndrome: ATRX syndrome represents a combination of α-thalassemia, mental retardation, and multiple associated developmental abnormalities. The gene defective in ATRX has been localized to the X chromosome and recently cloned. The ATRX gene encodes a gene product containing a SWI2/SNF2-type DNA-dependent ATPase domain. Thus, it has been hypothesized that ATRX could function in an ATP-dependent chromatin-remodeling complex and participate in regulation of gene expression. By immunoprecipitation from HeLa extract, we found that ATRX is in a complex with transcription cofactor Daxx. We demonstrate that this complex has ATP-dependent chromatin remodeling activity. Our study suggests that ATRX functions in conjunction with Daxx in a novel chromatin-remodeling complex. The defects in ATR-X syndrome may result from inappropriate expression of genes controlled by this complex.

Collaborators: Drs. Hans Joenje, Johan de Winter, Annette Medhurst, Quinten Waisfisz, Henri vad de Vrugt, Anneke Oostra, Free University, Netherlands; Drs. Alex Sobeck, Stacie Stone, and Maureen Hoatlin, Oregon Health and Sciences University; Drs. Richard Gibbons, Doug Higgs and Ian Hickson, Oxford University, UK; Dr. Jacques Cote, Laval University, Cancer Research Center, Canada; Drs. Jiemin Wong, Jun Qin, and Colin Bishop, Baylor College of Medicine, Houston, Texas; Drs. Everett Chen and Michael Cleary, Stanford University, California; Drs. Trevor Archer and Keji Zhao, NIH; Dr. Lei Li, Anderson Cancer Center, Texas; Dr. Alexander Varshavsky, California Institute of Technology, Pasadena, CA. Stephen West, Cancer Research UK, London, UK.

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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 National Institute on Aging in the Fall of 1998 to establish the Developmental Genomics and Aging Section within the Laboratory of Genetics. He is an Editor of DNA Research and Reproductive Biomedicine Online. He received the NIH Merit Award in 2001. His research accomplishments include the first demonstration of stochastic gene expression in a single cell, the first method to equalize/normalize cDNA library, and the construction of a whole cDNA catalog and its application to a genome-wide gene expression profiling. His group has generated and deposited nearly a half-million mouse cDNA/ESTs to the public database, including about half of all mammalian cDNA/ESTs from preimplantation embryos. In addition, his group has established three major resources: a 15,000 unique gene collection (NIA Mouse 15K cDNA Clone Set), a 7,400 unique gene collection (NIA Mouse 7.4K cDNA Clone Set), and a 60-mer oligonucleotide glass slide microarrays containing ~44,000 gene features. These resources have been provided to the research community and also facilitate some of the approaches in his research group.

Keywords:

stem cells
preimplantation embryos
cellular immortality and pluripotency
cDNA library
DNA microarray

Recent Publications:

Sharova LV, etal. *Dev Biol* 2007; 307: 446-459.

Falco G, etal. *Dev Biol* 2007; 307: 539-550.

Masui S, etal. **Nat Cell Biol** 2007; 9: 625-635.

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

1. Systematic Analysis of Gene Regulatory Networks: The goal of this project is to develop a method to monitor the expression levels of a large number of genes in various experimental conditions and to elucidate the global structure and behavior of a gene regulatory network in development and aging. In our previous work, we have constructed cDNA libraries from early mouse embryos and stem cells and generated a large number of expressed sequence tags (ESTs) (http://lgsun.grc.nia.nih.gov/cDNA/cDNA.html). In collaboration with the Agilent Technologies, we have developed a glass-slide microarray platform containing in situ-synthesized 60-mer oligonucleotide probes representing approximately 44,000 unique mouse transcripts. We have also developed four major bioinformatics tools/databases: (1) a web-based ANOVA-FDR software to provide user-friendly microarray data analysis (http://lgsun.grc.nia.nih.gov/ANOVA/); (2) an algorithm and a fully-automated computational pipeline for transcript assembly from expressed sequences aligned to the mouse genome; (3) a web-based browser to visualize all transcripts and alternative spliced forms of mouse genes (NIA Mouse Gene Index: http://lgsun.grc.nia.nih.gov/geneindex/mm9/); and (4) an web-based database and tool to visualize and map transcription factor binding sites of the mouse genome (CisView: http://lgsun.grc.nia.nih.gov/geneindex/mm6/cisview.html). We have developed a high throughput whole-mount in situ hybridization technique for preimplantation mouse embryos and ES cells. The unrestricted community access to the resource can accelerate a wide range of research, particularly in reproductive and regenerative medicine.

During the last year, we have undertaken a major initiative to generate and analyze up to 300 transcription factor-manipulable mouse ES cell lines. The goal is to dissect a complex web of transcription factors (TFs) and their target genes by perturbing the network -- overexpressing or repressing single transcription factors (TFs) and monitoring the impact on the entire gene set by the expression profiling and phenotyping. We expect this project to facilitate the analysis of global gene regulatory networks by providing resources to the research community and aiding in the identification of key genes governing pluripotency and self-renewal of ES cells, as well as key genes triggering or enhancing the differentiation of ES cells to specific cell lineages.

2. Preimplantation Mouse Development: Preimplantation development

is an important model system to study the pluripotency of mouse cells. Preimplantation development can be seen as a process in which totipotent stem cells (fertilized eggs) lose their totipotency. Preimplantation development also has many other interesting features as a biological system. For examples, it involves dynamic switching from a process governed by the activity of maternally stored RNA/proteins to a process governed by the genes of zygotic activation. Some oocyte mRNAs are translated, but fertilization triggers massive mRNA degradation. Transcription from the zygotic genome begins at the late one-cell to two-cell stage in mouse. The first cell differentiation event in mammalian development also occurs in preimplantation embryos. The process, "compaction," occurs at the 8- to 16-cell stage, when cells that were previously loosely associated begin to adhere in the tightly organized cell mass of the morula. This is the starting point for cell differentiation into Inner Cell Mass (ICM) (which eventually becomes the embryo) and Trophectoderm (which eventually becomes the placenta).

In our previous work, we carried out microarray-based global expression profiling of all preimplantation stages in mouse, which revealed and characterized the distinctive patterns of maternal RNA degradation and two major transient waves of de novo transcription. The first wave corresponds to zygotic genome activation (ZGA); the second wave, named mid-preimplantation gene activation (MGA), precedes the dynamic morphological and functional changes from the morula to blastocyst stage. We propose a cascade of gene activation from maternal RNA/protein sets to ZGA gene sets and thence to MGA gene sets ("waves of gene activation hypothesis"). Among the candidate genes identified in this study, we focused on Zscan4, which is expressed only in late 2-cell embryos and in the subpopulation (3 – 5%) of ES cells maintained in the undifferentiated condition. We have demonstrated that the downregulation of Zscan4 expression in 2-cell embryos by shRNAs delays the normal transition from 2-cell embryos to 4-cell embryos for about 24 hours, resulting in the abnormal blastocysts, which failed to implant to the uterus. This indicates a critical role of Zscan4 in preimplantation development.

3. Embryonic and tissue Stem Cells: Embryonic stem cells are derived from the inner cell mass (ICM) of the blastocyst and are pluripotent, i.e., give rise to all fetal tissues, including germ lines, *in vivo* and *in vitro*. The ES cells also have the capacity for "self-renewal," i.e., the capacity to undergo an unlimited number of symmetrical divisions without differentiation. Thus, they are naturally immortalized cells with stable

and normal karyotypes. Since the first establishment of mouse ES cell lines, these two features have been used to manipulate the mouse genome for functional studies of genes. Embryonic germ (EG) cells, which have similar characteristics, have also been derived from mouse primordial germ cells. Recent establishment of human ES and EG cells increases excitement about the possibility of using these embryonic stem cells for therapeutic purposes. For such applications, it is paramount to understand how the ES cells maintain their pluripotency and self-renewal, and how the ES cells differentiate into specific cell lineages *in vitro*.

The goal of this research project is to understand the nature of mouse embryonic and tissue stem cells, and to identify genes that are responsible for the maintenance of cellular pluripotency. We have been conducting global gene expression profiling with the mouse embryonic DNA microarrays developed in our laboratory. We have completed the expression profiling of mouse embryonic stem (ES) cells, trophoblast stem (TS) cells, adult neural stem (NS) cells, ES cells undergoing neural differentiation in culture, F9 embryonal carcinoma (EC) cells undergoing endoderm differentiation, and ES cells undergoing trophoblast differentiation. We are currently analyzing these data by Principal Component Analysis (PCA) and other statistical and bioinformatic analyses. We have also completed microarray profiling of gene expression in ES cells, in which the level of Oct3/4 - a gene critical for maintenance of undifferentiated ES cells - is controlled by tetracycline-inducible system, and have identified a number of downstream target genes of Oct3/4. These studies begin to identify and analyze gene regulatory pathways involved in the maintenance and differentiation of stem cells.

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Biography: Dr. Goldberg received his Ph.D. in Biochemistry and Cell Biology from the Johns Hopkins University School of Medicine in 1996. Following postdoctoral training in crystallography and virology at Harvard University, and image informatics at MIT, he joined the NIA in 2001. While at MIT, he founded the Open Microscopy Environment (OME: http://openmicroscopy.org) together with Drs. Peter Sorger and Jason Swedlow. The aims of OME are to provide open information interchange formats and open-source software infrastructure for the scientific imaging community. Currently, the IICBU continues to develop software and standards for OME, new approaches to pattern recognition in images, and new technology for image-based high throughput screening. All of this technology development drives the central theme of the IICBU: Using cell and tissue morphology as a biomarker for cell and organismal state.

Keywords:

open microscopy environment (OME)
image informatics
pattern recognition
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C. elegans
aging
osteoarthritis
knee X-rays

Recent Publications:

Johnston J, etal. *PLoS ONE* 2008; 3: e2821.

Macura T, etal. *In Microscope Image Processing.* 2008; 499-527.

Orlov N, etal. *Pattern Recognit Lett* 2008; 29: 1684-1693.

Shamir L, etal. *IEEE Trans Biomed Eng* 2008; in press.

Shamir L, etal. *Med Biol Eng Comput* 2008; 46: 943-947.

Image Informatics and Computational Biology Unit (IICBU): This program is designed to develop technology for quantitative imaging assays for studying age-related and disease processes at the cellular, tissue, and organism level.

1. Software and Standards for the Open Microscopy Environment (OME): OME is an open-source software project to implement image informatics infrastructure capable of analyzing, managing and organizing images and related information on a large scale (10^5 – 10^8 images per system) [1]. This is a collaborative project between four academic groups: The NIA IICBU, Jason R Swedlow, University of Dundee, Peter Sorger, Massachusetts Institute of Technology, Kevin Elicieri and John White, University of Wisconsin-Madison. The project currently comprises several hundred source files and nearly a half-million lines of code in Perl, C, Java, HTML, XML, and MATLAB. This ongoing project is in use world-wide in addition to the four collaborative groups, has active email lists for developers and users, produces at least one stable release per year, and has a live public code-base that receives a dozen commits per day. More information about OME, its history, architecture and technical documentation is available on its web-site at http://openmicroscopy.org.

Currently, IICBU is involved in four aspects of the OME project: 1) Curating the OME XML file format, which has gained acceptance by manufacturers of microscopy software and equipment. 2) Implementing

Publications-continued
Shamir L, etal. *Source Code Biol Med* 2008; 3: 13.

Orlov N, etal. *In Vision Systems: Segmentation and Pattern Recognition.* 2007; 221-242.

public image repositories based on OME that are cross-referenced with other public genomics and other "omics" datasources. 3) Developing end-user tools that work with OME's data model [8]; and 4) Maintaining and validating the OME Analysis Engine in machine vision and pattern recognition applications [4].

2. Quantitative Morphometry as a Biomarker for Cellular and Organismal State: Automated image analysis can be divided into two broad categories: model-based and model-free. In traditional model-based systems, a model of what is being imaged is manually constructed, and is then used as the basis to report quantitative information (e.g., an algorithm for finding "blobs" in an image that reports their size, shape, signal intensity, etc). The main advantage of the model-based approach is that one controls the aspects of the image that will be considered (e.g., the algorithm and parameters for finding the "blobs"). However, a different approach is needed in situations where the model can't be easily defined, or is completely unknown.

Model-free image-processing systems rely on training images to learn by example rather than making use of pre-conceived visual models. A model-free pattern recognition approach treats all images equivalently regardless of the imaging methodology or the image subject, which results in a more general approach to image analysis. The same operations are performed whether grading lymphomas, determining sub-cellular localization, sub-typing pollen grains, etc. Each image is reduced to a set of "signatures" (also called "features" in machine learning, or "image descriptors" in machine vision). Each feature is a numeric value produced by an algorithm sensitive to a specific type of image content, and can be thought of as a sensor for a specific image characteristic (e.g. various textures, intensity statistics, distribution of objects, etc). A large collection of features (>2000 in our case) ensures that there is a sufficient variety of sensors available for many kinds of images. Because the various features have different resolving power depending on the context of the imaging problem, they are automatically assigned weights based on a Fischer discriminant. The set of features can also be reduced by eliminating those with very low resolving power. The reduced set of weighted features can then be used to train standard classifiers developed for supervised machine learning. Currently, we've had the greatest success with our own modification to a nearest neighbor classifier. We've named the set of image descriptor algorithms and our classifier WND-CHARM (Weighted Neighbor Distance from a Compound Hierarchy of Algorithms Representing

Morphology). We have validated the generality and accuracy of this approach in over a dozen different "benchmark" imaging problems, showing that WND-CHARM is capable of automatically solving a variety of image classification tasks without sacrificing accuracy [5,7,8].

Of particular interest in our Institute has been the study of age-related muscle degeneration. Studying this process based on quantitative morphology of muscles required development of a technique to accurately measure similarity between images. Although image classification is a well understood problem in the pattern-recognition field, accurate measurement of image similarity is much less well understood. We have investigated several approaches to this problem using previously known techniques, but found that WND-CHARM consistently reports image similarity measurements much closer to what we can determine experimentally. We investigated the progression of age-related morphological change in the pharynx terminal bulb of the adult C. *elegans* worm. This muscular organ is used to pump food through the animal, and has previously been used to study muscle degeneration because it is can be easily imaged and its function can be easily measured by counting its pumping rate. To our great surprise, we were able to demonstrate that as this tissue ages, it undergoes discrete, step-wise transitions between three identifiable stable morphological states [3]. This was the first demonstration for any organism of stable post-developmental morphological states. If the aging process progresses through discrete states, it implies that the process is under some control and is not entirely stochastic. The existence of these transitions also implies that interfering with them may allow substantial alteration of the aging process, and not merely alleviating it or slowing it down by degrees.

We are also developing a high-content screening platform to make image-based morphological screens cheaper and easier to perform. This platform is based on microarray technology to print RNA-interference (RNAi) or gene-expression constructs at a high density on microscope slides. Once the slide is printed with 2000-5000 different gene-specific constructs, cells are plated on the entire slide. Cells that land on a printed "spot" will be altered relative to their neighbors depending on what was printed on that spot – either a single gene will be knocked-down if RNAi was printed, or a single gene will be over-expressed if an expression construct was printed. Current preliminary results indicate that we can print RNAi at adequate densities, grow cells on these slides, and observe predicted phenotypes depending on what was printed. Knocking down a gene required for

generated phenotype from slide to slide, and from day to day. We have already demonstrated that WND-CHARM is sensitive to morphological variations that are beyond our own abilities to discern, making precise reproducibility in these experiments a significant challenge. Recently we have identified several critical parameters necessary to ensure that the required level of repeatability is met.

The success of WND-CHARM has prompted us to investigate its use as a medical diagnosis tool. We have recently completed a study on differentiating three classes of Lymphoma based on biopsy sections stained with Hemotoxylin/Eosin (H+E). While we were able to differentiate the three lymphoma classes to a greater extent than pathologists are able to, we were not able to out-perform pathologists in diagnosis, i.e. determining the lymphoma type in an entirely new case. The study we contributed focuses on the various imaging and sample preparation parameters that help and hinder automated diagnosis.
Our second investigation of WND-CHARM as a diagnostic tool for medical imaging involved examining knee X-rays acquired in the BLSA study (Baltimore Longitudinal Study of Aging). In this case, WND-CHARM was able to agree with a diagnosis made by radiologists for both moderate (KL=3) and minimal (KL=2) osteoarthris with an accuracy of 91.5% and 80.5% respectively [6]. In this case, two radiologists performed each diagnosis independently, and a third adjudicated between the two in case of disagreement, indicating that WND-CHARM performs well compared to very well-determined clinical diagnosis.
Our investigations continue with a study on the predictability of future occurrence of OA, as well as applying WND-CHARM to knees imaged with MRI.

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The goals of the **Laboratory of Immunology (LI)** research program are aimed at uncovering information leading to a better understanding of fundamental cellular, genetic, and molecular mechanisms that contribute to changes in the immune system during the aging process and to diseases that are age-associated (e.g., increasing incidence with advancing age). Ultimately, there are seven major areas of concentration and long-term development with LI which include: 1) the molecular examination of telomere length and telomerase activity in lymphocyte populations; 2) the molecular analysis of differentially-regulated genes involved in lymphoid cell and organ development, differentiation, trafficking, and activation; 3) molecular mechanisms of memory lymphocyte formation, maintenance and activation; 4) the study and use of biological response modifiers to optimize and control leukocyte trafficking, activation, organ engraftment, and vaccine efficacy in normal and aging hosts; 5) induction of antigen-specific tolerance and use in transplantation and autoimmunity; 6) the cellular and molecular dynamics involved in thymic involution and regeneration; and 7) understanding the molecular and biological aspects of tumor cell development and metastasis.

The **Clinical Immunology Section** focuses on several important project areas including understanding the biological and molecular effects of chemokines on lymphocytes, the role of lipid rafts, cholesterol and adapter proteins in the maintenance of chemokine signaling and cellular activation, the immunoregulatory effects of pituitary and metabolic hormones in inflammation and immunity and understanding the various processes associated with age-associated thymic involution.

The *Cancer Biology Unit's* recent work has focused on using techniques of high throughput gene expression profiling to unravel pathways involved in cellular migration and metastasis of cancers such as melanoma, a highly

immune-modulated cancer. Especially important are those pathways associated with chemokine, cytokine and T-cell receptor signaling and pathways reflective of melanocyte development that go awry in metastasis, involving molecules such as Wnt5a.

The *Lymphocyte Cell Biology Unit's* recent work has focused on understanding the cell biology of lymphomas, tumor-induced immunosuppression, the roles of PTEN and mTOR in lymphocyte activation and function and defining the role of CD28-mediated costimulatory signal in immune responses particularly in cancer and autoimmune disease.

The *Lymphocyte Development Unit* is focused on understanding the role of Wnt-beta catenin-TCF signaling pathway in the development and function of T lymphocytes. Interaction of this signaling pathway with Notch 1 mediated signals as well as pre-TCR and TCR mediated signals will provide insight into the programs utilized by the bone marrow derived precursors as they commit to the T cell lineage, mature and age in mammals.

The *Immunotherapeutics Unit* concentrates on development of simpler and more potent vaccines for cancer and other clinically relevant diseases utilizing strategies, which targets Antigen Presenting Cells (APCs). Currently, the focus of the research is to assess a carrier potency and mechanism of antigen presentation of chemokine- and defensin-based vaccines, to search for alternative delivery methods for DNA vaccines (such as chemokine bearing empty protein particles) and to establish models to study therapeutic efficacy of newly found tumor associated antigens.

The **Lymphocyte Differentiation Section** is currently investigating the influence of age on telomere length and telomerase expression in peripheral blood T lymphocytes *in vivo*, regulation and function of telomerase in lymphocytes, and the molecular mechanisms involved in the generation and maintenance of memory T lymphocytes and their effector function.

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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 (SAIC) 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, Laboratory of Immunology as well as Director of the Clinical Core Laboratory and NIA Repository, CRB.

Keywords:

chemokines
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frizzled receptors
HIV
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neuroimmunology
Th17
Treg
immunosenescence
inflammation

Recent Publications:

Taub DD. *Cell Immunol* 2008; 252: 1-6.

Taub DD. *Vitamin Horm* 2008; 77: 325-346.

Redelman D, et al. *Cell Immunol* 2008; 252: 111-121.

Cholesterol and Lipid Rafts in T-Lymphocyte Signaling and Trafficking: Chemokine receptors (CRs) have drawn much attention since their description as human immunodeficiency virus (HIV) co-receptors by several groups in 1996. Prior to that time, HIV tropism was defined as either macrophage (M)- or T cell (T)-tropic, which corresponded to non-syncytia- or syncytia-inducing viruses, respectively. Today, the classification of HIV tropism is defined by chemokine receptor usage of CCR5, CXCR4, or both receptors. Chemokine receptors are a family of seven transmembrane spanning G protein-coupled receptors that are differentially expressed by a number of immune and non-immune cell populations. Certain CRs have been shown to be palmitoylated and targeted to cholesterol-and sphingolipid-rich membrane microdomains termed lipid rafts. Lipid rafts is a broad term for the collection of membrane microdomains enriched in cholesterol, sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins, and acylated signaling molecules. CCR5 and CXCR4 have been shown to be present in lipid rafts, colocalizing at the leading edge of migrating cells. We found that cholesterol extraction by beta-cyclodextrin (BCD) or oxidation of cholesterol significantly reduced the binding and signaling of CXCL12 and CCL4 using CXCR4- or CCR5-expressing T cells, respectively. Antibodies specific for distinct CXCR4 or CCR5 epitopes lost their ability to bind to the cell surface after cholesterol extraction and cholesterol oxidation. These results suggest that active ligand binding facilitates receptor association with lipid rafts or that raft association promotes a higher affinity conformation of chemokine receptors. Continuing efforts

Publications-continued
Dawson HD, et al. *BMC Immunol* 2008; 9: 16.

Yang H, et al. *Brain Behav Immun* 2008; 113: 575-584.

Ghosh M, et al. *Blood* 2008; epub.

Taub DD, et al. *Am J Med* 2008; 121: 1058-1064.

Weeraratna AT, et al. *Exp Cell Res* 2007; 313: 450-461.

Dawson HD, et al. *BMC Immunol* 2007; 7: 27.

Giri B, et al. *Eur J Immunol* 2007; 37: 2104-2116.

Lustig A, et al. *Cell Immunol* 2007; 245: 42-61.

Dixit VD, et al. *J Clin Invest* 2007; 117: 2778-2790.

have focused on the role of various adapter and signaling molecules in controlling CR localization in lipid rafts and understanding the biology of cholesterol in T cell biology. Given the large number of alterations in lipid and peroxidation and metabolism with age, changes in the types, saturation and levels of various membrane sphingolipids, fatty acids and cholesterol may result in specific changes in membrane fluidity, protein association and aggregation, cellular activation and function.

Flotillin proteins have recently been shown to be recruited to lipid raft microdomains upon cellular activation and have been implicated in neural cell regeneration, receptor signaling and lymphocyte activation. However, little is known about the relevance of the flotillin proteins in T cell responses to chemoattractant stimulation. To this end, cytoplasmic and lipid raft fractions from human T cells were analyzed for flotillin protein redistribution prior to and after CXCL12 stimulation. Flotillin-1 but not flotillin-2 redistributes to lipid rafts upon CXCR4 ligation. Moreover, in CXCL12-treated T cells, flotillin-1 also associates with several raft proteins including LAT, Lck, CD48 and CD11a. In addition, an increase in CXCR4 association with flotillin-1 in lipid rafts was observed after chemokine treatment. RNAi technology was also utilized to inhibit the expression of flotillin-1 resulting in an inhibition of CXCL12-mediated signaling, function and CXCR4 recruitment into lipid rafts. Together, these data suggest that the association of flotillin-1 with lipid raft during chemokine exposure may play an important role in chemokine receptor recruitment to and signaling in lipid rafts and possibly in leading edge formation. Overall, we believe that a greater understanding of the various signaling and cell surface proteins associated with lipid rafts may provide insight into age-related alterations in cell signaling and trafficking.

Additional studies have also revealed that dexamethasone (DM)-treated T cells demonstrate enhanced migration in response to the chemokine CXCL12, possibly through altering the cell membranes and rafts or through direct interaction with the chemokine and T-cell receptor signaling pathways. DM is a synthetic member of the glucocorticoid (GC) class of hormones that possesses anti-inflammatory and immunosuppressant activity and is commonly utilized to treat chronic inflammatory disorders, severe allergies and other disease states. While glucocorticoids are known to mediate well-defined transcriptional effects via GC receptors, there is increasing evidence that GCs also initiate rapid non-genomic signaling events in a variety of cell types. Here, we report that dexamethasone appears to induce the phosphorylation of Lck and the activation of other

down stream mediators including p59Fyn, Zap70, Rac1 and Vav in resting but not activated human T cells. DM treatment also appears to augment CXCL12-mediated signaling in resting T cells through its cell surface receptor, CXCR4 resulting in the enhanced actin polymerization and cell migration upon ligand exposure. Lck was found to be a critical intermediate in these DM-induced signaling activities. Moreover, DM-mediated Lck phosphorylation in T cells was dependent on the presence of both the glucocorticoid receptor and the CD45 molecule. Overall, these results elucidate additional non-genomic effects of DM on resting human T cells, inducing Lck activation and augmenting chemokine signaling and function.

Chemokines Differentially Regulate Wnt-Frizzled Gene Expression in Human T Cells: Chemokines have been shown to induce and direct adhesion, chemotaxis, activation, and degranulation of human and rodent leukocytes both *in vitro* and *in vivo*. CXCL12 and CCL19 are two important chemokines that regulate T cell motility and activation under normal and inflammatory conditions. Despite numerous reports examining the function of chemokines, little is known about the transcriptional events involved therein. Here, we performed microarray analysis on CXCL12- treated T-cells, and found that the Wnt family of proteins was significantly upregulated during CXCL12 treatment. Wnts are secreted glycoproteins, which are produced by different cell types. The Wnt family of proteins has over 19 members that are very closely related in structure. Wnt proteins signal through their specific frizzled (Fzd) receptors that, similar to chemokine receptors, signal via activation of G-proteins. The interactions between specific Wnts and Fzd receptors dictate which G-proteins are activated and in what manner the signals are transduced down stream. Confirmation of these results by real-time PCR and Western analysis revealed that the expression of Wnt5A and other members of the non-canonical Wnt pathway were specifically upregulated during CXCL12 stimulation, while beta-catenin and canonical Wnt family members were selectively downregulated. Wnt5A was found to augment signaling through the CXCL12-CXCR4 axis via the activation of protein kinase C (PKC). Moreover, our data has revealed that Wnt5A expression is required to mediate directional T-cell migration in response to CXCL12, and that the treatment of human T-cells with recombinant Wnt5A sensitized T-cells to CXCL12-induced migration. Furthermore, Wnt5A expression was also required for the sustained expression of CXCR4, both transcriptionally and translationally. These results were further supported *in vivo* using EL4 thymoma metastasis as a model of T-cell migration. Together, these

data demonstrate, for the first time, that Wnt5A is a critical mediator in CXCL12-CXCR4 signaling and migration in human and murine T cells. Interestingly, we also found that Wnt10A plays a role in CCL19 chemotaxis and in the maintenance of CCR7 expression on T cells. These findings may reveal a novel cooperative signaling network between various chemokine and Wnt receptors and ligands that may control cell polarization and directional migration.

Novel Connections Between the Immune and Endocrine Systems: Decrease in food intake (anorexia) is one of the most common symptoms of illness, injury or inflammation. Leptin is considered a critical sensory anorexigenic mediator that signals to the brain changes in stored energy, determined by an altered balance between food intake and energy expenditure and has been shown to exert certain proinflammatory effects on immune cells. In contrast, ghrelin, the endogenous ligand for growth hormone secretagogue receptors (GHS-R), is produced primarily from stomach serving as a potent circulating orexigen controlling energy expenditure, adiposity and GH secretion. We have recently found that ghrelin and several other orexigens are highly anti-inflammatory in nature, while leptin and other anorexigens demonstrated potent proinflammatory effects. The goals of our research are to define the role of these various hormones and their receptors on inflammation, immune cell activation, T cell differentiation and hematopoiesis. Furthermore, the circulating and lymphoid expression of these hormones appears to significantly diminish with age, especially in the thymus. Administration of ghrelin into aged mice results in a dramatic abrogation of age-associated inflammation and thymic involution, while leptin infusion into old mice enhances circulation inflammatory cytokines but also reverses thymic involution. Together, these data support the existence of a functional immunoregulatory network involving orexigenic and anorexigenic hormones that appear to play a significant role in cytokine regulation, cellular activation and survival. These data also support the potential therapeutic use of GH, ghrelin and GHS-R agonists in the management of wasting associated with chronic inflammation and cancer and in restoration of thymic function in immunocompromised individuals.

Molecular and Biological Mechanisms of Age-associated Thymic Involution: One of the consequences of an aging immune system is the process of thymic involution. The thymus undergoes a progressive reduction in size due to profound changes in its architecture associated with thymic epithelia atrophy and decreased thymopoiesis. This decline

is systemically followed by decreased numbers of circulating naive T cells and cell-mediated immune responses which may play a role in the increased tumorigenesis, autoimmunity, and infectious diseases observed within an aging host. Despite the extensive study of the pathophysiology of the aging thymus, the precise molecular mechanism involved in the involution process remains unclear. The loss of thymic function with age is believed to be due to diminished numbers of T-cell progenitors and the loss of critical cytokines and hormone mediators within the thymic microenvironment. To assess the molecular changes associated with this loss, we examined transcriptomes of progressively aging mouse thymi, of different sexes and on caloric-restricted (CR) vs. ad libitum (AL) diets. Genes involved in various biological and molecular processes including transcriptional regulators, stress response, inflammation and immune function significantly changed during thymic aging. These differences depended on variables such as sex and diet. Interestingly, many changes associated with thymic aging are either muted or almost completely reversed in mice on caloric-restricted diets. These studies provide valuable insight into the molecular mechanisms associated with thymic aging and emphasize the need to account for biological variables such as sex and diet when elucidating the genomic correlates that influence the molecular pathways responsible for thymic involution. Moreover, these results emphasize the need to account for biological variables such as sex and dietary interactions to elucidate the genomic correlates that influence the molecular pathways responsible for thymic involution. We are also currently completing the analysis and confirmation of these data from mice of various ages infused with various hormones that result in a partial restoration in thymocyte numbers. Array analysis of the thymi of such treated mice may yield valuable data on the common molecular processes involved in thymic regeneration. It is unclear whether certain lymphoid organs or cellular components play a critical role in longevity and lifespan. The specific goals of these studies are to indentify and further define novel proteins and signaling pathways associated within the thymus and thymic involution and to define how interventions such as caloric restriction or hormone infusion can modulate age-associated gene expression and thymic loss. Understanding the various gene and protein pathways involved in thymocyte development and thymus involution may provide a means to develop interventional strategies to boost thymic function in aged and immunosuppressed subjects.

Collaborators: Francis Ruscetti, Ph.D., National Cancer Institute, NIH; William Murphy, Ph.D., University of Nevada; James Lillard, Jr., Ph.D., University of Alabama, Birmingham; Dan L. Longo, M.D. and Arya Biragyn, Ph.D., Laboratory of Immunology, National Institute on Aging, NIH; Kevin Becker, Ph.D., Research Resources Branch, National Institute on Aging, NIH; Roy Smith, Ph.D., Baylor College of Medicine, Texas.

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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 29 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 700 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 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 every edition of Best Doctors in America.

Keywords:

lymphocyte
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CD28
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Recent Publications:

Heltemes-Harris LM, et al. *Mol Immunol* 2008; 45: 1799-1806.

Sasaki CY, et al. *Can Res* 2007; 67: 11218-11225.

Chen G, et al. *Blood* 2007; 109: 5301-5307.

Characterization of TGF-b Signaling In B-cell Lymphoma Cells

Transforming growth factor beta 1 (TGF-b1) induces growth suppression in a variety of cell types. In our laboratory, we are studying several B-cell lymphoma cell lines that are resistant to TGF-b1-mediated growth suppression. Our goal is to understand the mechanisms underlying the development of TGF-b1-resistant phenotype by some B-cell lymphoma cells. We are interested in two B-cell lymphoma cell lines, DB and RL, that are resistant to TGF-b1-mediated growth suppression. We have reported previously that low dose PMA rendered RL cells sensitive to TGF-b1, whereas DB cells remained insensitive. We have shown recently that the TGF-b1-mediated phosphorylation of Smad2 and Smad3 were absent in DB cells, whereas TGF-b1-induced phosphorylation of both Smad3 and Smad2 were observed in RL cells in presence of low dose PMA. Examination of the status of the TGF-b receptors (TbR) revealed that both RL and DB cells had TGF-b receptors I (TbRI) on their cell surface, whereas TGF-b receptors II (TbRII) were present only on the cell surface of RL cells. We have demonstrated that transfection of wild-type, but not a C-terminal truncated form of receptor II rendered the DB cells responsive to TGF-b1-mediated growth suppression. Analysis of the TRII gene revealed the absence of the receptor II message, which was reversed upon treatment with demethylating agent, indicating that the promoter methylation might be the cause of gene silencing. Promoter analysis revealed CpG methylations at -25 and -140 that correlated with the gene silencing. We have shown that the promoter methylation was also

involved in silencing TbRII gene in another B-cell lymphoma cell line, Akata. Regarding the unresponsiveness of RL cells to TGF-b1-mediated growth suppression, we have found that the transient TGF-b1 signaling is responsible for the resistance. Analysis of TbRII revealed ligand-induced receptor down-regulation in a time-dependent manner. With a low dose of PMA, RL cells restored the sensitivity to TGF-b1 by stabilizing TbRII and sustaining TGF- signaling. We are currently investing the mechanism of PMA-induced signal transduction responsible for the TbRII stabilization.

Rapamycin-sensitive pathway of T cell Activation

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 a pathway that is insensitive to 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 focus on three areas: (1) characterization of the CsA-resistant rapamycin-sensitive pathway of T cell activation; (2) examination of the physiological significance of this pathway; and (3) the effect of aging on the rapamycin-sensitive pathway. Our recent work has demonstrated that the immunosuppressive drug rapamycin selectively affects the CsA-resistant pathway. Our initial studies have focused on the mechanism of activation of the IL-2 gene in a CsA-resistant manner. We found that the effect of rapamycin on the IL-2 expression was due to alteration in IL-2 mRNA stability. More recently, we have also shown that activation of T cells by IL-12 is resistant to CsA, but sensitive to rapamycin. As the intracellular target of rapamycin is mTOR (mammalian target of rapamycin), we are investigating the mechanism of activation of mTOR during T cell activation. Regarding the physiological role of the resistant pathway, we have observed the effect of cytokine signaling, particularly the combination of IL-12 and IL-18 but not individual cytokine alone, in activating resting human peripheral blood in a CsA-resistant, but rapamycin-sensitive manner. γT cells in producing IFN-Interestingly, naive CD4+ T cells are more responsive to IL-12 plus IL-18 stimulation in comparison to memory CD4+ T cells. This cytokine-mediated activation of resting T cells is independent of antigen. We are currently investigating the *in vivo* physiological role of this cytokine signaling pathway.

Structure-function relationship of NFkB p65
The NFkB family of transcription factors plays a pivotal role in the regulation of inflammation and the development of the immune system. Abnormal alteration of the NFkB pathway has been implicated in cancer, dysfunction of the immune system, and perhaps aging. Recent evidence has shown that the post-translational modification of the NFkB members is involved in the activation of this pathway. The mechanism involved in the selective recruitment of the NFkB members to the numerous possible NFkB-responsive genes is not been fully elucidated. We are interested in understanding the role of protein phosphorylation in the activation of transcription and the selectivity of gene expression. We have previously demonstrated that the phosphorylation of p65 at serine 536 was differentially recruited to selective promoters following cell activation. We have recently demonstrated that the distance between the site of p65 binding and the transcription start site of a particular gene determines if p65 needs to be phosphorylated on serine 536. The phosphorylation of p65 was not involved in the formation of an enhanceosome, where the recruitment of histone modifying enzymes to proximal promoters was required. These findings suggested that the phosphorylation of p65 and the cis-acting elements of the promoter regulate the various NFkB responsive genes. We are currently investigating the role of various phosphorylation sites of p65 in controlling the chromatin architecture surrounding p65 responsive genes. We are also examining how the various IkB members regulate the nuclear translocation of phosphorylated p65. In addition to serine 536, the phosphorylation of serine 529 of p65 has been shown to regulate transcriptional activity. We are currently investigating the relationship between the phosphorylation of serines 529 and 536 in regulating the chromatin architecture. Furthermore, an alternative pathway has been described to activate the NFkB proteins and promote transcription. We are examining the role of p65 phosphorylation in controlling gene transcription induced by the alternative NFkB pathway.

Shaping the Pre-immune B Cell Repertoire: The immune response to PC (phosphocholine) is important because it has been shown to confer protection against infection by Streptococcus pneumoniae (S.pn.), a pathogen that poses a significant risk to elderly, very young and immunocompromised individuals. Recently, we have shown that the mouse VH1 gene is essential for immune response to PC and PC-mediated protection against infection by S.pn. Furthermore, by examining the associations between the VH1 gene and various light chains in PC-specific B cells, we have identified IgL chain structural determinants

that may explain differences in the relative affinity/avidity of VH1/VL combinations for different PC containing antigens. Our studies on these disease and mouse models are continuing and will provide insight into the complimentary contribution of interactions between VH and VL genes to a protective versus ineffective immune response to different pathogens.

An increase in the percentage of pneumococcal strains not represented in the current carbohydrate vaccines has been observed in individuals presenting with pneumococcal disease. In addition, the problem of emerging antibiotic resistant strains of many pathogens including S.pn. continues to worsen. We have recently developed and patented a novel, flexible and inexpensive synthesis strategy for preparing PC-derivatives that can be used to produce PC-conjugate vaccines against infection by S. pn. and other PC expressing pathogens. PC is an antigen found on virtually all strains of S. pn. as well as many other bacterial, fungal and parasitic protozoan pathogens. The relevancy of anti-PC antibodies to protection from challenge by various pathogens in humans is demonstrated by the observation that passive immunization of mice with anti-PC specific antibodies purified from humans provide the mice with protection from challenge by S. pn. We are continuing to develop and examine PC-conjugates as potential vaccines against S. pn. infection for use in humans and are developing mouse models for determining whether PC-conjugate vaccines may also provide protection against other pathogens which express PC. In addition, ongoing studies are underway to define the immune response to PC and other phospholipids in humans. These studies should provide further insight into factors which contribute to a protective and beneficial immune response and those which are harmful and detrimental to the host resulting in autoimmune pathologies.

The clonal selection theory and associated corollary (that a single cell expresses a single receptor with a single antigen specificity) has been a dominant tenet in shaping our thinking of the development of the immune system and immune response to antigenic challenge. Based on our earlier observations in PC transgenic mouse models we proposed that this corollary could be compromised and that dual receptor expression or “receptor dilution” was a mechanism by which a host can balance the necessity to avoid self reactivity (which could result in holes in the available repertoire) with the evolutionary pressure to provide protection against specific pathogens. We have recently demonstrated in wild type, nontransgenic C57BL/6 mice that dual receptor expressing B cells are a part of the normal wild type B cell repertoire. We continue to examine the VH and VK genes expressed by this small population of dual

isotype expressing B cells in wild type C57BL/6 mice and have noted that the inferred specificities for the expressed VH and VK genes are to both autoreactive antigens as well as antigens expressed on various pathogens. These observations suggest that coexpression may be a general mechanism for shaping this subpopulation of the B cell repertoire. Ongoing experiments should also provide additional evidence for the necessity to conserve the specificities expressed by these dual receptor expressing B cells as well as to delineate the developmental and molecular processes which result in generation and maintenance of this dual receptor expressing B cell population. Continued examination of the contribution these and other components play in shaping the immune repertoire will further expand our understanding of the mechanisms that distinguish between protective, ineffective and detrimental immune responses.

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Keywords:

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gene expression
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epigenetic changes
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Recent Publications:

Yang Y, et al. *J Immunol* 2008; 180: 3775-3781.

Araki Y, et al. *J Immunol* 2008; 180: 8102-8108.

Damjanovic AK, et al. *J Immunol* 2007; 179: 4249-4254.

Klaewsongkram J, et al. *J Immunol* 2007; 179: 4679-4684.

Research: The research interests of this laboratory are focused on two areas: (1) molecular mechanisms of memory T cell generation, maintenance, function, and aging, and (2) role of telomere and telomerase in lymphocyte lifespan and aging. In order to study the molecular features of memory T cells, we have analyzed and identified differentially expressed genes in naïve, effector, and memory T cells in both human and mouse. The identification of genes that are differentially expressed in memory T cells and other subsets of T cells at the resting state and after activation provides a starting point for elucidating the contribution of these differentially expressed genes in the process of memory T cell formation, maintenance, function and possibly aging. This finding leads to further analysis of 1) role of epigenetic changes in regulation of differentially gene expressions in T cell subsets particularly memory T cells and 2) role of specific differentially expressed gene in lymphocyte development and function. Telomeres and telomerase are the key factors that regulate cellular replicative lifespan. Recently, we have analyzed telomere length of lymphocyte from the caregiver of Alzheimer's disease, and telomerase activity in IL-7 mediated survival of T cells. Our results demonstrate that chronic stress from care giving is associated with altered T-cell function and accelerated immune cell aging as suggested by excessive telomere loss and that telomerase is also involved in the maintenance of IL-7 mediated survival of T cells. Currently, we are characterizing the roles of epigenetic changes and functions of differentially expressed genes in naïve and memory T cells, and determining the *in vivo* changes of telomere length and telomerase in lymphocyte with aging.

Molecular mechanisms of memory T cell generation, maintenance, function, and aging

A hallmark of the adaptive immune response is immunological memory, which involves the selection, differentiation, and proliferation of naïve T cells in response to antigen stimulation to become effector cells, and subsequently to form memory cells. Memory lymphocytes are long lived and are capable of undergoing extensive cell divisions to mount a rapid and effective immune response. Thus, the capacity of clonal expansion of lymphocytes, especially memory lymphocytes, is crucial for the success of sustained immune competency. Despite of the recent progresses in characterizing the differentially expressed genes in naïve and memory T cells, the mechanisms underlying the regulation of differential gene expression and function of these differentially expression in the generation, maintenance, function, and aging remain largely unknown.

- Histone acetylation facilitates rapid and robust memory CD8 T cell response through differential expression of effector molecules (Eomesodermin and its targets: perforin and granzyme B)

Immunological memory provides a fundamental basis of vaccination, yet it remains unresolved how the immune system achieves this long lasting enhanced function to re-encounters of the same pathogen. At the center of immunological memory are the memory lymphocytes that are capable of mounting a rapid and robust cellular response and have the stem cell like ability of self-renewal. These functional properties of memory T cells are acquired after activation of naïve cells in which transcriptional regulation of specific genes plays a central role in the process of memory cell generation and subsequent maintenance. To understand the mechanism regulating the rapid effector function of memory CD8 T cells, we examined expression and chromatin state of a key transcription factor (eomesodermin, EOMES) and two of its targets: perforin (PRF1) and granzyme B (GZMB). Accessible chromatin associated histone 3 lysine 9 acetylation (H3K9Ac) was found significantly higher at the proximal promoter and the first exon region of all three genes in memory CD8 T cells than in nave CD8 T cells. Correspondingly, EOMES and PRF1 were constitutively higher expressed in memory CD8 T cells than in nave CD8 T cells at resting and activated states. In contrast, higher expression of GZMB was induced in memory CD8 T cells than in nave CD8 T cells only after activation. Regardless of their constitutive or inducible expression, decreased H3K9Ac levels after treatment with a histone acetyltransferase inhibitor (Curcumin) led to decreased expression of all three genes in activated memory CD8 T cells. These findings suggest that

H3K9Ac associated accessible chromatin state serves as a corner stone for the differentially high expression of these effector genes in memory CD8 T cells. Thus, epigenetic changes mediated via histone acetylation may provide a chromatin memory for the rapid and robust transcriptional response of memory CD8 T cells.

- Krüppel-like factor 4 (Klf4) regulates B cell number and activation-induced B cell proliferation

Krüppel-like factor 4 (K*lf4*) is a transcription factor and functions in regulating cell differentiation, cell growth, and cell cycle. K*lf4* induces cell cycle arrest at the G1/S boundary in untransformed cells while acting as an oncogene by repressing p53 in transformed cells expressing RAS^{V12}. In addition, K*lf4* induces oncogenic transformation in the absence of functional cyclin D1 and the cyclin dependent kinase inhibitor (CDKI), $p21^{WAF1/Cip1}$. Although K*lf4* is expressed in lymphocytes, its function in lymphocytes is unknown. Here we show that the levels of Klf4 expression were low in pro-B cells and continuously increased in pre-B and in mature B cells. Upon activation, K*lf4* was rapidly decreased in mature B cells after 2 hr of activation. A modest decrease in numbers of pre-B cells in bone marrow and mature B cells in spleen were observed in K*lf4* deficient mice. In the absence of K*lf4*, fewer B cells entered S phase of the cell cycle and completed cell division in response to the engagement of BCR and/or CD40 *in vitro*. Furthermore, the delay in entering the cell cycle is associated with decreased expression of cyclin D2 in B cells that lack K*lf4* expression and we demonstrated that K*lf4* directly bound to the promoter of cyclin D2 and regulated its expression. These findings demonstrate that K*lf4* regulates B cell number and activation-induced B cell proliferation through directly acting on the promoter of cyclin D2.

Role of telomere length and telomerase in human lymphocyte lifespan and aging

Telomeres, the terminal structure of chromosomes, play an essential role in maintaining chromosomal integrity and regulate cellular replicative lifespan. Telomere consists of an array of tandem hexamer repeats, $(TTAGGG)_n$ and the binding proteins called shelterins. The inability of DNA polymerase to completely replicate the ends of chromosomes results in a loss of 50-200 base pair telomere repeats with cell division in normal human somatic cells. The cumulative loss of telomere from cell divisions could exhaust the telomere repeats, which in turn cause to stop

cell division. Thus, a minimal length of telomeres is essential for cellular replication and telomere reduction is a mechanism for limiting replicative lifespan in normal somatic cells. Despite accumulating evidence, mostly *in vitro* data, the *in vivo* significance of telomere and aging has not been demonstrated in human. Telomerase is a unique reverse transcriptase consisting of two essential components, telomerase RNA template (hTER) and telomerase reverse transcriptase (hTERT), and functions including synthesizing telomere repeats and cell survival. Telomerase protects integrity of chromosomes and prolongs replicative lifespan of cells. The selective presence of telomerase in the germline and malignant cells but not in most normal human somatic cells has been hypothesized as a basis for the immortality of the germline and of malignant cells. Our goals are to understand the regulation and function of telomere and telomerase in lymphocyte function, lifespan, and aging.

- Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients

The progressive deteriorating conditions of Alzheimer's disease patients put a considerable emotional and physical burden on their primary caregivers. The consequence of this chronic stress on biological and immunological function has begun to be understood. It has been shown that caregivers of Alzheimer's disease patients are associated with a decline of immune function. To assess the psychological and immunological changes of caregivers, we compared depressive symptoms, peripheral blood mononuclear cell (PBMC) composition, *in vitro* activation induced proliferation and cytokine production, and telomere length and telomerase activity of 82 individuals (41 caregivers and 41 age- and gender-matched controls). We found depressive symptoms were significantly higher in caregivers than in controls ($p<0.001$). Correspondingly, caregivers had significantly lower T cell proliferation but higher production of immune-regulatory cytokines (TNF-a and IL-10) than controls in response to stimulation *in vitro*. We examined the impact of these changes on cellular replicative lifespan and found that caregivers had significantly shorter telomere lengths in PBMC than controls (6.2 and 6.4 Kb, respectively, $p<0.05$) with similar shortening in isolated T cells and monocytes and that this telomere attrition in caregivers was not due to an increase of shorter telomere possessing T cell subsets in PBMC. Finally, we showed that basal telomerase activity in PBMC and T cells was significantly higher in caregivers than in controls ($p<0.0001$), pointing to an unsuccessful attempt of cells to compensate the excessive loss of

telomeres in caregivers. These findings demonstrate that chronic stress is associated with altered T-cell function and accelerated immune cell aging as suggested by excessive telomere loss.

- Telomerase is involved in IL-7 mediated differential survival of naïve and memory CD4 T cells

Balancing survival and death of lymphocytes is a vital task of the immune system necessary to maintain its function throughout life. Interleukin-7 (IL-7), produced by stromal cells, plays a key role in promoting proliferation and survival of T cells in periphery. However, the requirements for proliferation and survival for naïve and memory T cells appear different. Naïve T cells require IL-7 and a weak interaction between TCR and self-peptide/MHC to mediate survival and proliferation while survival and homeostatic proliferation of memory CD4 T cells can be supported by IL-7 in the absence of TCR signals. Furthermore, memory CD4 T cells appear to be faster in entering cell cycle than naïve CD4 T cells in response to IL-7 *in vitro*. The survival effect of IL-7 is thought to be mediated through regulation of Bcl2 family proteins. After a comparative analysis of IL-7 induced growth and cell death of human nave and memory CD4 T cells, we observed that more memory CD4 T cells underwent cell division and proceeded to apoptosis than nave cells in response to IL-7. However, IL-7 induced expressions of Bcl2 family members (Bcl2, Bclxl, Bax and Bad) were similar between nave and memory cells. Instead, we found that IL-7 induced higher levels of telomerase activity in nave cells than in memory cells, and the levels of IL-7 induced telomerase activity had a significant inverse correlation with cell death in CD4 T cells. Furthermore, we showed that reducing expression of TERT and telomerase activity significantly increased cell death of IL-7 cultured CD4 T cells. Together, these findings demonstrate that telomerase is involved in IL-7 mediated differential survival of nave and memory CD4 T cells.

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Keywords:

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TCF
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Recent publications:

Xu M, et al. *Mol Cell Biol* 2008; 28: 1713-1723.

Hossain MZ, et al. *Int Immunol* 2008; 20: 925-935.

Yu Q, et al. *J Immunol* 2008; 181: 3777-3783.

Yu Q, et al. *J Immunol* 2007; 178: 5028-5034.

Yu Q, et al. *J Immunol* 2007; 179: 126-131.

Research Description: Decline in the immune system is a feature of human aging. Reduction in naive T cell repertoire to combat novel pathogens stems from decreased function of the thymus where T cells develop. Within the thymus, stage-specific signal transduction and gene expression, resulting from reciprocal cell-cell interactions and locally produced cytokines and hormones, critically regulate T cell development. Cues from stromal cells regulate an exquisite balance of proliferation, quiescence, cell-death and cell-fate decisions in developing thymocytes. In turn, thymocytes regulate the maturation of thymic epithelial cells. Research in our laboratory is focused on understanding signaling events in T cell development and function and under normal and stressed conditions (e.g., aging). Current efforts are aimed at delineating the role of evolutionarily conserved Wnt-beta-catenin-TCF pathway. To this end, we have manipulated the beta-catenin gene, a major mediator of canonical Wnt signaling pathway. We have generated mice expressing transgenic beta-catenin (CAT-Tg) and mice with T cell-specific deletion (CAT-KO) of the gene. In the near future, we hope to address the role of other conserved signaling pathways in T cell development. Currently, the major effort of our laboratory is concentrated on four fronts:

1. The molecular mechanisms that regulate lymphocyte progenitor differentiation and transformation. We study the role of the pre-T cell receptor (pre-TCR) in the regulation of early T cell progenitor homeostasis, survival, proliferation and differentiation. More specifically, we are interested in signaling pathways involved in the development of T cell progenitors and their interaction with pre-TCR signaling. We have

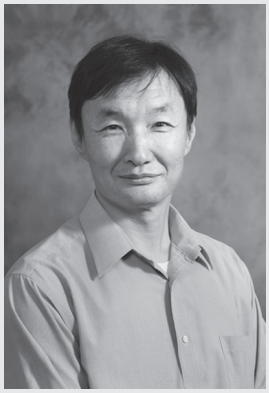
discovered recently that beta-catenin is a target of pre-TCR signaling. It is induced transiently by the pre-TCR and is down-regulated as the cells navigate the beta-selection checkpoint. Lack of beta-catenin expression impairs beta-selection while inability to down-regulate beta-catenin expression after beta-selection results in DNA damage, cellular senescence and p53-dependent apoptosis. In the absence of p53 function, beta-catenin expression leads to thymic lymphoma. Our future studies are aimed at the elucidation of the pre-TCR and beta-catenin signaling pathways and the understanding of their co-operation in normal T cell development and transformation.

2. The molecular mechanisms that regulate lineage commitment and positive selection of alpha-beta-TCR expressing thymocytes. It has been known, for some time, that mature CD4 single positive (SP) thymocytes mature with faster kinetics compared to CD8SP thymocytes. We have recently demonstrated that expression of beta-catenin regulates the timing of positive selection of CD8 T cells such that both CD4SP and CD8SP thymocytes mature at the same rate in CAT-Tg mice. Furthermore, CD8SP thymocytes benefit from IL-7R signals, which are enhanced in CAT-Tg mice and diminished in CAT-KO mice. Consequently, Cat-Tg mice have increased numbers of CD8SP thymocytes. Future studies aim to understand the molecular connections between signals from the TCR and beta-catenin.

3. The molecular mechanisms that regulate T cell function upon encountering antigen. Using retroviral expression of beta-catenin and CAT-Tg and CAT-KO T cells we have observed that beta-catenin regulates cytokine production as well as apoptosis and proliferation in activated T cells. In the future, we will delineate the molecular role for beta-catenin in TCR signaling using T cells expressing activate beta-catenin or lacking expression of this gene.

4. The molecular mechanisms involved in age-dependent thymic involution and aging. We have demonstrated that expression of beta-catenin results in accelerated age-dependent thymic involution and aging in CAT-Tg mice. Future efforts are aimed at understanding the molecular and cellular basis for accelerated thymic involution and aging in CAT-Tg and wild type mice.

Collaborators:
Dr. Marian Waterman (UC Irvine); Dr. Zhou Zhu (Johns Hopkins UNiversity School of Medicine); Dr. Arya Biragyn (National Institute on Aging; Laboratory of Immunology).



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Keywords:

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APC targeting
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immunotherapy
Tregs
DNA vaccines

Recent Publications:

Biragyn A, et al. *J Leukoc Biol* 2008; 83: 998-1008.

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Baatar D, et al. *J Immunol* 2007; 179: 1996-2004.

Biragyn A, et al. *J Immunol* 2007; 179: 1381-1388.

Dell'Agnola C, et al. *Expert Rev Vaccines* 2007; 6: 267-283.

Vaccine Development and control of tumor-mediated immunosuppression: The need for potent vaccines specifically designed for the elderly continues to be a high priority. Aging is often associated with a significant loss and alteration of immune responses to vaccines. In addition, the vaccines for elderly should be simple and least invasive. Thus, the focus of this laboratory is to study molecular mechanisms of immune responses for development of simple immunotherapeutic strategies for cancer and chronic diseases. Following projects are currently studied in the laboratory: 1) Development of cancer vaccines which target/deliver antigens to various subsets of antigen presenting cells (APCs), specifically immature DCs; 2) Development of novel formulations for preferential depletion or modulation of "bad" cells to enhance anticancer responses; and 3) To understand factors that control cancer metastasis and escape from immunosurveillance.

1. Chemoattractants as Vaccine Adjuvants. The laboratory develops various strategies that specifically control intricate interactions of immune cells through the use of chemokines. Chemokines are small peptides that regulate trafficking and activity of immune cells; and as such they can be used to modulate unique immune responses at will. As we reported in several our papers (Biragyn et al. 1999; 2001; 2002, 2004; 2007; Schiavo et al., 2006), chemoattractants are indeed potent inducers of antitumor immunity against variety of tumors in mice by targeted delivery of TAAs to chemokine receptors on APCs. Based on these results, we have

developed several chemokine-based vaccines, including one that target an embryonic antigen OFA-iLRP (a highly conserved the 37-kDa oncofetal immature laminin receptor that is specifically and highly expressed in a number of human malignancies). The vaccine is simple and it does not require the use of any adjuvants (Biragyn et al., 2007). The breadth of the chemokine-based vaccines is in their ability to efficiently utilize the MHC processing pathways to elicit CD4+ T helper and CD8+ T cell responses. Fusions with chemokines lead to significant augmentation of humoral immune responses to self- and foreign antigens, including beta-amyloid, HBsAg and Env of HIV-1. Modeling experiments with HBsAg-chemokine particles or chemokine-beta-Ab are being conducted to develop vaccines for the elderly and immunosuppressed subjects. Novel chemoattractant and antimicrobial peptide- based carriers that target and also activate APCs are currently being explored. For example, the immunological features of the TLR-4 activating antimicrobial peptide, murine b-defensin 2, are being examined.

2. Development of chemoattractant-based strategies that preferentially deplete or modulate "bad" cells. This topic is based on our recent observation that antigens can be efficiently delivered to the cytosol of cells, if they are fused/linked with chemokines. We have hypothesized that chemokines can be also utilized for delivering various inert moieties (such as toxins and RNases). Since chemokine receptors are differentially expressed on immune cells (and even by tumors), this strategy would allow us to specifically kill or regulate "bad" cells, such as tumors and suppressive immune cells. To do this, we have fused chemokines with human pancreatic RNase eosinophil-derived neurotoxin (EDN, a member of the pancreatic RNase superfamily); or with a truncated form of Pseudomonas exotoxin (PE38). The resulting formulations (chemotoxin) is able to efficiently deplete (both *in vitro* and *in vivo*) regulatory T cells (Tregs) acting via CCR4. In fact, utilizing this tool, we have recently demonstrated that CCR4 is a key chemokine receptor that regulates trafficking of a majority of so-called natural Tregs from human peripheral blood (Baatar et al., 2007a). We speculate that this technology will allow us to control immune responses during vaccinations through depletion of specific subsets of immune cells, such as Tregs. On the other hand, we have also demonstrated that chemotoxin can be efficiently used to combat leukemia in mice [where expression of CCR4 is often associated with a bad outcome of the disease] (Baatar et al., 2007b), indicating that it can be an effective treatment modality for human T cell lymphoma/leukemia.

3. To understand factors that control escape from immunosurveillance and cancer metastasis. Cancer metastasis is often a sign of progressive disease with the bad outcome. It is tempting to speculate that the disease might be efficiently stabilized or even combated by controlling cancer metastasis. However, despite significant efforts, there is little known about mechanisms of metastatic spread of cancer cells. In particular, the role of chemokine receptors in metastasis remains debatable due to difficulties of their detection on the surface of tumor cells. Here, we have hypothesized that breast cancer cells adopt and utilize an existing migratory pathway of immune cells to home into inflamed lungs. Experimenting with a highly metastatic breast cancer 4T1 model, we have demonstrated that their lung metastasis indeed depends on expression of CCR4. On the other hand, this inherent capacity to metastasize was not sufficient, as tumor cells required a help from CCR4-expressing Tregs to be protected from cytolytic responses of NK cells. These data also allowed us to successfully explore ways to control lung metastasis by targeting CCR4+ cells.

These data also indicate the important role of Tregs in regulation of antitumor responses. Despite significant efforts, little is known about mechanisms of suppressive activity exerted by Tregs. We have demonstrated that human PBL Tregs consist of at least two distinct subsets, memory-type CCR4+Tregs and naïve-type CCR4- Tregs (Baatar et al., 2007a). While freshly isolated CCR4+Tregs represent natural Tregs and appear to be primed to readily suppress T cell proliferation, CCR4-Tregs require TCR-mediated activation to render them fully active. Although Tregs-mediated regulation required cell contact-dependent process, it did not utilize perforin/granzyme-mediated process, a mechanism proposed by others for some Treg subsets. Instead, Tregs regulate T cell proliferation through a cell contact-dependent process involving FasL/Fas signaling. Furthermore, we have demonstrated that Th1-type of polarization and augmentation of antigen-specific T cell responses can be induced by the depletion of CCR4-expressing cells.

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The **Laboratory of Molecular Gerontology (LMG)** investigates processes related to DNA metabolism, such as genomic instability, DNA repair, DNA replication, and transcription. Accumulation of DNA damage in senescence is a major molecular change with aging, and these DNA lesions may eventually inactivate individual genes and lead to cellular dysfunction characteristic of the senescent phenotype. DNA damage is also believed to be a major cause of age-associated diseases, notably cancer. The goal of LMG is to understand the underlying mechanisms involved in DNA damage formation and processing, as well as the changes that take place with aging that render aging cells susceptible to cancer. DNA repair is likely to play a critical role, and we have a special interest in understanding the mechanisms involved in several DNA repair pathways, such as nucleotide excision repair, double strand break repair and base excision repair. We investigate the molecular mechanisms related to DNA repair and genomic instability in normal, senescent and cancer cells. Studies are carried out *in vivo* and *in vitro*, using purified proteins, fractionated cell extracts, intact cells in culture and animal models. We are also interested in the molecular processes that interact with DNA repair. These pathways include transcription, replication, targeted somatic mutation and mitochondrial
functions.

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

The area of oxidative DNA damage and its processing is of particular interest to us because oxidative lesions increase significantly with age in the mammalian gnome. Repair of oxidative DNA base lesions is investigated in whole cells, in mitochondria and in cancer cells. We are also studying the molecular deficiencies in human premature aging disorders using cell biological approaches and biochemistry. These hereditary progeriod disorders serve as model systems to study human aging and age-related diseases, including cancer. In particular, the laboratory studies DNA helicases, ATPases and exonucleases, such as the Werner syndrome, Bloom syndrome and Cockayne syndrome proteins. These enzymes are essential in maintaining genomic instability and we are investigating their function at a biochemical level and their interactions with other proteins. A major goal is to understand the role of these proteins in important DNA metabolic processes and to clarify their role in the normal aging process.

Telomeres are the ends of linear chromosomes. These repetitive DNA sequences shorten with age and telomeric instability has been directly associated with the aging phenotype. We investigate DNA repair pathways in the telomeres and how these premature aging syndrome proteins participate in maintenance of telomere length.

Our general interest in a better understanding of the processes that lead to genomic instability also focuses on the role of DNA polymerases in causing mutation. Recently, a number of new DNA polymerases have been discovered and some of these have low fidelity that can lead to mutation. Somatic hypermutation of antibody genes is a distinct process, which is central to the normal immune response. We are interested in its mechanism and how it relates to DNA repair, and whether it changes with age.

An interesting DNA structure that may arise in certain parts of the genome is the triple helix, which can lead to genomic instability. In addition, these structures can be used to mediate gene targeted DNA damage. We use this approach to introduce site specific inter-strand DNA cross-links and study the repair pathways for these lesions. Such cross links are extremely toxic to the cells and could be formed *in vivo* by aldehydes generated as by-products of normal cellular metabolisms.

We are also 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 they function in aging and premature aging disease. We are interested in the prevalence of genetic polymorphism in genes involved in DNA repair and in the potential relationship to premature aging syndromes and to age associated disease.

Restriction of caloric intake is the only intervention proven to expand both medium and maximum life span. The mechanisms by which calorie restriction modulates life span are still unclear, but decreased oxidative damage is believed to be an important factor. We study calorically restricted rodents with the aim of exploring whether this condition is associated with changes in the formation or repair of oxidative DNA lesions.

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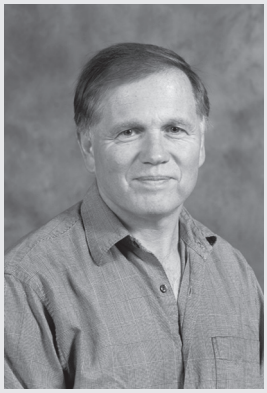

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Biography: Dr. Bohr received his M.D. in 1978, Ph.D. and D.Sc. in 1987 from the University of Copenhagen, Denmark. After training in neurology and infectious diseases at the University Hospital in Copenhagen, Dr. Bohr did a postdoctoral fellowship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

Keywords:

DNA repair
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Werner syndrome
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Recent Publications:

Cheng WH, et al. *Mol Biology Cell* 2008; 19: 3923-3933.

Maynard S, et al. *Stem Cells* 2008; 26: 2266-2274.

Kusumoto R, et al. *Biochemistry* 2008; 47: 7548-7556.

DNA Repair Processes: Many types of chemical modifications can occur in mammalian DNA. They are removed by several specialized DNA repair pathways. One is nucleotide excision repair (NER), which removes and replaces bulky lesions, such as UV-light induced pyrimidine dimers. Another important DNA repair pathway is base excision repair (BER), which removes a large number of non-distorting lesions from DNA, many of which are caused by oxidative modification. Other pathways include mismatch repair, homologous recombination and non-homologous recombination.

Oxidative DNA Damage and Mitochondrial DNA Metabolism: Reactive oxygen species (ROS) are generated in cells as by-products of cellular metabolism or exposure to environmental toxins. One major theory of aging holds that oxidative damage to cellular components, such as proteins, lipids and particularly DNA, accumulates with age, leading to the cellular dysfunction 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.

de Souza-Pinto NC, et al. *DNA Repair (Amst.)* 2008; 7: 1098-1109.

Muftuoglu M, et al. *PLoS ONE* 2008; 3: e1918.

Harrigan JA, et al. *J Biol Chem* 2007; 282: 36403-36411.

Weissman L, et al. *Nucleic Acids Res* 2007; 35: 5545-5555.

Because most reactive oxygen species are generated by the electron transfer reactions that occur in mitochondria, it is of great interest to understand the mechanisms processing oxidative DNA damage in these organelles. It is challenging to understand the mechanisms involved in the mitochondrial DNA repair process. We take several approaches to this, studying DNA repair *in vitro* with mitochondrial extracts and using transgenic animals that are defective in specific DNA repair genes involved in nucleotide excision repair or base excision repair to study the function of these gene products in mtDNA metabolism.

The mitochondrial DNA is not protected by histones and lies in close proximity to the electron transport chain, where most ROS are generated. Oxidative DNA base modifications are abundant in mitochondrial DNA and have been associated with aging and cancer. Although the notion has prevailed for many years that mitochondria cannot repair DNA damage (including the highly mutagenic lesion, 8-oxo-G) studies from our group and elsewhere have established that a number of lesions, including oxidized bases, are efficiently repaired from mitochondrial DNA. We have characterized several enzymes involved in BER in mitochondria, including the two major DNA glycosylases for oxidative DNA damage, OGG1 and NTH1. Recent work from our group showed that OGG1 can be phosphorylated *in vivo* by at least three distinct protein kinases, and that this event modulates its catalytic activity.

While much attention has been focused on the common oxidized base 8-hydroxyguanine (8-oxoG), we have recently demonstrated that other oxidized bases, fapyguanine and fapyadenine, are present in mouse DNA at comparable or higher levels than 8-oxoG. Using mitochondria obtained from mouse models lacking one or more DNA glycosylases, we found that these lesions are efficiently repaired in murine mitochondria and we detected a new DNA glycosylase in mitochondria, NEIL1, which could contribute to the repair of oxidative DNA damage in these organelles. Our studies demonstrated that most BER enzymes are not freely soluble in the mitochondrial matrix, but rather associate with the inner membrane through electrostatic interactions. These results imply that BER may be part of the newly identified nucleoid, a large complex of mtDNA and proteins that associate with the mitochondrial membranes. We have recently demonstrated that human mitochondria possess a mismatch DNA repair pathway. It was previously thought that this pathway only existed in the nucleus.

DNA Repair and Aging: The accumulation of unrepaired damage to DNA contributes to genomic instability and ultimately leads to cellular senescence. DNA repair efficiency may decline in normal human aging. This decline may be subtle and may reflect changes in specific DNA repair pathways. We are studying DNA repair pathways and transcription in normal aging, cells from patients with premature aging (segmental progeroid) disorders or age-associated diseases, aiming to identify which specific repair pathway may be defective. We are particularly interested in DNA repair changes in the aging brain, since the high energy demand of this organ makes it particularly vulnerable to oxidative DNA damage. We recently developed methodology to assess DNA repair activities in specific regions of the mouse brain and found a region-specific age-associated decrease in BER activities, particularly in mitochondria. We have found defective DNA repair of oxidative DNA damage in post mortem samples of brain tissue from AD patients and from patients with severe dementia (mild cognitive deficiency).

Alzheimer's disease (AD) is the leading cause of dementia in the old. Several lines of evidence indicate that AD may be associated with defects in DNA repair. Recent work from other laboratories suggested that cells from Alzheimer's disease patients are defective in the processing of DNA lesions induced by irradiation with fluorescent light. Specifically, we use mouse models of AD to identify the contribution of DNA repair defects in the progression of the disease.

The Baltimore Longitudinal Study of Aging (BLSA) provides a unique collection of biological material to investigate associations of genetic background with age. We are using samples from this cohort to try to identify genetic polymorphisms in DNA repair genes that are associated with shortening/extending life span.

Premature Aging Syndromes: A number of rare mutations and disorders in humans are associated with premature aging. The patients display many signs and symptoms associated with normal aging at much younger ages than the normal population. We are particularly interested in the Cockayne (CS) and Werner (WS) syndromes, which are good model systems for molecular studies of human aging. The WRN gene (mutated in WS), the CSB gene (mutated in CS), and other genes mutated in premature aging syndromes encode putative helicases. Therefore, further characterization

of the molecular defects in these disorders is a high and achievable priority in the understanding of normal aging. The functions of the CSB protein and of the WRN protein appear to be at the crossroads of aging, DNA transcription, replication, and repair, thereby nicely affording a combination of our interest in DNA function with our interest in aging. We have also been able to assess DNA repair in individual neurons.

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

The molecular basis of genomic instability in WS remains to be defined. Our laboratory is using several approaches to identify and characterize the molecular defect in WS cells. One approach is to compare the DNA metabolic activities of WS and normal cells. 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. Because of the non-isogenic nature of cells from WS patients compared to cells from normal individuals we generated two isogenic cells lines in which one line has a stable knockdown of the WRN protein. Using these lines we showed that WRN is involved in telomere maintenance, in a process that requires both catalytic activities (helicase and endonuclease) of the WRN protein. These observations reconcile the telomere shortening seen in WS cells.

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, and our studies indicate that WS cells are defective in two specific DNA repair pathways – base excision repair and recombination.

The gene defective in WS, the WRN gene, encodes for a member of the RecQ helicase family. Helicases play roles in a number of DNA related processes: transcription, replication, and DNA repair and chromatin structural organization. We have purified the WRN protein for use in a number of basic and complex biochemical assays. The protein has helicase and exonuclease catalytic activities. It interacts with replication protein A (RPA), both physically and functionally. RPA enhances the helicase activity when unwinding larger DNA duplex structures. WRN protein interacts with the Ku heterodimer, which stimulate its exonuclease activity, and this suggests that WRN may be involved in non-homologous end-joining, the pathway in which Ku exerts its main function. WRN also interacts with p53, possibly in the pathway of apoptosis, since WS cells have attenuated apoptosis. Further, we have discovered that WRN protein interacts functionally and physically with Flap endonuclease 1 (FEN-1), a protein involved in DNA replication and DNA base excision repair. This suggests that WRN protein plays a role in one or both of those processes. Recently, we have found more functional protein partners of WRN, further supporting that it has a role in DNA repair and recombination.

Several protein partners of the WRN protein are involved in BER, such as AP-endonuclease 1, polymerase β (pol β), PARP and FEN-1. These interactions suggest that WRN could play an auxiliary role in BER. We are testing this hypothesis and find that WRN stimulates pol β strand displacement activity and promotes long patch BER. In agreement with such role of the WRN protein, WS cells accumulate oxidative damage in their DNA, indicative of defective BER. Because of the role of WRN in telomere maintenance, we are also studying whether WRN's role in BER is particularly relevant in removing oxidative damage from telomeric sequences.

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

Cockayne Syndrome (CS): Cockayne syndrome is a rare human disease characterized by arrested post-natal growth, 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 sensitive to killing by ultraviolet radiation, as well as certain so-called UV-mimetic chemicals, such as 4-nitroquinoline-1-oxide and Nacetoxy-2-acetylaminofluorene. CS cells are defective in the enhanced rate of repair of the transcribed strand relative to the non-transcribed strand of transcriptional active genes. In recent experiments from this laboratory, we have demonstrated that mutations in the CSB gene are the cause of the transcription coupled repair defect.

The complex clinical phenotype of CS, however, suggests that DNA repair may not be the only defect. Moreover, recent evidence from our laboratory demonstrated that CSB cells are defective in RNA polymerase II (Pol II) transcription. Studies of transcription *in vitro*, in a plasmid-based system, demonstrate a significant transcription defect in CSB cells. We have generated stable human cell lines with functional domain knockout of different regions of the CSB gene. Mutations are introduced by site directed mutagenesis, in various motifs in the ATPase or helicase domain of the gene. The phenotypical alterations caused by these mutations are then examined and studies are also carried out using cell extracts from these cell lines. Further, the wild type CSB and mutated recombinant proteins are made from baculovirus constructs and studied biochemically. Mutations in the ATPase domain do not appear to affect the potential for oxidative DNA damage repair whereas certain mutations in the helicase domain markedly affect the capacity for DNA repair of oxidative DNA base lesions. Moreover, CSB interacts with and is polyribosylated by PARP-1 after oxidative stress. These results demonstrate that the CSB protein plays a role in base excision repair of oxidative DNA damage. Thus, this protein has several roles in DNA metabolism; it is involved in transcription, DNA repair, apoptosis and chromatin assembly.

Although CSB has putative helicase motifs, thus far only ATPase activity of the recombinant protein had been identified. Our recent studies identified novel enzymatic activities for CSB, single-strand DNA annealing and exchange activities. These enzymatic activities may have novel and important functions in transcription coupled repair and recombination. The function of the CSB protein is also investigated with microarray studies of gene expression. Here we find that several genes are under expressed in mutated CS cells, and that some of these confirm a substantial role for CSB protein in transcription and apoptosis.

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Keywords:

immunoglobulin
somatic hypermutation
switch recombination
DNA polymerases
mismatch repair

Recent Publications:

Martomo SA, et al. *DNA Repair* 2008; 7: 1603-1608.

Heltemes-Harris LM, et al. *Mol Immunol* 2008; 45: 1799-1806.

Alrefai RH, et al. *Mol Immunol* 2007; 44: 2800-2805.

Somatic Hypermutation of Immunoglobulin Genes: Somatic hypermutation occurs at a frequency that is a million times greater than mutation in other genes. Mutations occur in variable genes to increase antibody affinity, and in switch regions before constant genes to cause switching from IgM to IgG. Hypermutation is initiated in activated B cells when the activation-induced deaminase (AID) protein deaminates cytosine in DNA to uracil. Uracils are then processed to produce mutations by proteins in the base excision repair and mismatch repair pathways. We have studied the mechanism by examining mice that are deficient for the proteins, and by analyzing the biochemical interactions between the relevant proteins. Intriguingly, the hypermutation machinery has hijacked certain proteins from the mismatch repair pathway to create mutations, rather than to repair them.

Mismatch Repair Proteins: We first studied the role of the following mismatch repair proteins: MSH2, MSH3, MSH6, PMS2, and MLH1, since they would recognize mismatches. To see which of these are involved in hypermutation and class switch recombination, we and others have examined knockout mice. All of the repair-deficient mice had lower frequencies of mutation than their wild type counterparts, which may be due to direct (on hypermutation) or indirect (cells may divide slower) effects on the B cells. Most strikingly, two of the proteins, MSH2 and MSH6, were responsible for mutations of A: T base pairs (bp). The number of mutations at A: T bp and G: C bp is roughly equal in normal mice and in mice deficient for MSH3, PMS2, and MLH1 whereas mutations of A: T drop precipitously in mice deficient for MSH2 and MSH6. Thus, MSH2-MSH6 binds to a single mismatch, and recruits

exonuclease 1 to create a gap. The gap can then be filled in by a low fidelity DNA polymerase. The endonuclease that is needed to generate an initial nick for the exonuclease to act on is not known. However, for class switching, with the exception of MSH3, most of the mismatch repair proteins are involved, since we and others showed that mice deficient for MSH2, MSH6, PMS2, and MLH1 had less class switching. Therefore, unlike hypermutation, intact mismatch repair participates in resolving recombinational intermediates at the switch junctions.

Error-prone DNA Polymerases: If uracil remains in the DNA, it could be replicated by high fidelity DNA polymerases (pol), such as pols delta and epsilon, to produce C: G to T: A transitions. By convention, mutations are recorded from the nontranscribed strand, so equal frequencies of C and G transitions means that uracils are generated on both DNA strands. Low-fidelity polymerases must then generate the C: G transversions and A: T mutations that are abundant in immunoglobulin genes. We and others have studied the role of three polymerases in this process, using knockout mice. We first reported that pol eta is an A: T mutator after observing that the frequency of mutations of A and T dropped 4-fold in variable genes from patients with xeroderma pigmentosum disease, who lack pol eta. The frequency of mutations of G and C increased, so that the overall frequency of mutation was not changed compared to normal people, but the spectra were altered. This work was confirmed in mice deficient for pol eta, where the frequency of A: T mutations were also diminished in non-coding regions around V genes. An analysis of the switch regions in humans and mice deficient for the polymerase showed that pol eta synthesizes mutations of A and T there as well. We have also examined mice deficient for pols iota and theta and found no significant difference in the spectra or frequency of mutation compared to wild type mice. Since pol eta has such a dominant effect on A: T mutations, it may substitute in the absence of other pols to affect the pattern. We therefore bred mice that were doubly deficient for pol eta and either pol iota or pol theta, and measured mutation in these mice. Mice deficient for both pols eta and iota, and mice deficient for both pols eta and theta, had the same mutation spectra as pol eta-deficient mice—that is, fewer mutations of A and T. These findings confirm the major role of pol eta in somatic hypermutation. Class switch recombination was normal in mice deficient for pols eta, iota, and theta. Thus, these polymerases do not fill in the staggered ends generated during double-strand break repair, even though pol eta is clearly present and generating mutations in this region.

Interaction of MSH2-MSH6 and DNA Polymerase Eta: As noted above, mice deficient for MSH2, MSH6, and DNA pol eta all have the same hypermutation phenotype: fewer mutations of A:T. To see if they operate in the same mutagenic pathway, we tested their interactions biochemically. First, we showed that the MSH2-MSH6 heterodimer binds to a U: G mismatch, which suggests that it can enter the pathway right after deamination of C to U. Second, MSH2 and pol eta physically interact in cells, as shown by an antibody pull-down assay from cell extracts. Third, and most significantly, MSH2-MSH6 stimulated the catalytic activity of pol eta *in vitro*. These data support the hypothesis that these proteins work together during somatic hypermutation to produce mutations downstream of the initial U to fill in a gap created by exonuclease 1. As pol eta synthesizes in the repair patch, mismatches are generated opposite A and T. Repeated cycles of MSH2-MSH6 binding to the mismatches would further stimulate pol eta to make more mutations downstream of the original C deamination.

Targeting of AID to Immunoglobulin Genes: The most profound enigma in hypermutation and perhaps immunology; is how AID is targeted to a very small region of DNA in the immunoglobulin loci. Mutations are found in a 2 kb downstream of the promoter preceding rearranged variable genes and in a 4 kb region downstream of intronic promoters preceding the switch regions for constant genes. Why does mutation start and why does it stop? What co-factors guide AID specifically to these regions? We are currently addressing these questions by determining the role of transcription in bringing the AID protein to these regions, and by looking for proteins that interact with AID. Thus, in addition to its essential catalytic role, AID may play a coordinating, non-catalytic role in the generation of antibody diversity. Interactions of AID with known proteins in the pathway, such as MSH2-MSH6, will support the genetic basis of the mechanism, and lead to future studies on the biochemistry of the interactions. Novel proteins could be tested for function by deletion in cells and mice. There is clearly much to be solved in the targeting and mechanism of

hypermutation.

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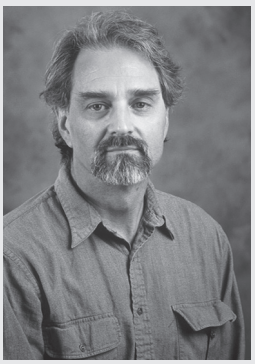

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Biography: Dr. Michael Seidman received his Ph.D. in biochemistry from the University of California, Berkeley, in 1975. He did postdoctoral work at the NIH and at Princeton University. He worked at the NIH and in the biotechnology industry before assuming his present position in the Laboratory of Molecular Gerontology, NIA.

Keywords:

gene targeting
DNA triple helix
DNA repair
crosslink

Recent Publications:

Majumdar A, et al. *J Biol Chem* 2008; 283: 11244-11252.

Thazhathveetil AK, et al. *Bioconj Chem* 2007; 18: 431-437.

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. We are also interested in understanding how one specific form of DNA damage- interstrand cross-links- are repaired.

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 cross-linker), which was designed to form a triplex with a sequence in the well-known cellular housekeeping

gene HPRT (hypoxanthine guanine phosphoribosyl transferase). This gene encodes an enzyme engaged in purine salvage. There is a simple selection procedure for cells, which lack the enzyme; consequently, the gene has become the most commonly used mutation marker gene in mammalian cells. The oligo was introduced in cells in culture and after photoactivation the cells were processed according to a standard mutation selection protocol. Mutations were found at the target site, and sequence analysis demonstrated that the majority were small deletions. This was the first evidence that chromosomal targets are accessible to triplex forming oligonucleotide reagents.

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

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. We have also found that the oligonucleotide–psoralen TFOs can be used to target gene knock in. This approach will be used to modulate genomic sequences via targeted gene knockout or knock in, with application in cell line and strain construction, and gene therapy.

Crosslink repair: We have developed an immunofluorescence based strategy for following crosslink repair in living cells. We synthesized antigen tagged derivatives of psoralen, a well known photoactive DNA cross-linking agent. We incubate cells in the compounds and then micro-irradiate a small region of the nucleus with a pulse laser. Cross-links form only in the irradiated region and can be detected by staining with fluorescent antibodies against the antigen tag. We have used this approach to characterize the crosslink repair competence of cells derived from individuals with DNA repair disorders.

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Biography: Dr. Robert Brosh received his Ph.D. in Biology from the University of North Carolina at Chapel Hill in 1996, his M.S. in Biochemistry from Texas A&M University in 1988, and his B.S. in Chemistry from Bethany College in 1985. He conducted postdoctoral training at the Laboratory of Molecular Genetics (NIA, NIH) and served as an adjunct faculty member at Towson University before assuming his present position at NIA in 2000. Dr. Brosh was appointed to Senior Investigator at NIH in 2006. He serves on editorial boards for several DNA repair and cancer journals, received a grant award from the Fanconi Anemia Research Fund, and is a Mentor for the NIH Summer Undergraduate Research Program.

Keywords:

helicase
genomic instability
DNA repair
replication
RecQ
RECQ1
Werner syndrome
Fanconi anemia

Recent Publications:

Wu Y, et al. *Mol Cell Biol* 2008; 28: 4116-4128.

Bugreev DV et al. *J Biol Chem* 2008; 283: 20231-20242.

Sharma S, et al. *Cell Cycle* 2008; 7: 989-1000.

Sharma S, et al. *PLoS ONE* 2007; e1297.

Sharma S, et al. *Mol Cell Biol* 2007; 27: 1784-1794.

Gupta R, et al. *Blood* 2007; 110: 2390-2398.

Peng M, et al. *EMBO J* 2007; 26: 3238-3249.

Roles of DNA Helicases in Genomic Stability: Helicases are molecular motor proteins that couple the hydrolysis of nucleoside triphosphate to nucleic acid unwinding. Enzymes of this class function coordinately with other proteins as a complex machine and play essential roles in pathways of DNA metabolism that include replication, DNA repair, recombination, transcription, and chromosome segregation. Despite considerable efforts to understand biochemical, structural, and genetic aspects of helicase function, the precise mechanisms by which helicases catalyze strand separation and perform their biological roles remain to be fully understood. The growing number of DNA helicases implicated in human disease suggests that these enzymes have vital specialized roles in cellular pathways important for the maintenance of genome stability.

RecQ Helicases as Caretakers of the Genome: Recent evidence indicates that mutations in genes of the RecQ family of DNA helicases result in chromosomal instability diseases of premature aging and/or cancer predisposition. Currently known, RecQ helicase-deficient disorders include Werner, Bloom, and Rothmund-Thomson syndromes. The WRN gene product, defective in Werner syndrome, is a helicase/exonuclease that presumably functions in DNA metabolism to preserve genome integrity. To understand the DNA structures and cellular pathways that WRN impacts, we have systematically examined the DNA substrate preferences of WRN helicase for unwinding and its interactions with human nuclear proteins. Our biochemical studies indicate that WRN preferentially unwinds DNA replication structures in a defined orientation and utilizes specific DNA structural elements for recognition. A real-time kinetic analysis of WRN helicase activity was used by our group to

characterize the mechanism of DNA unwinding by WRN. Biochemical studies were performed to investigate the mechanism for stimulation of WRN helicase activity by its auxiliary factor RPA. Our results indicate that the physical interaction between RPA and WRN plays a critical role in the functional interaction. To further understand the molecular functions of WRN protein, we have characterized the functional interaction of WRN with human Flap Endonuclease 1 (FEN-1), a structure-specific nuclease implicated in DNA repair, replication, and recombination. Our results indicate that WRN stimulates FEN-1 cleavage of important DNA intermediates by a unique mechanism whereby the efficiency of FEN-1 cleavage is dramatically enhanced. Our most recent work has elucidated a role for WRN in resolving stalled replication forks and recombination intermediates. Our hypothesis is that the aberrant mitotic recombination and genomic instability arises from inappropriate processing of replication/recombination intermediates in Werner syndrome cells. In vivo evidence for a role of WRN in cellular DNA replication was attained using a model genetic system for WRN structure-function studies. We are currently utilizing model systems to further understand the molecular functions and genetic pathways of WRN that are important for the cellular response to replicational stress.

Understanding the Molecular and Cellular Functions of Human RECQ1: Although the biochemical properties and protein interactions of the WRN and BLM helicases have been extensively investigated, less information is available concerning the functions of the other human RecQ helicases. We have focused our attention on human RECQ1, a DNA helicase whose cellular functions remain largely uncharacterized. RECQ1 was found to stably bind a variety of DNA structures, enabling it to unwind a diverse set of DNA substrates. RECQ1 was shown to catalyze efficient strand annealing between complementary single-stranded DNA molecules. To acquire a better understanding of RECQ1 cellular functions, we have investigated its protein interactions. Our results suggest a role of RECQ1 in regulation of genetic recombination by its interaction with mismatch repair factors. Currently, we are utilizing model systems to determine the biological functions of RECQ1. We characterized embryonic fibroblasts from RECQ1 knockout mice as well RECQ1-depleted human cells and discovered that RECQ1 has a unique and important role in the maintenance of chromosomal stability. Ongoing work involves the characterization of the roles and pathways of RECQ1 in the DNA damage response.

Unraveling the Linkage of DNA Helicases to DNA Repair: Our

recent work has focused on the roles of helicases in the DNA damage response. Mutations in the BRCA1-associated helicase BACH1 have been associated with early-onset breast cancer and cellular data suggest a role of the helicase in double strand break repair and checkpoint control. Recently, BACH1 (FANCJ) has been genetically linked to the chromosomal instability disorder Fanconi anemia (FA). To understand the molecular functions and biological substrates that FANCJ helicase acts upon, we have systematically evaluated the ability of purified recombinant FANCJ to unwind a panel of related DNA substrates with distinct tail variations including single-stranded versus double-stranded character, tail length, or backbone continuity. In addition, we have assessed the ability of FANCJ to catalytically unwind DNA structures proposed to be key intermediates of cellular DNA metabolism. Specifically, our recent work demonstrated that FANCJ unwinds G-quadruplex DNA structures in order to defend genomic integrity. The results from biochemical studies provide a platform to investigate the molecular interactions of the FANCJ helicase with its protein partners in double strand break repair by homologous recombination. Ongoing studies of FANCJ helicase explore the roles of the protein in FA-dependent and FA-independent pathways. Currently, we are conducting a structure-function analysis of the FANCJ helicase to determine the importance of clinically relevant mutations genetically linked to FA or associated with breast cancer.

Collaborators: Alex Mazin, Drexel University; Sharon Cantor, Department of Cancer Biology, University of Massachusetts Medical School, Worchester, MA; Alessandro Vindigni, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy; Ian Hickson, Cancer Research UK Laboratories, Oxford, UK; Perry Blackshear, National Institute of Environmental Health Sciences; Vilhelm Bohr, Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD; Michael Seidman, Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD

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Biography: Dr. David Wilson received his Ph.D. in Molecular Biology from Loyola University of Chicago, Stritch School of Medicine, in 1993. He performed his postdoctoral research training at Harvard University School of Public Health. In 1997 he became a Senior Biomedical Scientist at Lawrence Livermore National Laboratory in the Biology and Biotechnology Research Program. While at Livermore, he was also an adjunct Associate Professor in the Radiation Oncology Department at the University of California Cancer Center - Sacramento. Dr. Wilson started his position at NIA in March of 2002.

Keywords:

oxidative DNA damage
base excision repair
single-strand break processing
disease susceptibility and aging
cancer and neurodegeneration
therapeutic responsiveness

Recent Publications:

Kulkarni A, et al. Nucleic Acids Res 2008; 36: 5111-5121.

Lipton AS, et al. J Amer Chem Soc 2008; 130: 9332-9341.

Berquist BR, et al. J Mol Biol 2008; 379: 17-27.

Liu P, et al. Mol Cell Biol 2008; 28: 4975-4987.

Kulkarni A, et al. Amer J Hum Genet 2008; 82: 539-566.

Repair Mechanisms for Oxidative DNA Damage: Reactive oxygen species are formed as by-products of normal aerobic respiration. These metabolic products react with lipids, proteins and nucleic acids, and contribute to aging and age-related disease by promoting the gradual accumulation of macromolecular damage that leads to cellular dysfunction or cell death. Oxidative DNA damage – such as 8-oxoguanine, abasic (AP) sites and single-strand breaks (SSBs) – poses a mutagenic or cytotoxic challenge to the cell. The major system for correcting such damage is base excision repair (BER). The primary biochemical steps of BER are: (1) removal of a modified or inappropriate base, such as 8-oxoguanine, by a DNA glycosylase, (2) cleavage of the phosphodiester backbone at the resulting AP site by an endonuclease or lyase, (3) clean-up of the 3' or 5' terminal end, (4) replacement of the excised nucleotide by a polymerase, and (5) sealing of the final DNA nick by a ligase. The broad objective of my research group is to elucidate the molecular mechanisms of the BER pathway and establish a foundation for understanding the contribution of core and auxiliary BER proteins to disease manifestation and aging.

Using basic molecular and biochemical approaches, I and my collaborators have contributed to defining how specific human BER proteins recognize and process target lesions and/or coordinate with other components of the pathway. This research has centered largely on apurinic/apyrimidinic endonuclease 1 (APE1), the major mammalian protein for repairing abasic sites in DNA, and x-ray cross-complementing 1 (XRCC1), a key non-enzymatic scaffold protein that

Collaborators-continued
McNeill DR, et al. Mol Cancer Res 2007; 5: 61-70.

Wong H-K, et al. Nucleic Acids Res 2007; 35: 4103-4113.

facilitates the efficient execution of SSB repair (SSBR). In addition, we have increased our effort to delineate the role of Cockayne syndrome B (CSB) protein in the processing of endogenous DNA damage. Our studies in recent years have (i) uncovered novel biochemical properties associated with the APE1 repair protein, including its ability to incise at AP sites in certain biologically-relevant DNA configurations; (ii) identified APE1 as a potential target for the genotoxic and co-carcinogenic effects of lead, an important environmental toxin; (iii) established a novel dominant-negative form of APE1 that has potential utility in gene-therapy paradigms; (iv) described the biological significance of specific interactions of XRCC1 (e.g. with POLβ); (v) reported an interaction of XRCC1 with the replication/repair protein, PCNA, establishing a novel link between the DNA repair machinery and replication factories; (vi) determined the major biochemical repair defect(s) associated with XRCC1 deficiency in mammalian cells; (vii) characterized the repair capacity and genetic instability of human cells deficient for XRCC1 function using transient RNAi knockdown strategies; and (viii) demonstrated that CSB has a physical and functional interaction with APE1.

