# National Institute on Aging Intramural Research Program

# *Factbook 2006*

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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.

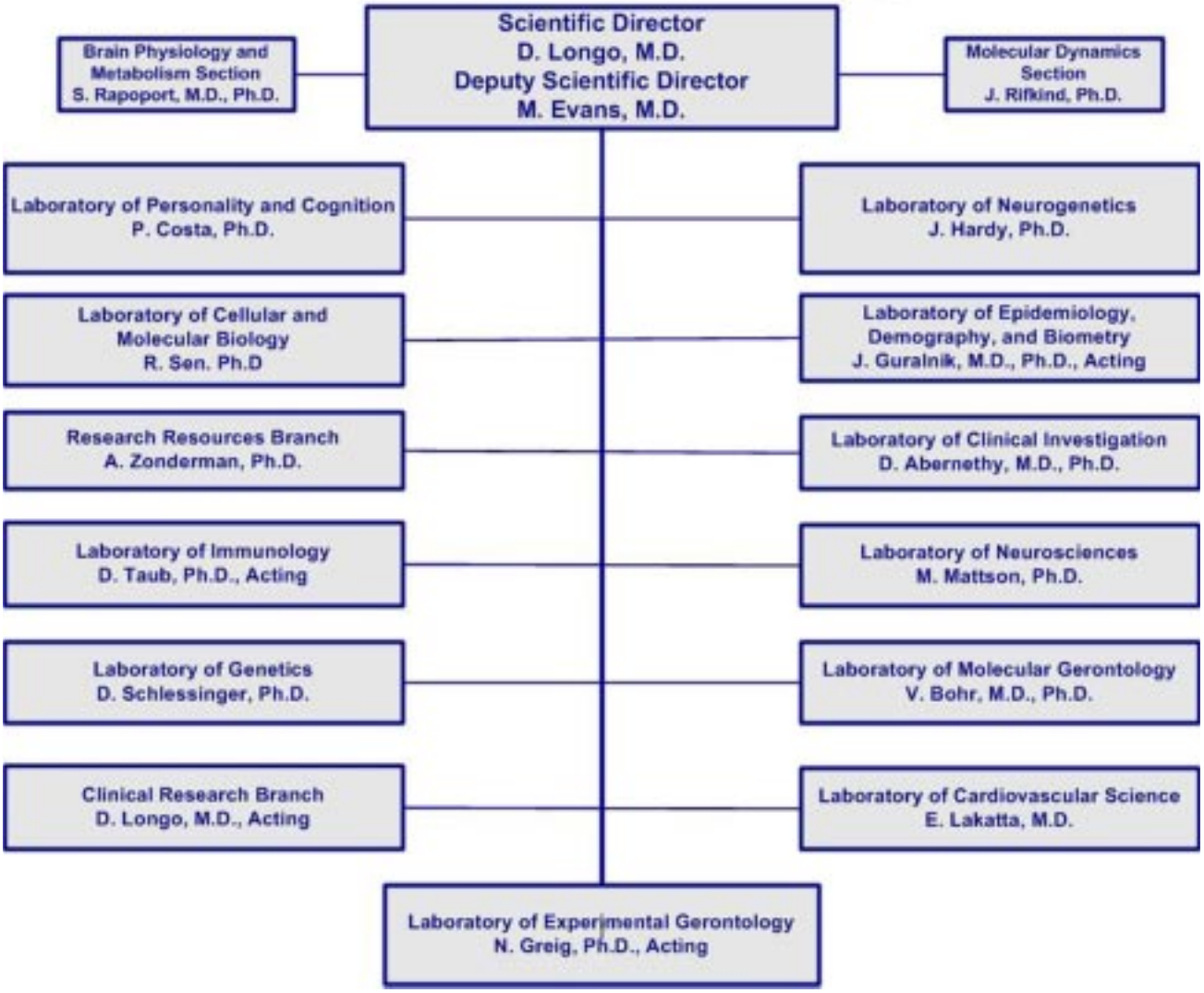
The Intramural Research Program (IRP) in the National Institute on Aging (NIA) comprises 11 scientific laboratories, a clinical research branch, a research resources support branch and 2 sections. The research program includes the scientific disciplines of biochemistry, cell and molecular biology, genetics, 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 Gerontology Research Center and the Triad Building 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 two sections: Cardiac Function and Behavioral Hypertension. The Cardiac Function Section, which comprised the entire LCS at its incipience, is organized into eight functional units, each headed by a tenured or senior scientist: the Cardiovascular Gene Therapy Unit, the Cardiovascular Biology Unit, the Cardioprotection Unit, the Cellular Biophysics Unit, the Hypertension Unit, the Receptor Signaling Unit, the Human Cardiovascular Studies Unit, and the Molecular Cardiology Unit. The Behavioral Hypertension Section had formerly been part of the Laboratory of Behavioral Science, and joined LCS in 1997.

The overall goals of the Laboratory of Cardiovascular Science are (1) to identify age-associated changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to 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<br>Vascular structure<br>Cardiovascular function at rest | Drugs<br>Postural reflexes<br>Exercise stress | Age, gender, race, socioeconomic status<br>Disease (CAD, hypertension), risk factors, and prevention<br>Genetics |
| **Intact Animals**<br>**Heart Failure**<br>**Hypertension**<br>**Aging**<br>**Preconditioning**<br>**Arterial Injury** | Arterial remodeling of aging<br>Cardiac remodeling post myocardial infarction, endogenous na/K ATPase ligands<br>Gene expression<br>VSMC proliferation and migration | Novel drugs | Age<br>Growth factors<br>Diet<br>Thyroid status<br>Local or systemic drug delivery<br>Gene therapy<br>Stem cell therapy |
| **Isolated Heart or Cardiac Muscle** | Myocardial contractile properties, excitation-contraction coupling, Ca2+ signals, action potentials | Ischemia<br>Anoxia, hypoxia<br>Free radicals<br>Neuropeptides<br>Novel drugs<br>Stretch | Age<br>Diet<br>Exercise<br>Hyperthyroid state<br>Cardiomyopathy<br>Heart failure |
| **Cardiac Cells**<br>**Myocytes**<br>**Fibroblasts** | Membrane ionic channel currents<br>Cardiac cell contraction<br>Cystolic Ca2+ signals<br>Mitochondrial Ca2+ signals<br>Sarcolemmal ion transport<br>Sarcoplasmic Reticulum function<br>Apoptosis | Receptor stimulated second messengers<br>Neuropeptides<br>Stretch<br>Anoxia, hypoxia<br>Free radicals<br>Novel drugs<br>Anesthetics<br>Growth factors<br>Novel endocardial factors<br>Novel endothelial factors | Development<br>Age<br>Disease<br>Heart failure<br>Hypertension<br>Diet<br>Growth factors<br>Hypoxia |
| **Vascular Smooth Muscle and Endothelial Cells** | Cystolic Ca2+ and pH regulation<br>Proliferation and secretion<br>Chemotaxis and invasion<br>Matrix regulation<br>Tubulin/microtuble dynamics<br>Differentation regulation<br>Angiogenesis | Shear stress<br>Receptor agonists/antagonists<br>Growth factors<br>Anoxia, hypoxia<br>Stretch<br>Anti-microtubule agents<br>Matrix degradation<br>Antisense inhibition and gene overexpression | Atherosclerosis<br>Arterial injury<br>Aging<br>Dedifferentation |
| **Stem Cells** | Mechanisms of pluripotency | Homing factors | Differentation into heart and vascular cells |
| **Sub-Cell Organelles** | Na/K transport systems<br>Sarcolemmal ion channels<br>Sarcoplasmic reticulum Ca2+ cycling<br>Mitochondrial membrane potential regulation, ATP K+ channels | Ionic composition<br>Adenine nucleotides<br>Neuropeptides<br>Ischemia, anoxia<br>Drugs<br>Reactive oxygen species | Age<br>Heart failure<br>Hypertension |
| **Molecules** | Genomics-SAGE cDNA assays<br>Control mechanism of gene expression in heart and vascular cells, ryanodine receptors, IP 3 receptors, G proteins<br>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<br>Stretch mechanisms<br>Growth factors<br>Neuropeptides<br>Nitric Oxide<br>Reactive oxygen species | Age<br>Hormones<br>Hypertension<br>Heart failure<br>Genetic manipulation |

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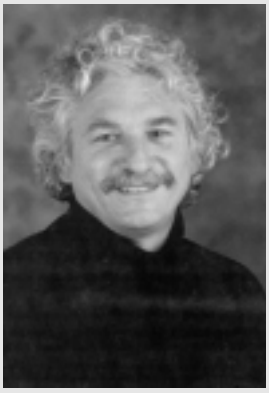

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**Biography:** Dr. Lakatta received his M.D., Magna cum laude, at Georgetown University School of Medicine. Following an internship and residency in Medicine at Strong Memorial Hospital, University of Rochester, Rochester, N.Y., he trained in basic research for two years at the NIH. Subsequently, he completed his cardiology fellowship at Georgetown and Johns Hopkins University Schools of Medicine. This was followed by a year of basic research training in the Department of Physiology, University College and the Cardiothoracic Institute, London England. Dr. Lakatta 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 270 original publications in top peer reviewed cardiovascular journals, written over 180 invited reviews/book chapters and delivered over 320 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., 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 two 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, 3 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
mitochondria
ischemia/reperfusion preconditioning
chemotaxis

**Recent Publications:**

Zorov DB, et al. *Biochim Biophys Acta* 2006; 1757(5-6): 509-517.

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.

**Publications-continued:**

Juhaszova M, et al. *Cardiovasc Res* 2005; 66(2): 233-244.

Juhaszova M, et al. *J Clin Invest* 2004; 113(11): 1535-1549.

**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 (MPT) 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 MPT is the end effector in these processes: the threshold for MPT induction by ROS being significantly reduced after ischemia reperfusion, but beneficially increased by preconditioning. The general mechanism of protection against oxidant stress damage was shown to be the convergence of more than one dozen distinct protection signaling mechanisms via inhibition of GSK 3β on the end effector, the permeability transition pore complex, to limit induction of the MPT. 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 two drugs applied to stents that have been shown to safely and effectively reduce the incidence of restenosis in humans. Paclitaxel eluting stents currently comprises ~60-70% of the drug eluting stents utilized worldwide. My lab is interested in novel strategies for vascular protection.

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

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

**Keywords:**

gene therapy
cardiac functions
hemodynamics
microcirculation
angiogenesis
heart failure
myocardial infarction

**Recent Publications:**

Moon C, et al. *J Pharmacol Exp Ther* 2006; 316(3): 999-1005.

Ahmet I, et al. *Heart Fail Rev* 2005; 10(4): 289-296.

Ahmet I, et al. *Circulation* 2005; 112(20): 3115-3121.

Wang M, et al. *Hypertension* 2005; 167(5): 1429-1442.

Moon C, et al. *Cardiovasc Durgs Ther* 2005; 19(4): 243-250. .

Ahmet I, et al. *Circulation* 2004; 110(9): 1083-1090.

Ahn D, et al. *Am J Physiol Heart Circ Physiol* 2004; 286(3): H1201-H1207.

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

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

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

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

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

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.

**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 established that the effective EPO dose can be reduced to 500 IU/kg, i.e. in the range of FDA doses approved for the treatment of anemia.

**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 β-adrenergic receptors (AR) subtype signaling in development of CHF is clearly important but purely understood. It is widely accepted now that β-1 AR activation is associated with development of CHF, thus, the use of β-1 AR antagonists became a recommended therapy for HF. The possible role of β-2 AR agonists remains debatable; however, it appears that similarly to β-1 AR, activation of β-2 AR during CHF is harmful. Recent research in the Laboratory of Cardiovascular Science using single myocytes indicated that β-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 β-2 AR agonist, fenoterol, and β-1 AR blocker, metaprolol, in rats starting 2 weeks after ligation of a coronary artery. Our results indicated that both, β-2 AR agonist and β-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. Our results suggest that beneficial effects of chronic treatment with β-2 AR agonists and β-1 AR blockers in CHF might be complimentary.

**Collaborators:** Richard Spencer, M.D., Nuclear Magnetic Resonance Section, Laboratory of Clinical Investigation, NIA, NIH; Maurizio Capogrossi, M.D., Institute of Dermatology, Rome, Italy; Piero Anversa, M.D., Cardiovascular Research Institute, Valhalla, NY; Irni Kovesdi, Ph.D., GenVec Inc., Rockville, MD; Edward G. Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH.

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**Biography:** Dr. Najjar received his bachelor's degree, *magna cum laude*, from Harvard College, and his M.D. degree from Yale University, where he was elected to the AOA honor society. He completed his internship and residency training at Johns Hopkins Hospital. He subsequently completed his cardiovascular fellowship at Johns Hopkins University, with advanced training in cardiomyopathy and heart transplantation. He joined the Laboratory of Cardiovascular Science in 2000, and became the head of the Human Cardiovascular Studies Unit in 2002.

**Keywords:**

cardiovascular aging
arterial stiffness
ventricular-vascular coupling
congestive heart failure

**Recent Publications:**

Spaeder J, et al. *Am Heart J* 2006; 151(4): e1-10.

Najjar SS, et al. *J Cardiovasc Magn Reson* 2005; 7(4): 657-666.

Najjar SS, et al. *Hypertension* 2005; 46(3): 454-462.

Sutton-Tyrrell K, et al. *Circulation* 2005; 111(25): 3384-3390.

Scuteri A, et al. *Diabetes Care* 2005; 28(4): 882-887.

Najjar SS, et al. *J Am Coll Cardiol* 2004; 44(3): 611-617.

The research agenda of the Human Cardiovascular Studies Unit includes three major programs. The first program focuses on exploring the age-associated changes in arterial structure and function, including arterial wall thickness and stiffness, how they interact with aging, lifestyle, the environment, and various disease states, and how they impact the structure and function of the heart. The second program investigates traditional as well as novel cardiovascular risk factors, with particular attention to arterial structure and function, in an attempt to elucidate the pathophysiological basis for the dominant role of age as a potent risk factor for cardiovascular diseases. The third program comprises clinical research studies in congestive heart failure, including a translational component that capitalizes on the exciting bench research findings and discoveries made in the Laboratory of Cardiovascular Science as well as other laboratories within the NIA.

Describing the age-associated changes in cardiovascular structure and function is one of the central tenets of the Laboratory of Cardiovascular Science. There is a growing body of evidence that increased thickening and stiffening of large arteries, endothelial dysfunction, and the ensuing increases in systolic and pulse pressure, in otherwise apparently healthy older individuals, formerly thought to be part of "normal" aging, precede and predict a higher risk for developing clinical cardiovascular disease. We are interested in characterizing the determinants of the age-associated changes in both vascular and cardiac structure and function, with particular emphasis on exploring the properties of the vasculature, including arterial wall thickness and stiffness, investigating how they interact with aging, lifestyle, the environment, and various disease states. We are also exploring how they

affect the structure and function of the heart. Indeed, ventricular-vascular interaction, or "coupling," is an important and largely under-appreciated determinant of cardiac performance. Normal ventricular-vascular coupling determines optimal left ventricular stroke work, cardiac efficiency, and ejection fraction. We therefore believe that much insight into the structural and functional alterations and adaptations of the cardiovascular system, as well as the cardiovascular reserve, may be gleaned from examination of the coupling between the heart and the vasculature. We are also studying interventions that modulate specific features of cardiovascular structure and function, especially those identified as deleterious or "risky."

Age is the dominant risk factor for cardiovascular diseases, yet the increased risk associated with aging has remained largely elusive. The accumulating evidence implicating the role of the age-associated changes in arterial structure and function as independent risk factors for cardiovascular diseases and outcomes, suggests that aging itself must alter the vascular substrate so as to promote the development, progression and manifestations of cardiovascular diseases. It is thus our hypothesis that the age-associated alterations in arterial structure and function may explain, in part, the increased cardiovascular risk associated with aging. We are therefore interested in studying the impact of traditional cardiovascular risk factors, as well as novel risk factors such as markers of inflammation and the metabolic syndrome, on cardiovascular structure and function. We are applying state-of-the-art imaging modalities to better characterize coronary as well as carotid arterial atherosclerosis, to evaluate the influence of vascular properties (including arterial thickness and stiffness) on the relationship between age and atherosclerosis. By relating the dissociation between physiologic and chronologic aging to atherosclerosis, we expect to define (and compare) "successful" versus "usual" versus "accelerated" cardiovascular aging.

We have a clinical interest in congestive heart failure. Congestive heart failure is a clinical syndrome that affects approximately 5 million Americans. It is estimated that 400,000 new cases are diagnosed every year. The annual expenditure is estimated at 15 to 40 billion dollars annually. The prevalence and incidence of heart failure increase exponentially with age. There is a ten-fold increase in the incidence of this syndrome between the fifth and the ninth decades of life, such that its prevalence is estimated at 10% among those over the age of 80. Our clinical research studies involve testing new preventive strategies and characterizing novel paradigms that could serve as therapeutic targets in the future.

**Collaborators:** Edward Lakatta, M.D., Angelo Scuteri, M.D., Ph.D., Mark Talan, M.D., Ph,D., Laboratory of Cardiovascular Science, NIA, NIH; Luigi Ferrucci, M.D., Ph.D., Shari Ling, M.D., Clinical Research Branch, NIA, NIH; Michele Evans, M.D., Laboratory of Cellular and Molecular Biology, NIA, NIH; Dan L. Longo, M.D., Laboratory of Immunology, NIA, NIH; Joseph Rifkind, Ph.D., Molecular Dynamics Section, NIA, NIH; David Schlessinger, Ph.D., Laboratory of Genetics, NIA, NIH; Alan Zonderman, Ph.D., Laboratory of Personality and Cognition, NIA, NIH; Gary Gerstenblith, M.D., David Kass, M.D., Wendy Post, M.D., Steven Schulman, M.D., Alan Schwartz, M.D., Bruce Wasserman, M.D., Johns Hopkins University.

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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

**Recent Publications:**

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

Zhou J AM, et al. *J Gen Physiol* 2005; 126(4): 301-309.

Stern MD, et al. *Cell Calcium* 2004; 35(6): 591-601.

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

The release of SR calcium is a tightly controlled and smoothly graded function of the trigger calcium; this is paradoxical since CICR is an intrinsically self-reinforcing process which might be expected to lead to an all-or-none response. A possible resolution of the paradox is based on the fact that the L-type trigger channels and the SR release channels are known to be localized to opposite sides of the 15 nm dyad junctions between the cell membrane and the SR membrane. This means that the trigger for CICR is not whole cell calcium, but rather the local calcium microdomain generated

in the neighborhood of the triggering channel. We have shown mathematically that the interaction between the stochastic gating of individual channels and the fluctuating calcium microdomains which they generate can give rise to smoothly graded and controlled calcium release in the whole cell aggregate, even though individual release events may be nearly all-or-none. This is the *local control* hypothesis, which implies that whole cell calcium release depends critically on the details of the gating and ion permeation of the trigger and release channels, and on the local geometrical relationship between them. Over the past several years, considerable evidence has accumulated showing that this is the case. We have recently constructed a similar local-control model of the role of CICR in skeletal muscle excitation-contraction coupling. This model successfully explains many paradoxical observations, and leads to the insight that collective behavior of mesoscopic arrays of calcium-coupled release channels, which we term couplons, may be the basic functional unit of EC coupling.

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

geometry which is predicted by the local control theory. A combined approach utilizing ES cell techniques, confocal calcium measurement, patch clamp and mathematical modeling will be used.

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

**Collaborators:** Kenneth Boheler, Ph.D., Edward G. Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Eduardo Rios, Department of Physiology and Molecular Biophysics, Rush University; Phillip Palade, University of Texas, Galveston; Michal Horowitz, Hebrew University, Jerusalem; Heping Cheng, Ph.D., Peking University, Beijing, China.

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**Biography:** Dr. Bagrov received his M.D. at Ivan Pavlov Medical University and Ph.D. at I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Leningrad, USSR. He subsequently completed his cardiology training and held clinical and academic appointments in St. Petersburg, Russia. In 1992-1994 and 1998-2001, he worked at the NIA as a Visiting Associate and NRC Senior Associate. He was appointed as a tenure track investigator in 2001.

**Keywords:**

Na, K-ATPase
endogenous inhibitors
hypertension
protein kinases

**Recent Publications:**

Kotova O, et al. *J Biol Chem* 2006; 281(29): 20085-20094.

Kennedy DJ, et al. *Hypertension* 2006; 47(3): 488-495.

Fedorova OV, et al. *J Hypertens* 2005; 23(8): 1515-1523.

Periyasamy SM, et al. *Kidney Int* 2005; 67(5): 1868-1877.

Fedorova OV, et al. *J Hypertens* 2005; 23(4): 835-842.

Akimova OA, et al. *J Biol Chem* 2005; 280(1): 832-839.

Bagrov AY, et al. *Front Biosci* 2005; 10: 2250-2256.