As part of our ongoing effort to delineate the molecular mechanisms of BER and SSBR, we have several central projects in the works. First, APE1 is the major mammalian enzyme responsible for the repair of abasic sites in DNA, yet has functions in SSBR and other cellular processes, including transcriptional regulation. We are in the process of establishing stable shRNA knockdown cell lines to dissect out the precise contribution of each proposed function of APE1 (i.e. its nuclease activity, redox regulatory role, etc.) to cell growth/viability and protection against DNA-damaging agents. Second, the contributions of XRCC1 to DNA damage responses, genomic stability and telomere maintenance are being evaluated in defined human cell lines using chronic shRNA knockdown strategies. Last, CS is a rare, autosomal recessive disorder characterized by poor growth, impaired development of the nervous system, cutaneous photosensitivity and premature aging, but no increased cancer incidence. The CSB protein harbors seven helicase-like ATPase motifs found within the SWI2/SNF2 superfamily of chromatin remodeling proteins, and interacts with a number of BER protein factors. We are presently examining the *in vitro* activities of CSB on key DNA and RNA transaction intermediates, and will elucidate the contributions of the unique N- and C-terminal portions of the protein that likely impart functional specificity.

BER in Cancer Susceptibility and Treatment: Recent studies indicate that significant inter-individual variation exists at the amino acid sequence level of BER proteins, and that variability presumably exists in the BER capacity of the general population. We have developed assays to determine the extent of inter-individual variation at specific steps of BER, and will elucidate whether variation in BER correlates with disease susceptibility and/or clinical agent responsiveness. Using these assays, we will also assess for age-dependent or gender-specific variation. The establishment of such techniques, and ultimately a high throughput BER pathway assay presently in design, will be necessary to evaluate the relationship of BER capacity to disease susceptibility among the Baltimore Longitudinal Study of Aging (BLSA) population.

Current strategies to eradicate cancer rely on the fact that malignant cells divide rapidly. Thus, to induce cell death, many anti-cancer agents interact with DNA to block replication and prevent tumor growth. Not surprisingly, cells with efficient repair of the cytotoxic DNA intermediates generated by anti-cancer agents are more resistant to cell killing. Hence, a goal has been to regulate strategically the repair capacity of cancer and/or normal cells to improve the efficacy of specific therapeutic paradigms. In particular, inhibiting the DNA repair capacity of cancerous cells has been an area of promising focus. We have identified a mutant form of the human APE1 protein – which we have termed ED – that enhances cellular sensitivity to a broad range of clinical alkylating agents and nucleoside analogues. We are presently designing adenoviral-based gene delivery systems to determine the effects of ED on modulating cancer cell survival. In addition, we are screening for small molecule chemical inhibitors of APE1, with the long term goal of creating high affinity inhibitors with potential clinical value. The establishment of adenoviral and complementary techniques will provide a foundation for more extensive investigations on the benefits of regulating cellular DNA repair capacity.

BER in Premature Aging and Neurodegeneration: The accumulation of endogenous DNA damage is thought to promote aging and a number of age-associated diseases, including neurodegeneration. An objective of my laboratory is to expand our understanding of the involvement of BER-related processes in the development of neurological dysfunction. As noted above, CS is a rare autosomal recessive disorder, characterized by growth defects, premature aging symptoms, and neurological

abnormalities. CS is divided into two complementation groups: CSA (mutation in CKN1) and CSB (mutation in ERCC6). Emerging evidence indicates a role for CSB in facilitating the BER response. For example, my laboratory, in collaboration with Dr. Vilhelm Bohr's group, recently identified a novel physical and functional interaction between CSB and APE1. However, the precise molecular contributions of CSB to the BER process remain poorly defined. We are currently determining the molecular functions of CSB in BER and how the protein's activities potentially contribute to DNA damage responses in disease manifestation.

XRCC1 is a scaffold protein that operates to coordinate SSBR, which is a sub-pathway of BER critical for repairing oxidative DNA strand breaks. Functional interactions with Aprataxin and TDP1 – proteins that are defective in hereditary spinocerebellar ataxias and that process complex SSB ends – imply a connection between XRCC1 (and SSBR in general) and neurodegenerative disease. Current data from our group and others implicate XRCC1, and more broadly SSBR, in the protection of non-dividing neuronal cells from the genotoxic consequences of oxidative stress. We are assessing the role of this protein in age-related pathologies using heterozygous mice, and will concomitantly evaluate the effect of XRCC1 haploinsufficiency on neurodegeneration and cancer proneness following defined insults.

Collaborators: Christopher P. Austin (MD) National Human Genome Research Institute, Rockville, MD; Vilhelm A. Bohr (MD, PhD) National Institute on Aging, Baltimore, MD; P. J. Brooks (PhD) National Institute on Alcohol Abuse and Alcoholism, Rockville, MD; Yung-Chi Cheng (PhD) Department of Pharmacology, Yale University, New Haven, CT; Bruce Demple (PhD) Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA; Ted L. DeWeese (MD) Department of Radiation Oncology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD: Paul Ellis (PhD) The Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA; Mark R. Kelley (PhD) Department of Pediatrics, Indiana School of Medicine, Indianapolis, IN; Mark P. Mattson (PhD) National Institute on Aging, Baltimore, MD; Karine Merienne (PhD) Department of Molecular Pathology, CNRS/INSERM/ULP, Strasbourg, France; Marit Otterlei (PhD) Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway Alice J. Sigurdson (PhD) National Cancer Institute, Bethesda, MD; Anton Simeonov (PhD) National Human Genome Research Institute,

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Biography: Dr. Yie Liu received her Ph.D. at Karolinska Institute, Sweden in 1995. Dr. Liu joined the University of Toronto as a postdoctoral fellow of the National Cancer Institute of Canada until 2001, and then became a Senior Research Scientist at Oak Ridge National Laboratory in 2002. Dr. Liu started at NIA as a tenure track investigator in 2006.

Keywords:

telomere
DNA repair
telomere proteins

Recent Publications

Wu J, et al. *J Cell Biol* 2008; 181: 475-483.

Zou Y, et al. *J Biol Chem* 2008; 283: 5728 –5737.

Giannone R, et al. *Biotechniques* 2007; 43: 296.

Repair and Maintenance of Damaged Telomeres in Mammals

Most eukaryotic chromosome ends terminate in structures of repetitive DNA sequences and associated proteins, called telomeres. Telomeres allow cells to distinguish natural chromosome ends from damaged DNA and to protect chromosomes against degradation and fusion. Telomere integrity in cells thus plays an essential role in controlling genomic stability. Loss of genetic material at chromosome ends ("telomere shortening") is frequently observed in elder populations, cellular senescence, and premature aging syndromes. Furthermore, telomere dysfunction contributes to genomic instability that leads to cell death, cell proliferation defects, and malignant transformation, which might in turn contribute to age related-disorders and a higher incidence of cancer during aging.

Accumulating evidence suggests that telomere integrity depends on the ability to maintain telomere length and/or the ability to mask telomeres from being recognized as damaged DNA. In mammals, telomeres normally exist in a loop structure with 3' single stranded telomeric DNA overhang concealed within the telomere double helix. This t-loop configuration is believed to protect chromosome ends from being recognized as broken DNA. Disruption of the telomere loop and subsequent exposure of the 3' overhang represents the uncapped state of telomeres. Telomerase and telomere associated proteins play essential roles in telomere length maintenance and telomere capping functions. Telomerase replenishes telomere loss due to incomplete DNA replication in almost all eukaryotes. Loss of telomerase activity leads to attrition of telomeric DNA, which, in turn, is known to trigger chromosome end-to-end fusions, genomic

instability, and cell arrest or death. Recent studies have shown that uncapped telomeres directly associate with many DNA damage response proteins in telomere-initiated cellular senescence, indicating that DNA damage repair proteins interact with telomere as a DNA damage check-point response during cellular senescence. Several DNA damage repair proteins are also critical in protecting telomere and chromosome integrity.

Oxidative DNA damage has been implicated in the etiology of aging and cancer and has been proposed to result in telomere attrition in aging. This hypothesis has not been experimental verified. Furthermore, it is unknown what types of oxidative DNA damage arise at telomeres in the course of aging and through what mechanism(s) they compromise telomere integrity. Oxidation induces a variety of lesions in DNA and oxidative base lesions are among the most common oxidative DNA damage and their repair occurs primarily via base excision repair pathway, initiated with their excision by DNA glycosylases. Our research focuses on elucidating the impact of oxidized base lesions and the role of DNA glycosylases at telomeres *in vivo*, using DNA glycosylase deficient model organisms. These genetic models are rich in certain spectrum of purine or pyrimidine lesions in dividing or non-dividing tissues, G or C strand telomere DNA, and single or double stranded DNA and thus allow us to examine unique base lesions. Dr. Liu's laboratory currently focuses on elucidating the impact of the most common form of oxidative DNA damage; oxidize purines and the role of its repair enzyme, OGG1 DNA glycosylase at telomeres using different model organisms. Her group found that oxidized guanine lesions accumulated at telomeres in *Ogg1* deficient yeast and mammalian cells. These base lesions led to changes in telomere length in both yeast and mouse cells. They also discovered the possible mechanisms of oxidized purines in compromising telomere length through SSBs, DNA replication defect, and *cis*-mechanism (telomere binding protein acts in cis at individual chromosome ends to control recruitment or access to telomerase).

Collaborations: Proteomics core and collaborative mouse cross core facility, Oak Ridge National Laboratory, Dr. Laura Haneline, University of Indiana, Dr. Hidetoshi Tahara, University Kausmi, Japan,. Dr. Kyungjae Myung, NCI.

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The **Laboratory of Neurogenetics (LNG)** is structured to be an integrated research laboratory working toward an understanding of the molecular basis of neurological diseases. The three primary research groups are the Molecular Genetics Section (Chief, Andrew Singleton), the Cell Biology and Gene Expression Unit (Chief, Mark Cookson) and the Transgenics Unit (Chief, Huaibin Cai); in addition to these groups the LNG contains a Computational Biology Core (Headed by Jaime Duckworth) and 2008 saw the creation of a Genomic Technologies Group (Headed by Dena Hernandez) that is in part the laboratories move to include a systems based approach in our research program.

The central aim of the LNG is to understand the molecular etiology and pathogenesis of neurological disorders using genetics; defining genetic variability that causes or contributes to neurological disease, understanding the role of normal genetic variation in gene expression, modeling risk/causative variants in cell and animal models and coming up with testable therapeutic points of intervention. The primary disease focus in the LNG is Parkinson's disease, but we also have active research programs in amyotrophic lateral sclerosis, dementia, dystonia, ataxia and stroke. Major research projects include:

Defining the genetic cause of diseases in patients with familial forms of neurological disorders; this work has primarily involved family based locus identification using high-density SNP based approaches

Defining genetic risk loci in Parkinson's disease, Alzheimer's disease, stroke and amyotrophic lateral sclerosis. This work has primarily involved genome wide association studies and candidate gene association studies.

Understanding the extent and effects of normal genomic variability in human populations and tissues. This work has involved characterization of genomic variability in diverse human populations and large-scale correlative analysis of the effects of genetic variation on gene expression in the human brain.

Modeling Parkinson's disease in cell-based systems; primarily focusing on the pathological consequences of LRRK2 mutation and PINK1 mutation in addition to elucidating the normal function of these proteins

Modeling Parkinson's disease and amyotrophic lateral sclerosis in transgenic animals; this work has focused on understanding the role of SNCA and LRRK2 mutation in Parkinson's disease in addition to the effects of p150glued mutations on ALS.

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Sampath Arepalli	Biologist

Neuromuscular Diseases Research Group

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Jennifer Schymick	Predoc IRTA Fellow
Yevgeniya Abramzon	Student IRTA
Shiao-Lin Lai	Special Volunteer

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J. Raphael Gibbs	Computer Info Scientist
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Xian Lin	Visiting Fellow
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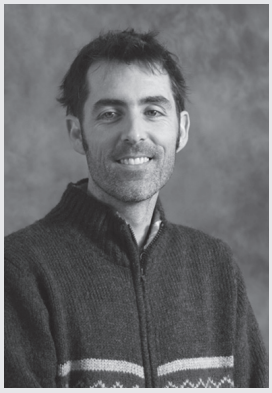

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Biography: Dr. Andrew Singleton is a human geneticist whose research interests focus on the genetics of neurological disease. Dr. Singleton received his B.Sc. (Hons) degree from the University of Sunderland, UK and his Ph.D. from the University of Newcastle upon Tyne, UK where he studied genetic causes and contributors to dementia. Dr. Singleton performed his postdoctoral training at the Mayo Clinic in Jacksonville, Florida, studying the genetic basis of neurological diseases such as dystonia, ataxia, essential tremor, stroke and Parkinson's disease. In 2001 he joined the NIA as an Investigator within the newly created Laboratory of Neurogenetics; in 2007 he became a Senior Investigator at NIA. Dr. Singleton's group investigates the genetic mechanisms underlying monogenic and complex neurological diseases.

Keywords:

neurogenetics
Parkinson's disease
parkinsonism
amyotrophic lateral sclerosis
ataxia
dystonia
stroke
dementia

Recent Publications:

Jakobsson M, et al. *Nature* 2008; 451: 998-1003.

Camargos S, et al. *Lancet Neurology* 2008; 7: 207-215.

Houlden H, et al. *Nature Genetics* 2007; 39: 1434-1436.

van de Leemput J, et al. *PLoS Genetics* 2007; 3: e108.

In recent years, an extremely successful approach to understanding disease has arisen from the study of rare familial forms of disorders related to more common "sporadic" disease. This is a research paradigm that was successful in Alzheimer's disease (AD). The identification of the APP, PS-1 and PS-2 mutations as causal of rare forms of early-onset familial AD led to a huge increase in our knowledge of the pathogenic mechanisms underlying the common late-onset form of AD. In the past we have successfully employed this approach to identify triplication of the SNCA locus and LRRK2 mutations as causes of Parkinson's disease (PD). The identification of the SNCA triplication showed simply increased expression of this gene and its cognate protein can lead to a Lewy body disease ranging from PD through to dementia with Lewy bodies. These data implicitly suggest the potential of a-synuclein lowering therapies in treating these diseases and shed light on the molecular mechanism of Lewy body diseases. The identification of LRRK2 mutations and subsequent work by us and others has shown simply that mutation of this gene is the most common known cause of Parkinson's disease, underlying disease in approximately 2% of all PD cases in North America. More recently we have applied novel techniques to allow rapid mapping and identification of genetic lesions underlying neurological disorders; this has lead to the discovery of several disease loci and gene mutations within our laboratory; most notably this includes the genetic cause of spinocerebellar ataxia 15 (SCA15), SCA11, SCA20, dystonia-parkinsonism 16 and parkinsonism-dystonia 14 (PARK14).

We have also begun work on the dissection of the etiologies of non-mendelian neurodegenerative diseases in general; however, the problems of identifying risk factor loci for diseases with complex modes of inheritance and in particular oligogenic (10 genes) and polygenic (>10 genes) disease are formidable. Given the huge socio-economic impact of some of the disorders of this nature such as Parkinson's disease and Alzheimer's disease, it is of paramount importance to design a viable strategy for the delineation of genetic predisposition in complex traits. We have approached this problem in several ways; first, as outlined above by studying rare familial forms of disease and then extrapolating the function of genes involved to related conditions. Second, using the Illumina technology, we have performed genome wide association studies in Parkionson's disease, stroke and amyotrophic lateral sclerosis. This work, which is ongoing, promises to identify risk loci involved in these diseases. In order to catalog and understand the effects of normal genetic variation we have also conducted a series of experiments using high-density SNP genotyping approaches. The first catalogs human genetic variation, including SNPs, haplotypes and copy number variants, in diverse human populations; the second set of experiments has examined the effects of common and normal genetic variation on the transcriptome in the context of the human brain. This work is designed to allow us to move from locus to effect as disease risk variants are detected.

Collaborators: Henry Houlden, University College Medical School, London, UK; Andrew Lees, University College Medical School, London, UK; Michael Okun, University of Florida, Gainesville; Okan Dogu, Mersin University, Turkey; Georgios Hadjigeorgiou, University of Thessaly, Greece; James Meschia, Mayo Clinic Jacksonville; Francisco Cardoso, Universidade Federal de Minas Gerais, Brazil.

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Biography: Dr. Mark R. Cookson is a cell biologist whose research interests include understanding the effects of mutations in the genes associated with neurodegeneration at the cellular and molecular level. Dr. Cookson received both his B.Sc. and Ph.D. degrees from the University of Salford, UK in 1991 and 1995, respectively. His postdoctoral studies included time spent at the Medical Research Council laboratories and at the University of Newcastle, Newcastle, UK. He joined the Mayo Clinic, Jacksonville, Florida, as an Assistant Professor in 2000 and moved to the NIA in February 2002. Within the Laboratory of Neurogenetics, Dr. Cookson's works on Parkinson disease, attempting to understand molecular mechanisms leading to neuronal damage.

Keywords:

Parkinson's disease
neurons
cell culture models

Recent Publications:

van der Brug MP, et al. *Proc Natl Acad Sci U S A* 2008; 105: 10244-10249.

Greggio E, et al. *J Biol Chem* 2008; 283: 16906-16914.

Deng J, et al. *Proc Natl Acad Sci U S A* 2008; 105: 1499-1504.

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Lewis PA, et al. *Biochem Biophys Res Commun* 2007; 357: 668-671.

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In **Parkinson disease (PD)** there are two major pathological hallmarks. First, there is a striking, although not entirely selective, loss of dopaminergic neurons in the substantia nigra. Second, there are deposits within the neurons that do survive called Lewy bodies, which are composed of the protein α-synuclein. Although most cases of Parkinson disease are sporadic, several rare forms have been found that are inherited in a Mendelian fashion. The challenge is to interpret what each of these genes tells us about the different pathological components of PD. This is not only intellectually challenging but may, one day, be used to underpin new treatments for PD and related disorders.

Five causal genes have been identified, which can be divided into recessive and dominant genes. The recessive genes are parkin, DJ-1 and PINK1. All of these are associated with parkinsonism and nigral cell loss and mutations are loss of function mutations. For DJ-1 and PINK1 this can be shown by the fact that some mutations dramatically destabilize the proteins. This leads to the hypothesis that these three genes are all neuroprotective and mutations cause a loss of this beneficial function. In our laboratory, we have largely focused on how mutations in DJ-1 affect cellular responses to oxidative stress. We have shown that a specific cysteine residue (C106 in human DJ-1) acts as a sensor for oxidative stress in the cells. We have also found that DJ-1 can bind RNA in an oxidative-stress responsive manner, suggesting that this single activity underlies the apparent involvement of DJ-1 in many cellular functions. However,

identification of a large number of mitochondrial RNA species suggests a preferential role for DJ-1 in controlling responses for this organelle, which is also a major source of free radicals in the cell. This potentially links DJ-1 with two other proteins involved in recessive parkinsonism, PINK1 and parkin, which have recently been shown to control mitochondrial morphology. Collectively, our results support a single underlying pathway for recessive parkinsonism that relates to how mitochondria respond to damage induced by oxidative stress, which might be one way to limit damage to neurons in these inherited disease and, perhaps, in PD more generally.

The two dominant genes, α-synuclein and LRRK2/dardarin, are linked by the observation that most cases with either mutation have PD-like symptoms and Lewy bodies. Most of our work in this area is currently focused on LRRK2, where we are exploring how each of the domains of this large, multifunctional protein, contributes to neuronal toxicity and protein deposition, mainly using cellular models. We have shown that LRRK2 is active as both a kinase and a GTPase. We have also shown that there are two different types of mutations; some increase kinase activity and some decrease GTPase activity. The two activities appear to be linked to each other through the formation of a dimer of LRRK2 and we are working actively on the structural basis of this observation. Furthermore, we reported that if we inactivate the kinase portion of the molecule its toxic effects are greatly decreased. This leads to the idea that we might be able to find small molecule kinase inhibitors, initially to test the hypothesis that kinase activity is required but also to provide a starting point for the development of novel therapeutic agents.

Collaborators: Rina Bandyopadhyay, University College, London, UK; Huaibin Cai, Laboratory of Neurogenetics, NIA, NIH; Junpeng Deng, Oklahoma State University; Myriam Gorospe, Laboratory of Cellular and Molecular Biology NIA, NIH; David Park, University of Ottawa, Canada; Gregory Petsko, Brandeis University; Andrew Singleton, Laboratory of Neurogenetics, NIA, NIH; Mark A Wilson, University of Nebraska, Lincoln; Benjamin Wolozin, Boston University.

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Biography: Jaime Duckworth is a computational biologist whose research interests focus on the application of informatics to biology and medicine. Jaime received her B.S. in Biology (minor Chemistry) with the highest distinction from Purdue University, Indiana, her B.S. in Electrical Engineering from Northern Jiao-Tong University and M.S. in Computer Engineering from the Chinese Academy of Science in Beijing. Before she became the facility head of the Computational Biology Section in the Laboratory of Neurogenetics in 2001, she was the appointed liaison between Bioinformatics Science and Engineering, responsible for the Scientific Computing in the Bioinformatics Department of GlaxoSmithKline Pharmaceutical Research and Development.

Keywords:

data integration
alternatively spliced
polymorphism
comparative analysis
structure homology modeling

Recent Publications:

Zody MC, et al. *Nat Genet* 2008; 40: 1076-1083.

Van der Brug MP, et al. *Proc Natl Acad Sci* 2008; 105: 10244-10249.

Greggio E, et al. *J Biol Chem* 2008; 283: 16906-16914.

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The **Computational Biology Facility** provides Bioinformatics Support for all research sections including the genotyping facility in the Laboratory of Neurogenetics and their collaborators. We act as translators and integrators between experimental science and digital technology. We integrate vast amounts of dynamic data from all sources such as sequence, genomic, genetic and proteomic data from the National Center for Biotechnology Information, NIH, Ensembl, EBI, and our own laboratory as well as scientific journals/literatures. We predict protein **structure** by **homology modeling** for unknown proteins. When the structure has been solved at high resolution, we try to identify small molecule interactors for the protein. We apply the most advanced bioinformatics tools to the data analysis, before we present our interpretation and hopefully a few workable leads to the bench scientists for further investigations. We help our lab researchers visualize multi-facet data and assist them in evaluating each line of evidence computationally. By doing so, we wish to expedite labor-intensive laboratory data analysis and provide ideas for good experimental designs, project prioritizations and management. The integrative and multi-species **comparative analysis** has shown promising leads in finding functional elements—coding or non-coding regulatory regions—among the genes closely examined by our laboratory such as DJ-1, a Synuclein and Tau genes as well as their **alternatively spliced forms** and **polymorphisms**.

In addition to Bioinformatics Support, our group has also been developing tools and interfaces to help the laboratory to digitalize biological data. Our intranet gives a centralized portal for browsing through internal data and yet having convenient links to external information. In an effort to eliminate duplicated patient data entry,

automate the genetic analysis pipeline and facilitate data mining for factors influencing longevity, health and age-associated disease, our group has been working closely with our clinical team and lab scientists in designing and developing an integrative system for Clinical Genetic Research and Analysis. This system will have the capacity of LIMS (Laboratory Information Management System) to handle large amounts of high-throughput genetic data with accuracy and convenience. It manages data flow, storage and retrieval in various aspects of clinical and genetic research on families and populations with Clinical Data Acquisition and Mining, Laboratory Sample Tracking, and Genetic Data Acquisition and Mining modules. It places special attention on extensibility, security, portability and ease of use. It aims to eliminate unnecessary paper medical records, sample mix-ups, heterogeneous data formats for genotyping, linkage/association and other downstream analysis. Through the reduction of these common inconveniences, the system can significantly increase research productivity, efficiency, effectiveness and robustness for large scale familial and association studies. Moreover, we expect the system to have the power, utility and accessibility as well as confinement over other conventional products available through the Internet. Its data organization and management facilities help researchers explore and discover both the genetic and environmental factors in determining normal and abnormal aging, by examining patient medical/family histories and cross group or population demographics. Meanwhile, its modularity, along with multi-level security, ensures the coherent **data integration** of sequences, genomics, proteomics and literature, without sacrificing the confidentiality of patient/laboratory data and the compliance of clinical research to the standard set by NIH.

Collaborators: Pankaj Agarwal, Computational Sciences and Biological Pathways, GlaxoSmithKline; Karen Kabnick, GlaxoSmithKline; Judith Rapoport, National Institute of Mental Health, NIH; Wesley Warren, Washington University School of Medicine; Rohan de Silva, Reta Lila Weston Institute of Neurological Studies, University College London; colleagues associated with the Laboratory of Neurogenetics, NIA.

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Biography: Dr. Huaibin Cai received his B.S. in Biology in 1991 from Peking University, Beijing, China and his Ph.D. in Neuroscience in 1999 from the Johns Hopkins University School of Medicine. He performed his postdoctoral training in the Division of Neuropathology, Department of Pathology at the Johns Hopkins University School of Medicine in Baltimore, Maryland. He joined the NIA Laboratory of Neurogenetics in 2003 as an Investigator in the Transgenesis Unit.

Keywords:

ALS
ALS2
dynactin p150
VAPB
Parkinson's disease
DJ-1
α-synuclein
LRRK2
neurodegenerative disease
mouse model

Recent Publications:

Wang L, et al. *J Neurosci* 2008; 28: 3384-3391.

van der Brug MP, et al. Proc *Natl Acad Sci U S A* 2008; 105: 10244-10249.

Cai H, et al. *Neurodegener Dis* 2008; 5: 359-367.

Hardy J, et al. *Mol Neurobiol* 2007; 36: 224-231.

van de Leemput J, et al. *Plos Genetics* 2007; 3: e108.

Lai C, et al. *J Neurosci* 2007; 27: 13982-13990.

Research Description: Studying the pathogenic mechanisms of neurodegenerative diseases provides a unique opportunity not only to learn how the nervous system functions but also to develop effective mechanism-based treatments for these devastating illnesses. Development of animal models of these diseases will provide a very useful tool for examining the *in vivo* consequence of the underlying genetic mutations and for testing potential therapeutics. I am particularly interested in exploring the molecular pathogenesis of Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) by using a combination of *in vivo* genetically engineered animal models and in vitro neurobiological approaches.

Research Program I: Amyotrophic Lateral Sclerosis (ALS) and Motor Neuron Diseases

First described in the nineteenth century by Jean-Martin Charcot, ALS is now recognized as the most common disorder of motor neurons. ALS lies within a spectrum of heterogeneous syndromes that lead to the selective degeneration of upper or lower motor neurons. The precise etiology underlying ALS remains unknown, though the variability in the rate of clinical progression suggests that the causative mechanism is likely to be multifactorial. ALS is largely sporadic, but in 5-10% of the cases, the disease is inherited through autosomal dominant or recessive genetic mutations. Mutations in the abundant free radical scavenging enzyme superoxide dismutase 1 (SOD1) were first described 15 years ago, and account for nearly 20% of all familial ALS. Since the identification of SOD1, four other genes causative for ALS have been identified, and at least six other loci have been mapped, with most the pedigrees carrying dominant modes of inheritance. These genetic links are excellent tools

for studying motor neuron diseases, because the sporadic and familial forms appear to share a common pathogenesis based on their similar clinical and histopathological phenotypes. We focus our research on 3 newly identified genetic mutations linked to ALS and other motor neuron diseases (MND), including mutations in *ALS2, DCTN1,* and *ALS8/ VAPB*. Interestingly, all three proteins are likely involved in intracellular protein/vesicular transport, suggesting a potential common pathway links these gene products in the pathogenesis of motor neuron diseases. To better understand how these three mutations contribute to motor neuron degeneration, we have successfully generated *ALS2* KO mice, *DCTN1* knock-in mice, and *VAPB* transgenic mice. We are interested in studying the potential alterations of protein/vesicle trafficking in neurons derived from these mutant mice.

Research Program II: Parkinson's Diseases

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra. It is the second most common neurodegenerative disorder after Alzhemier's disease (AD). Clinical symptoms of the disease can include classical extrapyramidal signs such as rigidity, resting tremor, postural instability and bradykinesia, with psychiatric and cognitive presentations appearing in some patients. Amelioration of some or all of the symptoms with treatment by dopamine precursor L-DOPA typically separates PD diagnosis from other neurological disorders. The cardinal neuropathological hallmarks of PD are the degeneration of nigrostriatal dopaminergic neurons and the presence of abnormal intracellular deposits of protein largely composed of ubiquitin and *α-synuclein* known as Lewy bodies (LBs) and Lewy neurites (LNs), although some types of pathology more commonly associated with AD such as tau aggregates can be found. Until the identification of mutations in *α-synuclein* in 1997, PD was thought to be one of the rare neurological disorders without a genetic component. Since then, two dominantly inherited and three recessively inherited genes have been shown to unambiguously cause PD in multiple families spanning a wide range of geographical regions of the world. Most of these monogenic forms, with the exception of *LRRK2* mutations, are rarely found in classical late-onset sporadic PD, and in sum, account for nearly 10% of all PD cases. The monogenic forms also appear to differ with respect to neuropathology. While patients with genetic alterations in *α-synuclein* and *LRRK2* typically have idiopathic PD pathology such as LBs, LNs, and dopaminergic cell death and sometimes other disease markers such as tau pathology, patients with parkin mutations typically

lack LB pathology. Currently, post-mortem examinations of patients with homozygous PINK1 and DJ-1 mutations have not been described, but there remains a strong possibility that different mechanisms exist in the etiology of recessively and dominantly inherited forms that lead to a common final disease phenotype. Since the initial identification of A53T mutation in *α-synuclein*, many lines of transgenic and KO mice have been generated to model PD-related dominant and recessive genetic mutations. Unfortunately, few of these mouse models develop significant degeneration of nigrostriatal dopaminergic neurons, the major pathological feature of PD. In addition, many questions remain to be addressed for the selective vulnerability of nigrostriatal dopaminergic neurons in PD. Therefore, our research on PD focuses on developing new mouse models that capture main properties of PD: dysfunction and degeneration of nigrostriatal dopaminergic system by selectively expressing disease-causing mutations in nigrostriatal dopaminergic neurons. We are also interested in studying the genetic and biochemical interactions among PD-related genetic mutations, which may provide important clues that lead to selective degeneration of nigrostriatal dopaminergic neurons.

Collaborators: Drs. Philip Wong, David Borchelt, Ted Dawson, Valina Dawson, Mark Cookson, Zuhang Sheng, Zheng Li, Brian Howell, Andy Singleton, Mark Mattson, Toni Shippenberg, Julius Zhu, Xiaoqin Yan, Gabriela Chiosis, Michael O'Donovan, Dave Lovinger, David Goldstein, Juan Troncoso, Susan Cheng, Mike Lenardo, and Don Price.

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Biography: Dr. Bryan Traynor is a neurologist whose research interests focus on the genetics of amyotrophic lateral sclerosis (ALS) and other neuromuscular diseases. Dr. Traynor graduated as a medical doctor from University College Dublin in 1993 and received his medical doctorate in the genetics and epidemiology of ALS in 1999. At that time, he moved to Boston where he completed his neurology residency training and fellowship at the Massachusetts General Hospital and Brigham and Women's Hospital. He received a Masters in Medical Science from Harvard University and Massachusetts Institute of Technology in 2004, and, in the same year, was appointed as a staff neurologist in Massachusetts General Hospital and as an instructor at Harvard Medical School. In 2005 he joined the NIH where he has been studying the genetic causes of ALS and other neurological diseases. In 2009 he was appointed as an Assistant Clinical Investigator. Dr. Traynor's group researches the genetic and cellular mechanisms underlying simple-Mendelian and complex neuromuscular diseases.

Keywords:

neurogenetics
genomics
amyotrophic lateral sclerosis
fronto-temporal dementia
myasthenia gravis
inclusion body myositis
neuromuscular disorders

Recent Publications:

Chiò A, et al. *Hum Mol Genet* 2009; epub ahead of print.

Guerreiro RJ, et al. *PLoS ONE* 2008; 3: e2450.

Chiò A, et al. *Neurology* 2008; 70: 533-537.

Cronin S, et al. *Hum Mol Genet* 2008; 17: 768-774.

Schymick JC, et al. *Hum Mol Genet* 2007; 16 Spec No. 2: R233-R242.

Laboratory: Tremendous progress has been made in identifying genetic mutations that cause disease; these discoveries have led to a greater understanding of the underlying biological processes in these disorders, and for several, such as Alzheimer's disease, the targeting of specific points in these pathways for testing of therapeutic intervention (e.g. amyloid-beta immunotherapy). This is the clear goal of genetic investigation of disease: to understand the pathogenesis of disease, and to use that understanding to halt or reverse the disease process.

The Neuromuscular Disease Research Group at the National Institute on Aging focuses on dissecting the genetic pathogenesis of neuromuscular diseases. Recent advances in genotyping technology have ushered in the era of genome-wide association studies, and the group has applied this powerful technique to identify variants underlying neuromuscular disorders, especially amyotrophic lateral sclerosis (ALS). In addition, the laboratory investigates the genetic and epigenetic determinants that underlie regional differences in gene expression within the central nervous system. A more complete understanding of regional gene expression patterns in normal tissue may shed light on why neuronal subpopulations are selectively damaged in neurodegenerative diseases such as the loss of motor neurons in ALS. Ultimately, this knowledge will serve as a road map for future genetic investigation of neurological disease.

Traynor BJ, et al. *Lancet Neurol* 2007; 6: 841-843.

O'Toole O, et al. *J Neurol Neurosurg Psychiatry* 2008; 79: 30-32.

Cronin S, et al. *Neurology* 2007; 68: 1002-1007.

Schymick JC, et al. *J Neurol Neurosurg Psychiatry* 2007; 78: 754-756.

Schymick JC, et al. *Lancet Neurol* 2007; 6: 322-328.

The Neuromuscular Disease Research Group also utilizes the high-density aspect of the latest genome-wide SNP assays to map Mendelian traits. Once genomic regions of interest are identified, positional cloning is undertaken to pinpoint the specific mutations underlying these diseases using sequence capture arrays followed by genomic sequencing on the Solexa Genome Analyzer II recently installed in the laboratory. This platform dramatically improves the efficiency and speed of sequencing. The Laboratory will continue to apply these methodologies as rapidly and expeditiously as possible to dissect the complex genetics of neuromuscular disorders and neurogenetics in general.

Determining the genetic variants that underlie complex diseases is only the beginning. To impact patient care, these discoveries need to be returned to everyday clinical practice as diagnostic tools and as therapy. However, translating what we learn about genetic variants for the population as a whole to the bedside of an individual patient is tremendously challenging. The resources of the NIH Clinical Center allow patients to be evaluated in a longitudinal, prospective manner. Establishing cohorts of patients with known diseases and genetic backgrounds will begin to address these issues in a meaningful, scientifically rigorous manner.

Collaborators: Adriano Chiò, University of Turin, Italy; Dan Drachman, Johns Hopkins University, Baltimore, USA; Kurt Fischbeck, National Institute of Neurological Disorders and Stroke, NIH; John Hardy, Institute of Neurology, Queen Square, London; David Heckerman, Microsoft Research, Redmond, WA; Hannu Laaksovirta, University of Helsinki, Finland; Giancarlo Logroscino, University of Bari, Italy; Albert Ludolph, University of Ulm, Germany; John Ravits, Benaroya Research Institute, Seattle, WA; Jeffrey Rothstein, Johns Hopkins University, Baltimore, USA; Pentti Tienari, University of Helsinki, Finland.

Laboratory of Neurosciences

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

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

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

Synaptic Signaling and Plasticity: Signaling at the synapse plays fundamental roles in both immediate brain functions such as visual recognition and responses, and body movements, and long-term changes such as learning and memory. Recent findings by LNS investigators suggest that alterations in synaptic signaling occur very early in the course of Alzheimer's disease and other age-related neurodegenerative disorders. The impact of oxidative stress, neurotrophic factor and cytokine signaling, and genetic aberrancies on synaptic physiology are being examined. By studying synaptic physiology, molecular biology and biochemistry in normal aging and in animal models of neurodegenerative disorders, LNS scientists hope to identify the specific alterations underlying neurodegenerative disorders.

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

DNA Damage Repair and Telomere Biology: Damage to nuclear and mitochondrial DNA accrues in neurons during aging and to a greater extent in neurodegenerative disorders. LNS investigators have shown that DNA damage can trigger cell death in neurons by mechanisms involving aborted cell cycle reentry and apoptosis. Impaired DNA repair occurs in Alzheimer's disease and may render neurons vulnerable to being damaged and killed by oxidative stress and amyloid. Dietary folic acid can improve DNA repair in neurons and may thereby protect against neurodegenerative disease. LNS scientists have recently discovered that proteins associated with telomeres (the ends of chromosomes) protect neurons against death in experimental models relevant to Alzheimer's disease and stroke. These findings suggest the possibility that stabilization of telomeres in neurons in the adult brain may protect against age-related neurodegeneration. Ongoing research is aimed at identifying the specific mechanisms whereby DNA repair and telomere function may be compromised in neurons during

aging. In addition, preclinical studies are underway to identify therapeutic interventions that target DNA repair and telomere-associated proteins.

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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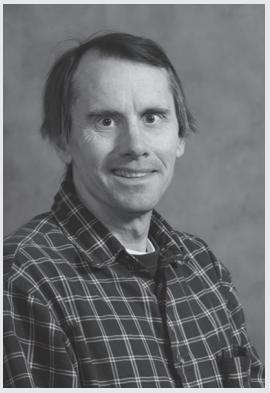

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Biography: Dr. Mattson received his Ph.D. in Biology from the University of Iowa in 1986. After 3 years of postdoctoral studies in Developmental Neuroscience at Colorado State University, Dr. Mattson took a faculty position at the Sanders-Brown Research 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 Neuromolecular Medicine and Ageing Research Reviews, and a Section Editor of the Journal of Neurochemistry and the Neurobiology of Aging. 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, the Alzheimer's Association Zenith Award and the Santiago Grisolia Chair Prize. 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 400 original research articles and more than 100 review articles. He is the most highly cited neuroscientist in the world.

Keywords:

neurodegenerative disorders
calcium and oxyradicals
signal transduction
synaptic plasticity
Alzheimer's disease
Parkinson's disease
Huntington's disease
amyotrophic lateral sclerosis
apoptosis
learning and memory
stem cells
neurogenesis

Recent Publications:

Stranahan AM, et al. *Nat Neurosci* 2008; 11: 309-317.

Tang SC, et al. *Proc Natl Acad Sci U S A* 2007; 104: 13798-13803.

Arumugam TV, et al. *Proc Natl Acad Sci U S A* 2007; 104: 14104-14109.

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 the animal 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

Publications-continued

Cheng A, et al. *J Neurosci.* 2007; 27: 1519-1528.

Xu X, et al. *Genome Biol* 2007; 8: R234.

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, PD and Huntington's diseases include transgenic mice that have been engineered to express mutant genes known to cause early-onset disease in humans.

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. Recent findings suggest that activation of adaptive cellular stress response pathways can be neuroprotective by a process called hormesis, which is an active area investigation in Dr. Mattson's laboratory.

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

synaptic physiology, molecular biology and biochemistry in experimental animal models of neurodegenerative disorders.

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

DNA damage increases in brain cells during aging and may be an important trigger of cell death in neurodegenerative disorders. A better understanding of mechanisms of DNA damage and repair may therefore provide a foundation for developing novel approaches for preventing neuronal degeneration. Investigators in Dr. Mattson's laboratory have identified genetic and environmental factors that may promote or prevent DNA damage and its adverse consequences in the nervous system. An example of recent findings include the demonstration that folic acid deficiency can sensitize neurons to DNA damage and death in experimental models of Alzheimer's disease and Parkinson's disease. Low levels of dietary folic acid result in an elevation of homocysteine levels. Homocysteine impairs the ability of neurons to repair DNA damage resulting in increased uracil misincorporation and oxidatively modified DNA bases. In another set of studies LNS scientists have shown that telomerase, a reverse transcriptase that prevents chromosome shortening in mitotic cells, can protect neurons against DNA damage-induced apoptosis. Additional studies have established roles for telomere associated proteins in brain development where it appears to promote neuroblast proliferation and the survival of early postmitotic neurons.

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

and associated sensory-motor dysfunction is employed in combination with studies of cultured brain cells. Three examples of ongoing major efforts are projects that target the tumor suppressor protein p53, mitochondrial ATP-sensitive potassium (Mito-KATP) channels and toll-like receptors (TLRs). By studying mice with targeted disruption of specific genes believed to play a role in the pathogenesis of stroke, investigators are working to identify additional therapeutic targets.

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

Sphingomyelin and cholesterol are important components of the plasma membrane of neurons where it functions in cellular signal transduction and cellular responses to stress. By analyzing spinal cord and brain tissues from human patients and mouse models, investigators in this section of the LNS have shown that profound abnormalities in sphingomyelin metabolism occur in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. The alterations, which include accumulation of long-chain ceramides and cholesterol esters, occur before neuronal degeneration and functional deficits in the mouse models. Moreover, agents that inhibit sphingomyelin synthesis or metabolism can protect neurons from being damaged and killed in experimental models of ALS and Alzheimer's disease, suggesting that the abnormalities in lipid metabolism are central to the disease process.

Collaborators: Vilhelm Bohr, Laboratory of Molecular Gerontology, NIA, NIH; Edward Lakatta, Laboratory of Cardiovascular Science, NIA, NIH; Josephine Egan, Laboratory of Clinical Investigation, NIA, NIH; Dennis Taub, Laboratory of Immunology, NIA, NIH; Ranjan Sen, Laboratory of Cellular and Molecular Biology, NIA, NIH; Dan Longo, Laboratory of Immunology, NIA, NIH; Jean Cadet, National Institute of Drug Abuse, NIH; Robert Tycko, National Institute of Diabetes and Digestive and Kidney Diseases, NIH; George Uhl, National Institute of Drug Abuse, NIH; David Baer, U.S. Department of Agriculture; Chris Earley, Johns Hopkins University; Solomon Snyder, Johns Hopkins; Frank LaFerla, University of California Irvine; Robert Brown, Harvard University; William Markesbery, University of Kentucky; Norman Haughey, Johns Hopkins; Avi Nath, Johns Hopkins; Michal Scwartz, Weissman Institute, Israel; Tim Bussey, Cambridge University, UK.

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Biography: Dr. Wolkow received her Ph.D. in 1997 in Molecular Biology and Genetics from the Johns Hopkins University School of Medicine where she studied target site selection by the bacterial transposon, Tn7. Moving to Boston, she carried out postdoctoral research as a research fellow of the Leukemia and Lymphoma Society with joint appointments at the Massachusetts General Hospital and Harvard University. During this period, Dr. Wolkow investigated longevity control by insulin-like signaling in *C. elegans*. This work forms the basis for current studies into genetic pathways that promote *C. elegans* longevity. Since joining NIA IRP, Dr. Wolkow's research program has expanded to investigate *C. elegans* functional aging and using *C. elegans* to identify prolongevity interventions based on natural products. Dr. Wolkow is a recipient of the Ellison Medical Foundation New Scholar in Aging Award (2004-2008).

Keywords:

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

Recent Publications:

Johnston J, et al. *PLoS ONE* 2008; 3: e2821.

Wilson MA, et al. *BMC Pharmacology* 2008; 8: 15.

Zou S, et al. *Mech Ageing Dev* 2007; 128: 222-226.

Iser WB, et al. *Devel Bio* 2007; 303: 434-447.

Iser WB, et al. *PLoS ONE* 2007; 2: e1240.

Genetics of Longevity in *C. elegans*: The nematode, *C. elegans*, has become a favorite organism for studying the genetics of longevity. This organism is easy to grow, reproduces readily and has a 2-3 week adult lifespan under laboratory conditions. These factors allow us to speedily assay lifespan in large populations and to quickly identify longevity genes. This organism can also be adapted to study prolongevity interventions. Molecular characterization of numerous longevity mutants has identified several pathways that govern lifespan in this organism. It is likely that a number of these pathways also affect longevity in people, due to the high degree of gene conservation between nematodes and humans. Thus, our studies of the genetic control of *C. elegans* longevity will reveal mechanisms that also promote longevity in humans.

Insulin Control of Longevity: Mutations disrupting *C. elegans* insulin/IGF-I-like signaling (IIS) dramatically increase lifespan and enhance stress resistance. Mutations in an insulin/IGF-I receptor-like protein, encoded by the *daf-2* gene, or PI(3)K, encoded by the *age-1* gene, significantly extend adult lifespan. IIS control of longevity has been observed in other species as well. Fruitflies with defective IIS survive longer than wild-type and mice lacking growth hormone display extended longevity.

The IIS pathway is well-conserved from *C. elegans* to humans. The

C. elegans genome contains 38 insulin-like genes, all potential ligands for the DAF-2 receptor. Once activated, the DAF-2 receptor signals intracellularly via IST-1, a homolog of vertebrate IRS proteins, to AGE-1/AAP-1, comprising the p110 and p55 subunits of PI(3)K. The lipid products of AGE-1 activate downstream S/T kinases, PDK-1 and AKT-1 and –2. DAF-18, a homolog of the vertebrate PTEN lipid phosphatase, antagonizes DAF-2 signaling. Signaling downstream of DAF-2 antagonizes the activity of the forkhead transcription factor DAF-16 which is phosphorylated by AKT-1/2. The phosphorylated form of DAF-16 is inactive and retained in the cytoplasm. Disruption of DAF-2 signaling relieves DAF-16 inhibition and allows DAF-16 to activate the expression of target genes required for long lifespan. A few DAF-16 target genes have been identified, including sod-3, encoding Mn-SOD. One hypothesis is that DAF-16 regulates the expression of target genes which stress resistance, thereby extending lifespan in *daf-2* mutants.

IIS within a variety of tissues can promote normal *C. elegans* lifespan via endocrine outputs. Developmental diapause is also regulated by IIS in a non-cell autonomous fashion via endocrine outputs. We hypothesize that IIS couples to secondary signaling pathway(s) responding to diffusible signals that coordinate DAF-16 activity throughout the body to promote developmental diapause and coordinate aging of multiple tissues. IIS also directly regulates cell-intrinsic outputs via direct control of DAF-16. We have recently described a new cell-intrinsic function of DAF-2/IIS, which is resistance to the induction of nutrient stress response in intestinal cells. In wildtype animals, nutrient stress induces the rapid redistribution of intestinal esterase activity from cytoplasmic vesicles to a nuclear or peri-nuclear localization. This redistribution can be easily viewed in fixed animals using a colorimetric assay for in situ esterase activity and we refer to this response as the FIRE response for Fasting-Induced Redistribution of Esterase. Mutations disrupting IIS promote resistance to the FIRE response under nutrient stress conditions, likely reflecting nutrient stress tolerance in these animals. Furthermore, FIRE response resistance in IIS-pathway mutants is cell-autonomously controlled by intestinal IIS. We hypothesize that intestinal DAF-16 directly activates the expression of target genes that promote intestinal nutrient stress tolerance and FIRE response resistance.