Our previous studies demonstrated that two endogenous sodium pump ligands (SPL), endogenous ouabain (EO) and marinobufagenin (MBG), coexist in mammalian tissues. MBG acts as a selective inhibitor of $\alpha$-1 isoform of Na/K-ATPase (NKA), the main sodium pump isoform in the kidney, vascular smooth muscle and adult cardiomyocytes. In Dahl salt-sensitive rats (DS) on a high NaCl intake, brain EO triggers peripheral MBG, which raises the blood pressure. In a model of preeclampsia, pregnant Sprague-Dawley rats on a high NaCl intake raise their blood pressure in parallel with an increase in MBG excretion. *In vivo*, administration of polyclonal and monoclonal anti-MBG antibodies to hypertensive DS and pregnant rats with NaCl-induced hypertension, lowered the blood pressure. During the last year our research efforts concentrated on (i) the study of the relationship between central and peripheral SPLs in DS with chronic salt-sensitive hypertension, (ii) on the development of monoclonal anti-MBG antibodies as a potential treatment of preeclampsia, and (iii) on the studies of mechanisms of MBG-induced NKA-mediated cellular signaling underlying pathogenesis of NaCl-sensitive hypertension.

**(i) Pathogenesis of NaCl Sensitive Hypertension:** Following acute NaCl loading of DS, an initial transient rise of EO in the hippocampus and amygdala, followed by an increase in EO in the supraoptic nucleus of the hypothalamus and pituitary, stimulates pituitary angiotensin II (ATII), and, via activation of sympathetic nervous system activates the renin-angiotensin system in the adrenal cortex. Adrenocortical ATII acting through AT1 receptors stimulates production of MBG. An increase in MBG production induces inhibition of the sodium pump in renal tubules and in cardiovascular tissues. During 2005 the above findings have been translated into the context of pathogenesis of chronic salt-sensitive hypertension in DS. In particular, administration of anti-ouabain antibody to DS prior to NaCl

**Publications-continued:**
Bagrov AY, et al. *Hypertension* 2004; 44(1): 22-24.

Fedorova OV, et al. *J Hypertens* 2004; 22(2): 389-397.

Fedorova OV, et al. *Hypertension* 2003; 41(3): 505-511.

loading markedly reduces MBG response to salt loading and attenuates NaCl-induced hypertension. Moreover, administration of ouabain to DS with a normal NaCl intake produces a sustained increase in renal MBG excretion and causes hypertension.

**(ii) Monoclonal anti-MBG Antibody as a Therapy for Preeclampsia:** We developed three monoclonal anti-MBG antibodies (Mab) with high affinity to MBG and low cross reactivity to digoxin and ouabain. We tested the ability of Mab: (a) to lower blood pressure (BP) *in vivo* in hypertensive DS rats on a high NaCl intake, (b) to lower BP in rats with pregnancy-associated hypertension, and (c) to reverse the inhibition of NKA in erythrocytes from patients with preeclampsia. The effects of Mab were compared to that of DIGIBIND. In 12 DS following two weeks of 8% NaCl intake, in which renal MBG excretion increased 4-fold vs. baseline levels, a single intraperitoneal administration of Mab lowered BP by 40 mmHg and restored the Na/K-pump activity in thoracic aorta by 51%. Plasma MBG concentration in 10 patients with PE (systolic BP: 151±3 mm Hg vs. 99±3 mm Hg in normotensive control), measured by competitive immunoassay based on Mab, exhibited a ten-fold increase vs. that in 10 normotensive pregnant women. The activity of erythrocyte NKA in patients with PE was inhibited by 50% as compared to that in normotensive pregnant women. *In vitro* treatment of erythrocytes from PE patients with Mab restored the NKA activity (95% vs. control), while DIGIBIND was less effective (75% vs. control). NaCl supplementation of pregnant Sprague-Dawley rats was associated with a 37 mm Hg increase in systolic BP, a 3-fold rise in MBG excretion, and a 25% inhibition of the NKA in the thoracic aorta, as compared to pregnant rats on a normal NaCl intake. A single intraperitoneal administration of Mab to pregnant hypertensive rats reduced the BP (25 mm Hg) and restored the vascular Na/K-pump activity.

**(iii) MBG-induced Cell Signaling and Mechanisms of Its Modulation:** In collaboratotion with Dr. Joseph Shapiro's laboratory at Medical University of Ohio, we demonstrated that MBG is implicated in the pathogenesis of uremic cardiomyopathy (Priyadarshi, et al, KI 2003). Rats subjected to MBG infusion (10 μg/kg/day, SM) or partial nephrectomy (PNx) develop cardiac fibrosis whereas PNx rats immunized against MBG were protected from this fibrosis. The expression of Fli-1, a transcription factor, which inhibits collagen synthesis was decreased by 50% in both the SM and PNx hearts. In primary culture of rat fibroblasts grown to confluence, physiological concentrations of MBG increased the incorporation of proline into collagen, caused a 2-fold increase in ROS production as well as a 150% increase in procollagen expression over 24 hours. Fibroblast Fli-1 expres-

sion was decreased by 80% by 10 nM MBG. These data suggest that MBG produces cardiac fibrosis in a process involving increased ROS and decreased Fli-1 expression. In rat aorta and renal medulla, we compared effects of ANP on NKA phosphorylation and on inhibition of NKA and sodium pump by MBG. MBG inhibited the NKA in renal and vascular membranes. ANP potentiated MBG induced NKA inhibition in the kidney, but reversed the effect of MBG in aorta. In a similar fashion, ANP modulated sodium pump inhibitory effects of MBG in rat aorta and in the proximal collecting tubules. In aorta, ANP dephosphorylated α-1 NKA, while in renal medulla ANP induced α-1 NKA phosphorylation. These effects of ANP on NKA phosphorylation and inhibition were mimicked by a protein kinase G (PKG) activator, 8-Br-PET-cGMP and prevented by a PKG inhibitor, KT5823. The fact that in the kidney, inhibition of α-1 NKA by MBG is enhanced via NKA phosphorylation by ANP, while in the aorta ANP exerts an opposite effect, may be explained by expression of two different PKG isoforms in these tissues. The concurrent production of a vasorelaxant, ANP, and a vasoconstrictor, MBG, potentiate each other's natriuretic effects, but ANP peptides may offset the deleterious vasoconstrictor effect of MBG.

Taken together, these findings demonstrate that MBG is an important factor in pathogenesis of hypertension, and open new pharmacological possibilities in the treatment of hypertension, including blockade of circulating MBG with a specific antibody and attenuation of NKA inhibitory effect of MBG on the NKA.

**Collaborators:** Joseph I. Shapiro, M.D., Medical College of Ohio, Toledo, Ohio; Sergei N. Orlov, Ph.D., Pavel Hamet, M.D., and Jean St-Louis, Universite de Montreal, Montreal, Canada; Nikolai Kolodkin, Ph.D., and Andrey Simbirtsev, M.D., Ph.D., Institute of Highly Pure Biopreparations, St. Peterburg, Russia; David E. Anderson, Ph.D., Laboratory of Cardiovascular Science, NIA, NIH; Natalia I Tapilskaya, M.D., School of Pediatric Medicine, St. Petersburg, Russia; Igor A. Zhuravin, Ph.D., Sechenov Institute of Evolutionary Physiology, St. Petersburg, Russia; Dan Darlington, Ph.D. and Donald Gann, M.D., Maryland Shock Trauma Center, University of Maryland School of Medicine.

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**Keywords:**

heart
development
embryonic stem cells
molecular biology

**Recent Publications:**

Li RA, et al. *J Stem Cells* 2006; In press.

Boheler KR, et al. *Methods Mol Biol* 2006; 329: 195-221.

Wiese C, et al. *Stem Cells* 2006; 24(9): 2085-2097.

Fu JD, et al. *FASEB J* 2006; 20(1): 181-183.

Wobus AM, et al. *Physiol Rev* 2005; 85(2): 635-678.

Elliott ST, et al. *Proteomics* 2004; 4(12): 3813-3832.

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 immuno-staining 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 knock-down 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 corre-

sponded 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 β-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. Together 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; Ronald Li, Stem Cell Institute, University of California; Professor Brenda Russell, University of Illinois, Chicago; Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany; Edward G. Lakatta, Laboratory of Cardiovascular Science, NIA, NIH.

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**Recent Publications:**

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

Zhu W, et al. *Circ Res* 2005; 97(6): 507-509.

Xu X, et al. *Cell* 2005; 120(1): 59-72.

Zheng M, et al. *FASEB J* 2005; 19(1): 109-111.

Zhu WZ, et al. *Circ Res* 2005; 97(3): 244-251.

Zheng M, et al. *Pharmacol Ther* 2005; 108(3): 257-268.

Her main scientific focus has been related to G protein-coupled receptors (GPCRs)-mediated transmembrane signal transduction in the cardiovascular system. In addition, considerable efforts have been put on cardiac aging and heart failure associated changes in GPCR signaling. The breadth of our work covers three intertwined programs: (1) Identification and characterization of cardiovascular disease-related genes; (2) β-adrenergic receptor subtype signaling in cardiovascular system; and (3) Modulation of cardiac excitation-contraction coupling by p38 MAPK or Ca/calmodulin-dependent protein kinase II (CaMKII) in normal and failing hearts. Most studies are designed to integrate information gleaned from genetic manipulations, including gene transfer by adenoviruses, transgenic and gene targeted animal models, in conjunction with physiological and pharmacological approaches, confocal imaging and cell biological techniques. The mechanistic and interdisciplinary nature of our research has made the past few years particularly fruitful.

**Identification and Characterization of Cardiovascular Disease-related Genes:** *(1) 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) (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.

*(2) 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 profoundly 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.

**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 in eukaryotes and in some prokaryotes. By activating their cognate heterotrimeric guanosine triphosphate (GTP) binding proteins (G proteins), GPCRs transduce stimulatory or inhibitory signals for a wide array of endogenous hormones and neurotransmitters, and ambient physical and chemical stimuli, as well as exogenous therapeutic reagents. ß-adrenergic receptors (ßARs) are archetypical members of the GPCR superfamily. There are, at least, both ß$_1$AR and ß$_2$AR present in heart muscle cells. Whereas both ßAR subtypes stimulate the classic G$_s$-adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade, ß$_2$AR can activate bifurcated signaling pathways through G$_s$ and G$_i$ proteins. Because of their distinct G protein coupling, these ßAR subtypes fulfill distinct, sometimes even opposite, physiological and pathological roles. Specifically, in the heart, whereas ß$_1$AR-generated cAMP signal can broadcast throughout the cell, the ß$_2$AR-stimulated cAMP signal is spatially and functionally compartmentalized to subsurface membrane microdomains by the concurrent G$_i$ activation, thus selectively affecting plasma membrane effectors (such as L-type $Ca^{2+}$ channels) and bypassing cytoplasmic regulatory proteins (such as phospholamban and myofilaments). Of potentially greater importance, the ß$_2$AR-to-G$_i$ pathway also delivers a powerful cardiac protective signal. As a consequence, ß$_1$AR and ß$_2$AR exhibit opposing effects on heart cell survival: ß$_1$AR activation can promote programmed heart cell death (apoptosis); in sharp contrast, ß$_2$AR activation can protect heart cells from a wide range of assaulting factors, including enhanced ß$_1$AR stimulation, hypoxia, and reactive oxygen species. The ß$_2$AR survival pathway sequen-

tially involves $G_i$, Gß, phosphoinositide 3-kinase (PI3K), and Akt. Furthermore, *in vivo* overexpression of $ß_1$AR, but not $ß_2$AR, induces heart muscle cell hypertrophy and heart failure in transgenic mouse models. 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.

In chronically failing heart, the $ß_2AR/G_i$ coupling is exaggerated. The enhanced $G_i$ signaling underlies the heart failure-associated dysfunction of $ß_2$AR. Based on the dual G coupling of $ß_2$AR, we conceptualize that receptor ligands may selectively activate a subset(s) of the post-receptor signaling pathways. By screening a variety of $ß_2$AR ligands, we have identified one ligand (fenoterol) that selectively activates $G_S$, bypassing the $G_i$ signaling. Strikingly, fenoterol is able to restore the markedly depressed $ß_2$AR contractile response in two experimental chronic heart failure models. Our most recent studies provide compelling evidence that stimulation of $ß_1$AR, but not $ß_2$AR, induces cardiac apoptosis. The anti-apoptotic effect of $ß_2$AR stimulation in cardiac myocytes is mediated by $G_i$-Gγ subunits-PI3 kinase-Akt signaling pathway. These studies not only reveal the diversity and specificity of ß-AR subtype and G protein interactions, but also provide new insights for understanding the co-existence and different functional roles of $ß_1$AR and $ß_2$AR in healthy and failing hearts.

**Modulation of Cardiac Excitation-Contraction Coupling and Myocyte Viability by p38 MAPK:** MAPK superfamily is one of the most important signal transduction systems conserved in all eukaryotes. There are three major subgroups identified, including the extracellular signal regulated kinase (ERK1/2), p38 MAPK and c-jun-$NH_2$terminal kinase (JNK). p38 MAPK is one of the most ancient signaling molecules involved in multiple cellular processes, including cell proliferation, cell growth and cell death.

In the heart, activation of p38 MAPK has been observed in pressure-overload or ischemia/infarction induced cardiac hypertrophy and heart failure in humans and animal models. In cultured cardiac myocytes, activation of p38 MAPK induces myocyte hypertrophy and apoptosis, and is also implicated in the preconditioning process and ischemia/reperfusion injury. Increasing evidence suggests that inhibition of p38 MAPK is able to improve cardiac contractility in ischemia/reperfusion-injured hearts.

The specific goal of this research program is to determine whether p38-MAPK activation modulates cardiac myocyte excitation-contraction coupling and if so, to explore the possible underlying mechanisms. We have examined the possible effects of p38-MAPK activation or inhibition on cardiac contractility at the single cell level, and verified the conclusion obtained from single myocyte experiments by *in vivo* studies in transgenic mice overexpressing activated mutants of p38 MAPK upstream kinases. In addition, we have examined the potential interaction between ßAR and p38 MAPK signaling pathways in regulating cardiac contractility, and the pathophysiological relevance of p38 activation in ischemic contractile dysfunction and cardiomyocyte injury.

Our *in vivo* and *in vitro* studies have demonstrated, for the first time, that inhibition of p38 MAPK leads to a positive inotropic effect, whereas enhanced p38 MAPK activation inhibits myocyte contractility and negates βAR/PKA-mediated positive inotropic effect. Furthermore, we have shown that inhibition of ischemia-induced, intracellular acidosis-mediated activation of p38 MAPK not only protects myocytes against ischemic death but also reverses ischemic contractile dysfunction. These findings reveal a novel function of p38 MAPK, and provide new insights for a better understanding of the coincidence of enhanced p38 MAPK signaling and cardiac contractile dysfunction under certain pathophysiological conditions, such as cardiac ischemic/reperfusion injury and chronic heart failure.

**Opposing Functional Roles of CaMKII-$\delta_B$ and CaMKII$\delta_C$ 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, $\delta_B$ and $\delta_C$, located in unclear and cytosol compartments, respectively. Our previous studies have shown that enhanced CaMKII-$\delta_C$ activation is both necessary and sufficient for $\beta_1$AR-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-$\delta_B$ remains elusive.The aim of the present study is to investigate the potential physiological and pathological functional roles of CaMKII-$\delta_B$ in the heart and explore its clinical implications. We have demonstrated that CaMKII-$\delta_B$ 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 inhibitory effects of MI and $H_2O_2$ on CaMKII-$\delta_B$ are fully prevented by ROS scavengers, indicating ROS constitutes a negative regulator of CaMKII-$\delta_B$ 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. Most importantly, overexpression of CaMKII-$\delta_B$ using adenoviral gene transfer substantially protects heart cells against ischemia- or oxidative stress-induced apoptosis. Moreover, several lines of evidence indicates that the CaMKII-$\delta_B$ cardiac protective effect is mediated by a Akt-dependent pathway. First, overexpression of CaMKII-$\delta_B$ leads to a robust elevation in Akt phosphorylation. Interestingly, the phosphorylated Akt is clearly enriched in nuclei, thus colocalized with CaMKII-$\delta_B$. Second, increased CaMKII-$\delta_B$ activation also profoundly augments the expression of a novel apoptosis repressor, ARC (apoptosis repressor with caspase recruitment domain), via a Akt-mediated mechanism, because inhibition of the PI3K/Akt signaling pathway with LY 294002 can block CaMKII-$\delta_B$ induced upregulation of ARC and the protective effect. These *in vivo* and *in vitro* data indicate that CaMKII-$\delta_B$ plays a pivotal role in protecting the heart against ischemia and oxidative insults through an Akt and ARC-dependent pathway, marking CaMKII-$\delta_B$ as a promising novel therapeutic target for the treatment of myocardial ischemic disease, the predicted number one killer in the world by 2020.

**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-$\delta_C$-mediated positive feedback regulation of the L-type $Ca^{2+}$ current ($I_{Ca,L}$). In freshly dissociated rabbit single SA node cells, specific CaMKII inhibitors, a peptide CaMKII inhibitor or KN-93 (0.1 - 3.0 μM), but not its inactive analog KN-92, depressed the rate and amplitude of spontaneous action potentials (APs) in a dose-dependent manner. Strikingly, 3 μM KN-93 or 10 μM CaMKII peptide inhibitor completely arrested SA node cells, which indicates that basal CaMKII activation is obligatory to the genesis of pacemaker AP 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-$\delta_C$ 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 dominant negative (DN) CaMKII-$\delta_C$. CaMKII activity was examined by determining the kinase activity and the level of CaMKII-specific phosphorylation of phospholamban at Thr-$^{17}$. Compared with β-gal adenovirus control, CaMKII-mediated phosphorylation of RyR, assessed by back phosphorylation, was reduced by DN-CaMKII-$\delta_C$, enhanced by CA-CaMKII-$\delta_C$, or unaltered by WT-CaMKII-$\delta_C$ expression. Concomitantly, spontaneous $Ca^{2+}$ sparks at 1mM $Ca^{2+}$ was hypoactive, hyperactive, or unchanged in CA-, DN- or WT-CaMKII-$\delta_C$ groups, respectively; $Ca^{2+}$ transients elicited by action potentials displayed accelerated, slowed or unchanged rate of relaxation in CA-, DN- or WT-CaMKII-$\delta_C$ groups, respectively, without affecting the amplitudes. Both WT- and CA-CaMKII-$\delta_C$ 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-$\delta_C$ increased $Ca^{2+}$ wave frequency at 20

mmol/L $Ca^{2+}$. Furthermore, activation of endogenous CaMKII during sustained $\beta_1$-adrenergic receptor stimulation (norepinephrine 100 nmol/L, 24h in the presence of $\alpha$-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-$\delta_C$ 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.

**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; 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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**Biography:** David E. Anderson received his Ph.D. in Clinical Psychology from the University of Oregon in 1966, and served a Postdoctoral Fellowship at the University of New York at Stony Brook, 1966-1967. His career interest in the behavioral origins of hypertension emerged while he was on the faculty at the Johns Hopkins University School of Medicine (1968-1981), where he developed an animal behavior model of hypertension. He elaborated this stress/salt interaction model while a Professor at the University of South Florida (1981-1987). He was a recipient of an NIH Research Career Development Award (1983-1987) and the Pavlovian Award for Biological Science in 1985. He joined the National Institute on Aging in 1987 as Chief of the Behavioral Medicine Section of the Laboratory of Behavioral Science, and became Chief of the Behavioral Hypertension Section, Laboratory of Cardiovascular Science in 1997.

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**Recent Publications:**

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Chesney MA, et al. *Int J Behav Med* 2005; 12(2): 50-58.

**Behavioral Medicine Research:** Behavioral medicine research is concerned with the application of behavioral principles and methods to the study of the origins of, and interventions in, medical disorders. The role of behavioral science in cardiovascular research is to clarify the nature of the contingencies on behavior that participate in the development of cardiovascular disorders, and to develop behavioral interventions for their prevention or reversal. It is understood that such chronic disorders are multi-factorial in origin, involving genetic and possibly other environmental/behavioral factors, including especially diet.

The mediating mechanisms by which behavioral factors participate in hypertension and coronary artery disease remain to be clarified, but are also likely to be distinct from each other. While coronary artery disease is clearly linked with anger and hostility (and associated activation of the sympathetic nervous system), the extent to which this mechanism mediates the development of sodium-sensitive forms of hypertension is far from established. The preponderance of evidence suggests that the physiological concomitants of emotional inhibition can play a significant mediating role, especially in interaction with high dietary sodium intake. It is with investigations of the mechanisms by which behavioral factors contribute to the pathogenesis of sodium-sensitive hypertension that the work of this section is dedicated.

**Stress, Salt and Blood Pressure:** Previous research in this laboratory found that a combination of behavioral stress and high sodium intake resulted in experimental hypertension in large laboratory animals over

periods of days. This form of hypertension was not prevented by adrenergic blockade or by renal denervation, but was accompanied by an inhibited breathing pattern that was conditioned to the experimental setting. Under these conditions, the inhibited breathing pattern increased $pCO_2$ and transiently decreased plasma pH. The respiratory acidosis expanded plasma volume by a variety of pathways, including increased sodium/hydrogen exchange. One consequence was an increase in plasma concentrations of a circulating endogenous sodium pump inhibitor, termed marinobufagenin, a substance found in the skin of toads. This compound promotes natriuresis, but also increases vascular tone. Subsequent experimental studies with healthy human subjects showed that voluntary performance of breathing suppression using a biofeedback procedure was accompanied by comparable effects on renal sodium regulation as in the previous studies with laboratory animals.

More recent research in our laboratory found that high resting end tidal $CO_2$ ($PetCO_2$) is a risk factor for blood pressure sensitivity to high sodium intake, particularly in older humans. In addition, high resting $PetCO_2$ was found to be an independent correlate of elevated resting systolic BP, especially in women who were low in trait anger. Thus, chronic hypoventilatory breathing pattern might be a risk factor for sodium sensitive forms of high blood pressure. We have also found that chronic stress is associated with slower breathing at rest than in others, especially women. Postmenopausal women were also found to have higher levels of an endogenous inhibitor of nitric acid that could contribute to their increased blood pressure sensitivity to high sodium intake. Taken together, these studies implicate daytime inhibited breathing pattern in long-term blood pressure regulation, and complement other findings that hypertension is potentiated by sleep apnea.

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

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

# Laboratory of Cellular and Molecular Biology

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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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**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:**

Mittal A, et al. *J Immunol* 2006; 176(4): 2183-2189.

Sen R, et al. *Curr Opin Immunol* 2006; 18(3): 237-242.

Banerjee D, et al. *Immunity* 2005; 23(4): 445-458.

**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_H$10 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_H$10 genes are activated by the *v-abl* tyrosine kinase. Our immediate objectives are to i) confirm the model that $D_H$-proximal $V_H$ genes are activated in response to $DJ_H$ recombination, ii) to identify the normal signals that activate $V_H$10 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 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 $V_H$J558 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 $V_H$ 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, 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
regulated mRNA turnover
RNA binding proteins
regulated translation
cell cycle control
stress response
senescence
microarray

**Recent Publications:**

Cunningham SC, et al. *Cancer Res* 2006; 66(11): 5560-5564.

Cunningham SC, et al. *Cell Cycle* 2006; 5(10): 1090-1093.

Kawai T, et al. *Mol Cell Biol* 2006; 26(8): 3295-3307.

Lal A, et al. *Mol Cell* 2006; 22(1): 117-128.

Mazan-Mamczarz K, et al. *Mol Cell Biol* 2006; 26(7): 2716-2727.

Fan J, et al. *Nucleic Acids Res* 2006; 34(5): 1492-1500.

**Research Summary:** Aging is characterized by a general decline in the ability of individuals to adequately respond to different stimuli from either environmental or endogenous sources. Changes in the expression of stress-response and proliferative genes is widely believed to play an important role in determining cell fate. While the transcriptional control of gene expression has been extensively studied, it is increasingly apparent that posttranscriptional regulatory mechanisms also critically influence gene expression. 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) 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.

**High-Throughput Analysis of mRNA Turnover and Translation:** We and others have shown that the expression of specific stress-response genes (p21, cyclin D1, gadd45, etc.) is strongly influenced by changes in mRNA stability. A major research initiative in the laboratory seeks to globally assess changes in mRNA turnover using cDNA arrays. We developed a method based on the use of cDNA arrays to investigate the global contribution of transcription and mRNA turnover and applied it to studies of stimulus-regulated gene expression patterns. This novel method compares large-scale hybridization patterns generated with steady-state mRNA with those

**Publications-continued:**

Lopez de Silanes I, et al. *Mol Cell Biol* 2005; 25(21): 9520-9531.

Lal A, et al. *Mol Cell Probes* 2005; 19(6): 385-388.

Lal A, et al. *EMBO J* 2005; 24(10): 1852-1862.

generated using newly transcribed RNA, synthesized and isolated using the nuclear run-on technology. By comparing the values from each analysis we ascertained the extent to which stimulus-regulated modifications in gene expression are due to transcriptional changes and to posttranscriptional changes. Following stress stimulation, changes in mRNA stability were found to contribute significantly to ~50% of all gene expression changes. Following T cell activation, mRNA turnover contributed to ~55% of the early changes in steady-state mRNA levels.

We have also developed a robust approach for the en masse investigation of the translational status of large subsets of mRNAs. Also using cDNA arrays as a transcript detection platform, we identified the relative distribution of mRNAs along polysome gradients by performing cDNA array analysis of each gradient fraction, and quantified the mRNA translational status by regression analysis. Using this strategy to study cultured cells exposed to stress agents such as short-wavelength ultraviolet light (UVC) irradiation or tunicamycin, we identified subsets of translationally induced mRNAs and subset of translationally repressed mRNAs following exposure to stress. We describe an effective approach for globally investigating changes in the translational engagement of mRNA collections and hence protein biosynthesis.

**Signaling Events Regulating HuR Function in Response to Stress:** Several recent studies have provided increasing support for the notion that mRNA stability is regulated through mechanisms akin to those controlling gene transcription, i.e., signal transduction pathways involving phosphorylation events. While transport of the RBP HuR from the nucleus to the cytoplasm is central to HuR function, the mechanisms underlying this process remain poorly understood. We identified the AMP-activated kinase (AMPK), an enzyme involved in responding to metabolic stresses, as a potent regulator of the levels of cytoplasmic HuR. Inhibition of AMPK increased HuR presence in the cytoplasm, enhanced binding of HuR to target mRNAs encoding p21, cyclin B1, and cyclin A, and elevated their expression and half-life. Conversely, activation of AMPK reduced cytoplasmic HuR, decreased the levels and half-life of mRNAs encoding p21, cyclin A, and cyclin B1, and diminished the association of HuR with the corresponding transcripts. We thus propose that AMPK regulates cytoplasmic HuR levels, which in turn influences the mRNA-stabilizing function of HuR and the expression of HuR target transcripts. Among the specific target molecules that were identified to mediate the transport of HuR through the nuclear pore is importin $\alpha$1. AMPK triggered the acetylation of importin $\alpha$1, a process dependent on the acetylase activity of

p300, and directly phosphorylated importin α1. Accordingly, expression of importin α1 proteins bearing point mutations in the relevant residues failed to mediate the nuclear import of HuR in intact cells. Our results point to importin α1 as a critical downstream target of AMPK and key mediator of AMPK-triggered HuR nuclear import.

**Role of HuR in Carcinogenesis:** Immunohistochemical analysis of paired tumor and normal tissue specimens revealed that the expression and cytoplasmic abundance of HuR increased with malignancy, particularly in colon carcinomas. Interventions to modulate HuR expression in human colon cancer cells altered gene expression profiles and identified α-catenin mRNA as a novel HuR target. Subcutaneous injection of HuR-overexpressing cells into nude mice produced significantly larger tumors than those arising from control populations; conversely, cells expressing reduced HuR developed significantly more slowly. These results suggested that HuR regulates genes important for cancer cell growth.

Moreover, while no HuR mutations have been found in cancer, a link between HuR and malignant transformation has been suggested in cancers of the breast, colon, lung, and ovary. Normal human cells become neoplastic by progressively acquiring mutations in cancer genes: gain-of-function mutations of oncogenes and loss-of-function mutations of tumor-suppressor genes and genome-maintenance genes. Together, these mutations provide the cell with a competitive growth advantage that is realized via at least five cancer-cell phenotypes: 1) enhanced cell division; 2) resistance to apoptosis; 3) maintenance of angiogenesis; 4) invasion of tissues and metastasis; and 5) and evasion of antitumor immune responses. In fact, HuR-target mRNAs encode proteins critical to the development of each of these traits of cancer cells, consistent with a central role of HuR in oncogenesis.

In this regard, the finding that HuR binds the mRNA encoding the anti-apoptotic protein prothymosin α (ProTα) and regulates its expression strongly supported the notion that HuR enables cancer cells to evade apoptosis (point 2 above). In HeLa cells, treatment with the apoptotic stimulus UVC triggered the mobilization of ProTα mRNA to the cytoplasm and onto heavier polysomes, where its association with HuR increased dramatically. Analysis of a chimeric ProTα mRNA directly implicated HuR in regulating ProTα production: ProTα translation and cytoplasmic concentration increased in HuR-overexpressing cells and declined in cells in which HuR levels were lowered by RNA interference. Importantly, the anti-apoptotic influence engendered by HuR was vitally dependent on ProTα

expression, since use of oligomers that blocked ProTα translation abrogated the protective effect of HuR. Together, our data support a regulatory scheme whereby HuR binds the ProTα mRNA, elevates its cytoplasmic abundance and translation, and thereby elicits an anti-apoptotic program.

**High-Throughput Identification of mRNAs Associated with RBPs:** To identify the collection of transcripts associated with a given RBP, we performed immunoprecipitation (IP) of the RBP in question using procedures developed by Keene and colleagues, whereby RBP association with endogenous transcripts is preserved. Following microarray-based identification of the bound transcripts, computational analyses were carried out in order to elucidate a common motif present among the target mRNAs. Using this general strategy, RNA motifs were predicted for TIA-1, an RBP involved in splicing regulation and translational repression, and for HuR. In both cases, the predicted motifs were U-rich, and formed a loop of variable size, although the specific motifs and their lengths were distinct for each RBP. These RNP interactions were validated by IP of endogenous mRNAs followed by reverse transcription and PCR-mediated detection, and by pulldown of biotinylated RNAs followed by Western blotting. The motifs successfully predicted additional targets for these RBPs in the genomic databases. Ongoing work is aimed at elucidating additional motifs for other RBPs involved in controling mRNA translation and stability.

**Regulation of mRNA Turnover During Cellular Senescence:** Cellular senescence is accompanied by alterations in gene expression patterns. Using three models of replicative senescence, we have described the influence of HuR in coordinately regulating the expression of cyclin A, cyclin B1 and c-fos, whose expression decreases during senescence. We demonstrated that HuR levels, HuR binding to target mRNAs encoding cyclin A, cyclin B1 and c-fos, and the half-lives of such mRNAs, were lower in senescent cells. We further showed that HuR levels directly influenced the senescent phenotype and that mRNA turnover played a critical role during the process of replicative senescence. In keeping with the notion that the cytoplasmic HuR levels (believed to be linked to HuR function) were inhibited by AMPK, treatment of human fibroblasts with AMPK activators such as AICAR, antimycin A, and sodium azide inhibited cell growth and lowered the expression of proliferative genes. As anticipated, AMPK activation also decreased both the cytoplasmic HuR levels and HuR's association with target radiolabeled transcripts encoding such proliferative genes. HuR function was previously shown to be implicated in the maintenance of a 'young cell' phenotype in models of replicative cellular senescence. We

therefore postulated that AMPK activation in human fibroblasts might contribute to the implementation of the senescence phenotype through mechanisms that included a reduction in cytoplasmic HuR levels. Indeed, senescent fibroblasts had 2- to 3-fold higher AMP:ATP ratios compared with young fibroblasts, and markedly elevated AMPK activity. Evidence that increased AMPK activity directly contributed to the implementation of the senescent phenotype was obtained through the use of AMPK activators, which triggered senescence characteristics in fibroblasts, and via infection of cells with adenoviral vectors that expressed active or inactive AMPK (which increased or decreased senescence-associated markers, respectively). Together, our results indicate that AMPK activation can cause premature fibroblast senescence through mechanisms that likely involve reduced HuR function.

Although the link between *in vitro* cellular senescence and human aging remains controversial, diminutions in proliferative capacity and stress responsiveness in senescent cell models are also a hallmark of *in vivo* aging. Therefore, knowledge of the mechanisms regulating gene expression during *in vitro* senescence is likely to aid in our understanding of *in vivo* aging, as well as contribute to our comprehension of age-related diseases such as cancer and hyperplasia, where control of proliferation is lost. Our findings further suggest that orchestrated gene expression during senescence may be regulated by RBPs that coordinately govern the stability of mRNAs encoding critical proliferation-, stress-, and senescence-associated proteins. The study of additional senescence-associated RNPs and their posttranscriptional fates is the major area of great interest within the RNA Regulation Section.

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**Biography:** Dr. Michele K. Evans, a board certified internist and medical oncologist, received her medical degree from the University of Medicine and Dentistry of New Jersey-The Robert Wood Johnson Medical School in Piscataway. She received her postgraduate training in internal medicine at Emory University School of Medicine and fellowship training in medical oncology within the Medicine Branch of the Clinical Oncology Program at the National Cancer Institute (NCI). Interest in human cancer prone disorders and DNA repair led her to study the role of DNA repair in cancer susceptibility as a Senior Clinical Investigator in the Laboratory of Molecular Pharmacology, NCI. At the National Institute on Aging (NIA), her major research interest centers on the clinical implications of eukaryotic DNA repair in cancer pathogenesis and aging. She also conducts epidemiologic work in the area of health disparities as head of the Health Disparities Research Section and co-principal investigator and medically responsible investigator for Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS). In addition, Dr. Evans serves as Deputy Scientific Director, NIA.

**Keywords:**

DNA damage
DNA repair
breast cancer
prostate cancer
oxoguanine-DNA glycosylase
base excision repair
genetic polymorphisms
health disparities

**Recent Publications:**

Evans MK, et al. *Oncogene* 2006; In press.

Hill JW, et al. *Nucleic Acids Res* 2006; 34(5): 1620-1632.

Sollers JJ 3rd, et al. *Biomed Sci Instrum* 2005; 41: 43-47.

Mager DE, et al. *Biomed Sci Instrum* 2004; 40: 337-342.

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

**DNA Repair in Breast and Prostate Cancer:** Several lines of evidence suggest that accumulation of DNA damage coupled with defects in DNA repair play an important role in breast and prostate tumorigenesis. Of primary importance are the base lesions caused by reactive oxygen species (ROS) particularly, Thymine glycol (Tg) and 8-hydroxyguanine (8-oxoG). Thymine glycol is a toxic lesion that blocks DNA replication and transcription, causing cell death. 8-oxoG is a premutagenic lesion. In order

**Publications-continued:**

Nguyen HT, et al. *Psychiatry Res* 2004; 126(2): 177-187.

Trzeciak AR, et al. *Carcinogenesis* 2004; 25(8): 1359-1370.

to avoid the harmful effects of these lesions, organisms have developed complex mechanisms for repairing this damage most notably Base Excision Repair (BER). The primary hypothesis of this work is that both oxidative DNA damage and BER pathways have specific roles in tumorigenesis in the breast and the prostate.

In breast cancer, studies using High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry have revealed increased levels of 8-oxoG in invasive ductal breast carcinomas relative to normal breast tissue implicating oxidative damage in the etiology of breast cancer. In addition several studies have found defects in the DNA repair capacity of specific DNA lesions in individuals with breast cancer and those at high risk for developing breast cancer. Mutagenic oxidative DNA base damage increases with age in prostatic tissue. Many factors may influence this increase including: increased production of reactive oxygen species, increased susceptibility to oxidative stress, alterations in detoxifying enzyme levels or defects in DNA repair. There is also a significant increase in the proportion of mutagenic oxidatively induced DNA base lesions, 8-hydroxyadenine (8-oxoA) and 8-hydroxyguanine (8-oxoG) present in malignant prostatic tissue as well as an increase in the levels of these lesions in benign prostatic tissue with aging.

Our studies have shown that mitochondrial extracts from both MCF-7 and MDA-MB-468 breast cancer cell lines are deficient in the removal of 8-oxoG. Both breast cancer cell lines exhibited more than two-fold decrease in their ability to incise 8-oxoG relative to the wild type. This defect was specific for 8-oxoG since the incision of Tg by the same mitochondrial extracts was comparable to that of wild type cells. We showed for the first time that mitochondria from human breast cancer cell lines are defective in the repair of 8-oxoG. This defective repair of 8-oxoG may imply that breast cancer cells have a high incidence of mtDNA mutations.

Further work in our laboratory on the repair of 8-hydroxyguanine (8-oxoG) in breast cancer cell lines has shown that HCC1937 cells have diminished nuclear repair of 8-oxoG relative to AG11134 (wt mammary epithelial cells). The repair defective HCC1937 cells also have no detectable 8-oxoguanine-DNA glycosylase (OGG1), the major enzyme for repairing these lesions. Nuclear DNA of HCC1937 and MCF-7 cells accumulated higher levels of 8'-hydroxyl-2'-deoxyguanosine (8-OH-d-G) after $H_2O_2$ treatment followed by a repair period compared to AG11134 cells as measured by High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry. The deficient repair is not associated with a genetic change in the *hOGG1* gene since the coding and the

promoter regions of this gene did not harbor any mutations. However, repair of 8-OH-d-G in HCC1937 cells was significantly stimulated by purified hOGG1. Furthermore, expression of hOGG1 by transfection of the *hOGG1* gene in HCC1937 cells resulted in increased repair of 8-OH-d-G. This study provides evidence for inefficient *in vivo* and *in vitro* repair of 8-OH-d-G in HCC1937 human breast cancer cells and directly implicates hOGG1 in this defect.

In prostate cancer, using LC/MS and GC/MS, we have shown increased levels of oxidative DNA base damage over the baseline in PC-3 and DU-145 prostate cancer cells following exposure to ionizing radiation and a repair period. Nuclear extracts of PC-3 and DU-145 prostate cancer cell lines have defective incision of the DNA base lesions, 8-hydroxyguanine (8-oxoG), 5-hydroxycytosine (5OHC) and thymine glycol (TG) when compared to the non-malignant prostate cell line. Mitochondrial extracts from PC-3 and DU-145 also have defective incision of 8-oxoG compared to the control. PC-3 mitochondrial extracts are severely defective in the incision of TG and 5OHC. Consistent with the incision data, NTH1 and OGG1 2a protein levels are decreased in mitochondria of PC-3 cells. The antioxidant enzymes, glutathione peroxidase (GPx), catalase, and superoxide dismutases (SOD1, SOD2) have altered expression patterns in the cancer cell lines. Genetic analysis of the OGG1 gene reveals that both PC-3 and DU-145 cell lines harbor polymorphisms associated with a higher susceptibility to certain cancers. These data suggest that the malignant phenotype in PC-3 and DU-145 cell lines is associated with defects in base excision repair (BER), alterations in expression of BER and antioxidant enzymes, and OGG1 genetic polymorphisms.

**Human Genetic Polymorphisms and Human 8-oxoguanine-DNA Glycosylase:** Our work on base excision repair in breast and prostate cancer highlighted the importance human 8-oxoguanine-DNA glycosylase (OGG1) in cancer cells. It is well known that a frequently occurring OGG1 polymorphism in human populations results in the substitution of serine 326 for cysteine (S326C). The 326 C/C genotype is linked to numerous cancers; however, the mechanism of carcinogenesis associated with the variant is unclear. We performed detailed enzymatic studies of polymorphic OGG1 and found functional defects in the enzyme. S326C OGG1 excised 8-oxoG from duplex DNA and cleaved abasic sites at rates 2- to 6-fold lower than the wild-type enzyme, depending upon the base opposite the lesion. Binding experiments showed that the polymorphic OGG1 binds DNA damage with significantly less affinity than the wild-type enzyme. Remarkably, gel shift, chemical cross-linking, and gel filtration experiments

showed that S326C both exists in solution and binds damaged DNA as a dimer. S326C OGG1 enzyme expressed in human cells was also found to have reduced activity and a dimeric conformation. The glycosylase activity of S326C OGG1 was not significantly stimulated by the presence of AP-endonuclease. The altered substrate specificity, lack of stimulation by APE1 and anomalous DNA binding conformation of S326C OGG1 may contribute to its linkage to cancer incidence. We are currently examining the functional properties of mutant proteins that result from other known human polymorphisms including other polymorphisms of hOGG1.

**Assessment of DNA Repair Capacity in Human Populations:** The direct role of DNA repair in cellular senescence, aging, sporadic disease susceptibility and incidence remains unclear except in the well known groups of heritable disorders associated with cancer susceptibility and premature aging such as Xeroderma Pigmentosum, Hereditary Non-polyposis Colorectal Carcinoma (HNPCC), and Werner's syndrome among others. DNA repair capacity (DRC) of individuals may be a useful clinical tool in identifying individuals at risk for sporadically occurring disease. The clinical applications of the COMET Assay are varied ranging from assessment of chemotherapy agent geno- and cytotoxicity to correlation of oxidative DNA damage levels in spermatozoa with male infertility. Use of the Alkaline Comet Assay to assess DNA repair capacity (DRC) in human population studies has been limited by difficulties in controlling for inter-experimental variability, developing appropriate internal standards and establishing a methodology for cryopreserved lymphocytes appropriate for use in this assay. The aim of this work is to develop an accurate, reproducible and efficient comet assay methodology for evaluating DNA repair in cryopreserved lymphocytes. Our work thus far shows that unstimulated human cryopreserved lymphocytes can be used to accurately measure DRC using the comet assay. These refinements have been used to assess DRC in a clinical cohort of individuals in the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study.

**Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study:** HANDLS is a community-based, epidemiologically driven multidisciplinary research effort designed to focus on evaluating health disparities in socioeconomically diverse Caucasians and African Americans in Baltimore. One of the domains of the HANDLS study examines the possible role of oxidative stress and defects in DNA repair in the development of age associated disease. 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 may also induce a phenotype of 'premature aging' or 'accelerated aging.' While we do not posit that health disparities result from genetic alterations in genes associated with the known heritable progeroid syndromes. We do hypothesize that in low SES populations with high rates of early onset age-associated disease the interaction of biologic, psychosocial, socioeconomic and environmental factors may result in a phenotype of accelerated aging biologically similar to these syndromes with increased susceptibility to oxidative stress, premature accumulation of oxidative DNA damage, defects in DNA repair and higher levels of biomarkers of oxidative stress. Health disparities therefore, may be the end product of this complex interaction in populations at high risk.

We are examining the repair of DNA damage induced by γ-irradiation in lymphocytes from four age-matched groups of male and female Caucasians and African Americans between ages 30-64. DRC is being assessed using parameters described in the literature including *half time of DNA repair* and *residual DNA damage after 30 min*. Our preliminary findings however include the definition of a new repair parameter that we call **"initial rate of DNA repair."** Our data suggest that the *"initial rate of DNA repair"* may approximate the fast component of DNA repair and that the *"residual DNA damage"* measure may correspond to the slow DNA repair component discussed in the basic science DNA repair literature. The clinical implications of these parameters require further investigation.

**Collaborators:** Alan Zonderman, Ph.D., Laboratory of Personality and Cognition, NIA, NIH; Charles Egwuagu, Ph.D., M.P.H., National Eye Institute, NIH; Miral Dizdaroglu, National Institute of Standards and Technology; Pawel Jaruga, National Institute of Standards and Technology; Neil Powe, M.D., MPH, Johns Hopkins University School of Medicine; Thomas LaVeist, Ph.D., Bloomberg School of Public Health, Johns Hopkins University; Marie Kuczmarski, Ph.D., University of Delaware; Alanna Moshfegh, Ph.D., U.S. Department of Agriculture; James Lepkowski, Ph.D., University of Michigan; Julian Thayer, Ph.D., Ohio State University; Shari Ling, M.D., Clinical Research Branch, NIA, NIH; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Jessica Kelley-Moore, Ph.D., University of Maryland at Baltimore County.

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**Biography:** Dr. Morin received his Ph.D. from Boston University in 1995. He then completed postdoctoral training at the Johns Hopkins Oncology Center before joining the National Institute on Aging in Baltimore, where he earned tenured in 2004. Dr. Morin also holds an Assistant Professor position at the Johns Hopkins School of Medicine, Department of Pathology.

**Keywords:**

ovarian cancer
gene expression profiling
drug resistance
SAGE
Claudin

**Recent Publications:**

Hewitt KJ. *BMC Cancer* 2006; 6: 186.

Honda H. *J Biol Chem* 2006; 281(30): 21433-21444.

Morin PJ. *Cancer Res* 2005; 65(21): 9603-9606.

Agarwal R, et al. *Cancer Res* 2005; 65(16): 7378-7385.

D'Souza T, et al. *J Biol Chem* 2005; 280(28): 26233-26240.

**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.

**Identification and Dissection of Pathways Important in Ovarian Cancer:** The myriad of genes abnormally expressed in ovarian cancer provides clues as to which molecular pathways may be relevant to ovarian tumorigenesis. We are using a variety of molecular biological tools to dissect the molecular pathways responsible for the aberrant gene expression. Of particular interest is the pathway involving the claudin tight junction proteins. Claudin-3 and claudin-4 are consistently elevated in ovarian cancer and may represent ideal targets for detection and therapy of this disease.

**Identification of Ovarian Cancer-specific Biomarkers:** In the past several years, we have examined genes expression in ovarian cancer and normal ovarian tissues using serial analysis of gene expression (SAGE) and microarrays. We have identified several thousand genes expressed in each tissue and found numerous genes differentially expressed between normal and malignant ovarian cells, including novel transcripts that we have named HOST (human ovarian-specific transcript). Genes whose expression is

elevated in ovarian cancer, especially those that encode secreted and/or surface proteins may become targets for early diagnosis and various therapeutic strategies, such as immunotherapy. We are evaluating promising candidates and generating antibodies to investigate their clinical potential.

**Analysis of Gene Expression Associated with Cisplatin 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 several experimental models of cisplatin resistance. 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 identified many genes encoding proteins of the extracellular matrix. We are interested in identifying the mechanisms of cell adhesion-mediated drug resistance (CAM-DR) in ovarian cancer. We are also investigating the roles of the PI3K pathway in ovarian cancer drug resistance.

**Collaborators:** Kathleen R. Cho, M.D., University of Michigan Medical School; Stanley Zucker, M.D., Temple University; Hongxiu Ji, M.D., Johns Hopkins School of Medicine; Michael Pazin, Ph.D., Laboratory of Cellular and Molecular Biology, NIA, NIH; James M. Mullin, Ph.D., Lankenau Institute for Medical Research.

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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:**

Fugmann SD, et al. *Proc Natl Acad Sci USA* 2006; 103(10): 3728-3733.

Unniraman S, et al. *Science* 2004; 305(5687): 1113-1114.

Fugmann SD, et al. *Eur J Immunol* 2004; 34(3): 844-849.

## 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. 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.

*Immunoglobulin Gene Conversion*: This Ig diversification process has been identified thus far in chicken, rabbits and sheep (but not in humans or mice). Sequence information from upstream pseudogenes is copied into the exon of the assembled Ig gene encoding the antigen binding domain to diversify the antibody repertoire. While the precise mechanism of this process is yet unknown, several proteins have been shown to play a critical role. The activation induced cytidine deaminase AID, an enzyme converting C to U within nucleic acids, is thought to create an initial DNA lesion within the respective Ig gene, and several DNA repair factors involved in homologous recombination act subsequently to patch the lesion using upstream pseudogenes as templates.

We use the DT40 chicken B-cell line as a model system to identify and characterize novel proteins complexes that are critical for Ig gene conversion. In addition we designed strategies to identify and characterize regulatory components (cis-acting DNA elements and trans-acting factors) that restrict and limit gene conversion to B-cells of a defined developmental stage and within those specifically to the Ig gene loci.

**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.

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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 our 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:**

Honda H, et al. *J Biol Chem* 2006; 281(30): 21433-21444.

Shogren-Knaak M, et al. *Science* 2006; 311(5762): 844-847.

Ishii H, et al. *J Biol Chem* 2006; 279: 7331.

**Research Summary:** Chromatin plays a critical role in regulating access to the information contained in the genome, as well as packaging the genome to allow it to fit within the nucleus of a cell. Chromatin structure can change rapidly when 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. We have identified a number of genes regulated by remodeling enzymes as well as binding sites for these enzymes in their target loci.

**B Cells and Immunoglobulin Heavy Chain Regulation:** Regulation of immunoglobulin loci is a complex process involving transcriptional regulation as well as gene rearrangement. It is believed that one important level of control is the accessibility of the loci, and that this may be regulated

through chromatin structure. In collaboration with Ranjan Sen, we are using a cell-free system to determine how chromatin remodeling regulates transcription and recombination. We have identified transcription factors and remodeling enzymes that regulate chromatin structure. Remarkably, we have found a remodeling event that erases a DNase I hypersensitive site without reverting the chromatin structure to the ground state; furthermore, the transcription factor that initiates formation of the hypersensitivity is not displaced. We believe remodeling plays a causal role in transcription in this system, as the structural changes precede and correlate with changes in gene regulation. Moreover, remodeling occurs in the absence of transcription, while transcription is impaired in the absence of remodeling.

**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:** Patrice Morin, Ph.D, Laboratory of Cellular and Molecular Biology, NIA, NIH; Ranjan Sen, Ph.D., Laboratory of Cellular and Molecular Biology, NIA, NIH; Craig Peterson, Ph.D., University of Massachusetts; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Weidong Wang, Laboratory of Genetics, NIA, NIH.

# Laboratory of Clinical Investigation

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The **Laboratory of Clinical Investigation (LCI)** is comprised of 4 Sections with 6 principal investigators. These are as follows: Bioanalytical Chemistry and Drug Discovery Section; Diabetes Section; Molecular and Clinical Pharmacology Section; and Nuclear Magnetic Resonance Section. The common theme and thread among these research groups is that of the identification and development of new therapeutic targets for the treatment of age-related disease. 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 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 quantitation 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 $\beta_2$–adrenergic receptor.

**Diabetes Section (DS):** (PI Dr. Josephine Egan) Type 2 diabetes mellitus and the identification of new targets for its treatment are the focus of this Section as it relates to drug discovery and development. Dr. Egan has 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 underway. The present focus is the study of the mechanisms of the release of enteric peptides that modulate insulin release, and an in-depth study of one of the peptides, GIP, and its receptor, to further understand the role of insulinotropic treatments for type 2 diabetes mellitus.

Dr. Bernier focuses on the insulin receptor. He works to elucidate 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.

**Molecular and Clinical Pharmacology Section (MCPS):** (PIs Drs. Nikolai Soldatov, Darrell Abernethy, and Nazli McDonnell) In the process of understanding gating mechanisms of the L-type calcium channel and the role of the intracellular domains of the channel in signal transduction, splice variations that change channel function in age and atherosclerosis were noted. Understanding the functional consequence of such splice variation and the changes in local cellular milieu associated with this splice variation has become a new focus of investigation. How these splice variant calcium channels change the pharmacodynamics of calcium channel antagonist drugs may provide understanding of intertissue and interindividual variation in response to these drugs. This is now a translational research effort as well to explore the clinical consequences of such splice variation. To advance methodology to study cell membrane microdomains and their dynamics with calcium channel and other receptor activation, analytic methods for characterizing such domains as identified by fluorescence resonance energy transfer are underway. A component of identification of a therapeutic target in addition to selection of optimal ligand(s) to move forward as drug candidates is understanding pharmacokinetic/pharmacodynamic properties of these compounds in animal models and man. Present efforts are to develop systems to characterize and predict dose/effect relationships that

have utility when neither drug concentration nor drug effect will be available at the time drug dose must be selected for an individual patient. The approach showing promise is with use of trained neural networks. A related area is that of understanding drug dose and concentration/effect relationships when the effect baseline is nonstationary (e.g. blood pressure, heart rate, mental status, WBC or platelet count). Beat-to-beat heart rate variability, and analysis of such data simultaneously in the time and frequency domains using wavelet analysis is the approach being developed. Dr. McDonnell studies the clinical genetics of connective tissue disorders such as Ehlers-Danlos Syndrome.

**Nuclear Magnetic Resonance Section (NMRS):** (PI Dr. Richard Spencer) This section has and continues to make important contributions in the study of chrondrocyte biology using tissue bioreactors in 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, study 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 transgenic animals that model human skeletal dysplasias, and with Dr. Soldatov in the Molecular and Clinical Pharmacology Section to study the consequences of variant L-type calcium channels on cardiac and vascular function are expected to produce animal models in which therapeutic interventions can be implemented and pharmacodynamics sensitively measured using *in vivo* NMR.

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| Mehmet Uluer | Student IRTA Fellow |
| Sharan Ramaswamy | Visiting Fellow |
| Holly Canuto | Visiting Fellow |
| Zhouli Zhang | Visiting Fellow |
| Dorota Bartusik | Visiting Fellow |
| Nancy Camacho | Special Volunteer |

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**Biography:** Darrell R. Abernethy, M.D., Ph.D. is Chief of the Laboratory of Clinical Investigation at the National Institute on Aging. Dr. Abernethy received his M.D. (AOA) and Ph.D. (Pharmacology) degrees from the University of Kansas School of Medicine in 1976. Further clinical training was in Internal Medicine at Jackson Memorial Hospital/University of Miami through Board Certification in Internal Medicine. He then did post-doctoral fellowship training in Clinical Pharmacology at the Massachusetts General Hospital. Dr. Abernethy joined the faculty at Tufts University School of Medicine as Assistant Professor of Psychiatry and Medicine in 1981. He moved to Baylor College of Medicine in 1983 where he advanced to Associate Professor of Medicine in the Division of Hypertension and Clinical Pharmacology. In 1986, he moved to Brown University School of Medicine as Chief of the Division of Clinical Pharmacology. He was subsequently promoted to Professor of Medicine at Brown. In 1994, Dr. Abernethy became the Francis Cabell Brown Professor and Director of the Division of Clinical Pharmacology at Georgetown University School of Medicine, where he served until 1999, at which time he assumed his current post. Dr. Abernethy has contributed to understanding of mechanisms of peripheral distribution of drugs and drug disposition and effect in obesity. He also contributes to the knowledge base in pharmacokinetic/pharmacodynamic relationships of cardiovascular drugs in aging and has advanced the concept that the pathophysiology of aging must be considered when interpreting drug effects in the aged patient. These efforts have resulted in 185 publications of original research and over 50 book chapters and reviews. Dr. Abernethy has participated in, and continues to participate actively in service and organizational activities which promote the safe and effective use of medications in aged patients. As an extramural investigator, he served on the NIGMS Pharmacological Sciences study section, the FDA generic drugs and cardiorenal advisory committees, and served as chair of the VA merit review Geriatrics subcommittee. As an educator, he served on the National Board of Medical examiners Pharmacology Test Committee, was chair of the NBME (Now called USMLE), Applied Pharmacology Committee, the Step 1 Test Committee and presently serves on the USMLE biostatistics task force. Editorial activities include membership on the editorial boards of *Clinical Pharmacology* and *Therapeutics*, the *Journal of Clinical Psychopharmacology, Drugs*, as Associate Editor of the *Journal of Pharmacology and Experimental Therapeutics*, and as Editor-in-Chief of *Pharmacological Reviews*. Organizational and public service have included serving as President of the American Society of Clinical Pharmacology and Therapeutics and on the Gerontology Committee of the United States Pharmacopeia, as member and chair. He also served on the USP Medicare Medication Guidelines committee, a group designated by the U.S. Congress to establish the basis for the Medicare Prescription Drug Benefit and in 2005, he was elected President of the USP Convention for the 2005-2010 cycle. In 2006, he was named head of the cardiovascular pharmacology section in the Faculty of 1000. Dr. Abernethy has been the recipient of the Rawls Palmer Progress in Medicine award of the American Society for Progress in Medicine, award of the American Society for Clinical Pharmacological Therapeutics and has served as visiting Professor at 15 different academic centers.

**Keywords:**

calcium
calcium antagonists
cellular microdomains

**Calcium Channel Variants in Aging and Disease:** Alternative splicing generates diversity of the calcium channel alpha subunit, but does not significantly change the overall topology of the protein, which is highly conserved in the regions of calcium antagonist drug binding. Instead regions

**Keywords-continued:**
pharmacokinetic/
pharmacodynamic
modeling
drug development

**Recent Publications:**

Beigi F, et al, *Chirality* 2006; 18(10): 822-827.

Mager DE, et al, *FASEB J* 2006; 20(6): 631-637.

Kobrinsky E, et al, *J Biol Chem* 2005; 280(13): 12474-12485.

Kobrinsky E, et al, *Biophys J* 2005; 88(5): 3625-3634.

Mager DE, et al, *J Pharm Sci* 2005; 94(11): 2475-2486.

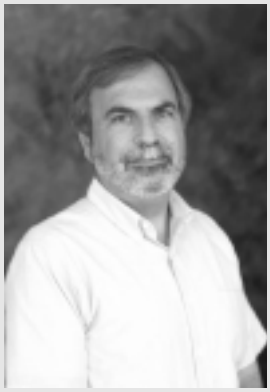
of diversity appear to regulate function of the calcium channel, in particular with regard to the rate of its inactivation following stimulation. The alternatively spliced variants of the calcium channel have been identified in different tissues, and appear to be expressed differently as a function of age. We are exploring the molecular correlates of calcium gating in this channel and how gating differs in the various naturally expressed channel variants. In addition we are studying the heterogeneity, distribution patterns and regulation of the splice variants in human cardiac and vascular tissues in relationship to age, hormonal, and pathological stimuli. L-type calcium antagonist drugs have become very important in cardiovascular therapeutics for the treatment of angina pectoris and hypertension. For further improvement of calcium channel targeting drugs, these studies will provide understanding of the molecular bases of regulation of the calcium channel.

**Dynamics of Cell Microdomains:** Characterization of the dynamics of cellular membrane and other substructure microdomains is essential to understand the functional relationship of specific structures and organelles to each other. Using fluorescent probes and fluorescence resonance energy transfer (FRET) experiments to study clustering of receptors and calcium channels, one-dimensional and two-dimensional wavelet analyses are being utilized to capture the dynamics of spatial and temporal associations of structures. Development of these methods and their extension to three-dimensional analysis is proving to be of considerable use in understanding the dynamic nature of interaction among cellular structures.

**Dose-Effect and Concentration-Effect Analysis in Drug Development:** Traditional pharmacokinetic/pharmacodynamic analytical methods require substantial amounts of concentration and drug effect information to be implemented; however, in practice such information is often not available. The use of neural networks to characterize dose-effect relationships for drugs with a narrow therapeutic index, but for which there is no drug concentration information and/or limited drug effect measures is being studied and has offered potentially therapeutically useful approaches for antiplatelet drugs such as abciximab. In contrast, in some instances much drug concentration and effect information is available. However, current approaches do not allow insight into the time dimension for changing physiological baselines. Study of beat-to-beat heart rate variability and drug interventions to change this parameter are being used to explore the use of wavelet analysis to capture both time and frequency elements of the pharmacokinetic/pharmacodynamic analysis, in contrast to the currently used Fast Fourier Transform methods that only capture one or the other.

**Drug Development for the Treatment of Congestive Heart Failure and Diagnosis of Alzheimer's Disease:** Potential therapeutic and diagnostic targets across the intramural research program are identified and for promising agents, a development program is outlined. At present the use of $\beta_2$-adrenergic agonists that have $G_s$ signaling selectivity is being evaluated. This concept, from the Laboratory of Cardiovascular Science, is being moved forward to a clinical development program, with Phase I studies of the first drug candidate planned within this year. Another concept from the Laboratory of Neurosciences, that of use of butrylcholinesterase inhibitors that have high affinity for regions of amyloid plaque as diagnostics, is in preclinical development. Such agents will be positron labeled and then evaluated for *in vivo* PET imaging diagnosis of amyloid plaque related dementing illness.

**Collaborators:** Nikolai Soldatov, Ph.D, Laboratory of Clinical Investigation, NIA, NIH; Irving Wainer, Ph.D., Laboratory of Clinical Investigation, NIA, NIH; Donald Mager, Pharm.D, Ph.D., University of Buffalo; Sarah Hilmer, M.D., Ph.D., University of Sydney; Rui-Ping Xiao, M.D., Ph.D., Laboratory of Cardiovascular Science, NIA, NIH; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Richard Spencer, M.D., Ph.D., Laboratory of Clinical Investigation, NIA, NIH; Mary Ann Mascelli, Ph.D., Centocor, Inc.; Desmond Fitzgerald, M.D., Royal College of Surgeons of Ireland; Linda Fried, M.D., M.H.S., Johns Hopkins University.



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**Biography:** Dr. Irving W. Wainer graduated from Wayne State University in 1965 with a B.S. in chemistry and then received his Ph.D. degree in chemistry from Cornell University in 1970. He did postdoctoral 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 300 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 doctotate in medicine 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.

**Keywords:**

cancer cachexia
drug metabolism
immobilized receptors
high throughput screens

**Recent Publications:**

Moaddel R, et al. *Anal Chem* 2005; 77(16): 5421-5426.

Moaddel R, et al. *Anal Chem* 2005; 77(3): 895-901.

Jozwiak K, et al. *J Med Chem* 2004; 47(16): 4008-4021.

**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.

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

measured in spot urines using capillary electrophoresis (CE). The presence of PIF in urine has been correlated with clinical status and with the identification of PIF in tumor biopsies. We have also correlated the presence of PIF in urine with treatment response and clinical relapse. A longitudinal study of the use of PIF as a disease marker has been designed and will be initiated this fall.

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

**Immobilized Receptors, Transporters and Enzymes:** We have developed liquid chromatographic stationary phases containing immobilized receptors, enzymes and transporters as an on-line, flow system for use in new drug discovery and in the characterization of lead drug candidates. These columns can range in size from standard lc columns to micro-columns, can be used to screen complex chemical mixtures, to characterize single compounds and to screen phage libraries. The columns can be used with characterized targets such as ligand gated ion channels (nicotinic, GABA, NMDA receptors), G-protein receptors (opioid and β-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 β-hydroxylase), as well as orphan receptors and other expressed proteins. In addition, the columns can be placed on-line with mass spectrometers or any other structure or activity detectors and provide real-time data. We also have data that demonstrate that this technique can give information that cannot be obtained using standard micro-titer plate approaches. For example, the immobilized nicotinic receptor column can be used to rapidly identify non-competitive inhibitors of this receptor. At the current time, non-competitive inhibitors can only be identified through functional ion-flux studies. The mechanism of non-competitive inhibitor binding in the central lumen of the nicotinic receptor has been studied and described using molecular modeling and chemometric techniques. Current research involves the use of immobilized nicotinic receptor columns to screen tobacco smoke condensates for CNS active compounds and the development of immobilized mast cell columns to study the inflammatory process.