An independent, but related, research direction has been to identify new IIS pathway components by studying second-site mutations able to compensate for the absence of *age-1* function. Mutations in

age-1, which encodes for the p110 catalytic PI3K subunit, lengthen adult lifespan and severe mutations cause constitutive entry into a developmental diapause, termed the dauer larval stage. We have characterized several second-site suppressors of the *age-1* dauer arrest phenotype and found them to encode gain-of-function mutations in two downstream components of the DAF-2 pathway, *pdk-1* and *akt-1*. Gain-of-function *pdk-1* and *akt-1* mutations fully suppressed the dauer-constitutive *age-1* phentoype but did not alter the longevity phenotype of *age-1* mutants. These findings suggest two models. One model proposes that *akt-1* and *pdk-1* preferentially regulate dauer diapause while a distinct effector pathway mediates *daf-2* regulation of longevity. An alternative model suggests that levels of *daf-2* pathway signaling determine phenotypic spectrum, such that low levels of *daf-2* pathway activity are sufficient to bypass dauer diapause but higher levels are necessary to promote normal longevity. In this case, we hypothesize that gain-of-function *akt-1* and *pdk-1* mutations only restored *daf-2* pathway activity to the level sufficient to bypass dauer diapause, but not to promote wildtype lifespan.

Prolongevity Intervention Screening in *C. elegans*: There is great interest in identifying compounds that that can promote longevity, stress resistance and/or disease resistance in humans. The IMGU has adapted *C. elegans* as a model for screening potential prolongevity interventions with particular focus on natural products and their derivatives. Polyphenolic compounds present in fruits and vegetables demonstrate a variety of effects *in vitro*, such as cell growth inhibition and enzyme activation. Using *C. elegans*, it is possible to study these effects in a whole organism and to use genetic analysis to identify specific targets. The IMGU has completed two investigations in this area. First, we identified prolongevity activity in a proanthyocyanin-enriched fraction of blueberry polyphenols. The prolongevity effect of blueberry polyphenols is not apparently due to antioxidant effects or induction of stress hormesis. Instead, the beneficial effects of blueberry polyphenols appeared to require a calcium-calmodulin signaling pathway previously implicated in pathogen resistance. Our second investigation in this area examined the effect of methoxylation on bioactivity of stilbenes, which are a related group of natural polyphenolic compounds. In contrast to the beneficial effects of blueberry polyphenols, we found methoxylated stilbenes to be toxic to adult *C. elegans* nematodes. In addition, methoxylated stilbenes were also able to inhibit growth of germcell tumors in a *C. elegans* cancer model. In contrast, non-

methoxylated stilbenes failed to exhibit any significant toxicity or tumor growth inhibition at comparable doses. These findings suggest that methoxylation either enhances stilbene interactions with *in vivo* targets or increases stilbene biostability in this model.

Functional aging in *C. elegans*: During normal aging, the body's tissues experience structural and functional declines. In both *C. elegans* and humans, one of the tissues where these declines are most evident is muscle. Aging is associated with loss of muscle strength and speed and increased structural disorganization and deterioration of muscle cells. However, the causes of muscle functional decline are poorly understood. Some proposed causes include proapoptotic changes in aging muscle, mitochondrial dysfunction and increased free radical stress, and mechanical damage from "wear-and-tear" mechanisms. The IMGU has studied tissue functional aging in the *C. elegans* pharynx, which is the neuromuscular organ in the head that ingests and mechanically disrupts the animal's food. These studies were conducted in adult animals, between young adulthood and midlife, when developmental processes are complete and before the development of severe aging-related deterioration. Using computer-based image classification analysis, we discovered that the pharynx in adult animals is morphologically dynamic during these stages, and not static as might have been expected. Furthermore, the pharynx exists in preferred morphology states that are characteristic for each age. Transitions between preferred morphologies characteristic of young adults, early midlife and later midlife were consistently observed. These findings suggest that postmitotic tissues in adults undergo morphology transitions that might reflect cellular homeostatic mechanisms induced as tissues begin to age. The characterization of age-associated morphological states and transitions between these states provide a novel biomarker for further studies of function aging.

Summary: The research program of the IMGU is targeted to discovering how aging affects the body's tissues and how IIS governs the rate of aging in each tissue. Nematodes will also be useful for identifying and characterizing prolongevity interventions that may offer therapeutic potential in humans. Together, this work will provide insight into challenges confronting the aging body as well as strategies for coping with them.

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Biography: Dr. Maudsley received his Ph.D. in 1997 in Pharmacology from the Department of Pharmacology at the University of Leeds where he studied the molecular mechanisms of tachykinin receptor activation and desensitization. With a Howard Hughes Medical Institute Fellowship, he moved to Duke University to work with Professor Robert J. Lefkowitz on the connectivity of G protein-coupled receptor (GPCR) signaling to tyrosine kinase pathways. During this period Dr. Maudsley developed new theories of GPCR signaling based upon the creation of higher order superstructures. Dr. Maudsley then accepted an Investigator position at the Medical Research Council at the University of Edinburgh in the United Kingdom. There he furthered the development of his concepts of the organization of GPCRs into discrete signaling structures for specific physiological functions. This work forms the basis of his research into the alteration of the GPCR signaling structures during healthy and pathological aging.

Keywords:

signal transduction
G protein-coupled receptor
protein complex
aging
Alzheimer's disease

Recent Publications:

Martin B, et al. *PLoS ONE* 2008; 3: e2750.

Martin B, et al. *PLoS ONE* 2008; 3: e2398.

Lopez de Maturana R, et al. *Mol Endocrinol* 2008; 22: 1711-1722.

Shin YK, et al. *J Neurochem* 2008; 106: 455-463.

Martin B, et al. *Ageing Res Rev* 2008; 7: 209-224.

Pawson AJ, et al. *Endocrinology* 2008; 149: 1415-1422.

Research Overview: For the majority of its experimental lifetime, information flow through G protein-coupled receptors (GPCRs) has been envisioned as unidirectional, i.e., changes in receptor conformation produced by extracellular agonist binding promotes the transfer of information from outside the cell inwards. Recent experimentation however, has demonstrated that receptor conformation is also controlled by protein-protein interactions occurring inside the cell. Receptor dimerization and interactions with intracellular scaffolding and signaling proteins can modify receptor structure and ligand selectivity and predetermine, from a menu of available options, which intracellular responses will predominate. In essence, the influences on receptor conformation are bi-directional; internal factors change the conformation of the receptor to reflect the status of the intracellular milieu, while extracellular factors, i.e., agonists, convey information to the cell about the external environment. This concept has critical implications for receptor theory and the design of therapeutics. Thus in complex physiological processes, e.g., aging or neurodegenerative disease, in which multiple proteins expression patterns are changed it is more likely than previously thought that GPCR signal conditioning could be affected. Therefore if indeed there is an alteration of GPCR pharmacology in these states then perhaps drug design should be targeted toward this new pharmacology rather than the standard models previously used.

Martin B, et al. *Histol Histopathol* 2008; 23: 237-250.

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Maudsley S, et al. *Curr Alz Res* 2007; 4: 3-19.

Johnson JB, et al. *Free Radic Biol Med* 2007; 42: 665-674.

G Protein-coupled Receptors and Their Therapeutic Importance: The heptahelical G protein-coupled receptors constitute the most diverse form of transmembrane signaling protein. Approximately 1% of the mammalian genome encodes GPCRs, and about 450 of the approximately 950 predicted human GPCRs are expected to be receptors for endogenous ligands. GPCRs allow organisms to detect an extraordinarily diverse set of stimuli in the external environment, from photons of light and ions to small molecule neurotransmitters, peptides, glycoproteins, and phospholipids. Emphasizing their importance as therapeutic targets, nearly 40% of all current drugs target GPCRs for their actions. Thus the manipulation of transmembrane signaling by GPCRs constitutes perhaps the single most important therapeutic target in medicine. Therapeutics acting on GPCRs have traditionally been classified as agonists, partial agonists, or antagonists based on a two state model of receptor function embodied in the ternary complex model. However many lines of investigation have shown that GPCR signaling exhibits greater diversity and 'texture' than previously appreciated.

Additional Protein Factors Add 'Texture' to Receptor Signaling: Signaling diversity from GPCRs arises from numerous factors, among them the ability of receptors themselves to adopt multiple 'active' states with different effector coupling profiles, the formation of receptor dimers that exhibit unique pharmacology, signaling, and trafficking, the dissociation of receptor 'activation' from desensitization and internalization, and perhaps most importantly the discovery that non-G protein effectors mediate some aspects of GPCR signaling. At the same time, clustering of GPCRs with their downstream effectors in membrane microdomains, and interactions between receptors and a plethora of multidomain scaffolding proteins and accessory/chaperone molecules confers signal pre-organization, efficiency, and specificity.

It is these interactions with proteins that organize GPCRs into greater signaling entities that are of prime interest for our laboratory as their effects upon GPCR signaling provide a gateway into new realms of therapeutic pharmacology. More importantly it is likely that alterations in the interactions of these proteins with GPCRs may occur in aging or neurodegenerative disorders, thus defining a distinct 'pharmacology' from that seen in younger organisms or normal physiology. In this context, the concept of agonist selective trafficking of receptor signaling, which recognizes that a bound ligand may select between a menu of 'active' receptor conformations and induce only a subset of the possible response profile, presents the opportunity to develop drugs that change the quality as well as the quantity of therapeutic efficacy. As a more comprehensive understanding of the complexity of GPCR signaling is

developed, the rational design of ligands possessing increased specific efficacy and attenuated side effects may become the standard mode of drug development. Therefore one of our primary goals is to specifically enhance these drug qualities for age-related disorders such as Alzheimer's, Huntington's and Parkinson's disease.

We are studying the ability of multi-protein complexes to condition receptor signaling in three major programs, these include the identification and classification of GPCR signaling complexes (also known as receptorsomes), the role of intracellular scaffolding proteins in the integration of multiple receptor inputs and the ability of lipid raft microdomains to control receptor signal transduction and neurotransmission.

GPCR Receptorsome Structure in Aging and Neurodegeneration: The functional unit of the GPCR has been hypothesized for many years as a ternary complex of stimulating hormone (agonist), receptor and the G protein effector. However both our research and that of many others has demonstrated that many other protein factors are required for the generation of the full spectrum of agonist-mediated intracellular signaling events. GPCRs have now been shown to physically interact with other GPCRs, receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) and scaffolding proteins such as PSD-95 (post-synaptic density protein of 95kDa). The multiprotein complexes the GPCRs that are involved in creating their full activity status are called receptorsomes and are now thought to be the true functional receptor signaling unit. Many of the factors that GPCRs interact with in these receptorsomes have been shown to fluctuate in expression during aging and neurodegeneration, e.g., β-arrestin and RGS (regulator of G protein signaling) proteins. As the pharmacology and signaling of the GPCR is dictated by the composition of the receptorsome, we are, therefore, studying, using proteomic screening technologies such as differential in-gel electrophoresis (DIGE) and tandem mass spectrometry, how the structure of receptorsomes of GPCRs implicated in neurodegenerative diseases (muscarinic acetylcholine, dopamine and serotonin) changes with age and pathophysiology. With this knowledge we are then attempting to alter currently existing therapeutics to enhance their activity at these different receptorsome states.

Mechanisms and Patterns of Complex Signal Integration in the Central Nervous System: In the central nervous system, there are up to 60 different identified neurotransmitters that are involved in modifying neuronal activity through direct synaptic transmission or neuromodulation.

For each neurotransmitter, there is a wide variety of specific receptors that it can interact with to affect neuronal cellular signaling. The presence of these receptors often typifies the specific neuronal type. At many synapses there is a co-release of several signaling hormones and over larger areas there is a diffusion of neuromodulating hormones and neurotrophic factors. Hence the activity of neurons is likely to be a function of the summated actions of multiple cellular inputs. However much of cellular neurophysiology has been studied employing *in vitro* scenarios in which the signal transduction activity of receptor signaling pathways is studied in isolation of other inputs. This approach has yielded a great understanding of the linear pathways of cell signaling yet it does not provide sufficiently reliable information with respect to how multiple inputs integrate to generate the eventual physiology of the neuron when exposed to multiple neurotransmitters or neurotrophic factors. We are attempting to identify how neuronal signaling is controlled by the application of multiple hormones to the cells in a progressive manner. An emerging principle underlying cellular physiology is that signal transduction cascades do not operate as self-contained linear units of information transmission, but rather function as integrative networks, interfacing at multiple levels both with themselves and with other signaling modules to effect context-appropriate functional outputs.

The molecular integration of these distinct multiple inputs (mediated by specific plasma membrane embedded receptors) occurs at the level of their associated signal transduction cascades. There has been over the past few years a realization that signal transduction cascades, including kinases, phosphatases and their substrates are actually pre-assembled into higher order structures by molecular scaffolds, e.g., AKAP (A-kinase anchoring protein), POSH (plenty of SH3 domains), JIP (c-Jun N-terminal kinase interacting protein), β-arrestins or 14-3-3 proteins. These proteins compartmentalize signaling pathways in the cell, enhance specificity of target-substrate interaction and improve the speed and efficiency of signal transduction. Conceptually we have, therefore, a funneling of the complex and diverse signaling inputs from hormones and their specific receptors at the plasma membrane into the higher order multi-protein signaling scaffolds attached either to cytoskeletal proteins or the plasma membrane itself. Thus the complex neuronal signaling traffic is likely to converge at cytoplasmic nexi, represented by these scaffolding proteins. Clustering of signaling molecules in multiprotein complexes eliminates delays that would otherwise occur as a result of random diffusion in the cytoplasm. An understanding of how multiple inputs works also may give us a more

true appreciation of how neurotransmitters/neurotrophic factors actually mediate intracellular signaling events in the physiological setting. Recent evidence has implicated many of these scaffolding proteins in mediating neurological disorders such as Parkinson's and Alzheimer's and therefore we are undertaking a detailed approach to understand how protein-protein interaction at these scaffolds both controls signal integration from cell surface receptors and also signal transfer within the cell. It is likely that both the qualitative and quantitative nature of hormonal effects on cells are dictated by the specific composition of these signaling nexi.

Summary: The laboratory's interest lies in the appreciation that receptor signaling systems do not have a static profile and their response to ligands and the downstream signals they create are plastic. Natural events such as aging as well as neuropathophysiology are likely to affect this plasticity to generate new pharmacological profiles for receptor systems. It is our primary thesis that it may be possible to use this knowledge to create therapeutic agents specific to these new pharmacological states.

Collaborators: Yuri Ushkaryov, Ph.D., Imperial College, London; Dan Donnelly, Ph.D., University of Leeds; Craig McCardle, Ph.D., Bristol University; Louis Luttrell, M.D., Ph.D., Department of Endocrinology Medical University of South Carolina; Robert J. Lefkowitz, M.D., Duke University; Chris Peers, Ph.D., University of Leeds; Adam Pawson, University of Edinburgh Medical Research Council.

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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; specifically, from the Pharmacology Department of the Royal College of Surgeons, 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. The company was launched on technology from Dr. Greig's program. 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 neurodegenerative diseases, with particular emphasis on Alzheimer's disease, and of type 2 diabetes. He heads the Drug Design and Development Section of the Laboratory of Neurosciences that extensively collaborates within NIA, academia and industry. 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, initiating companies (Axonyx Inc., New York, NY; QR Pharma, West Chester, PA), and are in preclinical and clinical development. New research within his program is providing both publications and patent applications to support potential drugs of the future.

Keywords:

drug design
acetylcholinesterase & butyrylcholinesterase
amyloid precursor protein & amyloid-β peptide
tumor necrosis factor-α
p53 inhibitors
apoptosis & anti-apoptotic agents
glucagon-like peptide-1 & exendin-4
Alzheimer's disease, Parkinson's disease & stroke
type 2 diabetes
anti-oxidants

Design of Drugs and Diagnostics: The goal of the Drug Design and Development Section is to develop novel agents against rate-limiting steps involved in the pathophysiology of diseases associated with aging with emphasis on nervous system diseases such as Alzheimer's disease (AD) to generate both pharmacological tools to aid elucidate diseases processes and experimental drugs to treat them.

Alzheimer's Disease:
Acetylcholinesterase Inhibition: Although the neuropathological quantification of β-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

Recent Representative Publications:

Luo W., et al. *Synthesis (Journal of Synthetic Organic Chemistry)* 2008; 21: 3415-3422.

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Perry T, et al. *Exp Neurol* 2007; 203: 293-301.

in memory processing. Additionally, there are numerous mechanistic-based interactions linking the cholinergic system to Aβ genesis, Tau phosphorylation, apoptotic cell death and inflammatory process that form a self-propagating cycle that drives AD pathogenesis. We have therefore focused our expertise on pivotal targets in each of these diverse but linked elements in order to develop mechanism-based strategies to not only slow or halt AD, but additionally to impact other neurodegenerative diseases.

Anticholinesterases: 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 (ACh) degrading enzyme, in brain. Extensive studies involving synthetic chemistry, X-ray crystallography, molecular modeling, 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 its sister enzyme, 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 as well as prophylactics for nerve gas poisoning (licensed to Cenomed, Irvine, CA, for use in counterterrorism and chemical defense).

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 a reversible drug/enzyme complex allows selective enzyme inhibition over a protracted time duration (numerous hours), which is independent of the pharmacokinetic half-life of the drug (often minutes). 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. It is difficult to achieve steady-state drug target levels and, indeed, when achieved, it generally results in a high body exposure to drug and potential toxicity. Our use of the former method, targeted enzyme inhibition, enhances specificity, lowers total body drug exposure and dramatically reduces toxicity. This is important in the elderly, which represents the fraction of the population afflicted with AD. The high

variability and slowing of drug metabolism, commonly associated with age, often results in a gradual overdosing and toxicity in the elderly as one dose is often administered before a prior one is fully cleared. The dissociation between pharmacokinetics and pharmacodynamics minimizes this, as drug clearance (measured in minutes) can change dramatically without impacting on drug action (measured in hours). Incorporating such concepts into our drug design has resulted in several novel compounds with dramatic sustained cognitive action for once or twice daily dosing with wide therapeutic windows and minimal toxicity. For example, the experimental drug, phenserine (licensed to TorreyPines Therapeutics, La Jolla, CA), a long-acting and brain-directed, selective AChE inhibitor, reached phase 3 clinical assessment in AD patients. It proved to be well tolerated in elderly individuals, particularly when compared to currently available prescription anticholinesterases. The agent has action on cognition and may positively impact Aβ generation (Ann Neurol. 63:621-31, 2008).

Other novel agents from SCIT are presently being developed as the first available reversible, nontoxic and brain-directed selective inhibitors of the enzyme BChE.

Butyrylcholinesterase Inhibition: Inhibition of AChE is a characteristic shared by all cholinesterase inhibitors currently approved for the treatment of AD. In the brain, AChE is primarily associated with neurons, where it hydrolyses acetylcholine (ACh) to terminate its biological action. Although overlooked for many years, a second cholinesterase, butyrylcholinesterase (BChE), is likewise capable of hydrolyzing ACh and may play an important role in the pathophysiology and symptomatology of AD. 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, where it co-localizes both with Aβ plaques and neurofibrillary tangles. The association of AChE and BChE with the AD neurotoxic peptide, Aβ, has been shown to dramatically modify 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.

Regarding its enzyme kinetics, an important feature distinguishing BChE from AChE is its kinetics toward concentrations of ACh. BChE is not inhibited by excess substrate. This is reflected in its K_m for ACh, which

makes it less efficient in its substrate hydrolysis at low concentrations but highly efficient at high substrate concentrations, at which AChE becomes substrate inhibited. Consequently, we hypothesize that one role of BChE in brain, particularly when associated with glia, is that of a supportive hydrolyzing enzyme for ACh. Under conditions of high brain activity, local synaptic ACh levels can reach μM levels, which are inhibitory for AChE activity. The close spatial relationship of glial BChE would allow compensatory ACh hydrolysis to occur. In addition, some 15% of cholinergic synapses in human brain have BChE rather than AChE as the metabolizing enzyme. A further important feature that distinguishes these two cholinesterase subtypes is that AChE is lost early in AD, by up to 85% in specific brain regions in line with the loss in presynaptic ACh, whereas BChE levels are elevated. This results in a mismatch between substrate and enzyme. Indeed, the ratio of BChE/AChE has been found to dramatically change in cortical regions from 0.2 to as high as 11. Clearly, such an altered ratio in the AD brain could jeopardize the normally supportive role of BChE to hydrolyze only excessive ACh, terminating its action too quickly. Selective inhibition of BChE may therefore be of value to normalize the BChE/AChE ratio in AD brain and augment cholinergic neurotransmission.

To elucidate the role of BuChE in AD, the first, reversible, selective carbamate inhibitors of BChE were developed (cymserine: (-)-4'-isopropylphenyl-carbamoyleseroline and analogues) and their effects on cognition were assessed by administering them to male aged Fischer-344 rats whose performance was quantitatively evaluated in a 14-unit T-Maze (Stone maze). This cognitive task has proved highly robust and sensitive in evaluating age-dependent declines in memory and pharmacological interventions in rodents. The action of selective BChE inhibition on brain levels of ACh, as measured by *in vivo* microdialysis, has also been studied, together with actions on the levels of AD neuropathological markers, amyloid precursor protein APP and Aβ peptide (PNAS 102:17213-8, 2005). Based on such studies, the experimental drug bisnorcymserine has been chosen to evaluate the therapeutic strategy of selective BChE in AD, and the agent has been preclinically developed in accord with US regulatory requirements to support its clinical evaluation.

Amyloid-β Precursor Protein (APP) and Amyloid-β (Aβ) Peptide Inhibitors: Another of our focuses to develop therapeutics for treating AD relates to reducing the production and secretion of Aβ. It is widely believed that Aβ plays a central role in the progressive neurodegeneration

observed in AD; diminishing the level of Aβ has therefore emerged as a critical goal in AD therapy. Aβ is generated from a larger protein, APP, by a group of enzymes collectively identified as secretases. Specifically, APP is proteolytically cleaved at specific amino acid by three secretases (α-, β- and γ-), to different protein fragments, including toxic Aβ and other C-terminal fragments that are implicated in the pathogenesis of AD. A major focus has hence been to develop agents to alter amyloidogenic processing to produce non-amyloidogenic by-products. The secretases as well as strategies to augment the clearance of Aβ are thus legitimate, albeit unvalidated, targets for drug discovery. Our program, together with collaborators (Prof. Debomoy Lahiri, Ph.D., Indiana University School of Medicine, Indianapolis, IN; Prof. Kumar Sambamurti, Ph.D., Medical University of South Carolina, Charleston, SC; and Prof. Jack Rogers, Ph.D., Harvard University, Boston, MA), is jointly engaged in studying various classes of agents that can reduce APP expression, as this is the precursor to all the Aβ toxic fragments, such agents can provide a new mechanism for lowering brain levels of Aβ.

In this regard, we have focused on the pharmacophore of our developed anticholinesterase, phenserine (a tricyclic hexahydropyrrolo[2,3b]indole with a phenylcarbamate), as in cell culture studies, phenserine lowered APP and Aβ levels in human neuroblastoma cells via a mechanism unassociated with its anticholinesterase action. In rats, it was shown to improve cognitive performance, and lower APP production in both naive and cholinergic lesioned animals. Likewise, in transgenic mice over-expressing human APP and Aβ, it was found to significantly lower both (for review: Curr Alzheimer Res 2:483-92, 2005; Expert Opin Investig Drugs 16:1087-97, 2007). Interestingly, phenserine's action to lower APP occurs through modulation of protein expression at the post-transcriptional level. In this regard, there are an increasing number of reports of post-transcriptional regulation of diverse gene products. For example, small molecules can significantly modulate post-transcriptional processes involved in the production of tumor necrosis factor-alpha (TNF-α). Phenserine's actions on APP are mediated through the 5' untranslated region (5'-UTR) of APP mRNA; the very same element previously shown to be up regulated in the presence of interleukin-1 and other cytokines that are elevated in AD brain. Post-transcriptional regulation of proteins such as APP by small molecules is hence a feasible approach to discover and develop new therapeutic agents that lower Aβ levels. Utilizing the pharmacophore of phenserine, we have developed a novel series of compounds to optimize action against APP and Aβ and

to set anticholinesterase activity within the concentration range of that optimal to lower Aβ. This approach was followed as the dose-limiting adverse actions of phenserine in humans (nausea and vomiting) are centrally mediated cholinergic actions. Of the novel compounds that achieved this action (J Pharmacol Exp Ther 318:855-62 & 320:386-96, 2007), the agent, posiphen, was chosen to evaluate in clinical trials. Posiphen readily entered brain and dramatically lowered both APP and Aβ levels in rodents at well-tolerated doses. Whereas the parent compound is devoid of anticholinesterase activity, it generates anticholinesterase-active metabolites following its N-demethylation. This slow generation of anticholinesterase activity avoids the initial burst of cholinergic action associated with administration of classic anticholinesterases that triggers their adverse actions. In phase 1 clinical trials, posiphen proved to be well-tolerated and allowed dose escalation to amounts in excess of 6-fold maximally-tolerated levels of phenserine. The achieved plasma concentration of posiphen in humans proved to be greater than those determined in rodents that were associated with dramatic reductions in brain Aβ levels. Future studies are aimed at elucidating the ability of posiphen to improve cognition and reduce Aβ in AD subjects.

Inflammation and TNF-α Inhibition: Inflammatory processes associated with the over-production of cytokines, particularly of TNF-α, accompany numerous neurodegenerative diseases, such as Alzheimer's disease and ALS, in addition to numerous systemic conditions that are common in the elderly, such as rheumatoid arthritis, as well as diseases such as erythema nodosum leprosum (ENL), septic shock, graft-versus-host and Crohn's disease (for review: Curr Alzheimer Res 4:378-85, 2007). TNF-α has been validated as a drug target with the development of the inhibitors Enbrel and Remicade as prescription medications. Both, however, are large macromolecules that require direct injection and have limited to negligible brain access. The classical drug, thalidomide is being increasingly used in the clinical management of a wide spectrum of immunologically–mediated and infectious diseases, and cancers. Its clinical value in treating ENL derives from its moderate TNF-α inhibitory activity. Structural modification of thalidomide was hence undertaken towards the discovery of novel isosteric potent analogues that would be of potential utility in the conditions described above. These were synthesized and evaluated for their TNF-α inhibitory activity against lipopolysacharide (LPS) stimulated peripheral blood mononuclear cells (PBMC) in cell culture as well as against immortal microglial cell lines. Cell viability was quantified to differentiate reductions in TNF-α secretion from cellular toxicity. Specific

analogues potently inhibited TNF-α secretion, compared to thalidomide. The mechanism underpinning this likely is post-transcriptional as they decreased TNF-α mRNA stability via its 3'-UTR, as determined by luciferase activity in stably transfected cells with and without the entire 3'-UTR of human TNF-α. The translational potential of the most active compounds has been evaluated in a time- and concentration-dependent manner in rodents whose TNF-α levels in plasma and brain were elevated by LPS administration. Specific compounds proved highly effective in lowering both brain and plasma TNF-α, and the activity of these is currently being evaluated in classical models of neurodegeneration (collaborators: Dr. Susanna Rosi, UCSF).

Neurodegeneration: Collaborative studies with Dr. Mark Mattson (Laboratory of Neurosciences, IRP, NIA, NIH) and Dr. Barry Hoffer (Cellular Neurobiology Branch, IRP, NIDA, NIH) 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 impede the translocation of the intracellular protein, p53, from the cytoplasm where it normally resides, to the nucleus and mitochondria where it triggers cell death. These compounds protect cells of neuronal origin from toxic concentrations of a variety of insults, including the AD Aβ peptide as well as glutamate excitotoxicity, in tissue culture. These actions translate to *in vivo* models, where our p53 inactivators not only protect the brain from ischemic insults in classical rodent models of stroke, but also aid in the regenerative process. Additional studies have demonstrated potency in a widely used model of Parkinson's disease and in a partial model of AD. The focus of our studies is to test the clinical utility of p53 inactivation with emphasis on neurodegenerative diseases such as AD, Parkinson's disease and stroke. However, p53 inactivators hold potential in protecting normal tissue from a wide variety of toxicities and insults, such as associated with chemotherapeutic agents and radiation therapy in cancer treatment, and these form a further focus of future research.

GLP-1 Receptor Agonists, Type 2 Diabetes and Neurodegeneration: Collaborative studies with Dr. Josephine Egan (Diabetes Section, Laboratory of Clinical Investigation, IRP, NIA, NIH) 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 and thereby lowers blood glucose levels. Like other endogenous hormones, it is short acting, as it is rapidly cleaved by plasma peptidases. 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 focused on the structure/activity relation of the GLP-1 amino acid sequence in relation to binding affinity, induction of cAMP levels and insulin release, as well as to metabolic processes involved in its cleavage and inactivation. Novel peptides have been synthesized around to cores of GLP-1 and Ex-4 to optimize the former processes and minimize the latter one by masking peptidase recognition sites. Additional research supported the transition of Ex-4 from the laboratory and into clinical trials as an experimental therapeutic for type 2 diabetes, and the agent is now approved for this indication.

Although predominantly located on pancreatic islet cells, numerous reports now document GLP-1 receptor (R) expression in both the rodent and human brain (for review see: Curr Alzheimer Res. 2:377-85, 2005). GLP-1 is produced by specific neural cells, and GLP-1 present in the bloodstream can enter brain; utilizing a blood-brain barrier peptide transport system. Intestinally derived peptides (incretins), such as GLP-1, are classified not only as hormones, but also as growth factors – peptides capable of regulating diverse cellular processes, including mitosis, growth, and differentiation. Our studies were the first to establish the neurotrophic/neuroprotective role of GLP-1 and analogues in the central and peripheral nervous system.

Following demonstration of the presence of the GLP-1R on neural cell lines, such as PC12 cells as well as primary rat hippocampal, cerebral cortical and ventral mesencephalic cells by RT-PCR analysis of RNA, we established the GLP-1R to be active by inducing increases in intracellular camp with GLP-1 and Ex-4 administration that revealed an affinity parallel

to that of pancreatic β cells. GLP-1R stimulation induced differentiation support of neural cells in a manner similar to nerve growth factor (NGF), which was reversed by co incubation with a selective GLP 1R antagonist. The cellular signaling pathways that are activated by GLP-1 in neural cells has been elucidated and remains a focus of current studies. In addition, GLP-1R activation by GLP-1 and Ex-4 provided complete protection against cell death induced by glutamate neurotoxicity in cultured primary neurons, as has been shown by other neurotrophic factors (e.g., NGF and BDNF), suggesting that GLP-1-like peptides play a significant role in protecting hippocampal neurons against excitotoxic damage and other types of brain injury. Protection, likewise, was afforded against Aβ as well as cellular oxidative stress and membrane lipid peroxidation induced by Fe^{2+}. Translational studies have been undertaken in classical animal models of acute and chronic neurodegenerative conditions. Amongst many, these include peripheral neuropathy and AD, and form the focus of current and future research.

Anti-oxidants as Therapeutics for Acute and Long-term Neurodegenerative Conditions

Uric acid is a major antioxidant in humans and its concentration in blood is positively correlated with increasing lifespan among mammalian species suggesting a role in retarding aging processes. Prior cell culture studies have shown that, by suppressing hydroxyl radical- and peroxynitrite-mediated damage, uric acid could protect neurons against oxidative and metabolic insults. Administration of extremely high doses of uric acid (500 mg/kg) have been reported to improve the outcome of focal ischemic brain injury as well as spinal cord injury in rodents. However, uric acid has a poor aqueous solubility that compromises its potential clinical use as it can crystallize in joints to cause inflammation as in gout. Nevertheless, uric acid provides a pharmacophore that can be manipulated by medicinal chemistry synthesis to optimize its assets and minimizes its deficits. Synthesis and preclinical development of neuroprotective methyl- and sulfur-containing analogs of uric acid was undertaken to generate agents with greater antioxidant activity and increased solubility (collaborator: Dr. Mark Mattson, Laboratory of Neurosciences, IRP, NIA, NIH).

In vitro and cell culture screening identified 6,8-dithiouric acid and 1,7-dimethyluric acid two analogs worthy of *in vivo* evaluation. When administered to mice, both lessened damage to the brain and improved functional outcome in an ischemia-reperfusion model of stroke. They, additionally, proved effective up to 4 h after stroke onset in a permanent

middle cerebral artery occlusion mouse model (Neuromolecular Med. 9:315-23, 2007.). Based on these studies, these agents are being assessed for toxicity in rodents and efficacy in a variety of neurodegenerative conditions.

Collaborators: Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Josephine Egan, M.D., Diabetes Section, Laboratory of Clinical Investigation, NIA, NIH; Barry Hoffer, M.D., Ph.D., IRP, NIDA, NIH; Yun Wang, Ph.D., IRP, NIDA, NIH; Donald Ingram, Ph.D., & Tada Utsuki, Ph.D., Pennington Biomedical Research Center, Baton Rouge, LA; Arnold Brossi, Ph.D., University of North Carolina, Chapel Hill, NC; Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Medical University of South Carolina; Jack Rogers, Ph.D., Harvard, Boston, MA; Jeffery Deschamps, Ph.D., Naval Research Center, Washington D.C.; Tony Giordano, Ph.D., Louisiana State University, Shreveport, LA; Kimi Sugaya, Ph.D., University of Central Florida; Mohammad Kamal, Ph.D., University of Sydney, Australia; Avigdor Shafferman, IIBR, Israel. Chaim Pick, Ph.D., University of Tel Aviv, Israel; Agneta Nordberg, M.D., Ph.D., Karolinska Institute, Sweden

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Biography: Henriette van Praag received her Ph.D. from the Department of Psychobiology at Tel-Aviv University in 1992 for her work studying the development of opiate receptor function in newborn rats with Dr. Hanan Frenk. She did her postdoctoral research on the role of nerve growth factors in developmental brain injury at Robert Wood Johnson Medical School in New Jersey with Dr. Ira Black from 1992-1997. She continued her research in brain regeneration as a staff scientist in the Laboratory of Genetics at the Salk Institute for Biological Studies in La Jolla, California with Dr. Fred Gage from 1997-2007. She researched the regulation of the birth of new neurons in the adult hippocampus, a brain area that is important in learning and memory. She investigated the physiological role of adult neurogenesis in mammalian brains, showing that newborn born cells become functional neurons in the hippocampal circuitry. Moreover, she demonstrated that the production of new neurons is strongly influenced by exercise.

Keywords:

neurogenesis
hippocampus
exercise
Huntington's Disease

Recent Publications:

van Praag H *Neuromolecular Med* 2008; 10: 128-140.

Mattson MP, et al. *Nat Cell Biol* 2008; 10: 249-250.

Toni N, et al. *Nat Neurosci* 2007; 10: 727-734.

van Praag H, et al. *J Neurosci* 2007; 27: 5869-5878.

Development and function of new neurons in the adult brain: It has become well-accepted that the adult mammalian brain can generate new neurons. The two neurogenic areas are the olfactory bulb and the hippocampus. The focus of our research is on neurogenesis in the hippocampus, a brain area important for learning and memory. Our aim is to determine whether neurogenesis plays an important role in normal memory function. To address this question we will use several different approaches. In one set of studies, we will house animals under exercise or enriched environment conditions and study levels of hippocampal neurogenesis and performance on a battery of learning and memory tasks. To determine whether enhanced neurogenesis facilitates performance on these tests the genesis of new cells will be ablated using hippocampal x-irradiation. In a second set of studies, we aim to determine how newborn granule cells contribute to hippocampal function by making a detailed analysis of their connectivity. To delineate new cell circuitry we will use a combination of viral vectors as anatomical tracers. We hope these studies will provide insight into the role of new neurons in the adult brain.

Exercise and brain function in a mouse model of Huntington's Disease: The overall goal is to determine whether exercise may become a therapeutic intervention in cases of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's Disease. Our current project focuses on a mouse model of Huntington's Disease. Huntington's Disease is a genetic neurological disorder caused by a trinucleotide CAG repeat

expansion in the gene that encodes for the Huntingtin protein. HD leads to progressive movement, psychiatric and cognitive disturbances. In normal rodents physical activity has been shown to enhance hippocampal neurogenesis, neurotrophin levels and learning. In our project we aim to determine whether onset and progression of HD may be slowed down by voluntary exercise in a running wheel. Specifically, we use transgenic N171-82Q mice that express a human N-terminal truncated huntingtin with 82 polyglutamine repeats driven by a mouse prion protein promoter to investigate the effects of voluntary exercise on motor function, hippocampal neurogenesis, learning, and lifespan.

Collaborators: Mark Mattson at NIA, Hoonkyo Suh, Fred Gage and Ed Callaway at Salk Institute for Biological Studies, La Jolla, CA. Ron Frostig at UC Irvine, Brian Christie at University of British Columbia

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, with Honors 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 the NIA to inaugurate a Stress and Coping Section. Since 1985 he has been Chief of the Laboratory of Personality and Cognition. Dr. Costa is also a Professor of Psychiatry and Behavioral Sciences at the Johns Hopkins University School of Medicine and a Clinical Professor of Psychiatry at Georgetown University School of Medicine. His enduring interests are in the structure and measurement of personality and in life-span development. Other research interests include health psychology—Compliance and disease progression in AIDS, Alzheimer's Disease, Predictors and Prognosis, Axis I and II mental disorders, and the neurobiology and molecular genetics of personality.

Keywords:

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

Recent Publications:

Löckenhoff CE, et al. *J Gerontol: Psychol Sci* 2008; 63B: P92-P99.

Terracciano A, et al. *BMC Psychiatry* 2008; 8: 22.

Bagby RM, et al. *Can J Psychiatry* 2008; 53: 361-370.

Terracciano A, et al. *Psychosom Med* 2008; 70: 621-662.

Research program

Background

Personality is central to understanding the individual person and it influences, to some degree, nearly all aspects of experience and functioning in everyday life. Personality psychology has made striking advances and breakthroughs in the past two decades. Today there is a real science of personality psychology -- an organized and growing body of knowledge -- in the form of a generally accepted model of personality traits. The fundamental taxonomy of personality traits, represented by the Five-Factor Model of personality (FFM), has facilitated rapid progress in understanding the origins, stability, predictive utility and universality of personality traits. These traits are rooted in biology, endure in adulthood, and influence an extraordinary range of psychological outcomes. My research program is organized around the FFM and broadly, the goals are to employ this comprehensive structural model of personality to investigate basic questions concerning aging and human development

This research is organized around the Five-Factor Model of personality (FFM) and broadly, the goals are to employ this comprehensive structural model of personality to investigate basic questions concerning aging and human development.

Below we briefly highlight progress on the following topics: (1) basic

research on (a) personality, age and emotion and (b) personality changes across the life span, (c) the FFM in traditionally understudied populations, and (d) the development of a new, more readable personality instrument to assess the FFM. (2) Contributions of the FFM as a unifying framework for research on personality and personality psychopathology with implications for the classification and diagnosis of personality disorders. (4), Application of the FFM to important life outcomes such as disease progression and stigmatization in HIV/AIDS, dietary habits, and stress. (5) Ongoing work on the genetic roots of personality traits and progress reports on the genome-wide association studies of personality in the Sardinian founder population.

Personality trait development in adulthood and old age.

Although the main outline of age changes in personality traits is known, many uncertainties remain. There is a need to clarify and refine the description of the longitudinal course of personality trait development in adulthood.

A recent study examined age trends in the five factors and 30 facets assessed by the Revised NEO Personality Inventory in Baltimore Longitudinal Study of Aging data (N = 1,944; 5,027 assessments) collected between 1989 and 2004 **(1).** Consistent with cross-sectional results, Hierarchical Linear Modeling analyses showed gradual personality changes in adulthood: a decline up to age 80 in Neuroticism followed by stability or slight increases in the very old; stability up to about age 40 and then decline in Extraversion – declines in E accelerate in the very old; steady linear declines in Openness from age 30 to 90; increases in Agreeableness were observed from age 30 to 90; and increase up to age 70 in Conscientiousness and then a small decline. Although most facets showed curves similar to the factor they define, some did not, particularly within the Extraversion domain. While the E4: Activity facet showed stability to age 40 then declines as the Extraversion domain showed, the E1: Warmth, E2: Gregariousness, E3: Assertiveness, and E6: Positive Emotions facets showed increases beyond age 40: Positive Emotions to age 50, Warmth to age 60, and Gregariousness and Assertiveness to age 65, followed by slight declines. The E5: Excitement-Seeking facet showed accelerating decline from age 30 on, stabilizing somewhat in very old age. Men and women showed similar curves and cohort effects were modest.

As important and impressive as those results are that study is not

without limitations. The longitudinal period was only 15 years. Whether similar or different trajectories would be found for a much longer longitudinal interval reveal, for example, 30 or 40 years is an important question that we attempted to answer in a follow-up study with another personality instrument. To address that important question, we examined developmental trends in personality traits over a 42-year time period from the longitudinal trajectories of Guilford-Zimmerman temperament survey scores from the Baltimore Longitudinal Study on Aging (N = 2,359; individuals aged 17-98), collected from 1958 to 2002 **(2).**

Hierarchical linear modeling analyses revealed cumulative mean-level changes averaging about 0.5 SD across adulthood. Scales related to extraversion showed distinct developmental patterns: General Activity declined from age 60 to 90; Restraint increased; Ascendance peaked around age 60; and Sociability declined slightly. Scales related to neuroticism showed curvilinear declines up to age 70 and increases thereafter. Scales related to agreeableness and openness changed little; Masculinity declined linearly. We found significant individual variability in change. Although intercepts differed, trajectories were similar for men and women. Attrition and death had no effect on slopes. This study highlights the use of lower order traits in providing a more nuanced picture of developmental change.

The results of these two longitudinal studies are consistent with cross-cultural patterns of age differences observed in cross-sectional studies, supporting the view that these maturational trends are universal. The study of normative trends provides a reference point against which to examine individuals with distinct patterns, which might be due to genetic factors, life experience, or disease, such as Alzheimer's disease or depression.

This research has helped clarify how personality changes over the life course. What remains a hotly debated question, however, is at what age personality reaches its greatest rank-order stability and whether this stability is seen over the remainder of the life course.

Two recent meta-analyses found that rank-order consistency: (1) Increased with age, even in adulthood, where those older than 50 years are most stable; (2) Increased up to age 50, then decreased thereafter. This is in contrast to other research which has concluded that "personality change is the exception rather than the rule after age 30" (McCrae & Costa, 1990). To address this issue, we examined rank-order consistencies within the

Baltimore Longitudinal Study on Aging (BLSA) for both the NEO-PI-R and GZTS **(3).** Results demonstrated that for 4 GZTS scales, in particular Ascendance or Assertiveness, rank-order consistency increased between the ages of 30 and 50, supporting the conclusion that rank-order stability increases to age 50. However, for the remaining GZTS scales and all NEO-PI-R factors, there was no such increase in consistency, supporting the perspective that rank-order consistency in personality remains largely the same after age 30.

Personality stability and change in the Baltimore Epidemiologic Catchment Area (ECA). In the study of personality stability and change, there is a need for longitudinal studies of ethnic minorities. There is also a need for research in the community or epidemiological investigations to study the causes of the modest changes that occur in personality traits in adulthood. To that end, we examined the personality traits and longitudinal change in traits in an ethnically and educationally diverse sample of 505 adults living in East Baltimore as part of the East Baltimore Epidemiologic Catchment Area (ECA) study **(4).** Our main objective was to assess the influence of demographic characteristics (i.e., gender, age, ethnicity – Black or White, and education) on five indices of personality stability and change over an average interval of 8 years. These five indices of stability consisted of:
Structural: Measurement invariance over time
Mean-level: Temporal stability of mean level of traits at the group level
Rank-order: The extent to which personality traits maintain similar relative positions in the distribution of personality scores over time
Reliable change: Individual-level change above and beyond chance fluctuations
Ipsative: Consistency in the configurations of traits within individual participants

In the full sample, examination of all five stability indices suggested that NEO-PI-R personality traits showed moderate to high levels of stability over time. There were few age and gender effects on temporal stability but rank-order, ipsative, and mean level stability were lower among Blacks and individuals with lower education.

Mean level trajectories for Whites and Blacks differed for the N factor and two of its facets (N1: Anxiety & N3: Depression), suggesting that Whites showed a slight increase in N whereas Blacks decreased. Mean level changes also differed for two facets of C (C1: Competence & C5:

Self-Discipline), with Blacks showing increases while Whites remained stable. Thus, while Blacks were generally less stable than Whites, they showed greater consistency with previously reported mean level changes. As for education, while more highly educated participants decreased in O, O1: Openness to Fantasy, and C2: Order, participants with lower education levels showed the opposite trajectories.

These findings support the view that indices of temporal stability differ by age, gender, ethnicity and education. However, it is important to keep in mind that across the different indices, demographic factors only accounted for a very small portion of the variability in personality plasticity (i.e., < 2% for mean level plasticity, ≤ 5% for rank order plasticity and reliable change, and < 10% for ipsative stability).

Personality structure of African-American older adults using the Baltimore Study of Black Aging (BSBA). Adding support to the ECA results concerning personality stability across ethnicity are results from the BSBA (N = 234; Age range 49–88, M = 67; 72% women; Education M = 11 years). The factor structure in this sample was compared with the census-matched normative NEO-PI-R factor structure **(5).** Principal components with Procrustes rotation was used to calculate factor, facet, and total congruence coefficients. Significant factor congruence coefficients at a 99% probability level or beyond were found, and only three facet-level congruence coefficients did not reach significance. With the exception of cross-loadings on a few facets, most primary and secondary loadings generally replicated the normative structure providing evidence that there are little differences among the African-American older adults and the largely European American normative structure.

NEO-PI-3: Cross-Sectional Comparisons from 12 to 90. Previously, we presented personality trajectories going as far back as the age of 12, and described research that demonstrated an instrument designed for use in adults (i.e., NEO-FFI) could also be applied in young samples. However, use of the Revised NEO Personality Inventory in adolescent samples has shown that a few respondents have difficulty with a subset of items. We identified 30 items that were not understood by at least 2% of adolescent respondents and 18 additional items with low item-total correlations, and we wrote 2 trial replacement items for each **(6).** We used self-report and observer rating data from 500 respondents aged 14 to 20 to select replacement items. The modified instrument, which we term the NEO-PI-3, retained the intended factor structure and showed slightly better

internal consistency, cross-observer agreement, and readability (Flesch-Kincaid grade level = 5.3).