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

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

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**Biography:** Dr. Josephine 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:**

Theodorakis MJ, et al. *Am J Physiol Endocrinol Metab* 2006; 290(3): E550-E559.

Kim BJ, et al. *J Clin Endocrinol Metab* 2005; 90(12): 6665-6671

Doyle ME, et al. *Endocrine* 2005; 27(1): 1-9.

**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 novel 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 found that Notch receptors and their ligands are present and functional in unstimulated beta cells. These play critical roles in regulation of proliferation, differentiation and apoptosis, in a context-dependent manner. Moreover, in unstimulated beta cells, Notch is cytoplasmic but with glucose and exenatide stimulation, Notch translocation occurred to the nucleus and led to upregulation of insulin promoter activity and IRS2 message and protein levels. We are presently focusing on understanding how Notch activation increases insulin promoter activity and upregulates the IRS2 gene and how Notch actually becomes activated on stimulation. We hypothesize that down-regulation of this pathway with aging may also be responsible for age-associated declines in beta cell replication rates. This may eventually lead to treatments, similar to exenatide, that would prevent age-related declines in beta cell turnover.

**Collaborators:** Maire E. Doyle, Dental Institute, University of Florida, Gainesville, Florida; 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. Nikolai Soldatov graduated from Moscow State University in 1974 with a B.S. in chemistry. He received his Ph.D. degree in bioorganic chemistry in 1981 from Shemyakin Institute of Bioorganic Chemistry of the National Academy of Sciences, Moscow, Russia. After postdoctoral training in the same institute, he worked in the Institute of Medical Biotechnology, Moscow (1986-1989), Rockefeller University, New York (1990-1993), University of Bern, Switzerland (1993-1996), and Georgetown University, Washington, D.C. (1996-1999). In 1999 he joined NIA as an Investigator. He is an adjunct Associate Professor of Georgetown University and a member of the Editorial Advisory Board of the *Journal of Pharmacology and Experimental Therapeutics*. Dr. Soldatov is an author of 70 scientific papers and chapters. The main direction of his study is investigation of molecular correlates of $Ca^{2+}$ channel regulation.

**Keywords:**

calcium signal transduction
human L-type calcium channel
mechanisms of inactivation

**Recent Publications:**

Kobrinsky E, et al. *J Biol Chem* 2006; 281(28): 19233-19240.

Kobrinsky E, et al. *Biophys J* 2005; 88(5): 3625-3634.

Kobrinsky E, et al. *J Biol Chem* 2005; 280(13): 12474-12485.

Morad M, et al. *Cell Calcium* 2005; 38(3-4): 223-231.

**Molecular Correlates of $Ca^{2+}$ Channel Regulation:** $Ca_v1.2$ calcium channels couple membrane depolarization to distinct cellular functions associated with regulation of gene expression, synaptic plasticity, exocytosis, cell survival and other processes. Among the ensemble of mutually coordinated determinants of inactivation identified in our study, the C-terminal tail of the pore $\alpha_{1C}$ subunit and annular determinant of slow inactivation in the cytoplasmic end of the pore are directly involved in this coupling, while the crucial correlates provided by $\alpha_{1C}$ N-terminus and $\beta$ subunits may add more specialization via genetic variation and alternative splicing. Physiological roles and molecular dynamics of this ensemble of regulatory determinant are in the focus of our research.

**Voltage-gated Differential FRET Microscopy of Ion Channels:** Green fluorescent protein (GFP) has become a unique tool of investigation in molecular biology because it can be genetically fused to many proteins without significantly affecting their functional properties. Spectral characteristics of the enhanced cyan (ECFP) and yellow (EYFP) variants of GFP are well suited for measurements of molecular rearrangements by fluorescence resonance energy transfer (FRET). Because FRET depends on the distance and angular orientation between the fluorescent partners, relative change of these parameters may be measured when a functional state of the $Ca^{2+}$ channel is stabilized by voltage clamp. This idea of differential voltage-gated FRET was successfully implemented by combining FRET microscopy with whole cell patch clamp to study molecular dynamics of the human vascular $Ca_v1.2$ calcium and $K_v2.1$ potassium channels in real time and in the live cell. This approach revealed

voltage-gated mobility of the cytoplasmic tails and their regulatory roles in intracellular signaling. Future investigation of the functional architecture of these and other ion channels using voltage-gated differential FRET microscopy of various functional parts and subunits will provide a unique insight into dynamic molecular changes associated with distinct functional states of ion channels in live cells.

**Small beta Subunits of the L-Type $Ca^{2+}$ Channel:** Coded by four different genes, β subunits are cytoplasmic proteins that facilitate voltage gating of $Ca_v1.2$ channels by impeding the block of the channel by the N-terminus of the pore-forming $\alpha_{1C}$ subunit. How they mediate these functions and whether β-specific sub-structural determinants may have therapeutic significance are important questions to be answered. The main determinant of β subunits is believed to be associated with the common central motif that has homology with the family of membrane-associated guanylate kinase (MAGUK) proteins that scaffold signaling molecules. Among the human cardiac and neuronal transcripts of the $\beta_2$ subunit we identified small $\beta_2$ splice variants lacking large central and N-terminal regions. In spite of the partial or complete loss of MAGUK, small $\beta_2$ subunits interact with the pore subunit and facilitate voltage gating of the channels. Thus, a tissue-specific splice variation of $\beta_2$ may generate subsets of human $Ca_v1.2$ channels in the cardiac muscle cells and neurons that rely on MAGUK-independent modulation. Structure-functional study of small $\beta_2$ splice variants may help to identify new therapeutic targets.

**Collaborators:** Evgeny Kobrinsky, Jo Beth Harry, Qu Zong Lao, Cheng Zhang Shi, Yuwei Zhang, Girma Asemu Sebrie, Darrell Abernethy, Laboratory of Clinical Investigation, NIA, NIH; Dennis Wray, University of Leeds, UK; Gregory Harms, University of Würtzburg, Germany; Martin Morad, Georgetown University, Washington, D.C.; Jung-Ha Lee, University of Seoul, South Korea.

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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:**

connective tissue
aneurysm
fibromuscular dysplasia
Chiari I malformation

**Recent Publications:**

McDonnell NB, et al. *Am J Med Genet* 2006; 140(2): 129-136.

**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, in collaboration with Drs. Bolognese and Milhorat, has 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.

**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), 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). Other projects on the horizon include siRNA treatment strategies for dominantly inherited genetic disorders.

**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:** Clair Francomano, Harvey Institute of Human Genetics, Greater Baltimore Medical Center; Andrew Singleton, Laboratory of Neurogenetics, NIA, NIH; Mark Talan and Samer Najjar, Laboratory of Cardiovascular Science, NIA, NIH; Shari Ling, Clinical Research Branch, NIA, NIH; Debbie Merke and Joan Marini, National Institute of Child Health and Human Development, NIH; Harry Dietz, Johns Hopkins Medical Institutions; Leena Ala-Kokko, University of Oolu; Paul Costa, Laboratory of Personality and Cognition, NIA, NIH; Ruth Altshuler, Massachusetts General Hospital; Thomas Milhorat and Paolo Bolognese, The Chiari Institute, NS-LIJHS; Mark Lavallee, Indiana University School of Medicine.

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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. He was a postdoctoral fellow with Professor Robert Griffin at the Francis Bitter National Magnet Laboratory of MIT before joining the NIH. Dr. Spencer joined the National Institute on Aging in 1991, as Chief of the Nuclear Magnetic Resonance Section. He completed internal medical residency training at Johns Hopkins Bayview Medical Center in Baltimore and is a Diplomate of the American Board of Internal Medicine, 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 80 articles and book chapters and is on the editorial board of several journals. He has been on the Faculty of the Internal Society of Magnetic Resonance in Medicine, delivering educational lectures on MRI hardware and signal processing. His research interests are primarily in imaging (MRI) and metabolic studies of three-dimensional cartilage grown from chondrocytes in culture with particular emphasis on biological response modifiers, spectroscopic studies of cardiac and muscle metabolism 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

**Recent Publications:**

Ramaswamy S, et al. *J Biomed Mater Res* 2006; 77(1): 144-148.

Spencer RG, et al. *Mag Reson Imaging* 2006; 24(1): 27-31.

Canuto HC, et al. *J Magn Reson Imaging* 2006; 23(5): 742-746.

Kim M, et al. *J Biomed Opt* 2005; 10(3): 1-6.

McConville P, et al. *Am J Physiol Endocrinol Metab* 2005; 289(3): E412-E418.

**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. 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 development 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 $O_2$ and $CO_2$ 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. Second, growth 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 will be of clear importance.

While arthroscopic biopsies provide such data, permitting assessment of the biochemical and histologic state of the tissue, it is clearly more desirable to utilize noninvasive assessment methods. MRI is becoming increasingly accepted as a noninvasive tool for the measurement of cartilage thickness and volume and of localized pathology while the ability of MRI to noninvasively assess cartilage quality is currently a topic of active research. The availability of a highly controllable system for generating cartilage with widely varying properties in a system permitting detailed MRI assessment would represent a clear advance in this effort. Finally, we note that the MRI-compatible bioreactor provides a flexible test-bed for current and future therapeutic agents and interventions. In summary, as a cellular system, the HFBR shares with other 3D culture systems the ability to support the hyaline cartilage type. Thus, one can evaluate the effect of growth conditions and therapeutics on hyaline cartilage tissue rather than fibrocartilage. As a tissue system, the HFBR permits true macroscopic growth. Thus, cell-matrix interactions and the effects of the matrix barrier to substrate delivery and metabolic product efflux are represented much more realistically than in monolayer systems. Finally, as a test bed for growth conditions and agents, the HFBR provides full control of substrate and perfusion conditions. All MRI studies for this work are performed on our 9.4Tesla system at the Gerontology Research Center.

We have successfully demonstrated that cartilage grown from chick sternal cells 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. We have also utilized 31P NMR measurements of pH, inorganic phosphate ($P_i$) and ATP to demonstrate that the developing cartilage in the bioreactor remains metabolically stable over the typical 4 week growth period. A major focus of our work has, in addition, been to demonstrate that MRI measurements of matrix fixed density correlate with measurements of dynamic and equilibrium compressive moduli. The MRI-derived FCD values correlate with S-GAG content but not with collagen content. These correlations were found to persist even in tissue which has undergone development in the presence of chondroitinase, acting as a catabolic agent on matrix proteoglycans. Noninvasive MRI evaluation of FCD therefore has

been shown to provide reliable information about cartilage matrix composition under the dynamic conditions of the HFBR in both control tissue and in tissue which has undergone degeneration analogous to that seen in osteoarthritis.

**Mouse Phenotyping Studies:**

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

The mouse model studied, the *oim/oim* mouse, exhibits a mutation resulting from abnormal collagen structure. This leads to bone fragility similar to that seen in patients with OI. We are investigating the skin of these animals, as skin structure and properties are also highly dependent upon collagen integrity and organization. MRI studies are performed on our 9.4Tesla system at the Gerontology Research Center. We have found significant abnormalities in the skin using MRI, histology, and Fourier transform infrared imaging. 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 $T_2$ values. Finally, we are addressing the question of whether MRI will be sensitive to skin abnormalities in OI patients.

***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. MRI studies are performed on our 9.4Tesla system at the Gerontology Research Center. 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.

**Collaborators:** Shari M. Ling, M.D., Clinical Research Branch, NIA, NIH; Edward Lakatta, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Nancy Pleshko Camacho, Ph.D., Hospital for Special Surgery, New York; Peter Torzilli, Ph.D., Hospital for Special Surgery, New York; Walter Horton, Ph.D., Northeast Ohio University College of Medicine, Darrell Abernethy, M.D., Ph.D. and Nikolai Soldatov, Ph.D., Laboratory of Clinical Investigation, NIA, NIH.

# Laboratory of Epidemiology, Demography, and Biometry

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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 (Section Chief's position is currently vacant) 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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| Andrea Garcia | Postbac IRTA Fellow |
| Antonia Coppin | Visiting Fellow |
| Marion Faulhaber | Visiting Fellow |
| Sandra Giannelli | Special Volunteer |
| Beth Han | Special Volunteer |

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| Rita Peila | Adjunct Investigator |
| Ann Scher | Adjunct Investigator |
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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 Acting Chief of the Laboratory since 2004.

**Keywords:**

epidemiology
chronic diseases
disability
functional status
physical activity

**Recent Publications:**

Pennix BW, et al. *J Gerontol A Biol Sci Med Sci* 2006; 61(5): 474-479.

Coppin AK, et al. *J Gerontol A Biol Sci Med Sci* 2006; 61(1): 86-91.

Rejeski WJ, et al. *Contemp Clin Trials* 2005; 26(2): 141-154.

Shumway-Cook A, et al. *J Am Geriatr Soc* 2005; 53(4): 700-704.

Volpato S, et al. *J Gerontol A Biol Sci Med Sci* 2005; 60(12): 1539-1545.

Guralnik JM, et al. *Blood* 2004; 104(8): 2263-2268.

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 Cohort Study.

**Physical Activity and Exercise:** A major research interest has been in examining the impact of physical activity and exercise on disability and other health outcomes in older people. Past work demonstrated the risk of incident disability related to sedentary lifestyle. Recent work using EPESE data has shown that active life expectancy, the number of years expected to be lived without disability, is strongly influenced by physical activity. We have also recently demonstrated that an active lifestyle is associated with both living to advanced old age and with dying with no major disability in the last year of life. Data from the WHAS have shown that many women

with difficulty walking continue to walk for exercise while nearly half of the women without difficulty don't walk at all for exercise. The amount of walking for exercise done by older women is strongly influenced by their level of disease and disability, but many psychosocial variables also influence the amount of walking these women do. Recent findings indicate that even very modest amounts of walking are associated with lower rates of disability onset. 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.

**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. Recently, 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 is being planned.

**The Pathway from Disease to Disability:** An important and ongoing area of research has been to develop an understanding of how the consequences of chronic diseases and the physiologic changes associated with aging cause important losses in functional status and affect the ability to remain independent in the community. A large amount of data collected in the WHAS and other studies provides the basis for empirical study of the steps in the causal chain of events in this pathway. A large research effort has gone into understanding muscle strength in older people and how it relates to functional limitations, disability and other outcomes. The impact on progression through the pathway of both specific conditions and co-

occurring multiple conditions (co-morbidity) has been a long-standing area of emphasis in our research. A large effort has gone into identifying biochemical markers of subclinical diseases and frailty that are strongly prognostic of mortality and other adverse outcomes. Our previous work demonstrated increased risk of mortality associated with low serum albumin level and also a graded risk of mortality across the full spectrum of serum albumin values. Research has assessed the impairments and functional limitations that result from diabetes and affect the steps in the pathway from diabetes to disability.

**Health Disparities:** We have had a long-standing interest in the impact of social class on health and have demonstrated that educational status and income are powerful predictors of disability onset and mortality. We have also shown that active, or disability-free, life expectancy is considerably longer in persons with higher levels of education. Race also plays a role in the health of older persons although its influence, after adjustment for education and income, has not been consistently demonstrated. Research has also been initiated on the impact of neighborhood characteristics on health outcomes. 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; Drs. Linda Fried, Karen Bandeen-Roche, Johns Hopkins Medical Institutions; 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. Jiska Cohen-Mansfield, Hebrew Home for the Aged, and George Washington University School of Medicine; Dr. Stephen Sayers, University of Missouri; Dr. Meredith Minkler, University of California, Berkeley; 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, 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 Epidemiology Section. Dr. Harris has developed the research of the Geriatric Epidemiology 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.

The role of the **Geriatric Epidemiology Section** is to integrate molecular and genetic epidemiology with interdisciplinary studies of functional outcomes, disease endpoints and mortality in older persons. This includes identification of novel risk factors and design of studies involving biomarkers, selected polymorphisms and exploration of gene/environment interactions. The Section has been particularly active in devising methods to integrate promising molecular or imaging techniques in ways that begin to explore the physiology underlying epidemiologic associations 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 Epidemiology Section initiated the Health, Aging and Body Composition Study (Health ABC) in 1996 to investigate these questions. The major study objective is to examine whether change in body composition, particularly loss of muscle, represents a common pathway by which multiple conditions contribute to disability. Since little was known about sarcopenia in an unselected population, the Health ABC population was selected as well-functioning and relatively health-stable, but at high risk of health transitions secondary to age, race and

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

The study was designed around the hypothesis that factors affecting body composition and loss of muscle would be consistent across all four race/sex groups and that factors in three key areas would modulate loss of muscle including: metabolic dysregulation, particularly inflammation or genetic factors; episodes of acute illness; and patterns of change in physical activity. A battery of detailed physiologic measurements and questionnaire material was developed to follow change over the 7-year period of examinations that is part of the study and that covers a period of rapid health transitions. All critical measures will be repeated during this time (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 Epidemiology Section has organized a series of studies to investigate this finding in more detail including collaborating with investigators who have a large library of full-body MRI scans to assess fatty infiltration by age, race and level of physical activity and molecular studies of muscle and fat tissue from several locations in the body.

The Geriatric Epidemiology 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 Epidemiology Section has been on the contribution of chronic low-level inflammation to health outcomes apart from cardiovascular disease, and to understanding what conditions and

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

**Assessing the Genetic Contribution to Diseases of Old Age:** The Geriatric Epidemiology Section initiated and works collaboratively with the Neuroepidemiology Section on the Age, Gene/Environment Susceptibility-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.

The Geriatric Epidemiology Section has also carried out studies of selected polymorphisms pertinent to inflammation and body composition measures in nested case-control studies in the Health ABC Study and in other datasets developed for this purpose. Efforts have been made to broaden the application of emerging techniques for genomic and proteomic studies to populations by development of new methods in collaboration with laboratory-based investigators. 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 in many epidemiologic studies of neurologic diseases including dementia and migraine headache. Dr. Launer joined NIA as Head of the Neuroepidemiology Unit in February 1999 and received tenure in 2005.

**Keywords:**

epidemiology
neurologic diseases
genetic and
environmental risk factors

**Recent Publications:**

Hartley SW, et al. *Neuroimage* 2006; 30(4): 1179-1186.

Peila R, et al. *Stroke* 2006; 37(5): 1165-1170.

Launer LJ. *Neurobiol Aging* 2005; 26(3): 335-340.

Petrovitch H, et al. *Ann Neurol* 2005; 57(1): 98-103.

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

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

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

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

As a further test of the hypothesis that vascular risk factors may contribute to brain disease in old age, we have initiated a sub-study to the NHLBI randomized ACCORD (Action to Control Cardiovascular Risk in Diabetes) Trial. This trial includes a large sample of type 2 diabetics over 55 years of age. It is designed to compare the effects on cardiovascular disease of standard versus intensive treatment of risk factors in diabetics. The trial design allows us to compare the effects of standard versus intensive treatment of hyperglycemia, hypertension, and dyslipidemia on cognitive function and brain structure as measured by magnetic resonance imaging.

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

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

**Collaborators:** Tamara Harris, M.D., M.S., Jack Guralnik, M.D., Ph.D., Laboratory of Epidemiology, Demography, and Biometry, NIA, NIH; Lon R. White, M.D., Pacific Health Research Institute, Hawaii; Alan Remaley M.D., NIH Clinical Center; Monique M.B. Breteler, M.D., Ph.D., Albert Hofman, M.D., Ph.D., Erasmus University Medical Centre, Netherlands; M. Ferrari, M.D., Ph.D., Mark van Buchem, M.D., Ph.D., Leiden University Medical Centre, Netherlands; Arthur Toga, Laboratory of Neuroimaging, UCLA; Oscar Lopez, University of Pittsburgh; S. Giampaoli, M.D., Institute of Health, Rome, Italy; Vilmundur Gudnason, Palmi Jonsson, M.D., Gudmundur Thorgeirsson, M.D., Ph.D., G. Sigurdsson, M.D., Ph.D., University of Iceland and Icelandic Heart Association, Iceland; M. Luster, Ph.D., NIOSH, W. Virginia; Mark Mattson, Ph.D., P. Scheltens, M.D., Ph.D., Laboratory of Neurosciences, NIA, NIH; J. Williamson, M.D., Wake Forest University; R. Lazar, Ph.D., Columbia University; A. Murray, M.D., University of Minnesota; M. Sullivan, M.D., Ph.D., University of Washington; Q-L. Xue, Ph.D., Johns Hopkins University; N.R. Bryan, University of Pennsylvania.

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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.

**Keywords:**

longitudinal studies
mathematical modeling
sleep
driving

**Survival Analysis and Modeling:** Recent work in longitudinal data analysis involves development and application of Cox proportional hazards models to study risk factors for onset of Alzheimer's disease (AD) and other dementias in the Honolulu-Asia Aging Study of dementia. Rarely is the date of onset for AD or other forms of dementia in epidemiological studies well established and available for the study of incidence and relative risks. Using the recommendations of several published papers we developed a standardized approach for establishing a date of onset for incident cases of dementia in the Honolulu-Asia Aging Study (HAAS) to investigate risk factors with Cox proportional hazards models. In this model, a date of onset is assumed to be the midpoint between two HAAS examinations in which a participant is free of dementia in the earlier examination and then receives a diagnosis in the subsequent examination. Participants without a subsequent examination due to death or to refusal are excluded from the analyses because the course of their cognitive status is unknown. Use of this Cox proportional hazard model has facilitated analyses of data from the midlife examinations which were gathered between 1965 and1975 on the HAAS cohort members as part of their prior recruitment into the Honolulu Heart Program to study cardiovascular disease beginning in 1965 among 8,006 Japanese-American men born 1900 to 1919.

**Prevalence and Consequences of Sleep-Disordered Breathing and Other Sleep Disorders:** For more than a decade, the epidemiological importance of obstructive sleep apnea and other forms of sleep-disordered breathing have gained attention as risk factors for cardiovascular disease and other adverse clinical endpoints including neuropsychological deficits. The estimation of the prevalence of sleep-disordered breathing, and in particular obstructive sleep apnea based on epidemiological studies, has been limited by small sample sizes due to the cost of overnight polysomnography in a

sleep laboratory for diagnosis. Consequently, a major initiative to develop portable polysomnography for unattended overnight sleep recordings in a person's home facilitated the launch of the large multi-centered Sleep Heart Health Study (SHHS) to assess cardiovascular consequences of sleep-disordered breathing among adults aged 40 years and older. The SHHS also provided support for use of the portable polysomnography in the HAAS.

Between 1999 and 2000, a total of 718 of the 1,524 surviving HAAS cohort members aged 79 to 97 years completed an overnight polysomnography using the SHHS protocol. This landmark study provides an opportunity to investigate the association between obstructive sleep apnea and cognitive impairment in a population of elderly men who are at high risk of developing dementia.

Self-reported data on sleep problems in several LEDB funded studies including the HAAS and the EPESE have provided epidemiological data on the prevalence, correlates and consequences of symptoms of insomnia and for symptoms of excessive daytime sleepiness among older adults. The earlier descriptive findings led to more recent initiatives to describe the epidemiology of chronic insomnia in the elderly as secondary to the onset and progression of chronic diseases including heart disease, stroke, arthritis and diabetes to name a few. Importantly, these findings highlight the need for advances in both cognitive-behavioral therapy and in long-term use of sleeping pills such as zolpidem and zaleplon.

**Epidemiology of Death and Dying:** Each year, nearly 2 million men and women age 65 years and older die from a variety of causes. Data from the National Mortality Followback Surveys and the Established Populations for Epidemiologic Studies of the Elderly (EPESE) provide opportunities to improve knowledge about mortality trends, particularly for Alzheimer's disease related deaths, and about dying trajectories. Currently, AD is among the 10 leading causes of death among the population age 65 years and older. In collaboration with lead investigators from the LEDB, Epidemiology and Demography Section, several distinct patterns of dying trajectories have been developed and examined using data from the EPESE.

**Aging and Driver Safety:** Older drivers have the second highest driver fatality rate in the nation while teen drivers have the greatest risk. Aging often corresponds with marked decrements in visual, cognitive and physical functioning that can compromise driving skills. Each year, over 600,000 elderly adults stop driving because of their health. The effects of vision impairments and dementia on driving skill are supported by numerous epidemiological studies in contrast to epidemiological studies of physical

impairments and driver safety. Importantly, the HAAS provides both upper and lower extremity performance measures for investigating the relationships among impairments, unsafe driving, and driving cessation. Because unsafe driving is based on self-reported crash histories, an initiative to acquire data from license and crash records maintained by the Hawaii Department of Transportation is planned for future analyses.

**Collaborators:** Dr. Lenore Launer, Neuroepidemiology Section, Laboratory of Epidemiology, Demography, and Biometry, NIA, NIH; Dr. June Lunney, Epidemiology and Demography Section, Laboratory of Epidemiology, Demography, and Biometry, NIA, NIH; Dr. Susan Redline, Division of Clinical Epidemiology, Case Western Reserve University, Cleveland; Dr. Andrew Monjan, Neuroscience and Neurospsychology of Aging Program, NIA, NIH; Dr. James Walsh, St. Luke's Hospital, St. Louis; Dr. Sonia Ancoli-Israel, University of California, San Diego; Dr. Donald Bliwise, Emory University, Atlanta; Dr. Maurice Ohayon, Stanford University; Dr. Michael Vitielo, University of Washington; Drs. Lon White and Kamal Masaki, Pacific Health Research Institute, Honolulu; Drs. John Eberhard and Jesse Blatt, National Highway Traffic Safety Administration.

# Laboratory of Experimental Gerontology

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The **Laboratory of Experimental Gerontology (LEG)** conducts basic research in experimental models focused on interventions that retard aging processes. Currently the LEG is comprised of the Behavioral Neurosciences Section (BNS) and two units, the Aging, Metabolism, and Nutrition Unit (AMNU) headed by Dr. Rafael de Cabo and the Functional Genomics Unit (FGU) headed by Dr. Sige Zou and the NIA Primate Aging Study. One of the major projects of the LEG is a longitudinal study being conducted in nonhuman primates to examine the potential beneficial effects of calorie restriction on mortality, morbidity, and aging processes. A second major project for the laboratory is the development of a standardized research program coordinated through the NIA extramural program to evaluate various aging interventions (pharmaceuticals, hormones, dietary supplements, genes) in mouse models to assess effects on lifespan, pathology, and functional capacity at older ages. Another important activity of the BNS is to develop behavioral assays of aging in rodents and nonhuman primates with focus on motor and memory performance and to conduct research to identify mechanisms of age-related decline in motor and memory performance. As a primary objective of research in LEG, investigations are directed toward preclinical development of pharmacological, genetic, and nutritional interventions that improve function at older ages.

## Laboratory of Experimental Gerontology Staff

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**Keywords:**

brain aging
behavioral performance
memory
neurotransmitters

**Recent Publications:**

Ingram DK, et al. *Aging Cell* 2006; 5(2): 97-108.

Devan BD, et al. *Psychopharmacology (Berl)* 2006; 183(4): 439-445.

Gregi NH, et al. *Proc Natl Acad Sci USA* 2005; 102(47): 17213-17218.

Mattison JA, et al. *Neurobiol Aging* 2005; 26(7): 1117-1127.

Maswood N, et al. *Proc Natl Acad Sci USA* 2004; 101(52): 18171-18176.

Roth GS, et al. *Science* 2004; 305(5689): 1423-1426.

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

Regarding age-related decline in memory performance, we have focused on the cholinergic and glutamatergic systems and their interaction. For cholinergic interventions, we have collaborated with Dr. N. Greig of the Laboratory of Neurosciences to develop a novel class of cholinesterase inhibitors, that are long-acting, highly specific for acetylcholinesterase and butyrylcholinesterase, with a wide range of therapeutic efficacy and low toxicity within this range. For glutamatergic interventions, we are examining manipulations of the glycine and polyamine sites on the N-methyl-D-aspartate (NMDA) glutamate receptor as well as generators of nitric oxide (NO) which is activated through the NMDA receptor. We have found that combinations of glycine agonists and polyamine agonists can act synergistically to improve learning performance. NO donors are also being assessed to overcome age-related learning impairments. The most recent line of research has focused on phosphodiaesterase inhibitors, which activate cAMP and cGMP. Collaborating with Drs. P. Mouton, M.A. Ottinger, and K. Manaye, we are examining the role of estrogen and estrogenic compounds, such as soy, in preserving memory and reducing glia-mediated inflammation in a mouse model of Alzheimer's disease. In addition to the behavioral analysis, the latter project is part of a larger project that involves quantitative morphometrics using unbiased stereology in a variety of mouse models. Specifically, we are assessing age-related changes in the numbers of neurons, synapses, and glia, in the hippocampus of mice from different genders and strains including transgenics and knock-outs. The objective is to relate specific neuromorphometric parameters to age or treatment-induced

changes in cognitive performance. We are also examining functional effects of a number of calorie restriction mimetics. These compounds are being evaluated for their ability to mimic the anti-aging effects of long-term calorie restriction without actual reduction in caloric consumption. Compounds currently under investigation include 2-oxyglucose, metformin, and resveratrol.

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

**Collaborators:** Nigel Greig, Ph.D., Laboratory of Neurosciences, NIA, NIH; Dan Longo, M.D., Laboratory of Immunology, NIA, NIH; Peter Mouton, Ph.D., Stereology Resource Center; Joseph Rifkind, Ph.D., Molecular Dynamics Section, NIA, NIH; Bryan Devan, Ph.D., Towson University; Mary Ann Ottinger, University of Maryland, College Park; Kebreten Manaye, Ph.D., Howard University.

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After receiving his B.S. and M.S. 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:**

caloric restriction
aging
oxidative stress
plasma membrane
bioenergetics
calorie restriction mimetics

**Recent Publications:**

Hunt ND, et al. *Ageing Res Rev* 2006; 5(2): 125-143.

Lopez-Lluch G, et al. *Proc Natl Acad Sci USA* 2006; 103(6): 1768-1773.

Hyun DH, et al. *Ageing Res Rev* 2006; 5(2): 209-220.

Liu D, et al. *Neuromolecular Med* 2006; 8(3): 389-414.

Bernier M, et al. *J Biol Chem* 2006; 281(5): 2551-2561.

Ingram DK, et al. *Aging Cell* 2006; 5(2): 97-108.

Lopez-Lluch G, et al. *AGE* 2005; 27: 153-160.

The **Aging, Metabolism, and Nutrition Unit (AMNU)** applies whole body physiological and 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.

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:** Myriam Gorospe, Ph.D., Laboratory of Cellular and Molecular Biology, NIA, NIH; Steve Sollot, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Peter Mouton, Ph.D., Stereology Resource Center; Joseph Rifkind, Ph.D., Molecular Dynamics Section, NIA, NIH; Paritosh Ghosh, Ph.D., Laboratory of Immunology, NIA, NIH; Mary Ann Ottinger, University of Maryland, College Park; Michel Bernier, Ph.D., Josephine Egan, M.D., Laboratory of Clinical Investigation, NIA, NIH; David Sinclair, Ph.D., Harvard University; Placido Navas, Ph.D., University of Pablo de Olavide, Sevilla, Spain; Jose Manuel Villalba, Ph.D., University of Cordoba; Ana Maria Cuervo, Ph.D., Albert Einstein College of Medicine, Bronx, NY.

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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. He performed his postdoctoral training at the University of California, San Francisco working on "Aging in *Drosophila melanogaster.*" He worked as a senior scientist with Bio-Rad Laboratories before coming to the NIA Laboratory of Experimental Gerontology in 2004 as an Investigator in the Functional Genomics Unit.

**Keywords:**

aging
microarray
tissue-specific
dietary restriction
*Drosophila melanogaster*

**Recent Publications:**

Ingram DK, et al. *Aging Cell* 2006; 5(2): 97-108.

McCarroll SA, et al. *Nat Genet* 2004; 36(2): 197-204.

Zou S, et al. *Proc Natl Acad Sci USA* 2000; 97(25): 13726-13731.

**Research Overview:** Aging is a fundamental and multi-factorial biological process that occurs in most eukaryotic organisms. Genetic analyses of model organisms have uncovered mutations in a number of genes that can affect lifespan. We have been applying genomic and genetic approaches to study mechanisms of aging at molecular, cellular and tissue levels and investigate prolongevity interventions that modulate aging processes.

To address how different tissues age, we have begun systematic identification of tissue-specific factors that are involved in aging processes. We have measured global transcription profiles of aging for seven tissues from the fly *Drosophila melanogaster*, including brain, muscle and tissues in the digestive and reproductive systems, which represent a wide range of functional tissues in flies. For each of these tissues, we have identified hundreds of genes showing significant changes at the transcript levels with increasing age. We are currently characterizing functions of these tissue-specific age-associated genes in modulating lifespan.

A number of mutations that can prolong lifespan in model organisms have been found in evolutionarily conserved genes, supporting the existence of evolutionarily conserved pathways to modulate lifespan and influence aging. Previously we found that repression of genes functioning in mitochondrial oxidative phosphorylation and up-regulation of genes involved in protein degradation pathways were conserved features of aging by comparing age-associated changes between *D. melanogaster* and *C. elegans* in collaboration with research groups at the University of California

at San Francisco. Some of these conserved genes have been shown to be involved in regulating lifespan in *C. elegans* by several research groups. We have been systematically investigating whether these genes regulate lifespan in *D. melanogaster*.

Lifespan has been shown to be influenced by a number of non-genetic factors, including dietary restriction and supplementations of chemical compounds or fruit extracts. In collaboration with Dr. Cathy Wolkow and Dr. James Carey, we have been using three evolutionarily distant invertebrates to investigate the effects of non-genetic factors in modulating lifespan. Using the nematode *C. elegans*, we have developed a novel paradigm to investigate dietary regulation of lifespan. Using *C. elegans*, *D. melanogaster* and Mexican fruit flies *A. ludens*, we have examined the effects of two isoforms of Vitamin E, alpha- and gamma-tocopherols on modulating lifespan. We have found both tocopherols have no or slight effects on lifespan of these species, suggesting that tocopherol supplements do not significantly alter lifespan. We will continue testing more compounds using the multi-species lifespan assays and investing molecular mechanisms of prolongevity interventions.

In summary, we have developed several approaches to conduct a systematic study on tissue-specific regulation of aging processes at the genomic level and to investigate evolutionarily conserved genes and compounds in modulating lifespan. This will lay a foundation for us and other researchers to speed up investigation on mechanisms of aging at the molecular and cellular levels and develop efficient aging intervention strategies for human.

**Collaborators:** Ming Zhan, Ph.D., Research Resources Branch, NIA, NIH; Catherine A. Wolkow, Ph.D., Laboratory of Neurosciences, NIA, NIH; David Schlessinger, Ph.D., Laboratory of Genetics, NIA, NIH; Dan L. Longo, M.D., Laboratory of Immunology, NIA, NIH; James Carey, Ph.D., University of California, Davis; Pablo Liedo, Ph.D., ECOSUR, Mexico; Hao Li, Ph.D., University of California, San Francisco; 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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Dr. Julie Mattison received a B.S. in Biology from the University of California, San Diego, 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 postdoctoral 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

**Recent Publications:**

Mattison JA, et al. *Neurobiol Aging* 2005; 26(7): 1117-1127.

Mattison JA, et al. *AGE* 2005; 27: 1-9.

Roth GS, et al. *Science* 2004; 305(5689): 1423-1426.

**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 collaborated with the Laboratory of Cardiovascular Science (LCS) to study additional projects of dietary interventions that more specifically affect cardiovascular aging.

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 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.

Two additional studies of age-related disease focus on cardiovascular disease and dietary interventions. These studies are being conducted in collaboration with the Laboratory of Cardiovascular Science (LCS). Although there is considerable evidence linking salt intake to hypertension, how this dietary variable is involved in remodeling of the vascular wall to affect arterial stiffness is still unknown. The effect of an incrementally increased salt load on vascular stiffness and modulation of this response by production of an endogenous ligand, marinobufagenin, is being studied in nine old normotensive male rhesus monkeys.

A second study in progress consists of monkeys over a broad age range eating either a diet moderately high in cholesterol or a low cholesterol control diet. The aims of this study are threefold: 1) To demonstrate links between the vascular changes present with aging and the early development and progression of atherosclerotic lesions; 2) To determine plasma biochemical markers which correlate with age-related vascular remodeling and atherogenesis; and 3) To validate the value of contrast enhanced-magnetic resonance imaging as a noninvasive diagnosis of atherosclerosis.

**Collaborators:** M.J. Novak, J.L. Ebersole, 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.

# Laboratory of Genetics

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The **Laboratory of Genetics (LG)** includes a Human Genetics Section, directed by David Schlessinger, a Genome Instability and Chromatin Remodeling Section directed by Weidong Wang, the Developmental Genomics and Aging Section under the direction of Minoru S.H. Ko, an Image Informatics and Computational Biology Unit led by Ilya Goldberg, and a Gene Recovery and Analysis Unit headed by Ramaiah Nagaraja.

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

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 totipotentiality and defined expression profiles of stem cells along trajectories to neural, endodermal, and placental lineages.

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 (a specific ubiquitin ligase) and the motive agent 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 and Manuela Uda, human geneticists at the University of Cagliari, Sardinia, and Goncalo Abecasis and Weimin Chen, statistical geneticists at the University of Michigan.

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.

The Laboratory is also equipped with other state-of-the-art resources for genomic approaches in the Gene Recovery and Analysis Unit, including large-insert clones and recovery methods, automated sequencing, chromatin analysis techniques, and site-specific modification of large-insert clones by recombineering techniques. Among the projects are the protein profiling of oocytes and selected tissues by mass spectrometry and the study of gene regulatory elements at a great distance from the transcription unit, focusing on PLAC1, a gene involved in placental development and well-being.

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 the Microarray Laboratory run by Kevin Becker (see Research Resources Branch) and Agilent Technologies, has developed gene expression profiling with microarrays bearing 44,000 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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**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:**

cartilage hair hypoplasia
ectodermal dysplasia
premature ovarian failure
circular chromosomes
open microscopy environment (OME)

**Recent Publications:**

Cui CY, et al. *Proc Natl Acad Sci USA* 2006; 103(24): 9142-9147.

Herrera L, et al. *Dev Biol* 2005; 279(2): 271-290.

Ottolenghi C, et al. *Hum Mol Genet* 2005; 14(14): 2053-2062.

Cui CY, et al. *Am J Pathol* 2005; 167(1): 89-95.

Uda M, et al. *Hum Mol Genet* 2004; 13(11): 1171-1181.

**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. The Unit has also generated knockout constructs for selected transcription factors involved in early embryonic development for the Developmental Genomics and Aging Section; and has implemented proteomics approaches, using mass spectrometry to profile gene expression at the protein level in mouse models. The study of regulatory processes is being extended to analyses of open and closed chromatin and histone modifications, projected for the genes recovered in chromatin form in artificial chromosomes. These techniques will be used, for example, to assess the role of the placental-specific gene (PLAC1) in fetal well-being and will be applied to analyze the long range regulation of PLAC1 and other relevant genes (see below).

**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.

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 mice 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.

***Cartilage hair hypoplasia.*** In this case, a gene encoding an RNA is mutated. The RNA functions in a complex with proteins to participate in the formation of ribosomal RNA and mitochondrial RNA species, and in the mutant, aberrant cartilage and defective, sparse hair are seen. Studies are defining the protein complement of the normal complex involved in RNA processing, and a mouse model is being constructed that reproduces features of the human condition, in order to define better the pathological defects caused by the mutation.

***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. The population is highly inter-related, including, for example, about 5,000 sib pairs. This has facilitated the determination that heritability of each trait is sufficient to anticipate the finding of any genetic locus that contributes 3% or more to the variance of trait values. As a first step to finding such loci and associated alleles in genes, full genome scans with up to 500,000 single nucleotide polymorphisms (SNPs) are now in progress for all participants.

**Collaborators:** Dr. Kuniya Abe, RIKEN Institute (Tokyo); 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.

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**Biography:** Dr. Wang was trained as a biochemist and a molecular biologist at both UCLA, where he obtained his Ph.D., and Stanford University, where he worked as a postdoctoral fellow. He joined the Laboratory of Genetics, 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 prestigious scientific awards, including the Presidential Early Career Award for Scientists and Engineers, and the Merit Award from the Fanconi Anemia Research Foundation. 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:**

Meetei AR, et al. *Nat Genet* 2005; 37(9): 958-963.

Yan Z, et al. *Genes Dev* 2005; 19(14): 1662-1667.

Yin J, et al. *EMBO J* 2005; 24(7): 1465-1476.

Meetei AR, et al. *Nat Genet* 2003; 35(2): 165-170.

**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. 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*. We have identified the subunits that provide such selectivity, and are in the process of investigating how these subunits target each complex to its chromosomal loci.

**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.

## Project II. 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 new protein, termed BLAP75, which is present in all BLM complexes. 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. Thus, these data suggest that BLAP75, BLM and Topoisomerase IIIa form a conserved core complex that guards genome integrity across eukaryotic species.

**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 four 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. Importantly, FANCL and FANCM proteins have enzymatic domains and activities. FANCL contains a ubiquitin ligase motif as well as the corresponding activity, and is required for FANCD2 monoubiquitylation *in vivo*. 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 may serve as an engine that translocates the FA core complex on DNA.

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 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, which possibly includes ubiquitination and the N-end-rule pathway.

**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.

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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:**

Aiba K, et al. *Stem Cells* 2006; 24(4): 889-895.

Yoshikawa T, et al. *Gene Expr Patterns* 2006; 6(2): 213-224.

Carter MG, et al. *Genome Biol* 2005; 6(7): R61.

Sharov AA, et al. *Genome Res* 2005; 15(5): 748-754.

**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.

**Publications-continued:**

Hamatani T, et al. *Hum Mol Genet* 2004; 13(19): 2263-2278.