We then set out to address the issue of the psychometric characteristics and suitability of the NEO-PI-3 to a life span sample of individuals from young adulthood to very old age **(7).** Data from adults aged 21 to 91 showed that the NEO-PI-3 functions as well or better than the NEO-PI-R in adults. Age trends from combined adolescent (n = 500) and adult (n = 635) samples confirmed previous cross-sectional findings: N and E decline cross-sectionally with age, whereas A and C increase; O shows a curvilinear trend peaking at age 19. Facets generally followed the trend of the domain to which they are assigned, but considerable variation was found in trajectories in the O and especially E domains. O1: Fantasy shows a steep curvilinear decline beginning in adolescence, whereas O5: Ideas does not decline with age. Among E facets, E1:Warmth increases, perhaps because it has a secondary loading on A, while E5: Excitement-Seeking declines dramatically. E3: Assertiveness, on the other hand, has a curvilinear shape peaking in middle age.

In general, age trends for the facets also resemble those found in other cultures (McCrae & Costa, 2006). Thus, the NEO-PI-3 facet scales appear to show the same pancultural developmental trends as the NEO-PI-R facet scales.

Having performed well in an adolescent sample ranging from 14 to 20, we sought to examine the performance of the NEO-PI-3 in a younger sample of 449 boys and girls aged 12 to 13, who described themselves or a peer **(8).** Our aims were twofold: substantively, to help bridge personality levels in adulthood with their developmental antecedents in childhood and practically, to examine whether normative data from older adolescents can be used to interpret scores from middle-school-aged children. Analyses of readability, reliability, factor structure, and convergent and discriminant validity suggested that the NEO-PI-3 can appropriately be used in this age group. Personality traits in children of this age closely resemble in structure and functioning the traits of older adolescents and adults. Most gender differences known from studies of adults are found in this age group, and mean levels show continuity with older groups.

Developmental psychology is based on the assumption that psychological processes change both qualitatively and quantitatively with increasing maturation. With respect to personality traits, however, the basic operation

of traits is already in place by age 12, and perhaps much earlier. Thus, knowledge about how personality affects many outcomes, including psychological well-being, academic achievement, and problems in living, can probably be generalized from adults to children as young as 12. Hypotheses developed in studies of adults can thus be meaningfully tested in early adolescence. Concretely, the NEO-PI-3 appears to be a useful instrument for such research, and potentially for clinical applications, in middle school-aged children.

Applied Research: Stress, Coping and Psychopathology

Personality disorders and the FFM. The limitations of the current categorical framework found on Axis II of the DSM has prompted a voluminous amount of research into alternative models of personality disorders. As discussed in my introduction, there is now a DSM-V workgroup currently considering the FFM as such an alternative model. Broadly, this work represents a growing consensus that the DSM will adopt a dimensional model of personality disorders.

With colleagues at the Johns Hopkins University (Drs Nestadt, Samuels, Bienvenu, Reti, and Eaton), I have explored such dimensional models in a sample of 742 community-residing individuals who participated in the Hopkins Epidemiology of Personality Disorders Study **(9, 10).** The presence of DSM-IV personality disorder traits was assessed by psychologists using the International Personality Disorder Examination (IPDE). Five factors were retained as the dimensional accounting for the personality disorder criteria. These factors were named Compulsive, Neurotic Avoidant, Aloof, Impulsive Callous, and Egocentric. Of the five factors, 4 were associated with evidence of diminished functioning. We examined the correspondence of these five personality disorder dimensions to the normal personality factors of the Five-Factor Model (FFM). The 5 PD factors each exhibited small to moderate correlations with several NEO dimensions, most notably the positive association between Neurotic Avoidant and Neuroticism (γ=.47), and the negative associations between Aloof and Extraversion (γ=-.43) and between Impulsive Callous and Agreeableness (γ=-.33). This indicates that the factorial structure of the PDs demonstrates a modest degree of correspondence to the structure of the FFM. However, the five personality disorder factors did not show a pattern of convergent and divergent correlations to the FFM that indicated the 2 factor structures share the same space, and thus, the two factor

structures should not be considered as being the same. Together, the NEO domain and facet scores explained a fifth to a third of the variance in PD dimensions.

Work continues in the effort to replace Axis II in the DSM-V with a 4-step process of personality disorder diagnosis based on the FFM, detailed initially in Widiger et al (2002): (1) Provide description of the person's personality traits with respect to the 5 domains and 30 facets of the FFM; (2) Identify the problems, difficulties, and impairments that are secondary to each trait; (3) Determine whether the impairments are clinically significant and, if so, diagnose the FFM-related disorders; (4) Determine whether the constellation of FFM traits matches sufficiently the profile for a particular personality disorder pattern.

Step 2 in the system of Widiger et al. (2002) requires that there be a list of problems associated with the poles of each factor and facet that can be used to guide systematic inquiry. These lists were generated rationally: the authors considered each pole of each trait in turn and proposed problems they believed would be commonly found in people with this characteristic. These rational decisions were, of course, based on a clear conceptualization of each trait and on a familiarity with the voluminous empirical literature on trait correlates that has appeared over the past 20 years. However, even if the Widiger et al. catalogue is entirely correct, there is no evidence so far that it is comprehensive. It is possible that there are clinically significant problems related to personality traits that Widiger et al. simply overlooked. A number of researchers have attempted to develop lists of problems and psychiatric symptoms, and a comparison of their lists with that of Widiger et al. may give some idea of how complete the latter system is, and how it might be improved by the addition of new items.

We used five problem checklists: The Computerized Assessment System for Psychotherapy Evaluation and Research (CASPER; Farrell & McCullough, unpublished manual); The Couples Critical Incidents Checklist (CCIC; Piedmont & Piedmont, 1996); The Inventory of Interpersonal Problems (IIP-64; Horowitz, Alden, Wiggins, & Pincus, 2000); The Personal Problems Checklist for Adults (PPCA; Schinka, 1985); and The Shedler–Westen Assessment Procedure (SWAP-200; Shedler & Westen, 1998). A single rater (Corinna Löckenhoff), familiar with the descriptions of the factors and facets of the NEO-PIR provided in the manual (Costa & McCrae, 1992), examined all 663 items in the five

instruments. She first judged whether the item was relevant to personality or not, and then whether it was specific enough to be associated with a factor or facet. Eighty-one items such as 'being attacked by an animal' were discarded as not relevant to personality (12% of all items); 36 items such as 'acting in an immature way' were discarded as being too ambiguous to allow meaningful classification (five per cent of all items). Most of the items in all five inventories were, however, classifiable (CASPER, 85%; CCIC, 98%; IIP-64, 100%; PPCA, 61%; SWAP-200, 93%). A comparison of the newly classified items from the five instruments with the problems was made to those by Widiger et al. (2002). Overall, the number of additions is relatively small; this suggests that Widiger et al. did a reasonably thorough job of identifying personality-related problems. The updated list of personality-related problems could help to streamline clinical assessments by allowing clinicians to focus their questions towards areas in which clients are most likely to experience problems **(11).**

Personality predictors of Disease Progression in Long-Term Survivors of HIV/AIDS. Earlier findings showing that NEO-FFI Conscientiousness is related to increased CD4 counts and decreased viral load over a 1-year followup in a sample of long-term survivors of HIV/AIDS. We also presented preliminary findings on the five personality factors of the NEO-PI-R over a 2-year span, showing that Conscientiousness and Openness to Experience were related to CD4 count trajectory, and higher Extraversion was related to faster decreases in viral loads **(12).**

Since then, we have examined the sample of long-term survivors over a 4-year period and confirmed the role of Openness in CD4 counts, and Extraversion in viral load (12). We did not find Conscientiousness related to CD4 counts over this longer time interval, but it was related to decreased viral load. Since we used the NEO-PI-R, an examination of the facets was also possible. The Extraversion facets of assertiveness, positive emotions, and gregariousness, Openness facets of ideas, aesthetics, and the Conscientiousness facets of achievement striving and order were all related to slower disease progression.

We also examined circumplex combinations (profiles) of the five factors (taken two at a time), which hold the potential to reveal associations that would not be seen when considering the domains separately and have been associated with health behaviors above and beyond their component domains. We found that personality styles which

seem to underscore the importance of remaining engaged (e.g., Creative Interactors (Combination of high E and high O), Upbeat Optimists (low N and high E), Welcomers (high E and high A), Go Getters (high C and high E), and Directed (low N and high C)) had slower disease progression, whereas the "homebody" profile (low E and low O) was significantly associated with faster disease progress. These findings may help identify those individuals at risk for poorer disease course and specify targets for psychosocial interventions **(13).**

Personality self-reports are concurrently reliable and valid during acute depressive episodes. Under the complication or scar model of personality and depression, personality is frequently seen to be scarred by or result from the residual effect of depression. A related issue under the complication model is the frequent criticism that personality traits assessed in those with depression are distorted by the ongoing depressive process and thus do not accurately represent the individual's true traits. This is frequently referred to as the state artifact hypothesis.

To test the state artifact hypothesis that the depressive state distorts the assessment of general personality traits 109 depressed patients completed the NEO-PI-R at baseline and after 14 to 26 weeks of antidepressant pharmacotherapy. 48 patients (49.5%) were identified as responders while 49 (50.5%) were identified as nonresponders (14). The remaining 12 patients were excluded because they met response criteria using one depression instrument but not for another at treatment completion. At baseline, NEO-PI-R scales showed high internal consistency and replicated the normative factor structure, suggesting that psychometric properties were preserved. Among non-responders, retest correlations were uniformly high (rs=.50 to .88) and mean levels showed little change, providing evidence for the consistency of personality self-reports during an acute depressive episode. NEO-PI-R scales showed construct validity in the concurrent prediction of a number of clinical criteria. Effective treatment had significant effects on the mean levels of neuroticism, which decreased, and extraversion, openness, and conscientiousness, which increased.

The results suggest that the effect of acute depression is to amplify somewhat the personality profile of people prone to depression. Rather than regard these depression-caused changes in assessed personality trait levels as a distortion, we interpret them as accurate reflections of the current condition of the individual. Personality traits have biological

bases, and when they are changed (by disease or therapeutic interventions) trait levels change.

Personality and Body Mass Index (BMI). Given the epidemic proportions of obesity in the US, an important research direction concerns identifying the correlates and causes of obesity. Personality has been studied as a predictor of weight loss in clinically obese samples, but far fewer studies have examined personality-obesity relations in non-clinical samples. With collaborators Beverly Brummet and her colleagues from Duke University Medical Center, we hypothesized negative associations between Conscientiousness and BMI across both genders, and gender-modified effects for Neuroticism and Extraversion. Personality predictors of Body Mass Index (BMI) and its change over a 14-year period were examined in a longitudinal sample of middle-aged community-residing participants **(15).** The personality domain of Neuroticism was positively related to average BMI, while Openness, Agreeableness, and Conscientiousness were negatively related. Relations for three domains were modified by gender. N was significantly related to BMI in females only. Extraversion (E) was positively related to BMI in males, whereas, this relation was non-significant in females. The relation between C and BMI was significant in males and females, however, the magnitude of the negative association was stronger in females. C also predicted change in BMI during midlife such that participants who were lower in C tended to show larger gains in BMI with age.

Personality and pathological gambling. Pathological gambling is recognized as an impulse-control disorder in the DSM-IV, and is characterized by "maladaptive gambling behavior that disrupts personal, family, or vocational pursuits". Past research linking personality to pathological gambling has been inconsistent, with some studies reporting a link to impulsivity, sensation-seeking, and related traits while other studies have not found this association. The goal of this investigation with collaborators from the Centre for Mental Health and Addiction (CAMH) in Toronto, Canada, was to examine the personality differences between non-treatment seeking pathological gamblers (PGs) and non-pathological gamblers (NPGs) using the domain and facet traits of the FFM, as measured by the NEO PI-R. Compared to NPGs, PGs scored significantly higher on the neuroticism domain and significantly lower on the conscientiousness domain **(16).** Significant differences between

PGs and NPGs also emerged for three of four FFM facet traits associated with impulsivity, with PGs scoring higher on impulsiveness and lower on self-discipline and deliberation facets. Both PGs and NPGs had equally high scores (relative to the norm) on excitement-seeking, the fourth facet associated with impulsivity, suggesting that excitement-seeking characterizes gambling behavior rather than pathological gambling. These findings suggest that the overall personality profile of the PG is one that combines high impulsivity with emotional vulnerability. Importantly, the results also suggest that excitement-seeking, a personality construct akin to sensation-seeking, may not be a specific marker of PG but rather a characteristic common to all those who gamble.

The Origins of Personality: Molecular Genetics
Genetic Architecture of Personality.

The Five-Factor Theory (FFT) posits the personality traits of the FFM as biologically-based basic tendencies. To explore this biological basis, we examined the heritability of NEO-PI-R domain and facet traits in a sample of 5,669 Sardinians **(17).** Across all 35 domains and facets, genetic effects explained 19% of the variance, ranging from 9.4% for the Agreeableness facet Tender-Mindedness to 32.8% for Openness to Experience factor. This demonstrates clearly a genetic, biological component to the FFM dimensions.

Moreover, we examined the genetic correlation coefficients between personality traits to better understand the genetic factor structure of the personality traits. In order to assess the similarity of this genetic factor structure to the American normative phenotypic structure, we rotated the obtained genetic structure toward normative structure and obtained factor and facet congruence coefficients. All factors had high congruence coefficients with the American normative structure (>.85), indicating that the genetic structure replicates the phenotypic structure well. This analysis suggests that the phenotypic covariation of personality traits is genetically rooted, or in other words, that shared genes underlie the facets that define each factor. This genetic underpinning of the five-factor structure explain the consistent finding of a –universal– five factor structure in samples from adolescents to old adults, male and female, general and clinical samples, and most remarkable, in sample from cultures around the world.

Taken together, the heritability estimates confirm the genetic roots of each

personality trait and the genetic structure work points to a biological basis for the universal five-factor structure of personality

Genetics of personality in Sardinia

The SardiNIA sample is a genetically homogeneous sample (N = 5,669) from the Ogliastra, an isolated region within Sardinia, Italy, and constitutes a founder population. The limited immigration and intermarriage with outside groups make founder populations like this one genetically more homogeneous or uniform, compared to general outbred populations. Given the genetic basis of personality, we may expect variability in personality to also be lessened.

The Italian version of the Revised NEO Personality Inventory showed good psychometric properties: Internal consistency reliabilities ranged from 0.80 to 0.87; the factor structure replicated the American normative structure; and associations with education and gender replicated cross-cultural patterns. The hypothesis that mean trait levels in the Sardinian founder population would differ from mainland Italian values was not supported. Phenotypic variation in this founder population was within the range found in other cultures. However, the hypothesis of restricted phenotypic variation was supported for all five factors and 28 of the 30 facets when a Sardinian subsample matched on age, sex, and education was compared to a mainland Italian sample (18). Given this result, the genetic homogeneity effect on the phenotypic expression of complex traits merits further exploration.

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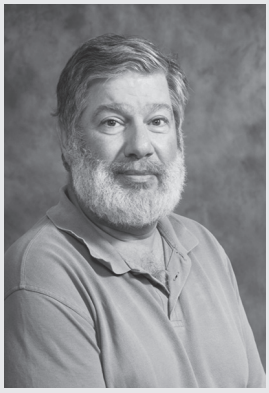

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Dotson VM, et al. Am J Geriatr Psychiatry 2008; 16: 318-330.

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. An important effort of research in the Cognition Section is focused on longitudinal research in the Baltimore Longitudinal Study of Aging (BLSA) and the Healthy Aging in Neighborhoods of Disparity across the Life Span (HANDLS) study. 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. HANDLS was initiated in 2004. The first follow-up examinations began in 2009.

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 that 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. Other effects include symptoms of depression, hypertension or other cardiovascular effects, and cerebrovascular effects such as stroke.

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.

In a follow-up to our findings that elevated systolic blood pressure was associated with poorer cognitive test performance on tests of confrontational naming and nonverbal memory, we examined the relation of cognitive performance with arterial stiffness2 measured by pulse pressure and pulse wave velocity. In a sample of 1,749 non-demented and stroke-free participants from the Baltimore Longitudinal Study of Aging with as many as 8 repeated cognitive assessments over 14 years, we found that increasing pulse pressure was associated with poorer performance on tests of verbal learning, nonverbal memory, working memory, a cognitive

screening measure. A subset of 582 participants had a single baseline measure of pulse wave velocity and as many as 6 repeated cognitive assessments over 11 years. Higher baseline pulse wave velocity was associated prospective decline on tests of verbal learning and delayed recall, nonverbal memory, and a cognitive screening measure.

Association of depressive symptoms with cognitive decline. In addition to physiological associations with age-associated cognitive changes, we also examined the association of depressive symptoms with cognition in participants of the Baltimore Longitudinal Study of Aging. This study investigated the effect of concurrent, baseline, and average depressive symptoms on cognitive functioning and decline, and examined the interactive effect of age and depressive symptoms on measures of learning and memory, attention and executive functions, verbal and language abilities, visuospatial functioning, and general cognitive status. Increased depressive symptoms were associated with poor cognitive functioning and cognitive decline in multiple domains. Concurrent, baseline, and average depressive symptoms had differential associations with cognition. Average depressive symptoms, a measure of chronic symptoms, showed the most widespread effects on cognitive abilities. Effects of depressive symptoms on some frontal functions were greater with advancing age. The results suggest that depressive symptoms are associated with poor cognitive functioning and cognitive decline, particularly with advancing age. The widespread impact of average depressive symptoms on cognition suggests that clinicians should consider depressive symptoms when evaluating cognitive functioning in older adults.

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.

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.

Clinical significance of Alzheimer's disease neuropathology.

Alzheimer's disease pathology is present in many individuals with no symptoms of dementia or cognitive deficits before death. The implications of this pathology are unknown. It either signifies the early presence of disease that fails to manifest behaviorally in cognitive performance. Or, it represents normal aging and is not disease, per se. If the former, these individuals would have eventually manifested symptoms of dementia had they lived longer. If the latter, our interpretation of Alzheimer's pathology may be incomplete. Using data from the Baltimore Longitudinal Study of Aging Autopsy Study, we examined participants who underwent prospective neuropsychological and neurological evaluations before autopsy. These participants and their families agreed to autopsy following death. They were followed with annual home examinations when they were unable to return to the National Institute on Aging. Our main objective was to determine whether cognitive trajectories differed between clinically normal participants with and without Alzheimer's disease neuropathology. In addition, we investigated whether clinically impaired individuals showed accelerated rates of cognitive decline compared with clinically normal elderly adults. We found that clinically normal elderly individuals with and without AD neuropathology had similar cognitive trajectories across several cognitive domains. In contrast, individuals with mild cognitive impairment or clinical Alzheimer's disease had steeper rates of longitudinal decline in several domains of cognition compared with clinically normal elderly individuals regardless of whether the latter had Alzheimer's neuropathology. Moreover, the cognitive differences between impaired and unimpaired groups could be detected years before a diagnosis of dementia. These results suggest that clinically normal individuals with and without Alzheimer's neuropathology do not differ in rates of cognitive decline across a number of cognitive domains.

Socioeconomic Status and Race: Little is known about the risks and rates of cognitive change as a function of socioeconomic status and race, particularly the extent to which health disparities moderate these relationships. We have initiated a new study, Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS). HANDLS is a multidisciplinary, prospective epidemiologic longitudinal study with which we hope to disentangle the relationships among race, socioeconomic status, and health outcomes. The study examines whether race and socioeconomic status influence health disparities in cardiovascular health, cerebrovascular health, and change in cognitive performance over time. HANDLS deploys a novel data collection

paradigm by using mobile medical research vehicles. These vehicles serve as community-based platforms for clinical research, and we use them as tools for creating effective methods for recruiting and retaining non-traditional research participants into age-related clinical research. Several pilot studies demonstrated the utility of using mobile medical research vehicles in the community. Baseline data collection began in 2004. The first follow-up examinations began in 2009.

Collaborators: Richard O'Brien, M.D., Johns Hopkins School of Medicine; Shari Waldstein, Ph.D., University of Maryland Baltimore County.

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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 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. Based on findings from these studies, she initiated the Women's Health Initiative Study of Cognitive Aging (WHISCA), an ancillary study to the Women's Health Initiative Memory Study (WHIMS) and the WHI randomized trials of the effects of hormone therapy.

Keywords:

memory aging
Magnetic Resonance Imaging
Positron Emission
Tomography
estrogen and cognition

Recent Publications:

Beason-Held LL, et al. *Neurobiol Aging* 2008; 29: 483-496.

Beason-Held LL, et al. *Neurobiol Aging* 2008; 29: 497-513.

Davatzikos C, et al. *Neurobiol Aging* 2008; 29: 514-523.

Fan Y, et al. *Neuroimage* 2008; 41: 277-285.

Sojkova J, et al. *J Nucl Med* 2008; 49: 1465-1471.

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. Since 2005, we have also been using PET scanning with 11-C-Pittsburgh Compound B (PIB) to measure the in vivo distribution of amyloid. A variety of risk and protective factors for cognitive impairment and dementia also are investigated.

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 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 have completed as many as 10 annual assessments.

Publications-continued
Moffat SD, et al. *Neurobiol Aging* 2007; 28: 914-920.

Maki PM et al. J Clin *Endocrinol Metab* 2007; 92: 4107-4114.

Resnick SM, et al. *Ann N Y Acad Sci* 2007; 1121: 562-575.

The specific goals of this study are: to determine the rate of brain changes with age, including increases in brain atrophy, ischemic/demyelinating white matter abnormalities, and amyloid deposition; 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.

Selected highlights of our publications to date are:

- The development of the RAVENS (Regional Analysis of Volumes Examined in Normalized Space) volume-preserving framework for voxel-based analysis of MR volumetric data. Goldszal et al. *Journal of Computer Assisted Tomography* 1998;22:827-837.

- Age differences in both gray and white matter volumes and one-year increase in ventricular volumes. Resnick et al. *Cerebral Cortex* 2000;10:464-472.

- Longitudinal declines over 4 years in gray and white matter volumes, even in healthy older adults. Resnick et al. *Journal of Neuroscience* 2003;23:3295-3301.

- Cross-sectional and longitudinal age effects on gray-white matter tissue contrast and local white matter signal intensity changes, indicating age changes in qualitative characteristics of tissue composition. Davatzikos and Resnick. *Cerebral Cortex* 2002;12:767-771.

- Sex differences in morphology of the corpus callosum and in patterns of correlations with cognitive performance. Davatzikos and Resnick. *Cerebral Cortex* 1998;8:635-640.)

- Faster rate of longitudinal hippocampal volume loss in Apolipoprotein E (APOE) ε4 carriers compared to non-carriers. Moffat et al. *Neurology* 2000;55:134-136.

- Longitudinal changes in aging brain function and their temporal patterns. Beason-Held et al, 2008a,b.

- Use of spatial patterns of abnormality for recognition of early Alzheimer's disease (SPARE-AD index) using MRI (Davatzikos et al, 2008) and PET (Fan et al, 2008) images.

- Association of amyloid deposition, measured with PET and 11-C-PIB, and longitudinal changes in regional cerebral blood flow. Sojkova et al, 2008.

Effects of Hormones on Cognitive Decline:

Postmenopausal Hormone Therapy: A major focus of our research program is the investigation of the potential modulatory role of hormone therapy (HT) 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 HT 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, HT appeared to protect against age-associated decline in memory. We have also compared HT users and nonusers who participate in our longitudinal imaging study. HT users and nonusers showed significant differences in the patterns of brain activation during the performance of memory tasks, and HT 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.

These findings, suggesting possible beneficial effects of HT in maintaining cognitive function, are challenged by the recent report from the Women's Health Initiative Memory Study (WHIMS) showing that daily doses of combination estrogen plus progestin doubled the risk for dementia in women randomized to receive HT after age 65. However, WHIMS did not address the effects of hormone treatment on specific cognitive

functions. To address this question, we initiated an ancillary study to the WHIMS and WHI in collaboration with the WHIMS investigators. This study, the Women's Health Initiative Study of Cognitive Aging (WHISCA), examined the effects of hormone treatment (combination conjugated equine estrogens CEE plus medroxyprogesterone acetate (MPA) (in women with a uterus and CEE only in women without a uterus) on longitudinal change in memory and other cognitive functions within the context of the large randomized intervention trial. The WHISCA findings in older women who had been randomized to HT were generally consistent with the WHIMS report of poorer cognitive function in women randomized to HT versus placebo. We found significantly poorer verbal memory over time in women randomized to CEE+MPA compared with placebo and a nonsignificant trend toward better performance on a figural memory test (Resnick et al, 2006).

Testosterone and Cognition: We found that an index of endogenous free testosterone was associated with performance on specific cognitive tasks in older men. Higher free testosterone index (FTI) was associated with better performance on tests of verbal and figural memory and attention, even after adjusting for age and medical conditions that influence endogenous testosterone levels (Moffat et al, 2002). We found that lower FTI was also associated with an increased risk for Alzheimer's disease (Moffat et al, 2004) and with increased regional cerebral blood flow in brain regions critical for memory and attention (Moffat and Resnick, 2007). Interestingly, these associations were not found for total testosterone and were specific to the FTI, which is more closely related to bioavailable testosterone and the fraction that may actually reach the brain to influence central nervous system functioning.

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 5 years of the study indicate substantial changes in brain volumes and ventricular CSF, but little overall cognitive change. 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 hormone therapy, and circulating hormones (DHEA, testosterone, cortisol) are being investigated as potential modulators of the relationship between brain and neuropsychological changes. The neuroimaging study will be 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. Through WHISCA, we will continue to investigate the effects of postmenopausal hormone treatment on specific cognitive function.

Collaborators: Christos Davatzikos, Ph.D., Dinggang Shen, Ph.D., University of Pennsylvania; Michael Kraut, M.D., Ph.D., Jerry Prince, Ph.D., Johns Hopkins University; Sally Shumaker, Ph.D., Steve Rapp, Ph.D., Mark Espeland, Ph.D., Wake Forest University; Alan Zonderman, Ph.D., NIA.

Brain Physiology and Metabolism Section

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The **Brain Physiology and Metabolism Section (BPMS)** of the NIA is located at the Bethesda Campus of the NIH. The Section's goals are to develop original *in vivo* animal models and *in vitro* cell models to understand and quantify dynamic aspects of brain lipid metabolism under normal conditions and in relation to aging, disease, diet and drug action, and to extend findings from these studies to examine human brain lipid metabolism in health and disease. Methods involve *in vivo* radiotracer techniques, neuropharmacology, mathematical modeling, chemical analytical procedures, molecular biology, analysis of enzyme activities and expression, microarray analysis, and quantitative autoradiography in rodent studies, as well as positron emission tomography (PET) in human studies. Animal models include unanesthetized wild type and genetically modified rodents. Studies are designed to elucidate neuroplasticity, neuroinflammation, and excitotoxicity, and *in vivo* brain signal transduction involving arachidonic and docosahexaenoic acids and the roles of enzymes that regulate their brain metabolism. Mechanisms of action and therapeutic effects of mood stabilizers and antidepressants, and of antipsychotic, anti-inflammatory and anti-excitotoxic agents are addressed, with an aim to develop new agents for treating patients with bipolar disorder and Alzheimer disease and for neuroimaging. The **Molecular Neuroscience Unit** of the BPMS, directed by Dr. Francesca Bosetti, focuses on cyclooxygenase (COX)-1 and COX-2 and their downstream signaling pathways in regulating brain function and resistance to inflammatory insults, and on other rodent models of neuroinflammation, as targets for drug development in treating neurodegenerative diseases with an inflammatory component. COX-1 and COX-2 knockout and other transgenic mouse models are studied to this end.

Why Study *In Vivo* Brain Lipid Metabolism? Phospholipids, their component polyunsaturated fatty acids (e.g., arachidonic and

docosahexaenoic acids) and fatty acid metabolites (eicosanoids and docosanoids) play critical and dynamic roles in brain development, aging and disease. They participate in signal transduction, synaptic membrane remodeling, gene transcription and brain blood flow, and can modify cognition and behavior. Phospholipid metabolism is abnormal in a number of human brain diseases, including stroke and vascular dementia, Parkinson and Alzheimer disease, multiple sclerosis, acquired immunodeficiency syndrome (AIDS), dementia, and bipolar disorder. Thus, having methods to quantify and image different aspects of lipid metabolism in animal models and humans, in relation to the enzymes and receptors that modify this metabolism, could help to elucidate and localize active roles of lipids in normal and diseased brain, and to develop appropriate treatments for disease states.

Collaboration and Communication

Scientists in the BPMS work on related and collaborative projects and are encouraged to generate new and relevant ideas, to test them, and to extend them for clinical studies. They present their plans and ongoing results to each other at weekly meetings. Collaboration is extensive with scientists at the NIH and at universities. Participation in national and international scientific meetings is frequent, and the publication rate of BPMS in high quality peer-reviewed journals is high.

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Biography: Dr. Rapoport received his M.D. from Harvard Medical School, was an Intern 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), Bethesda. He then was appointed as a tenured scientist at the NIMH, and later was named Chief of the Laboratory of Neurosciences, NIA. He currently is Chief of the Brain Physiology and Metabolism Section, NIA. He is a Fellow of the American College of Neuropsychopharmacology, the American Academy of Neurology, the American Society of Neurochemistry, and the Gerontological Society of America, and has received numerous honors.

Keywords:

arachidonic
lithium
bipolar disorder
brain
phospholipid metabolism
positron emission
tomography (PET)
diet
docosahexaenoic
phospholipase
Alzheimer disease
neuroimaging
autoradiography

Recent Publications:

Esposito G, et al. *J Nucl Med* 2008; 49: 1414-1421.

Igarashi M, et al. *J Lipid Res* 2008; 48: 152-164.

Lee HJ, et al. *J Lipid Res* 2008; 49: 162-168.

Rao JS, et al. *Mol Psychiatry* 2008; 13: 585-596.

Basselin M, et al. *J Neurochem* 2007; 102: 761-772.

Research Program

Phospholipids and their component polyunsaturated fatty acids (PUFAs), including n-6 arachidonic and n-3 docosahexaenoic acids, play critical and dynamic roles in brain development, aging and disease. They participate in neurotransmission, synaptic membrane remodeling, gene transcription and cerebral blood flow, and form cell membranes. Their metabolism is abnormal in a number of human brain diseases, including Alzheimer disease and bipolar disorder. These abnormalities may be related to the underlying pathophysiological processes of neuroinflammation and excitotoxicity, and be influenced by dietary PUFA composition as well as to liver PUFA metabolism. Thus, having methods to quantify, characterize or image different aspects of brain lipid metabolism in awake animals and in humans, and to examine their liver metabolism, could help to elucidate and localize active roles of lipids in normal and diseased brain, and to develop new therapies for disease states.

Dr. Rapoport's research group has developed such methods and is applying them to examine brain phospholipid and PUFA metabolism with regard to aging, drugs, diet and disease. One focus of his work concerns the effects drugs and bipolar disorder on brain lipid metabolism. Bipolar disorder affects 1-2% of the US population and has a 20% life-time incidence. Dr. Rapoport's group studied effects of lithium, valproic acid, carbamazepine and lamotrigine, mood stabilizers approved for treating bipolar disorder, whose mechanisms of action are not agreed on. When given chronically to rats, they showed that these agents reduced AA turnover in brain membrane phospholipids and downregulated the

Publications-continued

Rao JS, et al. *Mol Psychiatry* 2007; 12: 36-46.

Bhattacharjee AK, et al. *Neuroimage* 2007; 37: 1112-1121.

brain cytosolic phospholipase A2 (cPLA2) and cyclooxygenase (COX)-2 enzymes that regulate AA turnover, without affecting DHA metabolism or DHA metabolic enzymes. This common targeting by the mood stabilizers suggested that AA metabolism is elevated in bipolar disorder mania, and Dr. Rapoport's group is testing this hypothesis directly by examining postmortem bipolar brain. They also are showing that antidepressants that switch bipolar depressed patients to mania upregulate rat brain AA metabolism, consistent with the AA targeting hypothesis, and are examining effects of psychotic agents. Their in vivo rat method can be used to screen for potential antimanic agents that downregulate rat brain AA metabolism. Dr. Rapoport's group also has developed two animal models of upregulated brain AA metabolism that can be used for drug testing, an neuroinflammatory model caused by infusing bacterial lipopolysaccharide into the rat cerebral ventricles for 6 days, and an excitotoxicity model caused by daily administration to rats of the glutamatergic receptor agonist, NMDA.

With regard to diet and brain, Dr. Rapoport's group reported that 15 weeks of dietary n-3 PUFA deprivation in adult rats induced bipolar disorder-like aggression and depression, and upregulated brain AA metabolism and AA metabolic enzymes (cPLA2 and COX-2), suggesting a mechanism whereby dietary n-3 PUFA deprivation might exacerbate bipolar disorder. They also showed the rat liver is capable of synthesizing sufficient DHA to maintain brain DHA homeostasis, provided adequate DHA precursor, α-linolenic, is in the diet; they intend to test this conclusion in the clinic.

AA and DHA can participate as second messengers that in cholinergic, dopaminergic, serotonergic and glutamatergic neurotransmission. Dr. Rapoport's group use a quantitative autoradiographic neuroimaging method in awake rats that they developed to demonstrate how and in what brain regions AA and DHA participate in signaling, in response to pharmacological agents acting at neuroreceptors and reuptake transporters. They used this method to characterize AA involvement in dopaminergic signaling in a rat model of Parkinson disease, and are extending it to image AA and DHA signaling in the human brain with positron emission tomography (PET). With PET, they imaged the involvement of AA signaling in human volunteers during visual activation, and demonstrated increased brain AA metabolism in patients with Alzheimer disease, consistent with their finding upregulated AA metabolism in their rat model of neuroinflammation (see above),. PET now may be used to image PUFA signaling in the human brain, in relation to functional activation, diet, drugs, aging and disease.

Collaborators: Dennis Murphy M.D., National Institute of Mental Health, NIH; Joseph Deutsch Ph.D., School of Pharmacy, Hebrew University, Jerusalem, Israel; Richard Carson Ph.D., Department of PET Imaging, Yale University, New Haven, Connecticut; Margaret Weis Ph.D., School of Pharmacy, Texas Tech University, Amarillo, Texas; Mark Hallet M.D., National Institute of Neurological Disease and Stroke, NIH; Peter Herscovitch M.D., PET Department, CC, NIH; Robert C. Murphy Ph.D., Department of Pharmacology, University of Colorado Health Sciences Center, Aurora, CO; Richard Bazinet Ph.D., Department of Nutritional Sciences, University of Toronto, Toronto, Canada; Gene Alexander Ph.D., Clinical and Cognition & Neural Systems Programs, University of Arizona, Tucson, AZ.

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Biography: Dr. Bosetti received her Pharm.D. from the University of Pisa, Italy in 1996 and her Ph.D. in Molecular and Experimental Medicine in 2000 from "Sant' Anna School of Advanced Studies", Italy. She joined the National Institute on Aging as a tenure-track investigator in the Brain Physiology and Metabolism Section in 2004, where she is now the Head of the Molecular Neuroscience Unit. Her unit is investigating the involvement of arachidonic acid metabolism in inflammatory and excitotoxic brain damage. Dr. Bosetti is an associate editor of *Lipids*.

Keywords:

brain
cyclooxygenase
arachidonic acid
prostaglandin
excitotoxicity
neuroinflammation

Recent Publications:

Bonow RH, et al. *Pharmacogenomics J* 2008; in press.

Toscano CD, et al. *Neurotoxicology* 2008; 29: 1114-1120.

Aid S, et al. *J Neuroinflammation* 2008; 5: 17.

Toscano CD, et al. *Brain Res Bull* 2008; 75: 598-609.

Choi SH, et al. *FASEB J* 2008; 22: 1491-1501.

Bosetti F *J Neurochem* 2007; 102: 577-586.

Toscano CD, et al. *Genome Biol* 2007; 8: R14.

Research Interests: The focus of my research is to identify the role of brain arachidonic acid (AA) metabolism in animal models of neuroinflammation, excitotoxicity, and neurodegenerative diseases. The AA cascade involves the release of AA from membrane phospholipids by a phospholipase A_2 (PLA_2), and its subsequent conversion *via* cyclooxygenase (COX-1 and COX-2), lipoxygenases and cytochrome P450 epoxygenase to bioactive eicosanoids.

Glutamate neurotransmission, inflammatory mediators, and oxidative stress are increased in acute and chronic neurological and neurodegenerative diseases, as well as in normal aging. While the exact sequence of events that culminate in neuronal death are unknown, a better understanding of the genetic characteristics and molecular mechanisms that trigger excitotoxic and inflammatory cell death may offer therapeutic strategies for such disorders. The research goal of our group is to elucidate the role of the arachidonic acid cascade in the mechanism of neuroinflammation and neurodegeneration using knockout and transgenic mice models.

I. Role of Cyclooygenases in Excitotoxic Brain Injury: Release and metabolism of AA through the phospholipase A_2 (PLA_2)/ COX pathway is increased during excitotoxicity, a process that involves the over activation of brain excitatory neurotransmission. Excitotoxicity is thought to contribute to the progression of certain neurological, neurodegenerative, and psychiatric diseases. Investigations into the mechanism of excitotoxicity have suggested a role for COX, the primary target for the widely used non-steroidal anti-inflammatory drugs (NSAIDs). In fact,

selective inhibition or genetic deletion of COX-2 augments kainic acid (KA)- and N-methyl D-aspartate (NMDA)-induced seizure intensity and neuronal damage. In contrast, lindane, a persistent organic pollutant that indirectly causes seizures by antagonism of the inhibitory $GABA_A$ receptor, did not cause a different seizure response in COX-2 null mice compared to wild type mice. Our findings demonstrate that COX-2 regulates susceptibility to KA and NMDA excitotoxicity, which directly activate glutamatergic neurotransmission, but not to lindane, which indirectly alters glutamatergic neurotransmission. Furthermore, increased levels of prostaglandins after seizures are associated with consistent evidence of neuronal damage.

II. Changes in Gene Expression after Lipopolysaccharide-Induced Neuroinflammation: Neuroinflammation is a key component in the progression of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis. It remains to be elucidated whether inflammation is either the cause or the effect of the neuropathological changes associated with the diseases. Lipopolysaccharide (LPS) is a component of the bacterial wall of Gram negative bacteria, and is commonly used to induce inflammation in the periphery and in the central nervous system. It stimulates the innate immune system through interactions with CD14 and the toll-like receptor 4 (TLR4), which are expressed by microglia, the primary immunocompetent cells of the brain. Acute administration of LPS directly into the brain leads to neuronal death in the hippocampus. Moreover, behavioral studies have shown that object recognition and spatial memory are both impaired in animals treated with single doses of LPS. In line with these cognitive deficits, long-term potentiation (LTP) is largely blocked following acute LPS injections. To characterize the transcriptional changes that underlie these behavioral effects, we examined changes in gene expression in the mouse hippocampus and cerebral cortex 24 hours after a single intracerebroventricular (icv) injection of LPS using microarray technology.

Gene set analysis showed that gene ontology (GO) terms for inflammation, cytokine activity, chemokine activity, and cytoskeletal reorganization, were significantly enriched 24 hours following the injection, whereas GO terms associated with nervous system development, neuron migration, synaptogenesis, and learning and memory showed decreased expression. Using strict criteria for individual genes, we detected 224 changed transcripts in the cortex and 170 in the hippocampus. Among these,

expression of early growth response 1 (Egr1, also Zif268) and activity regulated cytoskeletal protein (Arc) mRNA were significantly lower in the cortex of LPS-treated animals. These effects are of interest because the protein products of Egr1 and Arc are induced during, and are required for, the consolidation of memory; reduced expression of these genes therefore may underlie some of the electrophysiological, behavioral, and cognitive changes observed in experimental neuroinflammation and in diseases with a marked neuroinflammatory component.

III. Role of Cyclooxygenases in Neuroinflammation: Microglia plays a pivotal role in the brain under normal and pathological conditions to maintain neuronal function. The activation of microglia may be deleterious, because they secrete pro-inflammatory cytokines, chemokines, and eicosanoids, all of which may exacerbate the pathology. In addition, in vivo and in vitro data have shown that activated microglia, upon activation of NADPH oxidase, produce reactive oxygen species (ROS), which may induce or exacerbate neurotoxicity by causing oxidative stress to neurons. COX-1 and -2 enzymes play a central role in the inflammatory cascade by converting AA to bioactive prostanoids. Therefore, it is important to determine the involvement of each isoenzyme in the mechanisms of neuroinflammation and neurodegeneration. To directly address the role of cyclooxygenase (COX)-1 and -2 in mediating neuroinflammatory responses and blood-brain barrier disruption, we assessed the effect of icv injection of LPS in COX-1 or -2 null and wild type (WT) mice, and in mice treated with specific COX-1 or COX-2 inhibitors. LPS specifically activates immune response in the central nervous system through a Toll-like receptor 4-dependent signaling pathway.

We showed that COX-1 null mice have a significant reduction in brain inflammatory response and oxidative damage after LPS. The protection was attributed to attenuation of microglial activation, a critical process in the initiation of inflammation, and to a reduction of inflammatory mediators such as PGE_2, IL-1β, TNF-α, and of protein oxidation, critical factors contributing to the secondary progression of the inflammatory reaction and oxidative damage. Translocation and activation of nuclear factor-κB (NF-κB) and signal transducer s and activators of transcription 3 (STAT3), important factors for signaling events during an inflammatory response, were also reduced in COX-1 KO mice. Administration of SC-560, a specific COX-1 inhibitor, prior to LPS injection, also attenuated the neuroinflammatory response by decreasing brain levels of prostaglandins, as well as the expression of pro-inflammatory cytokines and chemokines.

In contrast, LPS-induced expression of pro-inflammatory cytokines and reactive oxygen species-generating enzymes, such as iNOS and NADPH oxidase, was increased in COX-2 null compared to WT mice. Mice treated for 6 weeks with celecoxib, a selective COX-2 inhibitor, prior to LPS also exhibited higher brain levels of IL-1β and $p67^{phox}$, compared to untreated wild type mice. Since chemokines are involved in the trafficking and the recruitment of leukocytes into the inflamed brain, we are investigating blood-brain barrier integrity in COX-1 and COX-2 null mice, using quantitative magnetic resonance imaging. Our results suggest that COX-1 plays an important role in the regulation of microglial inflammatory responses in the central nervous system and that COX-1 inhibition might be beneficial to reduce the effects of neuroinflammation and related oxidative stress. In contrast, selective COX-2 inhibition may worsen neuroinflammatory responses, suggesting a neuroprotective function of COX-2 derived products and that selective inhibition of COX-2 is not beneficial in neurodegenerative disease with a marked inflammatory component. A full understanding of the physiological, pathological, and/or neuroprotective roles of COX isoforms may help to develop better therapeutic strategies for the prevention or treatment of neurodegenerative diseases with a marked inflammatory component.

Collaborators: Robert Langenbach, National Institute of Environmental Health Sciences, NIH; Kevin Becker, Research Resources Branch, National Institute on Aging, NIH; Afonso Silva, National Institute of Neurological Disorders and Stroke, NIH; Sharon Jackson, National Institute of Allergy and Infectious Diseases, NIH; Stefano Vicini, Georgetown University School of Medicine; Scott Turner, Georgetown University School of Medicine; Lawrence Marnett, Vanderbilt University School of Medicine; Eduardo Candelario-Jalil, University of New Mexico.

Molecular Dynamics Section

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The Molecular Dynamics Section (MDS) focuses on the interplay between structure and dynamics and how these influence biological function. The section is presently involved in studying the structural and dynamic factors in hemoglobin which regulate the binding of oxygen, the uptake and release of nitric oxide as well as autoxidation with its associated release of superoxide. The finding that autoxidation of hemoglobin is appreciably enhanced at reduced oxygen pressures, has lead to the proposal of a novel method for producing oxyradicals under hypoxic conditions. Studies are being performed on erythrocytes, interaction of erythrocytes with other tissues and with whole animals to determine to what extent this mechanism contributes to the pathophysiology of aging.

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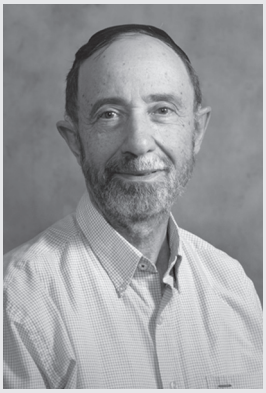

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Biography: Dr. Joseph M. Rifkind received his Ph.D. in Physical Chemistry from Columbia University in 1966. He obtained postdoctoral training in protein chemistry at the University of Minnesota and joined the Gerontology Research Center of what was then part of the 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
nitric oxide

Recent Publications:

Mohanty JG, et al. *Adv Exp Med Biol* 2008; 614: 29-35.

Kiefmann R, et al. *Blood* 2008: 111: 4205-4214.

Nagababu E, et al. *Blood Cells Mol Dis* 2008; 41: 60-66.

Nagababu E, et al. *Biochemistry* 2007; 46: 11650-11659.

Rifkind JM, et al. *Nitric Oxide* 2007; 16: 448-456.

Nagababu E, et al. *Free Rad Biol Med* 2007; 42: 1146-1154.

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. The red cell is responsible for the transport of oxygen through the circulatory system and the delivery of oxygen to the tissues. In the red cell, oxygen is reversibly bound to Fe(II) of hemoglobin with molecular oxygen released at reduced oxygen pressure. However, both oxygen and iron can undergo oxidative and reductive processes with the Fe(II) oxidized to Fe(III) and Fe(IV), while oxygen can be reduced to superoxide, hydrogen peroxide and hydroxyl radicals. The ramifications of these oxidative reactions in red cells have been the focus of the Molecular Dynamics Section.

A multipronged approach to red cell oxidative stress has been employed directed at understanding the source of this oxidative stress and its physiological ramifications. (1) We have investigated 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. (2) We have been 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. (3) Impaired red cell deformability found to be induced under hypoxia is also associated with subject aging. We are very interested in understanding altered deformability in the aged as well as other decrements in blood rheology. Our studies suggest a link with oxidative stress which could originate in hypoxic induced oxyradical production. Recent results indicate greater oxidation in venous blood than arterial blood confirming the production of oxyradicals as blood passes through the capillary bed at reduced oxygen pressures. The physiological ramifications of red cell oxidative stress are currently being investigated by probing physiological effects that result from injecting into an animal blood containing red cells unable to deal with oxidative stress.

We have recently expanded our studies of the detrimental red cell oxidative processes into two areas. (1) We have extended our understanding of the red cell oxidative processes and how hemoglobin–membrane interactions contribute to red cell oxidative processes by bypassing the cellular protective mechanisms. In the course of these studies, we have studied the secondary oxidative processes, which irreversibly damage the heme, and used the damaged high-spin rhombic heme and fluorescent degradation products as markers for the extent of red cell oxidative processes. (2) We have initiated a program directed at investigating the possibility that red cell interactions with amyloid fibrils may contribute to the toxicity of these fibrils and the pathophysiology of Alzheimer's disease.

At the same time, we have initiated a new program to investigate the relationship between hemoglobin oxidation and the role of the red cell in regulating nitric oxide delivery to the vasculature. This program has identified an important reaction between deoxygenated hemoglobin and nitrite that produces a labile reactive form of nitric oxide, which can improve the flow of blood through the microcirculation.

Collaborators: P.T. Manoharan, Ph.D., Indian Institute of Technology, Madras, India; Avraham Mayevsky, Ph.D., Bar Ilan University, Israel; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Jerome Fleg, M.D., National Heart, Lung, and Blood Institute, NIH; Donald Ingram, Ph.D., Pennington Biomedical Research Center, Baton Rouge, LA. Harry Silber M.D., Ph.D., Johns Hopkins University; Ryszard M. Pluta, National Institute of Neurological Disorders and Stroke; Jahar Bhattacharya, Columbia University; Mary E Fabry, Albert Einstein School of Medicine.

Clinical Research Branch

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The CRB is comprised of five distinct sections (Longitudinal Studies Section, Health Disparities Research Section, Translational Research and Medical Services Section, Clinical Support Section, and the Clinical Information and Data Management Section) which are coordinated through the Office of the Clinical Director (OCD).

The overarching goal of the CRB is to conduct, at the highest level, human subjects-based research aimed at understanding aging and age-related diseases and introducing novel interventions to enhance quality of life and independence in older people. To this end, the CRB supports: 1) the conduct of major longitudinal studies of aging including the Baltimore Longitudinal Study of Aging (BLSA) and the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS); and, 2) translational research with an emphasis on the transfer of findings from NIA IRP laboratories to clinical trials. Active translational research within the CRB includes studies in cardiology, neurology, endocrinology, immunology, rheumatology, genetics, hematology and oncology.

The CRB also provides service for the NIA Intramural Research Program as a whole for any research that includes human subjects, even if those subjects are seen or evaluated at other sites. Thus, the regulatory components of several large epidemiological and genetics cohorts, such as those in Iceland, Italy (Sardinia and Chianti), and throughout the United States are managed through the CRB Protocol Office. Furthermore, the Cytapheresis Unit, a component of the CRB Clinical Support Section, provides human blood fractions (primarily, mononuclear cells) to a number of NIA investigators for basic research in human genetics, molecular biology and immunology.

While resources are utilized in a coordinated way, and the interaction between basic and clinical investigators is vigorously nurtured, we maintain a robust emphasis upon patient safety. This involves a careful and thorough review of each proposed project with an eye on scientific merit, clinical importance and human subject safety. The CRB maintains both in-patient and out-patient evaluation units with a highly trained professional staff that includes nurses, nurse practitioners and physicians available to assure subject safety 24 hours/day. Furthermore, an onsite pharmacy is maintained for both efficient research purposes as well as immediate access to appropriate medications and supplies, should an emergency arise.

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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 31 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 750 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 *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 every edition of *Best Doctors in America*.

The **Office of the Clinical Director** and Branch Chief has the overall responsibility for the administration of the Clinical Research Branch and oversight of the clinical research program through the Protocol office as well as providing, through the Clinical Core Laboratory and Pharmacy Units, central support for laboratory and pharmacy services to all clinical trials requiring these services. Through the recently awarded MedStar Research Institute (MRI) support contract, support services including medical records, nursing and other patient care support are also provided. Patient travel in support of the BLSA and other protocols is also provided through use of central branch resources.

The **Protocol Unit** provides central protocol support including implementation through study initiation meetings, regulatory monitoring and physician credentialing services for all protocols supported within the NIA Intramural Research Program (IRP). The office provides a central site through which proposed clinical studies undergo initial concept review through the monthly Clinical Investigator's Meeting. The office provides support to the individual investigator for preparation of the protocol, necessary consents and HIPAA consents for IRB submission and review. All clinical investigator and regulatory training requirements are tracked by this office and certificates maintained on file for submission as needed to meet IRB documentation requirements. In addition, the office maintains the regulatory files on all protocols including all Institutional Review

Board correspondence, stamped consents, original and modified protocol submissions and on study registration via on study cards. The unit interacts with the Clinical Information and Data Management Section to complete IRP wide implementation of the Study Manager ™ program to permit monitoring of all trials within the IRP for protocol accrual, compliance and cost projection/ monitoring.

The **Research Pharmacy Unit** supports research pharmacy needs for protocols within the IRP. The unit, operated under the MRI-support contract, will operate a licensed on-site pharmacy at Harbor Hospital Center through which all investigational and support drugs are acquired and maintained consistent with FDA and other regulations and dispensed in response to specific protocol needs. The research pharmacist on staff participates in protocol development and safety evaluation as needed and provides pharmacy specific protocol support during and following protocol initiation.

The **Clinical Core Laboratory Unit** operates the CLIA certified clinical laboratory that provides basic as well as sophisticated monitoring for patients requiring clinical testing support including hematology, chemistries, virology screening as well as coagulation analysis. This provides cost effective support for all protocols requiring clinical and research monitoring. The unit, interacting with the Clinical Information and Data Management Section, is instituting a Laboratory Information System (LIS) that will provide IRP-wide support for clinical laboratory order entry, specimen processing and capture of clinical results from the instrumentation operated by the unit. This LIS will also directly interface with FDA and HIPAA compliant databases including Oracle Clinical ™ undergoing implementation at the present time.

The **Clinical Support Services Section** provides medical support services for all protocols within the NIA IRP. This includes protocol specific Clinical Research Coordination staff, many of whom are licensed RNs, research nursing staff, medical assistants, testing personnel (cardiovascular, EMG, DEXA), medical records and reception-scheduling staff. This staff is being constituted to provide flexible, adaptable support for the wide range of longitudinal and interventional trials ongoing or currently under development.

The **Longitudinal Studies Section** 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.

The **Health Disparities Research Section** has the primary objective to create a new representative longitudinal study of health status across the lifespan focused on investigating the differential influences of race and socioeconomic status on health in an urban population. This has led to the development of the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study, a community-based research effort designed to focus on evaluating health disparities in socioeconomically diverse African-Americans and whites in Baltimore. This study is unique because it is a multidisciplinary project that will not only assess physical parameters but also evaluate genetic, demographic, psychosocial and psychophysiological parameters over a 20-year period. It also employs novel research tools, mobile medical research vehicles to improve participation rates and retention among non-traditional research participants.

The **Clinical Information and Data Management Section** provides support for networking and management and analysis of clinical data. Major initiatives include implementation of Study Manager ™ in conjunction with the Protocol Office and Laboratory Information System (LIS) with the Clinical Core Laboratory Unit. In addition, with Oracle database programming support personnel through the MRI contract, we have implemented Oracle Clinical™ as the primary Clinical Research Form/Data Entry and Capture database within the NIA IRP clinical program. This provides a scalable secure environment for data storage and for generation of datasets for analysis by IRP staff.

The **Translational Research and Medical Services Section** supports clinical investigators on-site at Harbor Hospital. William B. Ershler, M.D. (Hematology/Oncology), Chee Chia, M.D. (Endocrinology), Nazli McDonnell, M.D. (Genetics), Demetrious Kapogiannis, M.D. (Neurology) Robert Fenton, M.D., Ph.D. (Oncology) and Madhav Thambisetty, M.D.,

Ph.D (Neurology) are current members of the section. They lead a variety of studies and participate jointly in multidisciplinary studies that cut across subspecialties. Investigators within this section work closely with other laboratories within the IRP to develop and support translational research programs utilizing basic laboratory developments within the IRP.

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Biography: Dr. Ershler is a graduate of Case Western Reserve University and the State University of New York Upstate Medical University at Syracuse. He received Internal Medicine, Hematology and Medical Oncology training at the University of Wisconsin in Madison and then was Assistant Professor (Hematology/Oncology) at the University of Vermont. He returned to the University of Wisconsin where he was Associate Professor and then Professor in the Departments of Medicine (Hematology and Geriatrics) and Human Oncology. While at the University of Wisconsin he was also Head of the Section of Geriatric Medicine and Director of the UW Institute on Aging. While at the Universities of Vermont and Wisconsin, Dr Ershler maintained a research program funded continuously by awards from the NIH, the Department of Veterans Affairs and the American Cancer Society. In December 2006 he joined the NIA/IRP as Deputy Clinical Director where he has centered his research upon the clinical translation of basic research.

Keywords:

anemia
frailty
inflammation
cytokines
Interleukin-6
lenalidomide

Recent Publications:

Makipour S, et al. *Sem Hematol* 2008; 45: 250-254.

Kanapuru B, et al. *J Am Geriatr Soc* 2008; 56: 1864-1865.

Boyd CM, et al. *J Gerontol Med Sci* 2007; 62: 286-295.

Ferrucci L, et al. Br *J Hematol* 2007; 136: 849-855.

Ershler WB. *J Appl Physiol* 2007; 103: 17-20.

Benz C, et al. *Cancer Res* 2007; 67: 4560-4563.

Yancik R, et al. *J Gerontol Med Sci* 2007; 62: 275-280.

Understanding the pathogenesis of late-life anemia: Among the elderly, anemia occurs with increasing frequency with each advancing decade. Unlike when it occurs in younger adults, its cause in the elderly is oftentimes not readily apparent or attributable to a single factor. However, this commonly observed age-associated anemia (termed Unexplained Anemia, or UA) can generally be dissected to its root causes, which include renal insufficiency, inflammation, testosterone deficiency and stem cell proliferative decline. Myelodysplasia (MDS) occurs commonly in this age group but can and should, for both diagnostic and therapeutic considerations, be distinguished from UA. We have established an anemia evaluation clinic within the Clinical Research Branch to conduct focused studies to define age-associated mechanisms accounting for UA and to provide the infrastructure for clinical trials directed at examining the effects of anemia correction on functional outcomes, such as walking speed, grip strength and quality of life.

Interventional trials to prevent frailty occurrence or progression: Older people who develop age-associated involutional changes long recognized as 'frailty' are particularly susceptible to negative health outcomes including falls, fractures, dementia, depression, progressive co-morbidities, hospitalization, nursing home placement and shortened survival. Frailty is not a universal phenomenon as it

Publications-continued:
Silverstein RL, et al. Blood 2007; 110: 3097-3101.

Ershler WB, et al. *Ann NY Acad Sci* 2007; 1112: 375-384.

Balducci L, et al. *Oncologist* 2007; 12: 1416-1424.

occurs in only a minority of older people, even at advanced ages. Studies to date have indicated that inflammatory processes may be involved. It has been our hypothesis that the dysregulation of certain inflammatory pathways occur with advancing age, and to a greater extent in those who are to develop the frail phenotype. Our research is aimed at identifying individuals who are at risk for developing frailty on the basis of clinical measures and evidence for dysregulated inflammation and to intervene with anti-inflammatory pharmacological agents.

Cancer in the frail elderly: Cancer prevalence increases dramatically with advancing age. Yet, large registry datasets have indicated a curious decline in cancer prevalence in the oldest-old (i.e., those over the age of 85 years). Although cellular and sub-cellular factors associated with advanced age might favor carcinogenesis, other features of a less fertile microenvironment may be unfavorable for tumor growth.

At the phenotypic level, frailty is becoming more objectively characterized, and dysregulated inflammatory processes are thought to figure prominently in its pathogenesis. Furthermore, a reduced capacity to produce or respond to angiogenesis or other growth factors might be a definable component of frailty. Thus, in frail individuals, the above factors may be responsible for a tissue microenvironment less conducive for tumor growth, and this may result in the appearance of less cancer in frail when compared to non-frail individuals of the same age.

To address this hypothesis we proposed there would be less cancer among those who reside in nursing homes because residents therein have a disproportionately higher representation of individuals meeting established criteria for frailty. We examined the Medicare Current Beneficiary Survey (MCBS), a continuous, multipurpose survey of a nationally representative sample of aged, disabled, and institutionalized Medicare beneficiaries. The sample included 40,125 persons who lived in the community and 2,190 persons who lived in a long-term care facility.

We found the prevalence of cancer to be lower among institutionalized beneficiaries in all age groups, reaching a level of statistical significance for those over 85 years when compared to age-matched individuals living in the community. The prevalence of cancer among elders in the community in the 75-84 years age group was 20.22% among those who live alone, 23.15% among those who live with spouse, and 19.42% among those who live with others. This was higher when compared to 5.16%

among the institutionalized. The difference was even greater for the oldest age group, those 85 years and older. Here, the prevalence of cancer in the community was 19.96% among those who live alone, 22.85 % among those who live with spouse, and 15.58 % among those who live with others. This was strikingly higher than the rate of 4.18% among those who reside in long term care facilities.

These observations support the premise that the development of the frail phenotype may somehow protect against cancer. Current investigations are underway in which we examine cancer development in a large cohort of elderly individuals enrolled in the EPESE (Established Populations for Epidemiological Studies in the Elderly) study, Participants were scored for 'frailty measures' and then followed for up to six years. If, like the nursing home data, we find less cancer among frail individuals, a logical next step would be to examine the questions of carcinogenesis and tumor growth in animal models of chronic inflammation, as this may lead to clues regarding the biology of frailty and the interrelationships of inflammation, cancer and aging.

Collaborators: Robert Fenton, MD, PhD, Translational Studies Section, Clinical Research Branch, Dan L. Longo, MD, Laboratory of Immunology and Clinical Research Branch, Dennis Taub, PhD Laboratory of Immunology, Arya Biragyn, PhD Laboratory of Immunology, Jack Guralnik, Laboratory of Epidemiology, Demography and Biometry, Kushang Patel, Laboratory of Epidemiology, Demography and Biometry, Luigi Ferrucci, Longitudinal Studies Section, Clinical Research Branch, Eleanor Simonsick, PhD, Longitudinal Studies Section, Clinical Research Branch, E. Jeffrey Metter, MD, Longitudinal Studies Section, Clinical Research Branch, Evan T. Keller, PhD, Department of Comparative Medicine, University of Michigan, Andrew S. Artz, MD Department of Medicine, University of Chicago, Stefan Gravenstein, MD Department of Medicine, Brown University

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Biography: Dr. Fenton received his undergraduate degree from the University of Cincinnati in 1976. After receiving MD and PhD degrees from NYU School of Medicine in 1983, he received Internal Medicine training at Brigham and Women's Hospital and Oncology training at the Dana Farber Cancer Institute. He spent 10 years at the NCI in Frederick, Maryland and then in 1998 moved to the University of Maryland Greenebaum Cancer Center. Since April of 2007 he has been on staff at the NIA, performing clinical research at Harbor Hospital and continuing work on acute leukemia in the laboratories at the BRC.

Keywords:

acute leukemia
DNA damage
PARP inhibitors
phase I trials
drug development
sarcopenia
metabolism
resveratrol

Recent Publications:

Badros A, et al. *Cancer* 2007; 110: 1042-1049.

Drug Development for Elderly Patients with Acute Leukemia: Despite therapeutic advances, treatment of adult patients with acute lymphocytic leukemia results in long term survival of only 30-40% of patients, with a significantly worse prognosis for patients over the age of 60. We have examined the activity of ABT-888, a potent inhibitor of PARP-1 and -2 in combination with several chemotherapeutic agents against a panel of human ALL and acute myelogenous leukemia (AML) cell lines. No enhanced killing was noted when ABT-888 was combined with VP-16 (topoisomerase II inhibitor), trichostatin A (histone deacetylase inhibitor), or alkylating agents. However, synergistic killing of ALL (but not AML) was observed when ABT-888 was combined with the topoisomerase I inhibitor topotecan, and to a greater extent with combination of ABT-888 and temozolomide as determined in a 48h WST-1 screening assay using temozolomide at concentrations from 1-200 μM combined with ABT-888 (5 μM). The pre-B ALL lines Reh, RS4;11, and SUP-B15 were all very resistant to temozolomide alone with IC50's > 200 μM, consistent with the high levels of MGMT expressed by these cell lines. Culture in ABT-888 alone did not inhibit cell proliferation. However, when pre-B ALL cells were incubated for 60 minutes in clinically achievable concentrations of temozolomide (50-100 μM), washed, and then cultured for 48h in the presence of ABT-888 (5 μM), the IC50's for the combination were 25-50 μM, demonstrating a dramatic synergy for this combination in B-lineage ALL. Interestingly, AML cell lines pulsed in temozolomide and then cultured in ABT-888 as described above showed no inhibition of cell proliferation. Pre-B ALL cells cultured in temozolomide + ABT-888, but not either drug alone, arrested in S-phase and subsequently

underwent apoptosis. Increased levels of γ-H2AX foci were noted in combination-treated cells, and this occurred prior to the onset of apoptosis as determined by PARP cleavage. These data are consistent with a model in which single strand DNA breaks are generated at sites of temozolomide-induced N^3-methyladenine adducts due to the actions of DNA glycosylase and APE1, however further repair via the base excision repair (BER) pathway is precluded by PARP inhibition. This leads to double strand DNA breaks during S-phase with replication fork collapse and subsequent apoptosis. We hypothesize that while AML cells can repair DNA breaks using homologous recombination, pre-B cells are relatively deficient in this pathway and thus exhibit selective sensitivity to this drug combination (synthetic lethal relationship). Experiments are underway to test this hypothesis. The in vitro results predict that the combination of temozolomide + ABT-888 could be an effective new treatment for adult ALL.

A Phase II Trial of Resvertrol in Patients over the Age of 60 to Identify Biomarkers and Physiologic Measures of Drug Activity: Resveratrol (3, 5, 4–trihydroxy-trans-stilbene), a polyphenol structurally similar to the estrogenic compound diethylstilbestrol, is a phytoalexin found in leaves and skin of grapes, in peanuts and roots of the plant polygonum cupsidatum. Although used since early years in Indian ayurvedic and Chinese medicine, it came into prominence in the 1990's as it was believed to be the major reason for the positive effect of wine on cardiovascular health and the so called 'French Paradox'. Since then studies have shown that resveratrol affects a number of key cellular pathways and molecular targets with wide range of biological effects. Noted among these are its cancer chemo-preventive properties, anti-platelet action and anti-inflammatory activities. Resveratrol also acts as a calorie restriction mimetic, perhaps through its action on Sirtuins, and is known to have beneficial effect on aging-related processes like glucose homeostasis and muscle strength.

Resveratrol action and pharmacology has been extensively studied in vitro and in animals. Recently, Phase I and II clinical studies in healthy human volunteers or in patients with type II diabetes mellitus have begun to elaborate possible roles for resveratrol as a pharmaceutical. Pharmacokinetic studies have identified the major metabolites of resveratrol. At oral doses of up to 5 grams per day, the drug appears to have little toxicity, at least in the patient populations thus far studied. However, there are no data available in elderly subjects.

The molecular target of resveratrol is unknown. Initial studies indicating an activation of SIRT 1 protein deacetylase activity have been questioned, although not completely dismissed. Other potential targets include AMPK, a Ser/Thr kinase that regulates a number of physiologically important metabolic pathways, and PGC-1a, a transcriptional co-activator involved in regulating expression of genes in fat cells and muscle (e.g. mitochondrial genes).

In order to determine in resveratrol does improve physiologic function in elderly patients, and to begin to identify potential biomarkers of these effects, we are planning a phase II study in which healthy patients over the age of 60 will receive 5g of resveratrol per day for 3 months. Initial trial design has endpoints as follows:

Primary endpoint: MVO2 peak as measured by treadmill studies.

Secondary endpoints:

A. Physiologic:
- Walking efficiency (standard treadmill, 5 min at constant rate, measure VO2).
- Resting metabolic rate (VO2)
- SPPB (measure of physical performance including lower extremity strength and balance)

B. Molecular/Genetic:
- Search for a relevant biomarker. We will test patient plasma or serum on the HepG2 cell line looking for molecular indicators of RSV or metabolite activity such as activation of Nrf2, AMPK, and or decreased acetylation of PGC-1α and FoxO1.
- Changes in MRI spectroscopy consistent with improved energy utilization (i.e. increased high energy phospho compounds (ATP, creatine-phosphate)
- Alterations in muscle structure as determined by EM and/or light microscopy (emphasis on mitochondrial content and structure).
- Alterations in GEP and specific signaling pathways as determined by microarray analysis performed before and after treatment (muscle biopsies, pre and post).
- Changes in plasma cytokine levels focusing on inflammatory cytokines and adiponectin.

Gene Expression Profiling of Dysplastic Bronchial Epithelium: Lung cancer arises in the bronchial epithelium in a stepwise fashion from normal, to dysplastic epithelium, to carcinoma in situ, and finally to invasive disease. Phenotypic progression is driven by acquired mutations that alter molecular pathways that govern cell behaviors effecting the cell cycle, apoptosis, invasion, angiogenesis, immune system evasion, etc. Little is known about the genetic lesions or the molecular pathways altered in the incipient stages of human lung cancer. Furthermore, it has been challenged that more dysplastic lesions are more likely to give rise to invasive cancer than more benign appearing lesions. The use of autofluorescent bronchoscopy (AFB) in the hands of an expert pulmonologist will provide the opportunity to obtain tissue samples of normal and dysplastic epithelium from patients with high risk of developing bronchial neoplasia. Gene expression profiling combined with pathway analysis of the data sets should provide insights into the changes that occur during progression from normal to neoplastic cellular phenotypes.

This protocol is designed to gather information about the natural progression of cancerous cells in the bronchial mucosa of the lung. In collaboration with Drs. William Krimsky and William McGuire of the Franklin Square Hospital Pulmonary and Oncology Divisions, we will receive normal and pathologic biopsy specimens obtained during screening bronchoscopies performed in patients at high risk for aerodigestive malignancies. The tissue will be obtained using a standard flexible bronchoscope that includes an AFB system with a camera unit. AFB is used in to detect malignant and premalignant lesions in the bronchial mucosa of the lung and has been shown to have a sensitivity rate of over 95%, often detecting lesions that are missed on sputum cytology. Biopsy specimens from premalignant and nearby healthy epithelium will be divided: half will be sent to Dr. Broussard of Franklin Square Department of Pathology, and the remaining half will be delivered to Dr. Kevin Becker at the National Institute on Aging. Gene expression profiling will be performed on normal and dysplastic specimens in an attempt to identify molecular pathways that are important for the development and progression of bronchial intra-epithelial neoplasia. This is a pilot study of paired biopsy specimens from 20 patients to determine the feasibility of obtaining reliable GEP data from very small tissue specimens, and to compare GEP data with pathologic classification of each specimen to determine if cellular heterogeneity within biopsies indicates the need to incorporate laser capture microdissection in future studies.

Collaborators: Dr. Ivana Gojo, University of Maryland Greenebaum Cancer Center, Baltimore MD 21201.

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Biography: Dr. Thambisetty is a Board-certified neurologist with sub-specialty training in cognitive/behavioral neurology and sleep disorders. He completed both residency and fellowship training in the Department of Neurology at Emory University School of Medicine in Atlanta. Prior to training in Neurology, he was awarded a PhD (DPhil) in Clinical Pharmacology from the University of Oxford where he pursued his doctoral studies on a Felix scholarship. His PhD thesis examined the role of synaptic remodeling in the actions of anti-depressant treatments. In 2004, he was awarded a three year research fellowship by the Alzheimer's Society of the United Kingdom to pursue research into 'Proteomic and Neuroimaging Approaches to Peripheral Biomarkers of Alzheimer's Disease' in Professor Simon Lovestone's group at the Institute of Psychiatry, King's College, London. His clinical responsibilities at the time included evaluation and care of patients in the Motor Nerve Clinic at King's College Hospital, London. He was also elected to the Emanoel Lee medical research fellowship at St. Cross College, Oxford in 2004. He joined the Clinical Research Branch in the intramural program of the NIA in 2007. His current research is based within the Laboratory of Personality and Cognition (LPC) and the Section of Brain Physiology and Metabolism (BPMS).

Keywords:

apolipoprotein-E (APOE)
insulin resistance
proteomics
Pittsburgh Compound-B (PIB)
Positron Emission Tomography (PET)
^{11}C-arachidonic acid
neuroinflammation
Alzheimer's disease (AD)

Recent publications:

Thambisetty M, et al. *J Neurol* 2008; 255: 1712-1720.

Greenberg N, et al. *Electrophoresis* 2008; in press.

de Jager CA, et al. *Neurol India* 2008; 56: 161-166.

Stefansson H, et al. *N Engl J Med* 2007; 357: 639-647.

Research Interests: Dr. Thambisetty's main focus of research is the application of Neuroimaging and Proteomic methods to the identification of Biomarkers for cognitive decline and dementia. A closely related goal is the development of novel PET methods to study neuroinflammation in conditions such as Alzheimer's disease (AD).

1. **Delineation of Risk Factors associated with decline in brain function during normal aging.** In the LPC, Dr. Thambisetty has used longitudinal [^{15}O]water PET data available from participants in the Baltimore Longitudinal Study of Aging (BLSA) to study the role of both genetic and environmental risk factors associated with cognitive decline in the elderly. Ongoing studies include investigating the effect of apolipoprotein-E (APOE) genotype and insulin resistance on longitudinal changes in regional cerebral blood flow (rCBF) during normal aging.

2. **Proteome-based plasma biomarkers associated with neuropathological correlates of dementia.** These studies in the LPC apply unbiased proteomic techniques to identify blood-based protein signatures associated with *in vivo* brain amyloid burden detected by PET imaging with ^{11}C-labeled Pittsburgh Compound-B (^{11}C-PIB).

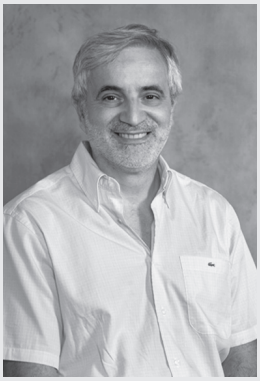
Lovestone S, et al. *Expert Rev Proteomics* 2007; 4: 227-238.

Thambisetty M, et al. *Neurology* 2007; 68: 229-232.

These experiments carried out within the neuroimaging substudy of the BLSA aim to identify antecedent peripheral biomarkers associated with core neuropathological features of Alzheimer's disease (AD) in pre-symptomatic individuals. In closely related studies, the role of pro-inflammatory cytokines as predictive markers of brain amyloid deposition are also under investigation.

3. **The utility of ^{11}C-Arachidonate-PET to study brain function in health and disease**. In the BPMS, Dr. Thambisetty's research is based on developing novel PET methods for imaging dopaminergic signal transduction in healthy humans as well as neuroinflammation in AD. These clinical protocols are based on applying ^{11}C-labeled arachidonic acid (^{11}C-AA) as a radioligand to image neural signaling events related to cytosolic phospholipase A2 (cPLA2) in the human brain. Ongoing studies have applied this method in healthy adult subjects to study dopaminergic neurotransmission following an apomorphine challenge. A new clinical protocol under preparation aims to study the utility of the ^{11}C-AA-PET method as a marker of neuroinflammation in patients with AD.

Collaborators: Susan Resnick, Alan Zonderman, LPC, NIA; Luigi Ferruci, Jeffrey Metter, CRB, NIA, NIH; Josephine Egan, LCI, NIA, NIH; Stanley Rapoport, NIA, NIH; Judith Rapoport, Jeih-San Liow, NIMH, NIH; Mark Hallett, NINDS, NIH; John Umhau, NIAAA, NIH, Raymond Scott Turner, Department of Neurology, Georgetown University; Simon Lovestone, Institute of Psychiatry, London, United Kingdom.



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Biography: Dr. Luigi Ferrucci is a geriatrician and an epidemiologist who conducts research on the causal pathways leading to progressive physical and cognitive decline in older persons. In September 2002, he became Chief of the Longitudinal Studies Section at NIA and Director of the Baltimore Longitudinal Study on Aging. He was appointed Editor of the Journal of Gerontology: Medical Sciences in 2004. Dr. Ferrucci received a Medical Degree and Board Certification in 1980, a Board Certification in Geriatrics in 1982, and a Ph.D. in Biology and Pathophysiology of Aging in 1998, all at the University of Florence, Italy. He spent a 2-year internship at the Intensive Care Unit of the Florence Institute of Gerontology and Geriatrics, and was for many years Associate Professor of Biology, Human Physiology and Statistics at the University of Florence. Between 1985 and 2002, he was Chief of Geriatric Rehabilitation at the Department of Geriatric Medicine, and Director of the Laboratory of Clinical Epidemiology at the Italian National Research Council on Aging. During that same period, he collaborated with the NIA Laboratory of Epidemiology, Demography, and Biometry where he spent several periods as Visiting Scientist. Dr. Ferrucci has made major contributions in the design of many epidemiological studies conducted in the U.S. and in Europe, including the European Longitudinal Study on Aging, the "ICare Dicomano Study," the AKEA study of Centenarians in Sardinia and the Women's Health and Aging Study. He was also the Principal Investigator of the InCHIANTI study, a longitudinal study conducted in the Chianti Geographical area (Tuscany, Italy) looking at risk factors for mobility disability in older persons. Dr. Ferrucci has refined the design of the BLSA to focus more on normal aging, age-associated frailty and factors associated with exceptionally healthy aging and longevity.

Keywords:

epidemiology
disability
frailty
inflammation
energetics

Recent Publications:

Ling SM, et al. *Osteoarthritis Cartilage* 2009; 17: 43-48.

Ruggiero C, et al. *J Gerontol A Biol Sci Med Sci* 2008; 63: 698-706.

Melzer D, et al. *PLoS Genet* 2008; 4: e1000072.

Research Interests: Aging is characterized by a global susceptibility for a number of different diseases and impairments that cannot be readily assessed by the currently available approaches. The mechanism that leads to such a susceptibility to disease and disability is poorly understood. One possible way of gaining a better understanding of the relationship between aging, morbidity and disability is to examine such a relationship in the context of longitudinal studies. Using this approach, scientists have started understanding that, the high prevalence of comorbidity in the elderly cannot be explained by a simple stochastic process (since the incidence and prevalence of many acute and chronic diseases increase with age, older patients are more likely to be affected by multiple conditions) but rather, results from a progressive dysregulation of the mechanism that maintains a stable homeostasis in the human organism. While aging, some individuals become more "frail" than others and, as a result of this process, they are at higher risk of developing comorbidity and disability.

In the geriatric literature, frailty had often been defined as a state of

"severe disability, typical of older persons affected by geriatric syndromes and resident in long-term care facilities." While individuals with these characteristics are likely to be frail, such a definition does not identify a late stage of the process that leads to full frailty and disability. On the contrary, frailty is a dynamic process that should be detectable evident earlier in life, when specific interventions are more likely to be effective. We hypothesize that frailty is a strong predictor of a number of negative outcomes including disability, hospitalizing, nursing home admission and mortality, and that it can be detected before any of these outcomes develop. Thus, a precocious detection of frailty may help in targeting preventive interventions to the people who are most likely to benefit from it. As a first approximation, we used mobility as a proxy variable for frailty. There are intrinsic advantages in using mobility as a proxy measure for frailty. Mobility is so important to life that efficient mobility has probably been a primary target for natural selection throughout human evolution. This has led to physiologic systems that not only are highly redundant but also are capable of functioning and interacting in a number of different ways to accomplish the same task. In our studies, we found that aging persons can use a number of compensatory strategies to maintain mobility even when many physiological systems are damaged. Only when this large functional reserve is exhausted, do problems in mobility emerge and can be clinically detected. We conducted a series of analyses on the longitudinal database of the EPESE study (Established Population for Epidemiological Studies of the Elderly) and found that in non-disabled older persons, poor performance in mobility and balance (performance-based tests of lower extremity function) is an independent, strong predictor of morbidity, hospital admission, incident disability, mortality and admission to a nursing home.

Perhaps the most interesting feature of gait as a marker of frailty is that even when the reduced physical activity that results from having gait problems is factored out, the prognostic value of gait speed is retained. This fact suggests that the pathophysiological process that leads to walking speed reduction mediates the outcome prediction, rather than the walking impairment directly. In other words, low walking speed is a robust marker of frailty. Having identified a robust proxy measure of frailty, it remained to be found why poor performance in lower extremity function is such a strong predictor of disability and other negative health outcomes. We conducted a series of studies in this direction. Taking a longitudinal perspective of the disablement process, we demonstrated that in 50% of older persons, disability results from an acute catastrophic

event that, within a short period of time, leads from full function to severe disability in activities of daily living. In the other 50% of older persons, disability develops slowly and progressively and often cannot be explained by acute pathological events, at least when looking at hospital admissions and discharge diagnoses over the same period. Progressive disability is more typical of the oldest old. Using data from the EPESE and the WHAS (Women's Health and Aging Study) studies, we demonstrated that high IL-6 serum level is one of the strongest independent predictors of accelerated decline of physical function. We demonstrated that the predictive value of IL-6 on accelerated functional decline could be explained by the catabolic effect of IL-6 on muscle metabolism. Using data from the WHAS, we also found that lower extremity muscle strength is associated with walking speed only below a certain threshold of strength and that there is a synergistic effect of reduced muscular strength and balance problems in causing severe walking disability. These findings demonstrated the existence of a large functional reserve that had been intuitively proposed but never demonstrated and suggested that muscular strength is the basic mechanism for compensating for the disabling effect of balance problems.

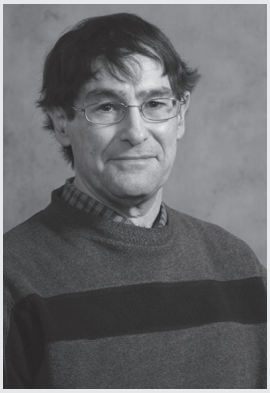
Recently, in the design of the InCHIANTI study, we outlined a reference model in which the impairments that may cause mobility problems are grouped into six main subsystems: 1. Central Nervous System; 2. Peripheral Nervous System; 3. Perceptual System; 4. Muscles; 5. Bones and Joints; 6. Energy Production and Delivery. However, preliminary data suggests that the two main predictors of poor lower extremity performance are the reduction of muscle power (secondary to sarcopenia) and dysfunctions (even minor) of the central nervous system, but also show many complex interactions between the anatomical integrity and functionality of the different subsystems. A similar paradigm is currently used in the refinement of the design of the Baltimore Longitudinal Study on Aging. In particular, we are 1) studying how the various physiological subsystems that are important for mobility interact with age in causing disability; 2) developing reference values for the integrity and functionality of the different physiologic subsystems that are implicated in mobility, to be used in clinical practice; 3) looking at risk factors for the development of "soft" neurological impairments in the absence of neurological disease that is already clinically evident; 4) identifying risk factors for accelerated sarcopenia and osteoporosis, including biomarkers of chronic inflammation, genetic polymorphisms and circulating levels of specific vitamins and hormones; 5) studying how nutritional intake of

macro- and micro-nutrients influence health status.

As mentioned above, our long-term objective is to unravel the biological pathways that lead to disability and comorbidity in older persons. This research topic is examined from different perspectives that can be envisioned as superimposed layers. On the surface is the behavior in the environment that is strongly conditioned by both physical and cognitive function. However, physical and cognitive performances require the integrity and functionality of multiple physiological systems, and, therefore, reduction of physical and cognitive function may result from multiple, possibly co-existing causes. Finally, loss of physiological function results from the incapacity of the organism to maintain the biological homeostasis and to provide quantities of energy compatible with environmental requests. These mechanisms include but are not limited to inflammation, oxidative stress, autonomic nervous system, hormones and the multiple adaptative mechanisms to physical activity. The study of the effect of aging independent of diseases on these biological mechanisms and their relationship with the development of disability is the main target of the new BLSA design.

Over the last year, the BLSA has been working on a new research project, namely the "Insight into Exceptional Aging and Longevity" (IDEAL). This project is aimed at enrolling into the BLSA 500 individuals both long-lived (age 80+) and healthy to identify factors associated with successful aging and maintaining successful aging in very old individuals. Enrollment for this project will start in 2010.

Collaborators: Linda P. Fried, Jeremy Walston, Paulo Chaves, Johns Hopkins University School of Medicine; Karen Bandeen Roche, Johns Hopkins University, Bloomberg School of Public Health; Jay Magaziner, Gregory Hicks, University of Maryland School of Medicine; Marco Pahor, Matteo Cesari, University of Florida College of Medicine; Stephen P. Kritchevsky, J. Paul Sticht Center on Aging, Wake Forest University School of Medicine; Stephanie Studenski, University of Pittsburgh; Mary M. McDermott, Feinberg School of Medicine, Northwestern University; Katherine L. Tucker, Jean Mayer, U.S. Department of Agriculture, Human Nutrition Research Center on Aging, Tufts University; Neil Alexander, University of Michigan Medical School; Gary Striker, Helen Vlassara, Mount Sinai School of Medicine, New York; Giovanni Paternostro, The Burnham Institute for Medical Research, California; Brenda W. Penninx, VU University Medical Center, The Netherlands; Heikkinen E. Finnish, University of Jyvaskyla, Finland; Stefania Bandinelli, Benedetta Bartali, Fulvio Lauretani, Annamaria Corsi, Italian National Institute on Aging, Florence, Italy; Niccolo Marchionni, Mauro Di Bari, Stefano Fumagalli, Institute of Geriatrics and Gerontology, University of Florence, Italy; Antonio Cherubini, Umberto Senin, Institute of Geriatric Medicine, University of Perugia; Stefano Volpato, Dipartimento di Medicina Clinica e Sperimentale, Università di Ferrara, Italy; Giorgio Valenti, Marcello Maggio, Gian Paolo Ceda, Geriatrics University of Parma; Maria Luisa Brandi, University of Florence, Italy; Giuseppe Paolisso, Michelangela Barbieri, Angela Abbatecola, Department of Geriatrics and Metabolism, University of Napoli.



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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 a physician for the Baltimore Longitudinal Study of Aging.

Keywords:

aging
longitudinal studies
neuromuscular
prostate

Recent Publications:

Roth SM, et al. *Eur J Hum Genet* 2008; 16: 391-394.

Metter EJ, et al. *Diabetes Care* 2008; 1026-1030.

Ruggiero C, et al. *J Gerontol A Biol Sci Med Sci* 2008; 63: 698-706.

Carter HB, et al. *Urology* 2007; 70: 685-690.

Talbot LA, et al. *Prev Med* 2007; 45: 169-176.

Ling SM, et al. *Osteoarthritis and Cartilage* 2007; 15: 1134-1140.

Prostate Aging and Disease: The Baltimore Longitudinal Study of Aging (BLSA) is characterizing normal aging in the prostate and identifying transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition, the research is using information about structure and function of the prostate to improve early detection of prostate disease. Clinical evaluations of prostate growth and function have been made in over 800 men with and without prostate disease and the availability of stored sera and genetic material. Prospectively, BLSA men aged from 30 to 79 have had physiological, clinical and imaging of their prostate. To date, the major accomplishments have come from analyses of prostate specific antigen (PSA) which show that PSA increases more over a period of years in men who develop BPH than in those who do not. The rate of change in PSA is even greater in men who develop prostate cancer, particularly starting 5-10 years prior to diagnosis. Furthermore, the ratio of free to total PSA is able to distinguish men who develop prostate cancer from, and those who do not, about 10 years prior to diagnosis. Analyses of a subset of the men who developed prostate cancer show that the free to total PSA ratio is lower in men who have clinically defined aggressive tumors. We have shown that normal levels of PSA can be used to stratify men at high risk of developing prostate cancer more than a decade prior to the time that prostate cancer is diagnosed. Further, PSA levels and the rate of change in PSA over time can identify men at risk for high grade, potentially lethal prostate cancer 10 to 15 years prior to the actual diagnosis of the cancer. Alterations in prostate structure or function are studied in relation to the possible development of prostate disease, particularly benign prostatic hyperplasia (BPH). Magnetic resonance

imaging of the prostate were performed 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. Current research is examining the natural history of development of prostate symptomatology and risk factors for the development of BPH. Recently, we have found that obesity, elevated fasting plasma glucose, and diabetes are risk factors for BPH. Further, PSA was not a good indicator for the development of prostate symptomatology.

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 currently has 2 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. We have shown that the age-associated declines are explained in part by change in muscle mass. However, other factors are also important including changes in nerve function and hormonal levels (e.g. testosterone). Age-asssociated changes in strength are related to functional performance as demonstrated by an association with walking

speed. However, in healthy individuals, a strength level is reached where no association is observed. This level implies the presence of excessive strength potential that acts as a reserve for walking performance. In addition, there is a complex relationship between muscle strength, muscle power, muscle mass and physical activity on mortality. We found that muscle strength and power and how they change over time are long-term predictors of longevity, independent of how much muscle is present and how active you are. We believe and are currently looking for evidence that age-associated changes in the central nervous system control of movement is a key contributor to the relationships between strength, power and longevity. We have found that muscle strength, muscle power, muscle mass, and movement speed are each independent predictors of mortality, arguing that both muscle and nervous system contributions to movement are important for survival.

2. 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 work 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.

Collaborators: Luigi Ferrucci, MD, PhD, NIA CRB; Shari Ling, MD, NIA, CRB; H. Ballentine Carter, MD, Johns Hopkins University, Baltimore; Jerome Fleg, M.D., National Heart, Lung, and Blood Institute, NIH; Robin Conwit, M.D., National Institute of Neurological Diseases and Stroke, Daniel Stashuk, Ph.D., University of Waterloo, Ontario, Canada; Stephen Roth, Ph.D., University of Maryland, College Park; Laura Talbot, R.N., C.S., Ed.D., Ph.D., University of North Carolina, Charlotte, NC.

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Biography: Dr. Nazli McDonnell graduated from University College Cork and Towson University with a B.A. degree in philosophy and a B.S. degree in chemistry and biology. She subsequently worked as a research assistant in Dr. Jeff Corden's laboratory at Johns Hopkins Medical School, Department of Molecular Biology and Genetics. She completed the M.D./Ph.D. program in University of Maryland Medical School in 1998. Her Ph.D. supervisor was Dr. Michael Summers in the Department of Biochemistry and Molecular Biology at the University of Maryland, Baltimore County. The focus of Dr. McDonnell's Ph.D. research was the study of protein-drug interactions by Nuclear Magnetic Resonance Spectroscopy. Dr. McDonnell's clinical training consisted of a residency in Internal Medicine at York Hospital, Pennsylvania, and a Medical Genetics Fellowship at the Metropolitan Washington D.C. Genetics Fellowship Training Program at National Human Genome Research Institute at the National Institutes of Health. In 2003, Dr. McDonnell joined Dr. Clair Francomano's laboratory at the National Institute on Aging, Laboratory of Genetics to study hereditary disorders of connective tissue. Upon Dr. Francomano's departure, she moved to the Laboratory of Clinical Investigation. Her professional memberships include Sigma Xi, American Medical Association, American Women's Medical Association, American Association for the Advancement of Science, American Society of Human Genetics, and Phi Kappa Phi.

Keywords:

genetics
connective tissue
aneurysm

Research Interests: Dr. McDonnell's research is focused on clinical and molecular investigations of hereditary disorders of connective tissue (HDCT). The disorders of interest are Ehlers-Danlos syndrome (EDS), Marfan syndrome, Stickler syndrome, hereditary aneurysm syndromes and fibromuscular dysplasia (FMD). Dr. McDonnell is investigating the natural history of these disorders at the NIA-ASTRA Unit, as well as studying genotype/phenotype correlations, molecular and cellular mechanisms and exploring treatment strategies in the laboratory.

Current Clinical Projects - Natural History of Hereditary Disorders of Connective Tissue: We are investigating the cardiovascular and musculoskeletal complications of hereditary disorders of connective tissue, including autonomic dysfunction observed in patients with EDS, incidence of aneurysms and cardiovascular abnormalities in patients with all forms of HDCT, incidence of spine abnormalities and bone density loss in patients with HDCT, and pain and quality of life issues associated with HDCT. These investigations have uncovered a predisposition to craniocervical junction abnormalities including Chiari I malformation in patients with HDCT. We are also enrolling a group of patients with a diagnosis of FMD in order to define this disorder clinically and discover

causative genes.

Principal Investigator, IRB approved protocol, National Institute on Aging: Project # 2003-86: "Clinical and Molecular Manifestations of Heritable Disorders of Connective Tissue." Patients with hereditary disorders of connective tissue have many early manifestations that usually afflict the elderly including osteoarthritis, loss of bone density, spinal disc disease, musculoskeletal weakness, arterial aneurysms, and alterations in vascular remodeling. Through clinical and laboratory evaluations in this group of patients, we expect to elucidate underlying mechanisms contributing to these common conditions associated with aging.

Collaborators (Intramural): Deborah Merke, M.D., NICHD, NIH; Andy Singleton, Ph.D., NIA-IRP; Josephine Egan, MD, NIA-IRP; Mark Talan, M.D., Ph.D., NIA-IRP; Samer Najjar, M.D., NIA-IRP; Dimitrios Kapogiannis, M.D. NIA-IRP; Paul Costa, Ph.D., NIA-IRP; Joan Marini, M.D., Ph.D., NICHD, NIH

Collaborators (Extramural): Clair A. Francomano, M.D. Greater Baltimore Medical Center; Harry Dietz, M.D., Johns Hopkins University; Leena
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Biography: Dr. Chia received her bachelor's degree and master's degree from the Massachusetts Institute of Technology and her medical degree from Northwestern University School of Medicine. After completing her internal medicine training at the University of Texas – Houston Medical School, she obtained her fellowship in endocrinology and metabolism from the Johns Hopkins University School of Medicine. She joined the Translational Research and Medical Services Section at NIA in September 2004.

Keywords:

growth hormone
thymic involution
rnteroendocrine hormones
neuroendocrine hormones
glucagon-like peptide-1
glucose-dependent
insulinotropic polypeptide
Type 2 diabetes mellitus

Recent Publications:

Chia CW, et al. *J Clin Endocrinol Metab* 2008; 93: 3703-3716.