Hamatani T, et al. *Proc Natl Acad Sci USA* 2004; 101(28): 10326-10331.

Hamatani T, et al. *Dev Cell* 2004; 6(1): 117-131.

**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). During the last year, we have accomplished the following additional goals. 1) We have developed a glass-slide microarray platform containing *in situ*-synthesized 60-mer oligonucleotide probes representing approximately 44,000 unique mouse transcripts. 2) We have produced web-based ANOVA-FDR software to provide user-friendly microarray data analysis. 3) We have developed an algorithm and a fully-automated computational pipeline for transcript assembly from expressed sequences aligned to the mouse genome. We have identified 191,946 genomic loci, which included 27,497 protein-coding genes and 11,906 additional gene candidates (e.g., non-protein-coding, but multi-exon). 4) We have developed a high throughput whole-mount *in situ* hybridization technique for preimplantation mouse embryos and ES cells. The nonrestricted community access to the resource can accelerate a wide range of research, particularly in reproductive and regenerative medicine. These resources and tools are now applied to the systematic analysis of gene regulatory networks in mouse ES cells.

**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.

First, it involves dynamic switching from a process governed by the activity of maternally stored RNA/proteins to a process governed by the genes of zygotic activation. Some oocyte mRNAs are translated, but fertilization triggers massive mRNA degradation. Transcription from the zygotic genome begins at the late one-cell to two-cell stage in mouse. Although it is well established that this transition is regulated by a “zygotic clock,” it is not known what type(s) of genes is activated first or how genes are activated.

Second, the first cell differentiation event in mammalian development occurs in preimplantation embryos. The process, "compaction," occurs at the 8- to 16-cell stage, when cells that were previously loosely associated begin to adhere in the tightly organized cell mass of the morula. This is the starting point for cell differentiation into Inner Cell Mass (ICM) (which eventually becomes the embryo) and Trophectoderm (which eventually becomes the placenta). Despite its importance, the molecular study of preimplantation development has been significantly delayed, mainly because of the scarcity of the materials for molecular biological/biochemical approaches.

In our previous work, we did 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 have selected and studied one novel gene, encoding a zinc finger binding protein that shows a transient expression in 2-cell embryos. The functional analysis of this gene, including a gene disruption study, is underway.

**3. Embryonic and Adult 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 the 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 adult 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 now 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.

**Collaborators:** Dr. Don Brown, Carnegie Institution of Washington, Baltimore, MD; Dr. S. K. Dey, Duke University, NC; Dr. Chen-Ming Fan, Carnegie Institution of Washington, Baltimore, MD; Dr. Andrew P. Feinberg, Johns Hopkins University, MD; Dr. Antonino Forabosco, University of Modena, Italy; Dr. Daniela S. Gerhard (NIH Mammalian Gene Collection, National Cancer Institute, National Human Genome Research Institute, NIH; Dr. Myriam Gorospe, Laboratory of Molecular and Cellular Biology, NIA, NIH; Dr. Brigid L.M. Hogan, Duke University, NC; Dr. Dan L. Longo, Laboratory of Immunology, NIA, NIH; Dr. Richard J. Maraia, National Institute of Child Health and Human Development, NIH; Dr. Hitoshi Niwa, RIKEN Center for Developmental Biology, Japan; Dr. Keiko Ozato, National Institute of Child Health and Human Development, NIH; Dr. Hans Scholer, Max Planck Institute, Germany; Dr. J. Christopher States, University of Louisville, KY; Dr. Colin Stewart, National Cancer Institute, NIH; Dr. Karl Swann, Cardiff University, UK; Dr. Catherine Verfaillie, University of Minnesota, MN; Dr. Angelo Vescovi, Institute For Stem Cell Research, Italy; Dr. Carl Wu, National Cancer Institute, NIH; Dr. Shinya Yamanaka, Kyoto University, Japan; Dr. Ryuzo Yanagimachi, University of Hawaii, HI.

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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: How cell and tissue morphology relate to cellular and organismal state.

**Keywords:**

open microscopy environment (OME)
image informatics
pattern recognition
*C. elegans*
high content screening

**Recent Publications:**

Orlov N, et al. *IEEE ISBI* 2006; 1152-1155.

Johnston J, et al. *IEEE ISBI* 2006; 1380-1383.

Goldberg IG, et al. *Genome Biol* 2005; 6:(5) R47.

Hocheiser H, et al. *IEEE CSBW* 2005; 381-387.

Swedlow JR, et al. *Science* 2003; 300(5616): 100-102.

**Image Informatics and Computational Biology Unit (IICBU):** This program is designed to develop technology for quantitative imaging assays for studying age-related 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 by many, 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 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; and 4) Maintaining and validating the OME Analysis Engine in machine vision and pattern recognition applications.

**2. Quantitative Morphometrics for Determining Cellular and Organismal State:** Automated image analysis can be divided into two broad categories: model-based and model-free. In traditional model-based systems, the model of what is being imaged is manually constructed, and is 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"); but a different approach is needed in situations where the model can't be easily defined, or is completely unknown.

Model-free systems make no assumptions of an underlying model and perform the analysis after training with a relevant set of images. Thus, a model-free pattern recognition approach is more widely applicable than a model-based approach. It treats all images equivalently, and performs the same operations 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 signature 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 (various textures, intensity statistics, distribution of objects, etc). A large collection of signatures (>1200 in our case) ensures that there is a sufficient variety of sensors available for many kinds of images. Because the vast majority of these signatures are irrelevant to a given imaging problem, all signatures with weak discrimination power are eliminated in a systematic automated way. The reduced set of signatures for a particular problem is then used to train standard network-based classifiers.

We have validated the generality and accuracy of this approach in over a dozen different imaging problems. For example, of particular interest in our Institute has been the application of the model-free approach to study the aging process in a quantitative as well as objective way. This required as a prerequisite a technique for continuous classification. We developed and validated two different approaches to solve this problem using images of *C.*

*elegans* body wall muscle and non-invasive imaging of the *C. elegans* pharynx terminal bulb. In both cases, a machine-built continuous classifier was able to report a value that correlated with the known age of the worm, and was able to correctly interpolate ages that were not used in training the classifier.

Continuous classification is being used to determine if tissue morphology at an early age is correlated with total life span. Ongoing experiments use non-invasive imaging of the worm's pharynx terminal bulb (the worm's eating organ), then track each individual worm to determine its total life span. The images are then grouped into "short-lived," "medium-lived" and "long-lived" depending on the life span. Given a sufficient sample size, the ability to train a classifier indicates that a correlation exists between early morphology and total life span on an individual basis. Preliminary results indicate that this may indeed be the case. If more data reinforces these results, follow-up experiments will use this classifier to segregate a genetically identical population into groups of long and short-lived worms at an early age. These sub-populations can then be compared using microarrays to determine the genes that are expressed differently in the two populations, and that may be exerting control over or are influenced by the aging rate.

As another application, a high-content screening platform is being developed to make image-based morphological screens cheaper and easier. 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 required gene results in a "hole" in the continuous lawn of cells wherever this gene's RNAi was printed on the slide. These control experiments are being followed up with a full-scale screen looking for genes whose expression levels affect nuclear and mitochondrial morphology.

Finally, we have embarked on a project to evaluate pattern recognition approaches and quantitative morphometry in machine-assisted medical diagnosis. This project is a collaboration with Dr. Elaine Jaffe at the National Cancer Institute, a world-renowned expert in classifying human

lymphoma. Currently, we are performing control experiments to determine how well our machine classifiers are able to reproduce diagnosis on three "classic" types of lymphoma: follicular, mantle-cell, and lymphocytic.

**Collaborators:** Dr. Peter Sorger, Massachusetts Institute of Technology; Dr. Jason Swedlow, University of Dundee, UK; Dr. Kevin Elicieri, University of Wisconsin-Madison; Dr. Catherine Wolkow, Laboratory of Neurosciences, NIA, NIH; Dr. Sige Zou, Laboratory of Experimental Gerontology, NIA, NIH; Dr. Elaine Jaffe, National Cancer Institute, NIH.

# Laboratory of Immunology

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The goals of the **Laboratory of Immunology (LI)** research program are to better understand 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). A common goal of these research programs is the elucidation of the age-related deficits in immune function that could be potentially targeted by various therapeutic strategies. There are several major areas of concentration and long-term development within LI including: (1) the molecular examination of telomere length and telomerase activity in lymphocyte populations; (2) the identification and characterization of differentially expressed genes in naïve and memory T cells and various aging lymphoid cells and organs; (3) the determination of the epigenetic basis for differential gene expression in various T cell populations; (4) the elucidation of the molecular and cellular alterations in T cell subsets with aging; (5) the study and use of biological response modifiers (such as cytokines, chemokines and hormones) to optimize and control leukocyte trafficking, activation, engraftment and vaccine strategies in normal and immunosuppressed hosts; (6) the induction of antigen-specific tolerance and use in transplantation and autoimmunity; (7) the cellular and molecular dynamics involved in thymic involution, regeneration and immunological aging; and (8) the molecular pathways underlying the shaping of pre-immune B cell repertoire. Additional efforts are aimed at elucidating the biochemical and molecular mechanisms regulating tumor cell growth, metastasis and progression and the potential factors and mechanisms associated with tumor-induced immunosuppression.

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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.

**Keywords:**

chemokines
T cells
aging
HIV
lipid rafts
ghrelin
neuroimmunology
Th1/Th2
immunosenescence
inflammation
thymic involution

**Recent Publications:**

Dixit VD, et al. *J Biol Chem* 2006; 281(24): 16681-16690.

Dixit VD, et al. *Exp Gerontol* 2005; 40(11): 900-910.

Taub DD, et al. *Immunol Rev* 2005; 205: 72-93.

Nguyen DH, et al. *Exp Cell Res* 2005; 304(2): 559-569.

**Chemokines, Inflammation and the Aging Immune Response:** The recruitment of lymphocytes into inflammatory sites requires several activation events including endothelial cell activation by inflammatory cytokines, the expression of adhesion molecules, cellular adhesion, diapedesis, and migration via established chemotactic gradients. Over the past 15 years, members of the *chemokine* super family have been shown to induce adhesion, chemotaxis, activation, and degranulation of human and rodent leukocytes and lymphocytes both *in vitro* and *in vivo*. We are currently examining a role for chemokines in lymphocyte activation and as immunoadjuvants in vaccine-based studies with hapten-carrier protein complexes. In addition, the laboratory is also examining the ability of various chemokines and other G-protein receptor ligands to modulate other T, B, and NK cell effector functions as well as antigen-presenting cell activities. Furthermore, studies examining the differential expression of various cytokines, chemokines and their cell surface receptors, post cellular activation via mitogens, hormones, lipids, and stress factors are also under investigation. We believe that a better understanding of the complexities of leukocyte extravasation and the mediators that induce cell trafficking and activation will greatly assist our ability to orchestrate, regulate, and control various pathological disease states associated with aging as well as enhance our understanding of normal leukocyte trafficking.

Besides chemotaxis, chemokines function as mediators in T-cell activation and in many lymphocyte biological responses. Detailed information about downstream signaling pathways is necessary to understand the role of chemokines in normal physiology and inflammation. We have utilized 44K oligonucleotide arrays to examine the ability of various chemokines, HIV-1 viral isolates, and gp120 proteins to directly induce gene expression in

young and old human T lymphocytes. We are currently verifying and characterizing several gene families that are highly expressed in T cells after migration in response to SDF-1, MIP-3β, gp120 and HIV-1 virus. We have identified some unique chemokine-induced gene transcripts (in particular, the Wnt-frizzled pathways) that may be associated with cell migration, adhesion and activation. A greater understanding of the transcriptional signals differentially induced by the ligation of various chemokine receptors may provide a means to dissect the pathways by which these chemoattractants induce cell migration and activation as well as any host transcriptional signals important in HIV entry and replication.

**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. Before 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. Lipid rafts are believed to be important signaling platforms enriched in many signaling proteins, including but not limited to src kinases, G alpha subunit, H-Ras, LAT, and NOS. CCR5 and CXCR4 have been shown to be present in lipid rafts, colocalizing at the leading edge of migrating cells. However, the role of cholesterol and these lipid rafts on T cell chemokine binding and signaling through CCR5 and CXCR4 remains unknown. We found that cholesterol extraction by beta-cyclodextrin (BCD) significantly reduced the binding and signaling of SDF-1 and MIP-1β using CXCR4- or CCR5-expressing T cells, respectively. Oxidized forms of cholesterol, known as oxysterols, are abundant in various food products and can be found naturally in membranes and mitochondria of a variety of cell types. Cholesterol oxidation was also found to result in the loss of chemokine binding and function in T cells and monocytes. Reloading treated cells with non-oxidized cholesterol restores chemokine binding and function in these situations. Antibodies specific for distinct CXCR4 or CCR5 epitopes lost their ability to bind to the cell surface after cholesterol extraction and cholesterol oxidation. Moreover, bindings studies with labeled chemokines have demonstrated extensive co-localization of ligand binding with the GM1 lipid raft marker while using

anti-chemokine receptor antibodies, we found the majority of chemokine receptors co-localize with CD59 and only partially with GM1. 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. Together, these data demonstrate that cholesterol and lipid rafts are important for the maintenance of the chemokine receptor conformation and are necessary for both the binding and function of this chemokine receptor. This cholesterol and lipid raft requirement for ligand binding may play a significant physiological role in controlling immune cell signaling and migration. More specific efforts are also underway examining the differences in the make-up of lipid rafts within the cell membranes of young and aged lymphocytes. 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.

**Novel Connections Between the Immune and Endocrine Systems:** Inflammatory cytokines released by immune cells have been shown to act on the central nervous system (CNS) to control food intake and energy homeostasis. Decrease in food intake or anorexia is one of the most common symptoms of illness, injury or inflammation. The adipocyte-derived hormone, 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. However, the functional role of ghrelin and GHS in immune cell function is unknown. Here, we report that GHS-R and ghrelin are expressed in human T lymphocytes, specifically localize in GM1-associated lipid rafts, and exert both specific and potent inhibitory effects on the TCR- and leptin-mediated expression of the proinflammatory cytokines via functional GHS-R and possibly a novel GHS receptor on the surface of human mononuclear and T cells. Moreover, ghrelin administration into endotoxin challenged mice significantly inhibits inflammatory cytokine protein and mRNA expression in the serum and various organs. Furthermore, the expression of ghrelin, leptin and their receptors as well as GH appears to be significantly diminished with age within specific immune subsets and lymphoid organs, including the thymus. Administration of ghrelin, GH and leptin into aged mice using implanted osmotic pumps resulted in a partial reversal of thymic involution and restored thymic GH and IGF1 expression. These hormone infusions appear to affect both

thymopoiesis and hematopoiesis with specific effects on lymphoid progenitor expansion. More detailed analysis of these findings is currently underway using GHS-R and GH-R KO mice as well as through the infusion of GHS-R and GH-R specific antagonists to determine the direct effects of ghrelin and the possible role of GH in the observed effects. We have recently generated T-cell-specific transgenic mice expressing GHS-R, ghrelin, leptin and leptin receptor as well as conditional KO mice for GHS-R and ghrelin for use in dissecting these regulatory pathways. 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 naïve 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. In an effort to profile molecular changes that occur within the aging thymus, microarray analysis was performed using RNA derived from thymus isolated from mice of varying ages. Using mRNA derived from the thymi of 2, 4, 6, 12 and 18 month old BALB/c mice, microarray analysis was performed using three distinct custom-made cDNA microarrays developed within our laboratory as well as an 18,000 gene cDNA murine arrays. The success of this project relies upon the reliability of the molecular profiling aged cells from defined aged sources, both from culture and freshly isolated aged cells. The first milestone will be the definitive characterization and selection of genes associated with thymic involution. We have performed serial analysis of gene expression (SAGE) in the thymi, spleens and bone marrow of mice of varying ages and have initiated studies examining expression differences in mice of distinct H-2 and genetic backgrounds. Our current data would suggest that thymic involution may be both strain- and gender-dependent and may, in part, be associated with distinct nutritional and stress-related factors rather than

simply aging. A number of genes associated with DNA repair, oxidative stress, apoptosis, and inflammation were found to be significantly upregulated with thymic aging. We are currently completing the analysis and confirmation of these data from mice of various ages given ad libitum or caloric restricted diets as well as from aged mice infused with various hormones that result in a partial restoration in thymocyte numbers. We have recently found that ghrelin and leptin infusions reverse age-associated thymic involution. 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 overall goal of this project is to produce a comprehensive gene expression profile in the thymus, spleen, and lymph nodes during the aging process to identify unique and common genes and functionally related groups of genes that are expressed in age-dependent manner in these different organ systems.

**Signaling Mechanisms of Melanoma Metastasis:** Dr. Ashani Weeraratna within the Clinical Immunology Section is currently investigating the signaling mechanisms involved in melanoma metastasis. Using gene expression profiling techniques such as SAGE and microarray, we are able to tease out pathways involved in melanoma progression. One such pathway involves the protein Wnt5a, which we have demonstrated to be significantly upregulated in motile melanoma cells. Current data from our laboratory also implies that this molecule is playing a role in the immune modulation of melanoma, by down regulating the expression of key immunogenic antigens, thus allowing the tumor cell to escape immune surveillance. Other consequences of Wnt5a overexpression in melanoma include the suppression of key metastasis suppressors, and also the transition of melanoma cells from the epithelial to mesenchymal phenotype, a hallmark of melanoma metastasis.

**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
immunosuppression
p53
cancer
CD28
aging
cell cycle
lymphoma
TGF-ß

**Recent Publications:**

Lee GD, et al. *Clin Cancer Res* 2006; 12(1): 198-205.

Sasaki CY, et al. *J Biol Chem* 2005; 280(41): 34538-34547.

Sakatani T, et al. *Science* 2005; 307(5717): 1976-1978.

Kusaba H, et al. *J Biol Chem* 2005; 280(2): 1037-1043.

Rezanka LJ, et al. *Cell Immunol* 2005; 238(1): 38-48.

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

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

**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 $V_H1$ gene is essential for immune response to PC and PC-mediated protection against infection by *S.pn.* Furthermore, by examining the associations between the $V_H1$ 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 $V_H1/V_L$ 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 $V_H$ and $V_L$ 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 $V_H$ and $V_K$ 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 $V_H$ and $V_K$ 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.

**Cyclosporin A-Resistant Costimulation of T Cells via CD28:** The CD28-mediated co-stimulatory signal plays a pivotal role in many immune responses including T cell responses against tumors, virus-infected cells, and transplanted alloantigens. Depending on the nature of primary stimulation, CD28 can initiate multiple intracellular signaling pathways that can be broadly classified into two groups: one is calcium-dependent and sensitive to cyclosporin A (CsA), and the other one is calcium-independent and resistant to CsA. The CsA-resistant pathway has been thought to be responsible for the ineffectiveness of CsA in the treatment of graft-versus-host disease following allogeneic bone marrow transplantation. Our primary objectives are focused on three areas: (1) characterization of the CsA-resistant co-stimulatory pathway; (2) examination of the physiological significance of this pathway; and (3) evaluation of the effect of aging on this pathway.

**Role of TGF-β-Receptor II in Resistance to TGF-β-mediated Growth Suppression in A B-cell Lymphoma Cell Line:** Transforming growth factor (TGF)-β1 is a member of the TGF-β superfamily that regulates cell growth and differentiation in a variety of cell types. TGF-β inhibits cell proliferation by arresting cells in G1 phase of the cell cycle. Resistance to TGF-β-mediated growth suppression in tumor cells is often associated with the functional loss of TGF-β1 receptors. We are studying a diffuse large B-cell lymphoma cell line, DB, which lacks TGF-β responsiveness with respect to growth suppression. Our goal is to identify the deficit in TGF-β signaling pathway in DB cells. Preliminary data indicate that DB cells lack functional TGF-β receptor II [(TβRII) on the cell surface in contrast to a TGF-β-responsive B-cell lymphoma cell line RL, whereas both cell lines carry comparable levels of receptor I (TβRI)]. Lack of functional TβRII correlates with the lack of TGF-β-induced nuclear translocation of phospho-Smad3 and phospho-Smad2, and lack of nuclear expression of $p21^{Cip1/WAF1}$ and down regulation of nuclear c-Myc in DB cells. Ectopically-expressed wild type, but not c-terminally- truncated TβRII, renders the DB cell line responsive to TGF-β1-mediated growth suppression, and correlates with up-regulation of nuclear $p21^{Cip1/WAF1}$ and down-regulation of c-Myc. Analysis of the TβRII gene reveals a truncated message in DB cells. We are currently investigating the nature of modifications in the TβRII gene.

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**Keywords:**

immunological memory
memory T cells
histone acetylation
telomere
telomerase
immune senescence
$CD28^{null}$ CD8 T cells
aging

**Recent Publications:**

Weng N-P. *Immunity* 2006; 24(5): 495-499.

Fann M, et al. *Immunol Rev* 2005; 205: 190-206.

Li Y, et al. *J Immunol* 2005; 174(7): 4019-4024.

Hess K, et al. *Int Immunol* 2004; 16(12): 1711-1721.