Chia CW, et al. *Clinical Geriatrics* 2007; 15: 27-34.

Research Interests:

Enteroendocrine hormones, metabolism, and aging: A primary function of the gut is to regulate food intake and to achieve efficient nutrient digestion and absorption. As part of this process, food ingestion leads to secretion of various enteroendocrine hormones including glucagon-like peptide-1 (GLP-1). Because intravenous glucose does not induce GLP-1 secretion, it appears that glucose within the gut lumen acts on the luminal surface to stimulate GLP-1 secretion. We had previously reported the expression of sweet taste receptors in human duodenal L cells in which the activation of these sweet taste receptors induces GLP-1 secretion from the L cells. In addition, we also found the presence of GLP-1 in the taste cells located in tongues. We are currently studying the mechanisms whereby nutrients induce enteroendocrine hormone secretion from both the taste cells in the intestine as well as the tongues of humans. Unraveling the underlying mechanisms of taste and hormone secretion may lead to a significant breakthrough in the understanding of metabolic dysfunction associated with aging and diabetes as both are associated with decrease in sweet taste sensation.

Novel connections between neuroendocrine hormones and changes in immune and metabolic systems: The decline in immune function with advancing age is well established. This altered immune system contributes to the increase susceptibility of older individuals to infections, autoimmune disease, and even cancer. In humans, thymic involution begins as early as the age of one year and continues throughout life where the thymus undergoes significant reduction in thymic mass and decrease in thymopoiesis. This decrease in thymopoiesis with aging is believed to be

a contributing factor to the decline in immune function with age. While the precise causes of thymic involution remain unclear, clinical observations and animal studies suggest that there may be a link between changes in the neuroendocrine hormones and the immune system. We have shown that growth hormone (GH) and ghrelin, a natural ligand of the growth hormone secretagogue receptor, are capable of increasing thymic size and cellularity in rodent studies. In addition, gonadotropin-releasing hormone (GnRH) agonist infusions in pregnant rats markedly attenuated pregnancy-induced thymic involution resulting in significant increases in thymic weight and thymocyte numbers. Others had reported that surgical or chemical castration of old rats led to regeneration of atrophied thymus. We are interested in finding potential therapeutic strategies, such as GH therapy, for rejuvenating the aging thymus in humans.

We are developing paradigms for GH replacement in the elderly. While it is well known that GH levels decline with age, it is not at all clear that replacing GH is beneficial and indeed evidence would seem to say it is detrimental to replace GH with development of insulin resistance and diabetes as adverse events. However, GH has been replaced in a non-physiological manner where daily bolus GH was instigated. We are now investigating paradigms whereby GH will be replaced in a pulsatile manner so as to mimic the usual physiological state.

We are also studying the immune function and metabolic complications in men with prostate cancer undergoing long-term androgen deprivation therapy (ADT). One of the complications of male hypogonadism is development of insulin resistance and type 2 diabetes, and testosterone replacement had been shown to improve insulin sensitivity in men with low testosterone levels. Since men undergoing ADT have castrate levels of androgens, they may be at a higher risk of developing these metabolic complications. Studying the thymic function of men undergoing ADT will provide us with a better understanding of mechanisms of thymic regeneration following ADT.

Collaborators: Josephine M. Egan, MD, Laboratory of Clinical Investigation, NIA; Dan L. Longo, MD, Laboratory of Immunology, NIA; Dennis D. Taub, PhD, Laboratory of Immunology, NIA; Luigi Ferrucci, MD, PhD, Longitudinal Studies Section, NIA; Shari M. Ling, MD, Clinical Research Branch, NIA; Shehzad Basaria, MD; Tamara B. Harris, MD, Laboratory of Epidemiology, Demography, and Biometry, NIA.

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Biography: Dr. Ngozi Ejiogu, a board certified internist received her medical degree (MBBS) from the College of Medicine, University of Nigeria. She completed her internship and 2 years of residency training in internal medicine at the Abia State University Teaching Hospital, Abia State Nigeria. In the United States she completed an internal medicine residency training at the Mount Sinai School of Medicine affiliate North General Hospital, New York, New York. She was junior attending/chief resident at the Mount Sinai/North General Hospital before going into primary care practice. She joined the Clinical Research Branch, Gerontology Research Center, at the National Institute on Aging in January 2003 as a staff clinician for the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study.

Recent Publications:

Trzeciak AR, et al. *Free Radic Biol Med* 2008; 45: 1631-1641.

Research Interest: Aging can be affected by differences in prevalence of disease risks and rates as they relate to pathological conditions. There are well documented differences in health status among groups defined by age, race, ethnicity, and socioeconomic status (SES). There are persistent disparities among African Americans and other minority groups in morbidity and mortality when compared to whites. The need to understand the driving factors behind persistent Black-white health disparities in overall longevity, cardiovascular disease, and cerebrovascular disease has led to develop the HANDLS study. Specifically, HANDLS is investigating the longitudinal effects of socioeconomic status and race on the development of cerebrovascular disease and cardiovascular disease; changes in psychophysiology, cognitive performance, strength and functioning, health services utilization, and nutrition, and their influences on one another.

The scientific research questions for this multidisciplinary epidemiologic study of minority health and health disparities are:

- Do race and SES influence health disparities independently or do they interact with several factors (race, environmental or biologic factors, and cultural or lifestyle practices)?
- What is the influence of SES and race on age-related declines in function in an urban population?
- What is the influence of SES and race on the incidence and natural history of age-related disease?

- Are there early biomarkers of age-related health disparities that may enhance our ability to prevent or ameliorate the severity of these diseases?

HANDLS was designed as a community-based, and epidemiologically-driven clinical research endeavor that specifically targets the evaluation of disparities that exist amongst Black and Caucasian inner city residents of Baltimore across the diverse range of socio-economic status. It's both fascinating and unique in the sense that it is a longitudinal study spanning a twenty-year duration and is multidisciplinary. In addition to the fact that it is designed to assess various physical parameters in the target groups in the population, it quests to evaluate their biologic, genetic, psychosocial, demographic, and psycho physiological parameters of participants from the groups across the higher and lower socio-economic status (SES) line. Its novelty derives from both the mobile research vehicles (MRV) and the other research tools that it employs to attract, improve and retain the rates of participation of particularly the non-traditional participants in the target population groups. HANDLS is designed to be conducted in phases. The pilot study was conducted in two phases and was completed within three years. The collection of study data is designed in two parts. In the primary part, data on the health status of participants their psychosocial factors, health service utilization, nutrition, demographics, and neighborhood characteristics are elicited during an in-house interview exercise involving the use of questionnaires. The second part of study data is collected when participants are invited to the MRV for medical and psychological examination. Such data include their medical history, physical conditions, dietary recall, and cognitive evaluation. Psychophysiology assessments that include arterial thickness, heart rate variability, carotid ultrasonography, muscle strength and bone density assessments; as well as laboratory measurements (hematology, blood chemistry, biomarkers of oxidative stress and biomaterials for genetic evaluations) are conducted at the time.

Collaborators: Abdul Adjei, M.D. - NIH-NIA-CRB; Dan E. Arking, Ph.D. - Johns Hopkins University School of Medicine; Darrell R. Abernethy, M.D. Ph.D. - USP; Malcolm Brock, M.D., M.P.H. - Johns Hopkins University; Craig Fletcher, DVM, PhD Johns Hopkins School of Medicine; M. Chris Gibbons, M.D., M.P.H. - Johns Hopkins Medical Institutions; Melissa H. Kitner-Triolo, Ph.D. - NIH-NIA-LPC; Marie T. Fanelli Kuczmarski, Ph.D., R.D., L.D.N. - University of

Delaware; Thomas A. LaVeist, Ph.D. – Johns Hopkins University; James Lepkowski, Ph.D., M.P.H. University of Michigan, Institute for Social Research; Shari Ling, M.D. – NIH-NIA-CRB; Samer Najjar, M.D. – NIH-NIA-LCS; Neil R. Powe, M.D., M.P.H., M.B.A. – Johns Hopkins Medical Institutions; Andrzej R. Trzeciak, Ph.D. – NIH-NIA-LCMB

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Biography: David E. Anderson's educational background includes a PhD in Clinical Psychology at the University of Oregon in 1966, and a Postdoctoral Fellowship in Behavioral Medicine at the State University of New York at Stony Brook in 1967. His University background includes faculty positions at The Johns Hopkins University School of Medicine (1968-1981) and the University of South Florida College of Medicine (1981-1987) where his research focused on animal models of hypertension. He was the recipient of an NIH Research Career Development Award (1983-1987), and the Pavlovian Award for Biological Science in 1985. He was appointed Professor in the Department of Psychiatry of the University of South Florida College of Medicine in 1986. He joined the National Institute on Aging Intramural Research Program in 1987 as Chief of the Behavioral Medicine Section, and is currently a Senior Investigator in the Clinical Research Branch.

Keywords:

blood pressure
breathing
hypertension
sodium chloride
sodium pump inhibitors

Recent Publications:

Anderson DE, et al. *Am J Physiol Regul Integr Comp Physiol* 2008; 29: R1248-R1254.

Anderson DE, et al. *Amer J Hypertens* 2008; 21: 1324-1329.

Behavioral Medicine Research: Behavioral medicine research is based on the evidence that environmental and behavioral factors interact with genetic predisposition to engender chronic disease states. Research in behavioral medicine is directed at the identification of behavioral factors that participate in the origins of diseases, and in the development of non-pharmacological interventions in them. A focus of much behavioral medicine research has been on cardiovascular disorders. For example, animal models have been developed that show that social stresses amplify the effects of high cholesterol intake on the development of atherosclerosis in primates, and that specific inhibitory behavioral stresses can potentiate the effects of high salt diet on blood pressure in canines. This work has been extended in research with humans to the study of behavioral and emotional factors in human hypertension and cardiovascular disease. Significantly, anger has been identified as important to the development of coronary artery disease, while repression of anger has been implicated in the development of hypertension. Work in this laboratory has been and continues to be concerned with the discovery of physiological pathways by which behavioral interactions contribute to chronic hypertension.

Stress, Salt and Blood Pressure: More than two decades ago, an animal model of hypertension was created in genetically-normotensive dogs on high salt diets by exposing them to intermittent aversive behavioral conditioning. The hypertension was not observed if either the high salt diet or the behavioral conditioning was imposed alone. This form of

hypertension was found to develop within 10 days, and to involve an increase in total body sodium levels. When the experiment was done on animals following bilateral renal denervation, the development of the hypertension was not prevented, indicating that the sodium retention was not a result of increased renal sympathetic activity. Subsequent research with micropigs showed that the behavioral procedures had induced a state of suppressed breathing that increased pCO2 and transiently altered acid-base balance (i.e. mild respiratory acidosis). It is known that the kidneys respond to decreases in plasma pH by increased reabsorption of sodium and circulating fluid volume. That the breathing inhibition led to an increase in plasma volume expansion was shown by increases in circulating concentrations of endogenous natriuretic factors that also inhibited sodium pump activity.

The implications of this animal model for human blood pressure regulation have since then been studied at the National Institute on Aging. Salt sensitivity of blood pressure of normotensive women has been found to be more likely in those who characteristically engage in a slow and shallow breathing pattern at rest, and this slow breathing pattern has been associated with high perceived stress levels. It has also been found that resting end tidal CO2 is higher in older women with higher resting systolic blood pressure, especially if they are low on trait anger. Recently, it has been found that the variability of breathing in women is positively associated with their 24-hr blood pressure level, supporting the conclusion that such individuals engage more frequently in daytime breath holding episodes that must be compensated by hyperventilatory episodes in order to maintain respiratory elimination of CO2 in balance with its metabolic production.

Ongoing Studies: A study is ongoing that tests the hypothesis that a guided breathing intervention that has been reported to be an effective behavioral treatment of human hypertension is mediated by hypocapnic breathing. Preliminary evidence supports this hypothesis, but also reveals the limited effectiveness of the intervention in reversing long-term elevations in 24-hr blood pressure levels. Therefore, an ambulatory breathing monitor is under development for retraining of breathing habits via immediate feedback of breath holding episodes in the natural environment, whose utility will be tested alone and in combination with dietary sodium restriction and meditative exercises. This miniaturized

device will enable continuous monitoring of end tidal CO2 in the natural environment. It will be an advance over existing technology in that current breathing monitors do not permit inference about whether changes in breathing are stimulated by changes in metabolic activity or are a component of emotional adaptations to the environment. Thus, the monitor will enable selective feedback for changes in breathing pattern that are consequent to changes in emotional state rather than biological need.

Collaborators: Alexei Y. Bagrov, M.D., Ph.D., Olga V. Fedorova, Ph.D., B. Gwen Windham, M.D., Margaret A. Chesney, Ph.D., University of Maryland School of Medicine

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Biography: Dr. Kapogiannis received his M.D. degree from the National and Capodistrian University of Athens, Greece. He completed his Internal Medicine internship at Northwestern University. Then, he completed his residency training in Neurology at the combined program of Massachusetts General/ Brigham and Women's Hospitals/ Harvard Medical School. He subsequently completed a fellowship in Cognitive and Behavioral Neurology at the National Institute of Neurological Disorders and Stroke. He joined the Clinical Research Branch in 2009.

Keywords:

dementia
cognition
religiosity
personality

Recent Publications:

Kapogiannis D, et al. *Proc Natl Acad Sci U S A* 2009; in press.

Huey ED, et al. *J Neuropsychiatry Clin Neurosci* 2008; 20: 390-408.

Kapogiannis D, et al. *Eur J Neurosci* 2008; 27: 1836-1842.

Kapogiannis D, et al. *Cent Nerv Syst Agents Med Chem* 2008; 8: 234-240.

Kapogiannis D, et al. *Neurology* 2008; 70: A124.

Research interests include frontotemporal dementia, Alzheimer's disease and mild cognitive impairment, personality and religiosity.

Frontotemporal Dementia (FTD) is a rare neurodegenerative disease that results in atrophy in frontal and temporal regions with predominant initial symptoms being personality change, inappropriate social behavior and apathy. Other cognitive domains disintegrate over time. No treatment currently exists for FTD, despite exciting new findings on its genetic and molecular basis. Our research objective is to perform a clinical trial on a drug which appears promising in altering the natural course of the disease. As part of this effort, we will follow several surrogate markers of disease progression by cognitive testing and neuroimaging, in collaboration with the Laboratory of Neurosciences and the Laboratory of Personality and Cognition.

Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI): AD is a neurodegenerative disease whose incidence continues to increase and a major Public Health issue. It is often heralded by MCI, which consists of mild and generally isolated cognitive deficits. In close collaboration with the Laboratory of Neurosciences and the Laboratory of Personality and Cognition, we will perform a clinical trial on a drug with the potential of altering the natural course of these diseases. As part of this effort, we will follow several surrogate markers of disease progression by cognitive testing and neuroimaging.

Personality and religiosity: It is increasingly recognized that personality

traits (several of which correlate with different aspects of religiosity) have a strong biological basis and are largely heritable. Nevertheless, the neural correlates of these traits are still largely unknown, as well as their potential role as disease modifiers. In collaboration with the Laboratory of Personality and Cognition, we will pursue functional and structural neuroimaging studies to define the neural correlates of various personality traits. Moreover, we will investigate their role in regards to response to treatments in the clinical trials we will conduct. In particular, having identified a common cognitive and neural architecture for religious belief, we will further investigate the cognitive and neural variability of modern religiosity and its relationship with personality traits.

Collaborators: Mark Mattson, Laboratory of Neurosciences, NIA, NIH, Paul Costa, Susan Resnick, Laboratory of Personality and Cognition, NIA, NIH, Alan B. Zonderman, Research Resources Branch, NIA, NIH, Chee Chia, Nazli McDonnell, Clinical Research Branch, NIA, NIH; Jordan Grafman, Cognitive Neuroscience Section, NINDS, NIH, Eric Wassermann, Brain Stimulation Unit, NINDS, NIH.

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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 six sections that focus on particular specialties or types of service. The Sections are Central Laboratory Services; Comparative Medicine; Instrumentation, Design and Fabrication; Networks, Computing, and Telephony; Visual Media; and Statistical and Experimental Design.

Central Laboratory Services is subdivided into Bioinformatics, Confocal Imaging, Flow Cytometry, Gene Expression and Genomics, and Proteomics and Analytical Biochemistry.

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.

Although this branch largely provides research services, there are several investigator-initiated projects conducted by RRB scientists. These projects include studies on the role of reactive oxygen species in ischemic preconditioning, bioinformatics, developing novel statistical models for survival analyses and predicting disease conditions, array based technology development, gene expression studies in rodents, humans, and other species, and the identification of novel markers in quiescent murine and non-human hematopoietic stem cells.

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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.

The **Central Laboratory Service Section (CLSS)** offers investigators specialized support to help them succeed in today's fast-paced and complex scientific environment. Established by NIA's Office of the Scientific Director and the Chief of the Research Resources Branch in 2000, this Section provides specific expertise, new technologies, and experienced staff to enhance the research efforts of all NIA investigators. High-throughput, cutting-edge analysis capabilities that can be found within CLSS include advanced sequencing, imaging, cell sorting, genetics, genomics, and proteomics technologies. The primary goal of the CLSS is to support the research interests and ongoing projects of various Laboratories within the IRP as well as to provide the expertise necessary to assist in the proper performance of specialized experiments and in the interpretation of obtained data. In addition to their service duties, some CLSS Unit Heads also perform hypothesis-driven, defined research projects within their laboratories.

The CLSS is currently divided into 5 service units:

(1) The **Bioinformatics Unit (BIU)** offers services in bioinformatic technology for both information management and the detailed analysis of genomic, proteomic, imaging, and clinical/epidemiological data.

(2) The **Confocal Imaging Facility (CIF)** provides investigators with state-of-the-art 3D optical confocal microscopy facilities for imaging of living and fixed cells and tissues and computational resources for visualization and extraction of quantitative information from images.

Various uses of this facility include simultaneous, sub-organelle level localization of proteins; two, three and four color protein co-localization; sub-micron level DNA damage by UV laser; Time-lapse, FRAP and ratiometric analysis of cellular processes in live cells; Intracellular protein trafficking and volumetric (3D) reconstruction of intracellular protein distribution.

(3) The **Flow Cytometry Unit (FCU)** provides cell sorting and enhanced fluoragraphic analysis in support of research at the GRC. In addition, the Shared Service technologist and Unit Head provide consultation to investigators in design and interpretation of flow cytometry and cell sorting studies. Various uses of this facility include measurements of antigen or ligand density, apoptosis, enzyme activity, DNA and RNA content, membrane potential, cytokine receptors and its synthesis, phagocytosis and viability obtained from cells, changes in cell cycle, intracellular pH, intracellular calcium, intracellular glutathione and oxidative burst.

(4) The **Gene Expression and Genomics Unit (GEGU)** provides technical support and training spanning the entire microarray process, from sample preparation through data analysis. Several gene expression array formats are available for use within this Unit including Illumina Bead arrays, microarrays for microRNA, and arrays for RNA splicing. Analysis of gene expression is performed with different analytical strategies and software depending on the application.

(5) The **Proteomics and Analytical Biochemistry Unit (PABU)** was formed in 2000 in response to a demand for high-sensitivity amino acid sequencing of purified and blotted proteins. The scope of this service Unit has been expanded to include the sequencing, identification, and determination of post-translational modification (PTM) of proteins from various cellular populations using state-of-the art mass spectrometers, phosphopep amino acid sequencing, MALDI-TOF mass spectrometry, and the phosphopeptide mapping of proteins from various cellular populations.

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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. 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:

cancer stem cells
stem cells
proliferation specific antigens
bone marrow progenitors
flow cytometry
cell cycle

Recent Publications:

Araki Y, et al. *J Immunol* 2008; 180: 8102-8108.

Tarasov KV, et al. *PLoS ONE* 2008; 3: e2478.T

Yamanaka S, et al. *Cell Tissue Res* 2008; 331: 5-22.

Core Laboratory Overview: The Flow Cytometry Laboratory functions as a core for the scientific mission of the laboratories located within the NIA Intramural Research Program in Baltimore providing analytical and cell sorting services and training NIA/IRP researchers in the capabilities and limitations of the instruments. Consistent with our mission and based upon the wide variety in the technical skills of investigators, we actively interact with users to provide innovative solutions to research problems, ranging from the optimization of users' protocols to complex data analysis including our application of the appropriate cell cycle modeling algorithms and strategies to remove debris and doublets for accurate cell cycle analysis. Typical cell sorting experiments include bulk and single-cell (for PCR) sorts of subpopulations of human peripheral blood lymphocytes, mouse thymocytes and spleen cells based on 2-6 color staining, the isolation of cells expressing reporter genes, sorting cell cycle fractions, and rare event sorts (where the frequency of the desired cells is <0.05% of the total population) of human bone marrow side population progenitor cells based on Hoechst 33342 staining and antigen expression, and stained rat brain cells, mouse and human ES cells and ES cell-derived cardiomyocytes. The laboratory is equipped with state-of-the art equipment, including a Becton-Dickinson (BD) LSRII, FACSCanto II (3-laser, 8-color), FACSAria II (special order 4- laser), Beckman Coulter (Cytomation) MoFlo (3-laser), and an iCyt HAPS system (3 heads, 9 laser 40+parameter system housed in a Baker biological safety cabinet) for sorting live, biohazardous specimens.

Research in the Flow Cytometry Laboratory is focused on the application of new techniques in the field of cytometry to a wide range of interdisciplinary projects, with an interest in the basic cell biology of stem and cancer stem cells. In many instances, the impetus for present and past projects, such as the effect of doublet discrimination on cell cycle analysis arise from issues borne out of practical problems associated with the techniques developed for users of the core facility or the unambiguous analysis of client data and its' relevance to the scientific mission.

Cancer Stem cells: The concept of cancer stem cells and their potential impact on chemotherapeutic responses is an area of intense interest in cancer research. Inhibitors of the epidermal growth factor receptor (EGFR) are currently used for treatment of non-small cell lung cancer (NSCLC), however, their response is incomplete in even highly sensitive tumors and the basis for this is not known. To test the hypothesis that resistant sub-populations of cells of may be responsible for this incomplete response, human NSCLC cell lines were sorted on the basis of CD24 and CD44 surface marker expression and drug resistance tested. Interestingly, cells lacking both CD24 and CD44 expression (CD24-/CD44-) were the most drug resistant in clonogenic assays. Neither the CD24-/CD44- phenotype nor relative drug resistance seems to be associated with increased efflux of Hoechst 33342 dye, suggesting that this assay for putative stem cells could not explain drug sensitivity. We are currently testing the hypothesis that non-small cell lung cancers may consist of heterogeneous populations of cells, with variations in cell signaling activity and responses to chemotherapeutic agents.

Immunophenotypic Fluorochrome Selection: Polychromatic immunophenotypic analysis has the capability to potentially resolve new clinically import lymphocyte subsets, while at the same time minimizing sample processing and the costs associated with the use of redundant antibodies in multiple tubes. Generally, the rule of thumb for selecting the appropriate fluorochrome combination is to assign the brightest label (PE, APC, or PE-Cy5) to the antibody that reacts with the protein of interest having the lowest expression. Multiple factors can affect the perception of brightness including the biophysical properties of the fluorochrome itself and instrument-dependent variables. Peridinin chlorophyll protein (PerCP), a subunit of the photosynthetic complex of the dinoflagellate

Glenodinium sp., has an extremely high quantum efficiency and large Stokes shift, yet it photobleaches easily, thus limiting it usefulness in high power jet-in-air flow cytometers and restricting its use to low-power, fixed beam systems. PerCP is used for multicolor analysis since its spectral overlap is minimal with other common fluorochromes; however it commonly thought to be more suited for antigens expressed at medium or high densities. In the course of designing and testing a hybrid 4-color panel for routine immunophenotypic analysis of the major lymphocyte subsets (CD4 and CD8 T-cells, B, and NK cells), PerCP-conjugated antibodies could be readily utilized without any sacrifice in accuracy for common antigens expressed at relatively low densities, as in B-cells. However, staining was significantly altered when PerCP-conjugated antibodies to CD19 were used in stabilized blood samples. Additionally, cell fixation and permeabilization is a requirement for the measurement of intracellular antigens, as in the case of ZAP-70 expression, a surrogate marker in chronic lymphocytic leukemia disease progression and survival. Under these conditions, the accurate enumeration B-cells is preferentially and significantly altered, whether measured with PE-, PerCP, or PEAF700-conjugated fluorochromes. Overall, this diminished sensitivity leads to a poorer separation between the background fluorescence of unlabeled-(control) and labeled-cells, thereby confounding the accurate measurement of B-cell numbers and introducing error in the validity of the clinical value of these markers stained under these conditions.

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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 Gene Expression and Genomics Unit at the NIA in November of 1998.

Keywords:
microarray
bioinformatics
autoimmunity
gene expression
genetic association

Recent Publications:

Long JM, et al. *Cell Cycle* 2008; 7: 3062-3073.

Mazan-Mamczarz K, et al. *Cancer Res* 2008; 68: 7730-7735.

Cheadle C, et al. *BMC Med Genomics* 2008; 1: 43.

Zhang P, et al. *Curr Biol* 2008; 18; 1489-1494.

Martin B, et al. *PLoS ONE* 2008; 3: e2750.

Mazan-Mamczarz K, et al. *Oncogene* 2008; 27: 6151-6163.

Pearson KJ, et al. *Cell Metab* 2008; 8: 157-168.

Lee CT, et al. *PLoS Med* 2008; 5: e117.

Thiriet N, et al. *Brain Res* 2008; 1222: 31-41.

Dahiya N, et al. *PLoS ONE* 2008; 3: e2436.

The **Gene Expression and Genomics Unit** is involved in the application, and analysis of DNA microarrays 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 bioinformatic applications that integrate genetic and gene expression studies with complex biological systems.

Recent gene expression studies using microarrays have included aging and caloric restriction, drug abuse, T cell induction, cancer; among others. Recent bioinformatic projects and applications include development of the Genetic Association Database (http://geneticassociationdb.nih.gov), Human and mouse disease phenotype analysis, and the development of gene set analysis tools.

Collaborators: Dr S. Alex Wang, Center for Information Technology, NIH; Dr. Tomas Guilarte, Johns Hopkins University Bloomberg School of Public Health; Dr Ryan Miller, Department of Pediatrics, Johns Hopkins University School of Medicine; Dr. Paul Drew, University of Arkansas; Dr Stuart Kim, Stanford University; Dr. William Freed and Dr Jean Lud Cadet, National Institute on Drug Abuse, NIH.

Rong-Fong Shen, Ph.D., Staff Scientist/Facility Head
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Central Laboratory Services Section

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Biography: Dr. Shen received his Ph.D. degree in Pharmaceutical Sciences from the University of Kentucky. His dissertation focused on purification, characterization, and immunological studies of porcine thromboxane synthase. He did postdoctoral research at Howard Hughes Medical Institute, Baylor College of Medicine on transcriptional regulation of liver-specific gene expression. Following the training, Dr. Shen served 11 years as an Assistant/Associate Professor in the Division of Human Genetics, University of Maryland School of Medicine (1989-2000) investigating gene structures, regulation, and polymorphism of mammalian thromboxane synthases. Dr. Shen was an established investigator grantee of the American Heart Association from 1997 to 2000. In 2001, he joined the Proteomics Division of Thermo Finnigan, Inc. as a Senior Scientist. There he utilized 2D LC-MS system in shotgun protein sequencing and phosphoprotein capture and analysis. From 2003 to 2008, Dr. Shen headed the NHLBI Proteomics Core Facility. He joined NIA in September of 2008.

Keywords:

proteomics
mass spectrometry
biomarkers
sample preparation
complexity reduction

Selected Recent Publications:

Wu WW, et al. *Biochem Biophys Res Commun* 2008; 367: 7-13.

Alves G, et al. *J Proteome Res* 2008; 7: 3102-3113.

Yu MJ, et al. *Amer J Physiol-Cell Physiol* 2008; 295: C661-C678.

Wu WW, et al. *J Proteome Res* 2007; 6: 2447-2459.

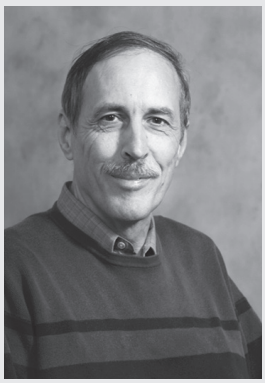
Noda Y, et al. *Proc Natl Acad Sci* 2007; 104: 18456-18460.

Research Interests: Dr. Shen's research interests can be divided into two major areas. One concerns with methods development for mass spectrometry-based proteomics. Briefly, he is interested in developing methods that may facilitate biomedical applications of mass spectrometry. This line of research includes sample preparation (e. g., complexity reduction and fractionation), membrane protein analysis, proteomic quantification, and the analysis of posttranslational modifications. The other area of his research interest deals with biomarker discovery for revealing potential pathways underlying cell malignancy, differentiation, or apoptosis, as well as proteomic changes due to genetic alterations or drug treatments. He is particularly interested in phosphoproteome changes in kinase A-deficient cells, and aging-associated change in the functions of regulatory proteins.

The **Proteomics and Analytical Biochemistry Unit** (PMSU) is equipped with state-of-the art mass spectrometers and other instruments for mass spectrometry-based proteomics and biochemical analysis. Its main mission is to support proteomic endeavors undertaken by NIA investigators. Toward this aim, the Unit provides proteomic project consultation, mass spectrometric analyses of samples, and data collection and interpretation. In addition, the Unit serves as a platform where interested scientists and unit staff may exchange ideas and discuss

options of project approaches, and research fellows and students may learn relevant technologies and instrumentation. The Unit members keep abreast of instrument and technology development in proteomics to ensure up-to-date methods are adopted for research conducted at NIA.

Collaborators: Dr. Paul Insel, University of California, San Diego; Drs. Terry Rogers and Marvyn Monteiro, University of Maryland School of Medicine; Dr. Seung Joon Baek, University of Tennessee; Drs. John Park (NINDS), Steven Shaw (NCI), Yi-Kuo Yu (NCBI), Boon Chock (NHLBI), Mark Knepper (NHLBI), and Maurice Burg (NHLBI) of NIH.



Larry J. Brant, Ph.D., Staff Scientist/Facility Head
Chief, Statistical and Experimental Design Section

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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 Biostatistics 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:

Hourani R, et al. *Am J Neuroradiol* 2008; 29: 366-373.

Strasak A, et al. *Clin Chem* 2008; 54: 273-284.

Strasak AM, et al. *Int J Cardiol* 2008; 125: 232-239.

Strasak AM, et al. *Cancer Res* 2008; 68: 3970-3977.

Strasak, AM, et al. *Arterioscler Throm Biol* 2008; 28: 1857-1865.

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 investigation of various longitudinal models involving the modeling of individual ages and measurement times in the study. In addition, methods for the prediction or classification of preclinical disease states are being developed using longitudinal measurements of biological markers and multilevel models. Initially, models utilizing a single marker have been extended to multivariate prediction models using several biological markers simultaneously. Data analyzed and reported using these prediction methods include information from the Baltimore Longitudinal Study of Aging, the Framingham Offspring Study and the Vorarlberg Health Monitoring and Promotion Program of Austria. 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. 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, and Dr. Hanno Ulmer, Department of Medical Statistics, Informatics and Health Economics, Innsbruck Medical University.

Ming Zhan, Ph.D., Staff Scientist
Facility Head, Bioinformatics Unit, Central Laboratory Services Section

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Biography: Dr. Zhan received his B.Sc. in 1982 from the University of Qinghai and his M.Sc. in 1985 from the Chinese Academy of Sciences, China. He received his Ph.D. in molecular biology in 1993 from the University of Western Ontario, Canada.

Keywords:

bioinformatics
comparative transcriptomics
gene regulatory network
RNA motifs for post-transcriptional regulation
aging
stem cells

Recent Publications:

Li H, et al. Bioinformatics 2008; 24: 1874-1880.

Zhan M. Front Biosci 2008; 13: 276-283.

Sun Y, et al. Plos One 2008; 3: e3406.

Li H, et al. Front Biosci 2008; 13: 263-275.

van der Brug MP, et al. Proc Natl Acad Sci USA 2008; 105: 10244-10249.

Liu Y, et al. BMC Dev Biol 2008; in press.

Ling SM et al. Osteoarthritis Cartilage 2008; epub ahead of print.

Zhan M, et al. Genome Res 2007; 17: 1236-1243.

Research Interests: Dr. Zhan's research addresses the fundamental requirements of bioinformatics by biomedical research, and implements computational molecular biology studies on aging and embryonic stem cells. The bioinformatics research focuses primarily on deciphering the regulome, the cell's regulatory program. The following aspects are particularly addressed:

a) Transcriptional modules and gene co-regulation: A new algorithm based on two-stage matrix decomposition is developed for transcriptional module discovery. The algorithm takes into account the nonlinear structure in the data, does not assume that genes of the same pathway or similar functions share similar expression profiles, and can partition one gene into multiple modules. In comparison with other similar methods, the new algorithm shows a higher performance in uncovering biologically relevant transcriptional modules. The algorithm is also extended to incorporate ChiP and promoter data and to cross-species transcriptional analysis to examine gene co-regulation and to facilitate comparative transcriptomics studies.

b) Dynamic behavior of biological pathways: Biological networks or pathways behave only in certain ways and controlled manners in response to disease, aging, development, or external stimuli. A new algorithm is developed to characterize the dynamic behavior of biological pathways for identification of how disease or cellular phenotypes arise from the connectivity or networks of genes and their products. In this algorithm, gene expression profiles and pathway topologies are modeled by a finite-state Markov chain, and the behavior transition of a pathway in instances such as disease development or cell differentiation is assessed through a series of transcriptional interventions conducted *in silico* on each gene or gene combination. The particular value of this study is in its ability to *in silico* simulate the pathway behaviors which may not be easy

Publications-continued:
Zhan M. Genomic Medicine 2007; 1: 19-28.

Li H, et al. Bioinformatics 2007; 23: 473-479.

Sun Y, et al Genomics 2007, 89: 22–35.

Li H, et al. *J Bioinf Syst Biol* 2007; 27: 1-10.

Xu X, et al. *Genome Biol* 2007; 8: R234.

Kim HS, et al. *Mol Cell Biol* 2007; 19: 6806-6817.

Shin S, et al. *Stem Cells* 2007; 25: 1298–1306.

Norgate M, et al. *Biometals* 2007; 20: 683-697.

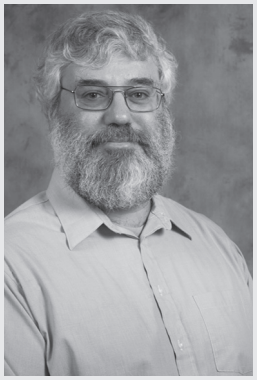
to recreate in vitro, and the derived hypotheses could then be tested via independent experiments.

c) Cross-species transcriptomic profiling: Evolutionary conservation and variation of the transcriptome are examined across species by utilizing a suite of mathematic algorithms, including generalized singular value decomposition, independent component analysis, sparse matrix factorization, comparative partition around centroids, and correlation of transcriptional responses to aging, development, environmental perturbation, etc. The analyses are applied to pre-defined gene sets that show similar functions or biological processes, or to compare the global organization of transcriptomes across species.

d) RNA motifs for posttranscriptional regulation: Post-transcriptional regulation of genes arises through protein-RNA and RNA-RNA interactions. The interactions are dependent on the presence of RNA motifs that are conserved on the structure and, in a looser extent, on the sequence. A computational method is developed for the discovery of RNA motifs and genome-wide scanning of target genes of RNA binding proteins. The program requires no prior knowledge about sequence alignment or structural features of a motif. Using the method, RNA motifs and subsequent target genes have been identified for HuR, TIA1, TIAR, AUF1, RNase-L, and other RNA-binding proteins, and the validity of the identifications are confirmed by independent biological assays.

The computational molecular biology studies conducted by Dr. Zhan utilize newly developed bioinformatics algorithms and theories to examine molecular mechanisms controlling aging or stem cell pluripotency. Comparative studies between normal and longevity mutants as well as between different species are conducted to elucidate fundamental patterns or tissue- or species-specific changes. Longevity-related or stem-cell-critical pathways are particularly examined for *in silico* identification of genetic perturbations (*e.g.* gene knock-out) and external stimulations (*e.g.* diet restriction) that impact longevity or pluripotency.

Collaborators: Dr. Myriam Gorospe, Laboratory of Cellular and Molecular Biology, NIA, NIH; Dr. Sige Zou, Laboratory of Experimental Gerontology, NIA, NIH; Dr. Mahendra Rao, Invitrogen Inc.; Dr. Xianmin Zeng, Beck's Institute for Aging Research; Dr. Henry Yang, Bioinformatics Institute, Singapore.



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Biography: Dr. Indig received his M.Sc. *Summa Cum Laude* in 1987 and Ph.D. in 1992 from Tel-Aviv University. After post-doctoral research at the Scripps Research Institute and Salk Institute he joined the NIH in 1999 and has been the head of the Confocal Imaging Facility at the NIA since 2004.

Keywords:

VCP
p97
CDC48
AAA ATPase
nucleolin
DNA damage
UV laser
intranuclear trafficking
nuclear domains
endoplasmic reticulum
proteasome
endosome
protein-protein interaction
protein trafficking
RNA stress granules
immunological synapse

Recent Publications:

Ghosh MC, et al. *Blood* 2009; 113: 575-584.

Leotlela PD, et al. *Oncogene* 2007; 26: 3846-3856.

Jiao R, et al. *Oncogene* 2007; 26: 3811-3822.

Thazhathveetil AK, et al. *Bioconjug Chem* 2007; 18: 431-437.

Dissanayake SK, et al. *J Biol Chem* 2007; 282: 17259-17271.

Research Interests: I have a long-standing interest in the structural and functional analysis of multi-component protein complexes, the regulation of their formation and the intracellular protein trafficking steps that lead to their creation. These functional groups of proteins are the machinery of life. They are responsible for most of cellular physiology, from signal transduction to DNA replication, and play key roles in cellular processes that lead to cancer and aging.

In the pursuit of greater understanding of these intracellular interactions, I have employed state of the art confocal imaging techniques combined with biochemical and immunological approaches that are at the forefront of Cell Biology research. My recent studies, done in collaboration with researchers interested in various aspects of aging, are described below.

(1) Intracellular Protein Trafficking. We use the Zeiss LSM 510 Meta and LSM 410 to obtain confocal fluorescence images of immunochemically stained cells and tissue to determine the sub-cellular localization of three to four proteins simultaneously. These images are then used to reconstruct intracellular and intranuclear protein trafficking both in time (time-lapse) and in space (volumetric [3D] reconstruction). Examples are trafficking of MHC class I antigens (Schiavo et al., *Blood* 107: 4597, 2006; Biragyn et al., *J Immunol*. 179:1381, 2007); Claudin-1 expression in melanoma cells (Leotlela et al., *Oncogene* 26:3846, 2007); regulation of Claudin-4 in tight junctions (D'Souza et al., *Exper. Cell Res.* 313:3364, 2007). These techniques were successfully utilized for the technically challenging imaging of intracellular RNA-protein complexes (Lopez de Silanes et al., *Mol. Cellul. Biol*. 25:9529, 2005).

Publications-continued:
Imam, SZ, et al. *Nucl Acids Res* 2007; 35: 4941-4951.

Biragyn A, et al. *J Immunol* 2007; 179: 1381-1388.

D'Souza T, et al. *Exper Cell Res* 2007; 313: 3364-3375.

(2) DNA Damage and Repair Mechanisms. Using high resolution microscopy coupled with deconvolution and 3D reconstruction of intranuclear protein distribution, we established the novel nuclear localization of the AAA ATPase VCP complex and furthermore, that the VCP complex interacts with the Werner Syndrome protein helicase (Partridge et al., *Mol. Biol. Cell* 14:4221, 2003; Indig et al., *J. Struct. Biol.* 146:251, 2004). We have developed imaging-based techniques for analysis of intranuclear trafficking of repair proteins before and after DNA damage. Using those tools, we were able to show the following relationships of intranuclear proteins: between Werner helicase and Topoisomerase I (Laine et al., *Cancer Res.* 63:7136, 2003); Base excision repair proteins (Ahn et al., *JBC* 279:53465, 2004); Cockayne Syndrome group B protein interaction with PARP-1 (Thorslund et al., *MCB* 25:7625, 2005); Werner helicase and chromatin assembly factor 1 (Jiao et al., *Oncogene* 26:3811, 2007); Cockayne Syndrome B protein and C-Abl tyrosine kinase (Imam et al., *Nucl. Acids Res*. 35:4941, 2007). To analyze DNA damage repair in real time, we have developed a cutting-edge technique to utilize the continuous scanning UV laser of the 510 Meta confocal in order to cause specific sub-micron UV damage to live cell DNA (Thazhathveetil et al., *Bioconjug. Chem.*, 282:17259, 2007).

(3) Multi-component Protein Complex Analysis. We have utilized advanced imaging techniques coupled with biochemical approaches such as specific chemical cross-linking to perform structural and functional analysis of novel multi-protein complexes and their components. Those include: the tetraspanin CD9 and the integrin GPIIb-IIIa complex (Indig et al., *Biochem. J.* 327:291, 1997); ER membrane fusion complex (Patel et al., *Cell* 92:611, 1998); the AAA ATPase VCP complex and Werner Syndrome protein helicase (Partridge et al., *Mol. Biol. Cell* 14:4221, 2003; Indig et al., *J. Struct. Biol.* 146:251, 2004); Cockayne Syndrome B protein and C-Abl tyrosine kinase (Imam et al., *Nucl. Acids Res.* 35:4941, 2007).

Collaborators: Dr. Myriam Gorospe, LCMB, NIA, NIH; Dr. Josephine M Egan, LCI, NIA, NIH; Dr. Michel Bernier, LCI, NIA, NIH; Dr. Arya Biragyn, LI, NIA, NIH; Dr. Ashani T Weeraratna, LI, NIA, NIH; Dr. Vilhelm A Bohr, LMG, NIA, NIH; Dr. Michael M. Seidman, LMG, NIA, NIH; Prof. Eric Grote, Johns Hopkins University; Prof. Ella Englander, UTMB Galveston, Texas

Training Opportunities

National Institute on Aging
Intramural Research Program

Postdoctoral Training Program

Intramural Research Training Award Program - The Intramural Research Training Award (IRTA) Program provides advanced training and research experience to physician and Ph.D. level investigators who are at the beginning stages of their professional research careers. Participants engage in research studies under the close guidance of a senior NIA investigator who serves as a supervisor during the appointment period.

Postdoctoral IRTA Fellowship: Candidates must be a U.S. citizen or permanent resident with a doctoral degree and have 5 years or less of relevant postdoctoral research experience. Initial IRTA commitments are made for two years with appointments made in one-year increments which may be renewed.

Pharmacology Research Associate Program: The Pharmacology Research Associate (PRAT) Program is a competitive postdoctoral fellowship program to pursue research in one of the laboratories of the National Institutes of Health (NIH) or the Food and Drug Administration (FDA). It is intended for individuals with backgrounds in the basic or clinical sciences who wish to obtain advanced experience in an area of pharmacology, or for those who are already pharmacologists to gain experience in new fields.

Visiting Fellowship Program: Visiting Fellowships are awarded to foreign (non-U.S.) scientists to support advanced postdoctoral research and training in NIA's Intramural Research Program laboratories. Visiting Fellows must have a doctoral or equivalent degree in the sciences and five years or less of relevant postdoctoral research experience.

Current openings for postdoctoral positions can be found at: http://www.grc.nia.nih.gov/vacancies/vacancy.htm

To apply for the program, please send the following items to the address below to the attention of Dr. Jaron Lockett:

1) Curriculum vitae
2) Bibliography
3) Three letters of recommendation
4) Statement of research goals
5) Official copy of transcript
6) Summary of doctoral dissertation

National Institute on Aging
Intramural Research Program
251 Bayview Boulevard, Suite 100
Baltimore, MD 21224

Direct Questions to:

Dr. Jaron Lockett
NIA IRP Postdoctoral Recruitment Specialist
Phone: 410-558-8470
E mail: lockettj@mail.nih.gov

Training Opportunities
National Institute on Aging
Intramural Research Program

Predoctoral Training Program

Predoctoral IRTA Fellowship: To provide practical research training and experience to students, by supplementing academic course work and/or encouraging pursuit of professional careers in biomedical research to: 1) students enrolled in doctoral degree programs in biomedical sciences. The research experience that frequently involves dissertation research, is undertaken as an integral part of the student's academic preparation and will involve close cooperation and planning between NIH and the academic institution; 2) students who are enrolled in graduate, other doctoral or medical degree programs and who have written permission from their school to interrupt their current schooling and to return within one year to their degree granting program. Students must be U.S. citizens enrolled in doctoral degree programs in the biomedical sciences. Awards are granted for 1-year periods, with annual 1-year renewals up to a total of 3 years.

Postbaccalaureate IRTA Fellowship: Provides opportunities for recent college graduates to spend a year engaged in biomedical investigation. Postbaccalaureate fellows are also expected to initiate the application process for graduate or medical school. The duration of the program is normally one year, but the fellowship can be extended for an additional year provided the performance of the trainee is satisfactory and continued support by the laboratory is available. Candidates must be U.S. citizens or permanent residents and have graduated from an accredited U.S. college or university.

Summer Research Training Program - The program offers many excellent training opportunities in both laboratory and clinical medicine with a wealth of valuable resources. The Intramural Research Program is actively seeking students to participate in NIA's Summer Research Training Program. There are limited opportunities available so please apply early.

Summer Internship Program: The Summer Internship Program in Biomedical Research offers a unique opportunity for high school, college and graduate students to develop skills in scientific research. In this program, students receive hands-on experience. Summer internships generally last from eight to ten weeks, beginning in late May and ending in mid-to-late August. Some flexibility exists to accommodate individual student needs. Students must be enrolled at least half-time in an accredited U.S. high school, college, or university. In addition, candidates must be U.S. citizens or permanent residents and at least 16 years of age.

Training Opportunities
National Institute on Aging
Intramural Research Program

Summer Research Fellowship Program: The Summer Research Fellowship Program is open to students from any of the nation's medical and dental schools. This program is intended to expose students to research procedures in a unique environment devoted exclusively to biomedical research and training. With guidance from scientists in the Intramural Research Program, students conduct research in selected areas of laboratory investigation. In addition to participating in research projects, students attend lectures and seminars to enhance their education and develop investigative skills. The program runs for a minimum of ten weeks, usually from early June to the end of August; some flexibility exists to accommodate individual student needs.

Minority Access to Research Careers (MARC): MARC Undergraduate Student Training in Academic Research (U*STAR) Awards provide support for students who are members of minority groups that are underrepresented in the biomedical sciences to improve their preparation for graduate training in biomedical research. These minority groups include, but are not limited to, African Americans, Hispanic Americans, Native Americans (including Alaskan natives), and natives of the U.S. Pacific Islands. The program can also support efforts to strengthen the faculty, science course curricula, and biomedical research training programs and infrastructure at institutions with significant enrollments of minority students.

Awards are made to colleges and universities that offer the baccalaureate degree. Only one grant per eligible institution will be awarded. The institutions select the trainees to be supported. Trainees must be honors students majoring in the sciences who have an expressed interest in a biomedical research career and who intend to pursue postgraduate education leading to the Ph.D., M.D.-Ph.D., or other combined professional degree-Ph.D. The period of appointment to the MARC U*STAR Program is 2 years at the junior/senior level.

Prospective candidates should apply on-line: http://www.training.nih.gov.

Direct Questions to:

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NIA IRP Predoctoral Recruitment Specialist
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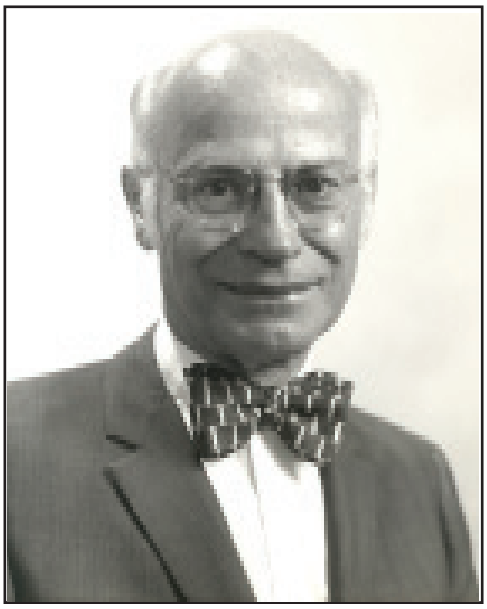
Nathan W. Shock Memorial Lecture

The National Institute on Aging initiated the Nathan W. Shock Annual Lecture in 1990 in honor of Nathan W. Shock, former NIA Scientific Director and NIH Scientist Emeritus, to pay tribute to his pioneering efforts in the field of gerontology. This award provides an opportunity to recognize a scientist, who has made significant contributions to our understanding of the basic mechanisms of aging.



Nathan Wetherell Shock, Ph.D. (1906-1989)

Dr. Shock was recognized as the dean of American gerontologists, and the father of American gerontology. He was founder of the Baltimore Longitudinal Study of Aging started in 1958. Over his career, Dr. Shock was directly involved in the postdoctoral training of over 200 gerontologist and geriatric researchers, many of whom are now heading their own aging programs across the country.

Lecture Winners:

1990 - Philip W. Landfield, Ph.D., Department of Physiology and Pharmacology, Bowman Gray School of Medicine, “The Glucocorticoid Hypothesis of Brain Aging: New Evidence on Possible Mechanisms.”

1991 - Phyllis Wise, Ph.D., Professor, Department of Physiology, University of Maryland School of Medicine, “Changing Neurotransmitter Rhythms: Insights into the Aging Brain.”

1992 - Richard A. Miller, M.D., Ph.D., University of Michigan, Institute of Gerontology, “Defects in Calcium Signals and Protein Kinase Pathways in T-Lymphocytes from Old Mice.”

1993 - Arlan Richardson, Ph.D., University of Texas Health Sciences Center, San Antonio, “Gene Expression Changes Key to Dietary Restrictions Benefits?”

1994 - Steven N. Austad, Ph.D., Associate Professor of Zoology, Department of Biological Science, University of Idaho, “Size and Aging: The Biomedical Implications.”

1995 - Thomas E. Johnson, Ph.D., Associate Professor Psychology and Fellow of the Institute for Behavioral Genetics at the University of Colorado in Boulder, “Identification and Function of Gerontogenes in C. elegans.”

1996 - Vincent M. Monnier, M.D., Professor of Pathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, “From Bjorksten to Kohn: The Collagen Theory of Aging in Light of the Maillard Reaction.”

Nathan W. Shock Memorial Lecture

1997 - S. Michal Jazwinski, Ph.D., Professor, Department of Biochemistry, Louisiana State University Medical Center, New Orleans, Louisiana, "Longevity, Genes, and Aging: The View Provided by a Genetic Model System."

1998 - Calvin Harley, Ph.D., Geron Corporation, Menlo Park, California, "What Can Immortality (of the cell) Teach You?"

1999 - Olivia M. Pereira-Smith, Ph.D., Huffington Center on Aging, Baylor College of Medicine, Houston, Texas, "Identification of a Novel Gene Family of Transcription-like Factors: A Role for Cell Aging."

2000 - Richard Weindruch, Ph.D., Department of Medicine, University of Wisconsin, Madison, Wisconsin, "Caloric Intake, Oxidative Stress, and Aging."

2001 - Rudolph E. Tanzi, Ph.D., Department of Neurology (Neuroscience), Director, Genetics and Aging Research Unit, Massachusetts General Hospital, Harvard Medical School, "Alzheimer's Disease: From Genes to Drugs in the 21st Century."

2002 - Gordon J. Lithgow, Ph.D., Associate Professor, The Buck Institute for Age Research, Novato, California, "The New Biology of Aging - Worms, Flies and Age Related Disease."

2003 - Nir Barzilai, M.D., Associate Professor of Medicine, Albert Einstein College of Medicine, New York, "New Insights Into the Biology of Longevity."

2004 - Christiaan Leeuwenburgh, Ph.D., Associate Professor, University of Florida and Director, Biochemistry of Aging Laboratory, "Oxidative Stress, Cell Death and Aging: Role of Exercise and Calorie Restriction."

2005 - Gerd Kemperman, M.D., Max Delbruck Center for Molecular Medicine, Germany, "New Neurons for Old Brains: Lifelong Neuronal Development in the Adult Hippocampus."

2006 - Ana Maria Cuervo, M.D., Ph.D., Associate Professor, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, New York, "Autophagy and Aging: When the Cleaning Crew Goes on Strike."

2007 - Anna Csiszar, M.D., Ph.D., New York Medical College, "Vascular Inflammation in Aging."

2008 - Bret H. Goodpaster, Ph.D., University of Pittsburgh, "The Loss of Function with Aging: Shifting Toward a Lipocentric Perspective"

Nathan Shock Trainee Award

Each Spring NIA's Intramural Research Program (IRP) sponsors a Scientific Retreat to 1) foster collaboration among individuals in the IRP; 2) train junior staff in oral and poster presentation of scientific work; and 3) learn about new and ongoing areas of research.

In conjunction with the scientific retreat, IRP fellows (postdoctoral, predoctoral and visiting fellows) compete for the Nathan Shock Trainee Award by presenting their research. The winners, selected by the Scientific Director, Deputy Scientific Director, and Laboratory Chiefs, receive a travel award and plaque. The research competition is sponsored by the Nathan W. and Margaret T. Shock Aging Research Foundation.

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Intramural Research Program

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Invited Speaker Seminars - 2007

NIA-Intramural Research Program

JANUARY

Drs. Susan Bonitz and Raphael Mannino, BioDelivery Sciences International, Inc. "Cochleate Delivery of Molecules."

Dr. Peter Rapp, Associate Professor, Co-Director, Graduate Training Program in Neuroscience, Mount Sinai School of Medicine. "New Horizons on Neurocognitive Aging."

Michelle L. Demory, Department of Microbiology, University of Virginia. "Epidermal growth factor receptor localization to the mitochondria is regulated by c-Src, endocytosis and a mitochondrial localization signal."

Dr. Johannes D. Veldhuis, Professor of Internal Medicine, Consultant, Clinical Investigator, Endocrine Research Unit, Mayo Clinic College of Medicine. "Regulation and Replacement of Growth Hormone."

Giancarlo Pepeu, M.D., Emeritus Professor of Pharmacology, University of Florence. "The Role of the Brain Cholinergic System in Cognitive Processes."

Amy Kenter, Ph.D., Associate Professor, Department of Microbiology and Immunology, University of Illinois College of Medicine. "Long-range interactions between germline transcript regulatory elements promotes S/S synapsis during lg class switch recombination."

Dr. Florian H. Pilsczek, Max-Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany. "Gamma delta APC's express CD1 for lipid presentation in tuberculosis and activation of gamma delta effector T cells."

FEBRUARY

Maureen L. Upton, Duke University, Department of Biomedical Engineering. echanbiology and Cell-Matrix Mechanics of the Knee Joint Meniscus."

Franz Hofmann, M.D., Chairman and Professor, Institut fur Pharmakologie und Toxikologie, Medical Faculty, Technische Universitat, Munchen, Germany. "Analysis of L-type calcium channel function by tissue-specific deletion."

Kim Sutton-Tyrrell, Professor, Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh. "Vascular Aging: Consequences for the Brain and Kidney."

Georgia M. Dunston, Ph.D., Founding Director of the National Human Genome Center (NHGC) at Howard University, and Director of Molecular Genetics in the NHGC, Professor and former chair of the Department of Microbiology, Howard University College of Medicine. "DNA Variation in Human Identity and Health Disparities."

Yong Zhong Wu, Ph.D., Virginia Commonwealth University, Richmond, VA. "The Regulation of Vimentin Gene in Human Breast Cancer Cells and Mouse Muscle Cells."

Yun Lin, M.D., Dana-Farber Cancer Institute, Boston, MA. "Effective Graft versus Leukemia is associated with the presence of nucleic acid-immunoglobulin complexes that stimulate TLR8 and TLR9."

Penelope Bonnen, Ph.D., Laboratory of Molecular Genetics, The Rockefeller University, NY. "Leveraging population genetics for mapping human disease genes."

Dr. Vladmir Larionov, Laboratory of Biosystems and Cancer, National Cancer Institute, NIH. "TARgeting of genome: insight into gene function, chromosome organization and evolution."

Patricia C. Jessamy, Baltimore City States Attorney. "Telling Their Story: African Americans in Service to Their Country."

MARCH

Marjorie Oettinger, Ph.D., Department of Molecular Biology, Massachusetts General Hospital. "Epigenetic control of antigen receptor gene assembly."

Dr. Katsumi Matsuzaki, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan.

Dr. Katsumi Matsuzaki, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan. "Ganglioside cluster as a platform for the aggregation of Alzheimer's amyloid beta-protein."

Invited Speaker Seminars - 2007

NIA-Intramural Research Program

Dr. Adong Yu, CBS/Cardiovascular Branch, NHLBI, NIH. "Genome-wide search susceptibility genes of complex diseases in aging."

Howard T. Petrie, Ph.D., Scripps Institute, Jupiter, Florida. "Dissecting novel stromal signals for lymphocyte development in the post-natal thymus."
Professor Paolo Bernardi, Director, Department of Biomedical Padova. "Mitochondria and cell death: The permeability Leveraging population genetics for mapping human disease."

Bernard E. Flucher, Ph.D., Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria. "Old and new functions of auxiliary calcium channel subunits in excitable cells."

Dr. Krzysztof Jozwiak, Assistant Professor at the Faculty of Pharmacy, Medical University of Lublin, Poland. "The Familial Alzheimer's Disease mutations in Presenilins as a Guide in Resolving the Molecular Structure of Gamma-secretase."

Subbarao Bondada, Ph.D., Professor, Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky. "Role of B cell receptor signaling in normal B cell development and B lymphoma growth."

Somesh Baranwal, University of Pennsylvania School of Dental Medicine, Department of Pathology, Philadelphia, PA. "Actinobacillus actinomycetemcomitans Leukotoxin (Ltx)/LFA-1 interaction."

Anthony J. Capobianco, Ph.D., Associate Professor, Molecular and Cellular Oncogenesis Program, The Wistar Institute. "Notch Signaling and Tumorigenesis."

Dr. John O'Shea, Scientific Director, NIAMS, NIH. "Cytokines, Lymphocyte Development and Function."

Abbe N. de Vallejo, Ph.D., Associate Professor of Immunology and Pediatrics, University of Pittsburgh School of Medicine. "Immune Remodeling During Aging and in Chronic Disease."

Xiaochun Chen, Ph.D., NIH, NICHD. "A novel small RNA represses nod gene expression on transcriptional level."

John Tainer, Ph.D., The Scripps Research Institute, La Jolla, California. "Solution and crystal structures of DNA repair complexes: Master keys to the structural cell biology of cancer and aging."

APRIL
Akira Orimo, M.D., Department of Biology, Whitehurst Institute, MIT. "Tumor Promoting Properties of Stromafibroblasts in Invasive Human Breast Carcinomas."

James J. Werner, Ph.D., Assistant Professor, Department of Family Medicine, School of Medicine, Case Western Reserve University. "Developing Practice-Based Research Networks."

Gerald Rameau, Ph.D., Research Associate Faculty, Department of Urology, Johns Hopkins School of Medicine. "NMDA Receptor Regulation of nNOS Phosphorylation, AMPA Receptor Trafficking and Cell Death."

Dr. Bing Xia, Dana-Darber Cancer Institute and Harvard Medical School, Boston, Massachusetts. "BRCA2 and PALB2 in Breast Cancer and Fanconi Anemia."
David M. Dietz, Fellow, Florida State University. "Behavioral Sensitization to Amphetamine: The Role of Stress and Individual Differences."

Qin Wan, Ph.D., Department of Pharmacolocy, University of Maryland, School of Medicine. "Burst-dependent Protection from Homosynaptic Depression in Aplysia."

I-Feng Peng, Ph.D, Department of Biological Services, University of Iowa. "Neuronal Electrical Activates and Development in Drosphila Central Neurons - A Genetic and Physiological Approach."

Gunars Duburs, Ph.D., Head of Laboratory, Latvian Institute of Organic Syntheses, Riga, Latvia. "1,4-Dihydropyridines as biomimetics and bioprotectors."

Carl June, M.D., Professor, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine. "New Strategies in Human Adoptive T cell Therapy."

Invited Speaker Seminars - 2007

NIA-Intramural Research Program

Akira Sawra, M.D., Ph.D., Director, Program in Molecular Psychiatry, Department of Psychiatry and Behavioral Sciences, Department of Neuroscience, Graduate Program in Cellular and Molecular Medicine, Johns Hopkins School of Medicine. "A novel function of an old enzyme GAPDH: a sensor of oxidative stress towards chromatin remodeling."

James Osborn, Medical Robotics Technology Center, Carnegie Mellon University, Pittsburgh, Pennsylvania. "Quality of Life Technology."
Suzanne Topalian, M.D., Professor of Surgery and Oncology, Johns Hopkins Medicine, Director, Melanoma Program, Sidney Kimmel Comprehensive Cancer Center. "The Molecular Interface between Melanoma and the Immune System."

Tanya Barrett, Ph.D., NCBI/NLM/NIH. "NCBI GEO: tools to explore and mine public microarray data."

Benjamin Sredni, CAIR Institute, Bar Ilan University, Israel. "AS101 sensitizes solid and hematological tumors to taxol and abraxane via inhibition of IL-10-stat3-survivin signaling."

Dipanjan Chowdhury, Ph.D., CBR Institute for Biomedical Research, Department of Pediatrics, Harvard Medical School. "Role of Phosphatases in Removal of Gamma-H2AX."

Yunbo Ke, Ph.D., Research Assistant Professor of Physiology, University of Illinois at Chicago. "Function of P21 activated kinases-1 in cardiovascular cells."

Wei-Men Chen, Ph.D., Postdoctoral Fellow, Statistical Genetics, Center for Statistical Genetics, University of Michigan. "Efficient Use of Family Data in Genome-Wide Association Scans."

Dr. Cris Kamperschroer, Trudeau Institute. "SAP Function in T-cell help for B cells."

Dr. Eugene Drigalenko, Department of Neuropsychiatry and Behavioral Sciences, Texas Tech University Health Sciences Center. "Genome screen of Alzheimer disease families by a new multipoint linkage analysis method."
Dr. Adayabalam S. Balajee, Center for Radiological Research, Department of Radiation Oncology, Columbia University Medical Center. "Role of Tumor Suppressor Genes in Chromosome Stability."

John H.B. Bridge, Ph.D., Research Professor, Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah. "Brain Na channels appear to be essential for excitation-contraction coupling in heart: Studies on the structure and function of couplons."

Teresa Seeman, Ph.D., Professor of Medicine and Epidemiology, Associate Chief for Research, Division of Geriatrics, Geffen School of Medicine at UCLA. "Exploring a Biopsychosocial Model of Aging: Linking Social Experience to Biology."

Livio Mallucci, Ph.D., Professor, Kings College London, Pharmaceutical Sciences Research Division, London, UK . "PI3K targeting by the beta-GBP cytokine in cancer therapeutics and beyond. Down regulation of signaling and gene silencing."

Barbara Birshtein, Ph.D., Professor, Department of Cell Biology, Albert Einstein College of Medicine. "Good Fences Make Good Neighbors: Regulation of Antibody Heavy Chain Gene Rearrangements and Expression by a 3' lgh Regulatory Region."
Andrew S. McCallion Ph.D., Assistant Professor, McKusick-Nathans Institute for Genetic Medicine Johns Hopkins University School of Medicine. "Unraveling critical genetic mechanisms in development and disease."

JULY
Soren Brage, Ph.D., Associate Professor, University of Southern Denmark. "Measurement of Physical Activity in the Epidemiological Setting-Balancing Validity and Feasibility."

Ernest Beutler, M.D., The Scripps Research Institute. "Ethnic Variations in Hematologic Laboratory Values."

Invited Speaker Seminars - 2007

NIA-Intramural Research Program

George W. Huntley, Ph.D., Associate Professor, Fishberg Department of Neuroscience, The Mount Sinai School of Medicine, New York. "More missions for matrix metalloproteinases: novel roles extracellular proteolysis in remodeling synaptic connections."

Rick Flannery, Ph.D., Yale University. "CNG channels in olfactory cilia/Bcl-xL and synaptic regulation."

Antonio Rangel, Ph.D., California Institute of Technology. "The Neuroeconomics of Simple Choice."

Gerald S. Shadel, Ph.D., Department of Pathology, Yale University School of Medicine. "Mitochondrial Gene Expression in Aging and Disease."

Dana Spence, Michigan State University. "New Roles for Red Cells in the Circulation: Implications for Diabetes, Cystic Fibrosis, and More."
Christopher Austin, M.D., NIH Chemical Genomics Center, National Human Genome Research Institute. "The NIH Chemical Genomics Center: Translational Chemical Tools for the Genome Era."

Akhil Bhalla, Ph.D., Department of Physiology, University of Wisconsin. "Role of synaptotagmin in SNARE-catalyzed membrane fusion."

Dr. Chih-Chung Lu, National Taiwan University, Department of Microbiology, College of Medicine, Taipei, Taiwan. "The role of uracil-DNA glycosylase in lytic DNA replication of Epstein-Barr virus."

Dr. Youngkyoo Jung, Magnetic Resonance Imaging Research Laboratory, University of Wisconsin-Madison. "Rapid 3D MRI With Multiple Echo Radial Steady-State Acquisition."
Dr. Qinghua Chen, Sanders-Brown Center on Aging. "Studies of Aging and Alzheimer's Disease."

Christopher A. Ross, M.D., Ph.D., Professor of Psychiatry, Neurology and Neuroscience; Director, Division of Neurobiology; Director, Baltimore Huntington's Disease Center, Johns Hopkins University School of Medicine. "Genetics and Pathogenesis of Huntington's disease."

SEPTEMBER
John M. Dopp, PharmD, Associate Professor of Pharmacy, University of Wisconsin School of Pharmacy. "Sleep Apnea and Vascular Dysfunction: Strategies for Prevention and Treatment."

Olle Melander, M.D., Ph.D., Clinical Research Center, Malmo University, Sweden. "Renal sodium reabsorption and genetic variation in hypertension and cardiovascular disease."

Damineh Morsali, Department of Pharmacology, Wolfson College, Oxford, UK. "Cross-talk between the periphery and injured central nervous system; involvement of hepatic macrophages."

Melitta Schachner, Ph.D., New Jersey Professor of Spinal Cord Research, Keck Center for Collaborative Research, Department of Cell Biology and Neurosciences, Rutgers University. "Adhesion molecules and the rejuvenating nervous system."

Susan Smith, Associate Professor, Skirball Institute of Bimolecular Medicine, NYU School of Medicine. "The role of tankyrase 1 in sister telomere cohesion and mitotic progression."

Drs. Lesley Colby and Bob Dysko, University of Michigan at Ann Arbor. "Commissioning and Occupancy of New Vivaria at the University of Michigan."
Martin F. Flajnik, Ph.D., Professor, Microbiology and Immunology, University of Maryland. "Serendipity and Evolution of the Adaptive Immune System."

Thais Moreira, Ph.D., Postdoctoral Fellow, Department of Pharmacology and experimental Therapeutics, University of Maryland School of Medicine. "Angiotensin II Modulates Nodose Neurons Excitability."

Charles G. Drake, M.D., Oncology, Immunology, and Urology, Johns Hopkins Sidney Kimmel Comprehensive Cancer Center. "Immunotherapy for Prostate Cancer."

Invited Speaker Seminars - 2007

NIA-Intramural Research Program

OCTOBER

John A. Hanover, Ph.D., Laboratory of Cell Biochemistry and Biology, NIDDK, NIH. "The 'O-GlcNAc code': A nutrient sensing pathway linked to diabetes and neurodegeneration."

Judy Hwang, Biophysics Graduate Program, University of California, Berkeley, CA. "Mapping Metabolic Pathways in the Metal Reducer, Shewanella Oneidensis MR-1."

Dr. David Le Couteur, Professor of Geriatric Medicine, The University of Sydney, Director, Centre for Education and Research in Aging. "Old Age and the Hepatic Sinusoid."

Zackary I. Cleveland, Graduate Research Assistant, Department of Chemistry, Colorado State University, Fort Coliings, Colorado. "Hyperpolarized 83kr MRI and NMR: Prospects for Pulmonary Biomedicine."

Dorothy E. Shippen, Ph.D., Professor, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas. "End games: Telomere dynamics and evolution in Arabidopsis."

Michael Zimmerman, Office of the Dean, College of Liberal Arts and Sciences, Butler University. "The Clergy Letter Project: Building Bridges to Enhance Science Literacy."

Marcus P. Cooper, M.D., Instructor of Medicine, Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA. "Metabolic control through LRP130: Implications for diabetes and obesity."

Bradley T. Hyman, Ph.D., M.D., Director, Massachusetts Alzheimer Disease Research Center, Massachusetts General Hospital, Boston, MA. "The Pace of Alzheimer's Disease."

Michela Gallagher, Ph.D., Department of Psychological and Brain Sciences, Johns Hopkins University. "Individual Differences in Neurocognative Aging."

Dr. Siliva Naitza, SardiNIA Study. "IRAK-M is involved in the pathogenesis of early-onset persistent asthma."

Dr. Laura Crisponi, SardiNIA Study. "Crisponi syndrome is caused by mutations in the CRLF-1 gene and is alleli to cold-induced sweating syndrome type 1."

Dr. Serena Sanna, SardiNIA Study. "Statistical genetics of highly inter-related founder population cohorts."
Ravi Varadhan, Ph.D., Johns Hopkins University, Department of Medicine. "Predictive Learning Methods in Observational Studies of Geriatric Outcomes."

NOVEMBER

R. Stephen Lloyd, Ph.D., Professor and Senior Scientist, Department of Molecular and Medical Genetics, Center for Occupational and Environmental Toxicology, Oregon Health and Science University, Portland, OR. "Threading a knot through the eye of a needle: Replication bypass of interstrand DNA crosslinks."

Michael Griswold, Ph.D., Johns Hopkins University, Bloomberg School of Public Health. "Much Ado About Nothing."

Professor Christian Roussel, University Paul Cezanne, Marseille, France. "Accessing the Expanded Chiral Pool using ChirBase Molecular Database."

Onyi Irrechukwu, Georgia Institute of Technology, Atlanta, Georgia. "The Role of Matrix Composition and Age in Diffusion within Articular Cartilage."

Jose A. Cancelas, M.D., Ph.D., Division Director of Research, Hoxworth Blood Center, Associate Professor of Pediatrics, Division of Experimental Hematology, Cincinnati Children's Hospital Medical Center. "Rac activation: a drug target in leukemia."

Amina S. Woods, Ph.D., Cellular Neurophysiology Section, National Institute of Drug Abuse/NIH. "The Biological Magnets: A Study of Phosphorylated compounds interactions."

Dr. John Atack, Chief, Neuroscience Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, La Jolla, CA. "H3 Receptors and Cognition."

Invited Speaker Seminars - 2007

NIA-Intramural Research Program

Barry P. Sleckman, Ph.D., Associate Professor of Pathology and Immunology, Washington University School of Medicine. "Lymphocyte Development: Taking Cues from Damaged DNA."

Dr. Janusz Pawliszyn, Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada. "In-vivo Applications of New Sampling/Sample Preparation Technologies."

Lars-Oliver Klotz, Ph.D., Associate Professor, Department of Molecular Aging Research, Institute of Research in Environmental Medicine. "Stress-induced modulation of FoxO transcription factors: implications for selenium homeostasis."

Gang-Ming Zou, Ph.D, Department of Pathology, Johns Hopkins University School of Medicine. "The Redox Regulatory Role of Apel in Hematopoietic Stem Cells and Their Niche."

Roberto Agis-Balboa, The Psychiatric Institute, The University of Illinois at Chicago. "Down-regulation of neurosteroid biosyntheses in corticolimbic circuits mediates social isolation-induced behavior in mice."

Deepta Bhattacharya, Ph.D., Institute for Stem Cell Biology and Regenerative Medicine. "Cell extrinsic control of hematopoietic homeostatis."

Hava Avraham, Ph.D., Associate Professor of Medicine, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine. "Angiogenic and Cell Survival Functions of VEGF and VEGFR-1 in Breast Cancer Cells: Application to Anti-Angiogenesis Therapy."

Karen Bandeen-Roche, Ph.D., Johns Hopkins University, Bloomberg School of Public Health. "An Introduction to Latent Variable Analysis."

Roberto Cabeza, Ph.D., Center for Cognitive Neuroscience, Duke University. "Title To Be Determined."

Kwang Seok Ahn, Ph.D., Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center. "Inflammation as a Target for Prevention and Treatment of Cancer."

DECEMBER

Scott Wakefield, Asawari Samant The MathWorks, Natick, Massachusetts. "MATLAB: Bioinformatics Capabilities and Systems Biology."

Yusen Liu, Ph.D., Associate Professor of Pediatrics, Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Department of Pediatrics, Columbus, Ohio. "Regulation of innate immune responses by MAP kinase phosphatase-1: It is the time to shut down the inflammation."

Christopher Price, Orthopedics Research Laboratory, Department of Orthopedics, Mount Sinai School of Medicine, New York. "Systems based approaches to identifying the effect of genetic variability on bone growth and functionality-fragility and fracture."

Meenhard Herlyn, D.V.M., D.Sc., Professor and Program Leader, Molecular and Cellular Oncogenesis Program, The Wistar Institute. "Understanding melanoma biology to develop new strategies for therapy."

Solomon Snyder, Ph.D., Johns Hopkins University School of Medicine. "Novel Neural Messengers."

M. Daniele Fallin, Ph.D., Johns Hopkins University, Bloomberg School of Public Health. Genetics of Aging Disorders."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

JANUARY

Kathleen Griffioen, Ph.D. "Cellular Mechanisms of Central Cardiorespiratory Responses to Hypoxia."

Edward D. Huey, M.D., Assistant Clinical Investigator, Cognitive Neuroscience Section, NINDS, NIH. "Frontal lobe syndromes in dementia."

Ke-Yong Li, Ph.D., Department of Anesthesiology, University of Medicine and Dentistry of New Jersey. "Nanamolar Propofol Stimulates Transmission to Dopamine Neurons: A Possible Mechanism of Abuse Potential."

Nicholas Mitchell, Ph.D., Visiting Assistant Professor of Biology, Lynchburg College, Lynchburg, VA. "High and Low Affinity Ampa Receptor Subunit Combinations Reveal the Importance of Binding-Site Occupancy to Ion Channel Function."

Ted M. Dawson, M.D., Ph.D., Leonard and Madlyn Abramson Professor in Neurodenerative Diseases, Johns Hopkins University School of Medicine. "Molecular Mechanisms of Neurodegeneration in Parkinson's Disease: Looking Forward to Tomorrow's Therapies."

Daniel Procissi, Ph.D., California Institute of Technology. "Intergrating MRI and MRS in Biomedical Research. The role of an MRI Scientist."

Jagan M.R. Pongubala, Ph.D., Research Assistant Professor, Department of Molecular Genetics and Cell Biology, The University of Chicago. "Transcriptional control of B cell fate commitment."

Hugo R. Arias, Ph.D., Department of Pharmaceutical Sciences, Midwestern University, Glendale, AZ. "Interaction of novel ibogaine analogs with nicotinic receptors: Potential anti-addictive therapy."

Roger Reeves Ph.D.,Core Faculty, McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University School of Medicine. "Therapies for and from Down's Syndrome."

Robert C. Young, M.D., Chancellor, Fox Chase Cancer Center, Philadelphia, PA. "The Global Burden of Cancer."

FEBRUARY

Dr. Guo-Min Li, Professor, Department of Toxicology, Joint Appointment in Department of Pathology, University of Kentucky, Lexington, KY. "DNA Loop Repair in Human Cell Extracts."

Bob Hous, Ph.D., Memorial Sloan-Kettering Cancer Center. "Quantitative Perfusion, Diffusion, and Functional MRI of a Brain."

S. Sendhil Velan, Ph.D., The Center for Advance Imaging at West Virginia University. "Investigation of Lipid Metabolism in Human Subjects Using Novel Magnetic Resonance Spectroscopy Techniques at 3 Tesla."

Leslie Miller, M.D., Walters Chair of Cardiovascular Medicine, Director of Cardiology Programs, Washington Hospital Center & Georgetown University Hospital. "Myocardial Recovery with the Novel Beta-2 Agonist, Clenbuterol."

Douglas C. Wallace, Ph.D., Donald Bren Professor of Molecular Medicine, Director, Center for Molecular and Mitochondrial Medicine and Genetics (MAMMAG), University of California, Irvine. "The Mitochondrial Role in Aging and Age-Related Diseases."

Angelique Regnier, Ph.D., Doctoral School of Physiology and Biology, University Paris VII-Denis Diderot Medical School, Paris, France. "Neuronal damage in the temporal lobe after thalamicinduced generalized seizures in the rat."

Dr. Odile Gabay, Department of Physiology and Pathophysiology, University Pierre and Marie Curie Paris, France. "Mechanotransduction in cartilage and bone under compression."

Natasha Caplen, Ph.D., National Cancer Institute, NIH. "RNAi in mammalian cells - small RNAs to large screens."

Yang Shi, Ph.D., Professor of Pathology, Harvard Medical School. "Histone demethylases and dynamic regulation of histone methylation."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

MARCH

Dr. Takashi Yonetani, University of Pennsylvania. "Dynamic Allostery Model of Hemoglobin: Integration of structure, function, allostery, dynamics, and energetics."

Wei Yang, Ph.D., NIH, NIDDK, Laboratory of Molecular Biology. "Stop-action movie of UvrD helicase unwinding DNA one base pair per at a time."

Dan Stashuk, Ph.D., Professor, University of Waterloo, Department of Systems Design Engineering, Ontario, Canada. "Electrophysiological Characterization of Muscle Using Quantitative EMG Measures."

Rong-Fong Shen, Ph.D., Head, Proteomics Core Facility, National Heart, Lung and Blood Institute, NIH. "Mass spectrometry-based proteomics and biomedical applications."

Sangram Sisodia, Director, The Center for Molecular Neurobiology, University of Chicago. "Function and Dysfunction of Presenilins in Alzheimer's Disease."

Ryuji Kobayashi, Ph.D., Professor, Molecular Pathology, MD Anderson Cancer Center, University of Texas. "A New Approach for Proteomics."

Heather Marton, Ph.D., Johns Hopkins Medical School. "Two tales of factors involved in RNA polymerase II transcription."

Sharon Milgram, Ph.D., Director, NIH Office of Intramural Training and Education. "Creating and Presenting a Dynamic Poster."

David M. Smalley, Ph.D., Assistant Professor, Mellon Medical Biomarker Discovery Laboratory, Cardiovascular Research Center, University of Virginia, Charlottesville, VA. "Selection of Highly Informative Subproteomes for Biomarker Discovery."

Hiroe Kikuchi, M.D., Ph.D, Postdoctoral Researcher, Educational Physiological Laboratory, Graduate School of Education, University of Tokyo, Japan. "Dynamic Organization of Our Behavior: Methods, Analyses, Interpretation and Future Directions."

Edward J. Goetzl, M.D., Director, Immunology and Allergy Research, University of California, San Francisco. "T Cell and Macrophage Alterations in Mammalian Immunosenescence."

Markus Hardt, Ph.D., University of California San Francisco. "Proteolytic Systems through the Lens of Mass Spectrometry."

Ryuji Kobayashi, Ph.D., Professor, Molecular Pathology, MD Anderson Cancer Center, University of Texas. "A New Approach for Proteomics."

Eric Guervin, President, Lutronic North America. "Nonatec Micro-Transponders and Traceability Solutions."

APRIL

Dr. Quentin Li, National Cancer Institute, NIH. "Epigenetic regulation of mitogen-induced immediate early gene in T lymphocytes."

Dr. Julia Pridgeon, United States Department of Agriculture. "Molecular Mechanisms of Neurodegenerative Diseases."

Kurt G. Beam, Ph.D., Professor, Department of Physiology and Biophysics, University of Colorado at Denver & Health Sciences Center. "Probing the cytosolic structures unnderlying excitation-contraction coupling with biotin and fluorescent proteins."

Christopher Peers, Ph.D, Professor of Cellular Physiology, Academic Unit of Cardiovascular Medicine, Leeds University, Leeds, UK. "Hypoxia and Neurodegeneration."

Stefan Gravenstein, M.D., M.P.H., Clinical Director, Quality Partners of Rhode Island, Providence, Rhode Island. "Influenza, Heart Attacks and Strokes: Linking Inflammation and Aging."

Dr. Roger Kautz, Director, NMR Facility, Principal Research Scientist, Barnett Institute of Chemical and Biological Analysis, Northeastern University. "An Optimized LC-MS+NMR Strategy Using a Microcoil NMR Probe with Normal-bore LC."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

MAY

Jiukuan Hao, Ph.D., M.D., School of Pharmacy, Texas Tech University, Amarillo, TX. "Therapy of Stroke with Novel Neuroprotective and Anti-inflammatory Agents."

Leslie Miller, M.D., Walters Chair of Cardiovascular Medicine, Director of Cardiology Programs, Washington Hospital Center & Georgetown University Hospital. "Myocardial Recovery with the Novel Beta-2 Agonist, Clenbuterol."

Dimitrios Kapogiannis, M.D., Clinical Fellow, National Institute of Neurological Disorders and Stroke, NIH. "Reward processing in humans: lessons learned from the animal literature."

Zahide Ozer-Aras, Ph.D., Staff Scientist in Biochemistry, Group Leader in Protein Chemistry, Parker Hughes Cancer Institute, St. Paul, MN. "DNA-Dependent Protein Kinase component of Ku Protein Associate with Ikaros Isoforms and Enhances Their DNA Binding Affinity."

Dr. Shukdeb Sen, Professor of Biology, Bethune Cookman University, Daytona Beach, FL. "Cross talk between Nitric Oxide and Hemoglobin influencing seed germination."

Sara Farquhar, PT, Doctoral Candidate, Biomechanics and Movement Science Program, University of Delaware. "Changes in Strength and Function After Unilateral Total Knee Replacement."

JUNE

Yoshiharu Yamamoto, Ph.D., Associate Professor, Educational Physiological Laboratory, Graduate School of Education, University of Tokyo, Japan. "Complexity in Daily Life: Toward Understanding of Out Behavioral Organization."

Dr. Isao Shimokawa, Professor, Department of Investigative Pathology, Unit of Basic Medical Science, Graduate School of Biomedical Sciences, Nagasaki University. "A potential role for RoxO! in the effect of calorie restriction."

Nicole Noren-Hooten, Ph.D., Staff Scientist, Burnham Institute for Medical Research, La Jolla, CA. "Multiple Roles for the EphB4 Receptor in Breast Cancer."

Ranga N. Venkatesan, Ph.D., Senior Research Fellow, Joseph Gottstein Memorial Cancer Research Laboratory, University of Washington Department of Pathology. "Consequences of mutation at the active site of DNA polymerase delta."

Lee Zou, Ph.D., Assistant Professor, Harvard Medical School. "Sensing of DNA damage by the checkpoint kinases ATR and ATM."

Jeffrey Field, Ph.D., Associate Professor, University of Pennsylvania, School of Medicine. "Sensing of DNA damage by the checkpoint kinases ATR and ATM."

Sarah Rothman, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA. "Spinal inflammation in radicular pain."

JULY

Dr. Zhong Wang, Massachusetts General Hospital, Harvard Medical School, Richard B. Simches Research Center, Boston, MA. "ATP dependent chromatin remodeling in embryonic and cardiac stem cell development."

Richard F. Loeser, Jr. M.D., Head, Section of Molecular Medicine, Wake Forest University School of Medicine. "Aging and Osteoarthritis: It's Not Just Wear and Tear."

Shintaro Seto, Ph.D. "Mycobacterium tuberculosis inhibits phagolysosome biogenesis in macrophages by modulating localizations of Rab GTPase proteins on it phagosome."

Dr. Howard G. Shertzer, Professor of Environmental Health & Center for Environmental Genetics, Director of Division of Environmental Genetics and Molecular Toxicology, University of Cincinnati Medical Center. "The amazing biochemical and pharmaceutical properties of tetrahydroindenoindole."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

Mark Obrenovich, PhD, Department of Pathology, Case Western Reserve University, Cleveland, OH. "Glycation by ascorbic acid (ascorbylation), relevance to health disease and aging."

Dr. Catherine C. Fenselau, Professor of Chemistry and Biochemistry, Department of Chemistry and Biochemistry, University of Maryland College Park. "Residue-specific checmical cleavage for proteomics."

Yulan Liang, Ph.D., Department of Biostatistics, School of Public Health and Health Professions, University of Buffalo, the State University of New York. "Statistical Methods in Human Genomic Research for Complex Diseases."

Fengyu Zhang, Ph.D., MSM, MS, Senior Statistical Geneticist (Contractor), National Institute of Mental Health, NIH. "Overview and Applications of Statistical Methodology in Genetic Study of Complex Human Disorders/ Traits."

AUGUST

J. Abigail Woodroffe, Graduate Student Research Assistant, Department of Ophthalmology and Center for Statistical Genetics, University of Michigan. "Genetic Epidemiology of Complex Diseases: examples from Primary Open Angle Glaucoma and Schizophrenia."

Benjamin Sredni, M.D., CAIR Institute, Bar Ilan University, Israel. "Interference with the interaction of VLA-4 and fibronectin in AML cells by the Tellurium compound AS101 results in their sensitization to chemotherapy."

Linda Malkas, Ph.D., Vera Bradley Chair of Oncology, Indiana University School of Medicine . "Altered Human Cell DNA Synthesis: Implications for Biomarker and Therapeutic Discovery."

Stacey S. Cherny, Ph.D., Department of Psychiatry, The University of Hong Kong. "Searching for Huntington's Disease modifier genes in the Venezuelan HD kindreds."

SEPTEMBER

Trent Woodruff, Ph.D., School of Biomedical Sciences, University of Queensland, Queensland, Australia. "Role of the complement factor 5a (C5a) in neurodegenerative disease: inhibition of neuro-inflammation with a specific c5a receptor antagonist."

Dr. Emily Patterson, Johns Hopkins Medical School. "Convergence and Complexity of the Hepatitis C Virus-Specific CD8+ T Cell Response."

Valter Longo, Ph.D., Associate Progessor, Hanson Chair of Biogerontology, School of Gerontology and Department of Biological Sciences, University of Southern California, Los Angeles . "Turning Anti-aging Genes Against Chemotherapy."

Jenna Carroll, Neuroscience Department, University of Southern California, Los Angeles, California. "Sex Steroid Hormones and Alzheimer's Disease."

David Fox, University of Washington, Department of Biochemistry, 2008 NIH National Graduate Student Research Festival Award. "BARD1 Ankyrin Repeat Domain and Its Functional Consequences."

Albert Gjedde, M.D., D.Sc., PET Center, Aarhus University Hospital, Aarhus, Denmark. "Steady-state cerebral blood flow rate: How faithful a marker of neuronal activity?"

Christine Van Broeckhoven, Ph.D., University of Antwerp Institute Born-Bunger, Flanders Institute for Biotechnology, Antwerpen, Belgium. "A genetic approach to neurodegenerative dementias."

Keji Zhao, Ph.D., Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute. "Characterization of human epigenomes."

George Kunos, M.D., Ph.D., Senior Investigator, Neuroendocrinology Section, Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism. "Endocannabinoids and Energy Homeostasis."

OCTOBER

Dr. Shiro Kanegasaki, University of Tokyo. "Development of Effective Drugs for Cancer and Inflammatory Diseases."

David M. Holtzman, M.D., Andrew and Gretchen Jones Professor and Chair, Washington University School of Medicine, Department of Neurology, St. Louis, MO. "From man to mouse: Amyloid beta metabolism and the pathogenesis, diagnosis, and treatment of Alzheimer's disease."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

Elizabeth Murphy, Ph.D., Chief, Cardiac Physiology Section, Translational Medicine Branch, National Heart, Lung and Blood Institute, NIH. "Male-Female Differences in Cardioprotection."

Jun Yao, M.D., Ph.D., Department of Neurology, Mount Sinai Medical Center, New York. "Role of A beta-binding Alcohol dehydrogenase in A beta-induced cell street in Alzheimer's disease."

Silvio Monfardini, M.D., Chief, Geriatric Oncology Program, Instituto Oncologico Veneto. "Medical Cancer Treatment in the Elderly."

Jonathan P. Rast, Ph.D., Scientist, Sunnybrook Health Sciences Center, Assistant Professor, Department of Medical Biophysics and Department of Immunology, University of Toronto. "Complex Innate Systems in the Sea Urchins and the Origins of Vertebrate Immunity."

NOVEMBER

Alfredo Kirkwood, Ph.D., Associate Professor of Neuroscience, The Solomon H. Snyder Department of Neuroscience and Mind/Brain Institute, The Johns Hopkins University. "A Switch in Synaptic Plasticity Mechanisms May Prevent Cognitive Decline with Aging."

Alfredo Kirkwood, Ph.D., Associate Professor of Neuroscience, The Solomon H. Snyder Department of Neuroscience and Mind/Brain Institute, The Johns Hopkins University. "Sulfiredoxin specifically catalyzes the deglutathionylation of 2-Cys peroxiredoxin: An in vitro and in vivo study."

Michal Schwartz & Noga Ron, Professor and Student of Neuroimmunology, Department of Neuroimmunology, The Weizmann Institute of Science, Rehovot, Israel. "Immune over brain: Innate and adaptive immunity contrinute to central nervous system plasticity and repair."

Alexader Deten, M.D., Fraunhoder Institute for Cell Therapy and Immunology, Leipzig, Germany. "Cord blood cells to treat myocardial infarction? Experimental studies in rats."

Zoltan Ungvari, M.D., Ph.D., Associate Professor, Department of Physiology, New York Medical College, Department of Physiology, Valhalla, New York. "Oxidative stress and inflammation: at the Crossroad of Aging, Diabetes and Cardiovascular Disease."

Hongjun Liu, Ph.D., Postdoctoral Fellow, Translational Medicine Branch, NHLBI, National Institutes of Health. "The Role of Wnt Signaling in Mammalian Aging."

Dr. Livio Mallucci, School of Health and Life Sciences, Kings College London, London, UK. "Cytokine Property of the Regulatory Protein beta-GBP."

Jeonga Kim, Ph.D., Research Assistant Professor, Department of Internal Medicine, University of Missouri-Columbia, MU Diabetes and Cardiovascular Center. "Insulin Resistance and Endothelial Dysfunction: role of pro-inflammatory signaling and potential therapeutic targets for age-related diseases."

Carmen Vivar-Estudillo, Postdoctoral Fellow, Center for Research and Advanced Studies, Department of Physiology, Biophysics and Neurosciences. "Effects of the Mossy Fiber GABAergic Transmission on Pyramidal Cells and Interneurons."

David A. Steinman, Ph.D., Professor, University of Toronto. "Image-Based Hemodynamic Modeling of the Carotid Bifurcation in the Validate Study."

Melanie Willingham, Ph.D., Medical Education Unit Tutor, Department of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, Australia. "Investigating Roles and Mechanisms of Proneurotrophin Signaling."

Adil Khan, Ph.D., Staff Research Investigator, The Buck Institute for Age Research. "Neuronal Death Signaling and Neuroglobin."

DECEMBER

Dr. Sandford P. Markey, Chief Laboratory of Neurotoxicology, NIMH, NIH. "Proteomics in the study of normal and aberrant neuronal structure and function."

Invited Speaker Seminars - 2008

NIA-Intramural Research Program

Shaday Michan, Ph.D., Postdoctoral Fellow, Pathology Department, Harvard Medical School. "The Multifaceted role of sirtuins in aging: from cancer to cognition."

Mark Baxter, Ph.D., Welcome Trust Senior Research Fellow in Basic Biomedical Science, Department of Experimental Psychology, Oxford University. "A cholinergic explanation of dense amnesia?"

Shih-Chieh Lin, M.D., Ph.D., Postdoctoral Fellow, Department of Neurobiology, Duke University. "The roles of non-cholinergic basal forebrain neurons in top-down attention."

Takashi Sato, M.D., Ph.D. Postdoctoral Fellow, Janelia Farm Research Campus. "Imaging the response properties of single cortical neurons identified by their targets."

Bruce Howitz, M.D., Ph.D., Division of Immunology Research, Department of Pathology, Boston, MA. "Regulation of Mucosal Inflammation by NF-kB."

Index of Principal Investigators

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
National Institute on Aging