**Research:** The research interest of this laboratory is to understand the mechanisms of memory T cell generation, response, and aging. Specifically, we have focused our efforts on the three areas: 1) identification and characterization of differentially expressed genes in memory T cells, 2) determination of the epigenetic basis for differential gene expression in memory T cells, and 3) elucidation of the molecular and cellular alterations of memory T cells with aging. As the pattern of gene expression and silencing defines the cellular characteristics and functions, one major research goal of our laboratory is to identify genes that are differentially expressed in memory T cells and to further characterize the roles of those genes in the generation, function and homeostasis of memory T cells. We have identified dozens of differentially expressed genes in memory T cells using the microarray method. To further understand the basis of differential gene expression in memory T cells, we investigate the contribution of chromatin structure, particularly the modification of histone, in regulation of gene expression. We found that the acetylation levels of histone H3 lysine 9 (H3K9) are higher in memory cells than in naïve cells at resting and activated states. We are also interested in age-associated changes of memory T cell function and replicative lifespan. Short telomeres affect cellular replications but the rate of telomere attrition *in vivo* is unknown and whether the levels of telomerase activity decline in T cells with age *in vivo*. A major research goal in our laboratory is to investigate the regulation of telomere length and telomerase activity in T cells with age and to understand their roles in memory T cell function and replicative lifespan. Using primary human T cells, we are conducting molecular and cellular analysis as well as longitudinal studies to elucidate the changes of T cell function with aging. We anticipate that knowledge derived from these experiments will further our understanding of the mechanism of memory T cell formation, response and age-associated decline of memory T cell functions. These insights are

essential for the rational design of vaccines for prevention of infections as well as for cancer and autoimmune diseases in general and elder populations.

**Identification and Characterization of Differentially Expressed Genes in Memory T Cells:** 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 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 recent progress in characterizing the functions of lymphocytes, the mechanisms underlying the generation and maintenance of memory lymphocytes remain largely unknown. In the past few years, we have applied microarray methods to identify genes that are differentially expressed in memory T cells of both human and mouse. With the completion of human and mouse genome sequences, it has become feasible to assess the gene expression profile at the whole genome scale. We have conducted whole genome gene expression analysis of mouse memory CD4 T cells and human memory CD8 T cells using whole genome gene chip (~40,000 unique genes or sequences for both human and mouse) and identified dozens of differentially expressed genes in memory T cells. We are currently investigating the role of a few selected differentially expressed genes in memory T cell generation and response. In addition, we also compared gene expression differences between naïve and memory T cells after *in vitro* activation and identified activation-related genes that were preferentially expressed in activated memory T cells. Some of these activation-induced genes were involved in effector functions such as cytokines and enzymes. The basis of enhanced effector function of memory T cells is not known. We are investigating the role of chromatin structure in regulating gene expression in memory T cells.

**Association of Histone H3 Lysine 9 (H3K9) Acetylation and Differential Gene Expression in Memory T Cells:** Modification of chromatin structure via covalent chemical changes (acetylation, methylation, phosphorylation, etc.) of histone amino-terminal tails has become increasingly recognized as critical to controlling gene expression. Accumulating evidence suggests that specific modifications of histone tails or combinations thereof can define the actual or potential transcriptional states. Acetylation of histone H3 lysine 9 and 14 and H4 lysine 8 is associated with accessible chromatin structure for transcription, while methylation of H3 lysine 9 is associated with gene

silencing. Histone hyperacetylation was observed in the loci of interleukin 4 (IL4) and interferon gamma (IFNγ) genes during T cell differentiation to effector cells, where it was associated with elevated transcription. However, the role histone modification in memory T cells is unknown. As modified histones can transmit epigenetic information from one cell to its descendants, this mechanism has the potential to transmit memory during clonal expansion.

To understand the molecular basis for the rapid and robust memory T cell responses, we examined gene expression and chromatin modification by histone H3 lysine 9 (H3K9) acetylation in resting and activated human naïve and memory $CD8^+$ T cells. We found that, although overall gene expression patterns were similar, a number of genes are differentially expressed in either memory or naïve cells in their resting and activated states. To further elucidate the basis for differential gene expression, we assessed the role of histone H3K9 acetylation in differential gene expression. Strikingly, higher H3K9 acetylation levels were detected in resting memory cells, *prior* to their activation, for those genes that were differentially expressed following activation, indicating that hyperacetylation of histone H3K9 may play a role the selective and rapid gene expression of memory $CD8^+$ T cells. Consistent with this model, we showed that inducing high levels of H3K9 acetylation resulted in an increased expression in naïve cells of those genes that are normally expressed differentially in memory cells. Together, these findings suggest that differential gene expression mediated at least in part by histone H3K9 hyperacetylation may be responsible for the rapid and robust memory $CD8^+$ T cell response.

**Gene Expression, Generation and Growth of $CD28^{null}$ $CD8^+$ Memory T Cells by IL-15 and Its Induced Cytokines:** Accumulation of $CD28^{null}$ $CD8^+$ T cells is a hallmark of the age-associated decline of T cell function in humans. However, the causes, growth, and precise changes of $CD28^{null}$ $CD8^+$ T cells are not fully understood. We first analyzed the transcriptional changes in $CD28^{null}$ $CD8^+$ T cells using microarray method and identified genes that were differentially expressed in $CD28^{null}$ and $CD28^+$ $CD8^+$ T cells. Based on their known functions, these differentially expressed genes can be divided into four groups: 1) cell surface receptors, 2) cytokines and their receptors, 3) effector molecules, and 4) transcriptional regulators. Alteration of cell surface receptor expression is one of the most consistent changes in lymphocytes with aging, e.g. loss of co-stimulatory receptor CD28 expression in CD8 T cells. One striking finding is the gain of expression of a variety of stimulatory NK cell receptors in CD28- CD8

memory T cells. These NK cell receptors include 1) KIR2DL2 (NKAT6), KIR2DS2, and NCR1 of the Ig-like NK cell receptor family, 2) KLRC3, KLRC4, KLRD1 (CD94), KLRF1, KLRG1 and KLRK1 (NKG2D) of the C-type lectin-like NK cell receptor family, and 3) CD16 and CD244. It has been proposed that gain of NK receptor expression in CD28- CD8 T cells may facilitate their effector functions as compensation for impaired proliferation.

Elevated expression of chemokines and cytokine receptors was also found in both human CD28- CD8 T cells and CD4 T cells from aged mice. Chemokine receptors (CX3CR1, CCRL1) and chemokine-like receptor 1 (CMKLR1) were significantly more highly expressed in human CD28- CD8 memory T cells compared with their $CD28^+$ counterparts. CCR1, 2, 4, 5, 6, and 8 and CXCR2-5 were highly expressed in CD4 T cells from old mice relative to CD4 cells from young mice. Reduced expression of interleukin 7 receptor (IL7R) and interleukin 12 receptor β2 (IL12RB2) was found in CD28- CD8 memory T cells. CD28- CD8 memory T cells expressed higher levels of interleukin 12A (IL12A), interleukin 13 (IL13), chemokine (C-C motif) ligand 4 (CCL4, MIP1-β), and effector proteins such as perforin, granzymes B and H (GAMB and GZMH) than their $CD28^+$ counterparts. Finally, elevated expression of transcription factors in CD28- CD8 memory T cells was also identified, including T-box 21 (TBX21, T-bet), which functions in initiating Th1 lineage development and Ig class switching and Eomesodermin (EOMES), which induces the production of IFNγ, perforin and granzyme B in CD8 T cells. On the other hand, MYC, an important regulator of cell proliferation, differentiation, and apoptosis, was down-regulated in CD28- CD8 memory T cells.

Although it is believed that $CD28^{null}$ $CD8^+$ T cells were derived from $CD28^+CD8^+$ T cells after repeated antigenic stimulation and had proliferative defects in response to antigenic stimulation, it is not clear how these cells accumulated with age. We analyzed the growth of $CD28^{null}$ and $CD28^+CD8^+$ memory T cells in response to the homeostatic cytokine IL-15 *in vitro* and found that there was no proliferative defect of $CD28^{null}CD8^+$ memory T cells in response to IL-15 compared to their $CD28^+$ counterparts. In addition, we showed that IL-15 induced stable loss of CD28 expression in those frequently dividing $CD28^+CD8^+$ memory T cells, suggesting that homeostatic cytokine, such as IL-15, can also be a cause for generation of $D28^{null}$ $CD8^+$ T cells. We further demonstrated that IL-15 induced TNF-α (down-regulating CD28 expression) and MIP-1β (inhibiting the growth of $CD28^{null}$ cells) had opposite effect in generation and growth of $CD28^{null}$

$CD8^+$ T cells. Together, these findings demonstrate that $CD28^{null}CD8^+$ memory T cells proliferate normally in response to IL-15 and that IL-15 and its induced cytokines regulate the generation and growth of $CD28^{null}CD8^+$ T cells, suggesting a possible role of IL-15 in the increase in $CD28^{null}CD8^+$ T cells that occurs with aging.

**Collaborators:** Richard J. Hodes, M.D., National Cancer Institute and National Institute on Aging, NIH; Susan L. Swain, Ph.D., Trudeau Institute; Kevin Becker, Ph.D., Research Resources Branch, National Institute on Aging, NIH; Ron Glaser, Ohio State University.

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**Keywords:**

thymus and spleen
signal transduction and gene expression
thymic involution with age
ß-catenin and Wnts
dexamethasone

**Recent Publications:**

Xu Y, et al. *Nat Immunol* 2003; 4(12): 1177-1182.

Xu Y, et al. *Eur J Immunol* 2003; 33(1): 12-18.

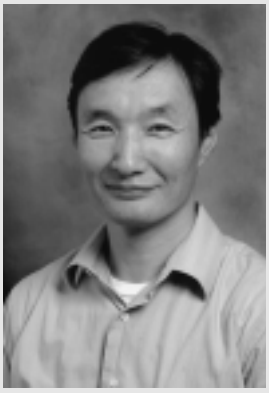
**Research Description:** The immune system is seriously impaired under various clinical situations and in older people. The long-term goal of our research is to define molecules that are significant in the reconstitution of a functional immune system in adult mouse. T cell development in the thymus is a direct consequence of stage specific signal transduction and gene expression, resulting from reciprocal cell-cell interactions and locally produced cytokines and hormones. Our research is focused on analyzing signal transduction mediated by p38 MAP kinase, NFκB and c-myc in the survival and differentiation of T cells in the thymus. Currently we are excited about the study of the role of Wnt-β-catenin signaling pathway in T cell development using mice deficient in these molecules and transgenic mice expressing mutant forms of β-catenin. Described below are three projects currently ongoing in the laboratory.

**Wnt-β-Catenin Pathway in the Development and Function of Immune Cells:** Because the Wnt pathway-associated transcription factors, TCF-1 and LEF-1, have been shown to be critical for T cell maturation, we hypothesized that Wnt signals may be important in the thymus. We have determined that Wnt-1, -3A, -4 and -5A are expressed in thymocytes. In collaboration with Andy McMahon's lab, we have shown that Wnt-1 and Wnt-4 are required for generating thymic cellularity. In the future we will determine if the reduced cellularity seen in the absence of Wnt signaling results from diminished proliferation or impaired survival of thymocytes. We would also like to know if Wnt-1 and Wnt-4 activity is supplemented by Wnt-3A and Wnt-5A as we have shown that these growth factors are also expressed in developing thymus.

Wnts bind Frizzled receptors and alter gene expression through transcription factors TCF-1 and LEF-1 by stabilizing their co-factor β-catenin. Wnt-β-catenin signaling has been shown to be critical for the development of many organs, such as skin, gut and the brain, that involve a balance of proliferation and cell fate decisions mediated by cell-cell interactions. To investigate the role of Wnt-β-catenin-TCF-1, signaling we have generated mice expressing a stabilized mutant of β-catenin (βCat-Tg) in thymocytes. βCat-Tg mice exhibit enhanced positive selection. In particular, they show a 3- to 7-fold increase in the number of mature CD8 cells and 1 to 2-fold increase in mature CD4 thymocytes. Extensive mechanistic analysis has shown that the increase in CD8 SP thymocytes is likely due to increased generation of these cells during development. However, the role of β-catenin in T cell migration is of great interest and is one focus in the lab. Tissue-specific deletion of β-catenin drastically reduces the number of splenic T cells and mature thymocytes. Mechanistic studies suggest this loss results from impaired T cell development at the important checkpoints. Cellular proliferation and survival are also affected by β-catenin deletion.

**Signals Mediated by NF-κB and c-Myc and the Survival of Thymocytes:** Immature DP thymocytes are very sensitive to glucocorticoid hormones. We have shown that this sensitivity is modulated by NF-κB and c-Myc *in vivo*. To determine target genes activated in immature thymocytes by NF-κB and c-Myc, we are using gene-array technology. In collaboration with scientists at the Whitehead Institute Center for Genome Research and McGill University, we are pursuing a bio-informatics approach to study response of thymocytes to treatment with dexamethasone *in vivo*. This project will help understand the molecular basis for sensitivity of immature thymocytes to glucocorticoid hormones and will identify targets of NF-κB and c-Myc that mediate cell survival signals. Several genes identified in the initial analysis are only known as ESTs. Further analysis of these genes will involve cloning full length genes and manipulating them *in vivo* and *in vitro* to study their function in cell survival. The unifying theme of our research is to explore how stage-specific gene expression resulting from cell-cell interactions between thymic epithelial cells and thymocytes that modulate cell-survival, cell-death and differentiation in the thymus. Similarly, we are interested in how signals from cell-cell interactions between antigen presenting cells and T cells modulate immune response in the peripheral immune system.

**Collaborators:** Andy McMahon, Ph.D., Harvard University; Jill McMahon, Ph.D., Harvard University; Thomas J. Hudson, M.D., McGill University; Walter Birchmeier, Ph.D., Max Delbrück Center.



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**Keywords:**

chemokine
defensin
APC targeting
cancer
immunotherapy
DNA vaccines

**Recent Publications:**

Schiavo R, et al. *Blood* 2006; 107(12): 4597-4605.

Cha SC, et al. *J Immunol Methods* 2006; 312(1-2): 79-93.

Biragyn A. *Curr Protein Pept Sci* 2005; 6(1): 53-60.

Chertov O, et al. *Expert Rev Proteomics* 2005; 2(1): 139-145.

Biragyn A, et al. *Blood* 2004; 104(7): 1961-1969.

**Vaccine Development:** Our current research program is focused on the development of simpler and more potent vaccines for cancer and other clinically-relevant diseases utilizing a chemoattractant-based antigen delivery strategy and self-particulated viral antigen carriers. The vaccine modeling studies are performed using murine tumors, such as 38C13, A20, BCL1 and MOPC315, weakly immunogenic B cell tumors that adequately represent human B cell malignancies. Using such vaccine strategies, non-immunogenic tumor self-antigens were rendered immunogenic and therapeutic antitumor immunity was elicited when the antigens were taken up by antigen-presenting cells (APCs) through chemokine receptors. This strategy permits the modification of antigen-specific immune responses towards either humoral or cellular responses depending on the chemokine utilized (e.g., depending on the type of APC being targeted). Furthermore, the breadth of this vaccine strategy is in the ability of the vaccine formulation to efficiently utilize the MHC class I and II antigen processing and presentation pathways. Naked DNA immunizations are also currently being explored using various methods of delivery, such as a gene gun and electroporation. Several vaccine formulations that express novel tumor-associated antigens are currently being tested for the possible initiation of a clinical vaccine trial for lung cancer patients.

**Chemoattractants as Vaccine Adjuvants:** Fusions with chemokines lead to significant augmentation of humoral immune responses to self- and foreign antigens, including beta-amyloid and HBsAg, 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 β-defensin 2, are being examined.
Dr. Biragyn has previously demonstrated that murine β-defensin 2 binds and signals through TLR-4 up-regulating expression of costimulatory molecules and the production of inflammatory Th1 cytokines by immature dendritic cells (iDCs). This work is currently being expanded to examine the molecular signaling pathways of β-defensin-mediated iDC activation. At present, a wide variety of human and mouse defensin-like genes have been cloned to search for functionally similar peptides, which can also be utilized for cancer vaccine studies.

**Generation of Immune Response Modifiers to Boost Vaccine-induced Immune Responses:** The laboratory has also focused efforts to examine the mechanisms of T regulatory cell (Tregs) suppression. Dr. Biragyn has developed novel formulations designated "chemotoxins," which are recombinant chemokines linked to RNase or toxin. Using these chemotoxins, vaccine-induced T cell responses can be significantly boosted via the specific depletion of Tregs that express the chemokine receptor, CCR4. Chemotoxins are also being tested for their applicability for the treatment of cutaneous leukemias based on their preferential expression of certain cell surface chemokine receptors.

**Collaborators:** Vladimer Larionov, Ph.D., National Cancer Institute, NIH; Sam Hwan, M.D., Ph.D., National Cancer Institute, NIH; Michael Lerman, Ph.D., National Cancer Institute, NIH; Joost J. Oppenheim, M.D., National Cancer Institute, NIH; Dennis D. Taub, Ph.D., Laboratory of Immunology, National Institute on Aging, NIH; Dianne Newton, SAIC-Frederick; Michael Agadjanyan, Ph.D., D.Sc., The Institute for Molecular Medicine; Yukio Koide, M.D., Hamamatsu University School of Medicine; Livio Mallucci, Ph.D., King's College, London.

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

The area of oxidative DNA damage and its processing is of particular interest to us 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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### Unit of Structure and Function in Base Excision Repair

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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
oxidative damage
Cockayne syndrome
Werner syndrome
mitochondria
telomeres

**Recent Publications:**

Bischoff C, et al. *Twin Res Hum Genet* 2005; 8(5): 425-432.

Hu J, et al. *Nucleic Acids Res* 2005; 33(10): 3271-3282.

Lee JW, et al. *J Biol Chem* 2005; 280(47): 39627-39636.

**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.

**Publications-continued:**

Opresko PL, et al. *J Biol Chem* 2005; 280(37): 32069-32080.

Stuart JA, et al. *Nucleic Acids Res* 2005; 33(12): 3722-3732.

Thorslund T, et al. *Mol Cell Biol* 2005; 25(17): 7625-7636.

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.

**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.

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. We are assessing various pathways of DNA repair in Alzheimer's cells to characterize this possible defect, which could be etiologically linked to the disorder. 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 understanding 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.

**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. Some WS cells are defective in transcription coupled DNA repair, but no other DNA repair defects have been demonstrated. Experiments with intact cells and cell extracts suggest that WS cells may have a defect in basal transcription. Cells from WS patients grow more slowly and become senescent at an earlier population doubling than age-matched normal cells, possibly because the WS cells appear to have accelerated losses of the telomeric ends of their chromosomes. Telomeric shortening is an established marker of cellular senescence. Because of the non-isogenic nature of cells from WS patients compared to cells from normal individuals we generated two isogenic cell 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, although the actual biochemical defect remains unknown.

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 recently discovered that WRN protein interacts functionally and physically with Flap endonuclease 1 (FEN-1), a protein involved in DNA replication and DNA base excision repair. This suggests that WRN protein plays a role in one or both of those processes. Recently, we have found more 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 being made, the nature of the defect(s) in WS is still a mystery, as is the nature of the processes that occur in cellular senescence and normal human aging. Our ongoing and future studies will be directed towards elucidation of the causes of the accelerated aging phenotype in WS, with hope that this knowledge can also be applied to our current understanding of both the aging of cells and organisms in general.

**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 abnormally sensitive to killing by ultraviolet radiation, as well as certain so-called UV-mimetic chemicals, such as 4-nitroquinoline-1-oxide and N-acetoxy-2-acetylaminofluorene. CS cells are defective in the enhanced rate of repair of the transcribed strand relative to the non-transcribed strand of transcriptionally active genes. In recent experiments from this laboratory, we have demonstrated that mutations in the CSB gene are the cause of the transcription coupled repair defect.

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

We have generated stable human cell lines with functional domain knockout of different regions of the CSB gene. Mutations are introduced by site-directed mutagenesis, 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 underexpressed in mutated CS cells, and that some of these confirm a substantial role for CSB protein in transcription and apoptosis.

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**Biography:** Dr. Patricia Gearhart received her Ph.D. in Immunology from the University of Pennsylvania in 1974. She performed postdoctoral training at the Johns Hopkins University and was a staff associate at the Carnegie Institution of Washington until 1982. She then became a faculty member at the Johns Hopkins University until 1995, when she moved to the NIA.

**Keywords:**

immunoglobulin
somatic hypermutation
heavy chain class recombination
DNA polymerases
base excision repair
mismatch repair

**Recent Publications:**

Martomo SA, et al. *Proc Natl Acad Sci USA* 2005; 102(24): 8656-8661.

Xeng X, et al. *J Exp Med* 2004; 199(7): 917-924.

Martomo SA, et al. *J Exp Med* 2004; 200(1): 61-68.

Gearhart PJ. *J Immunol* 2004; 173(7): 4259.

**Proteins Involved in Somatic Hypermutation of Immunoglobulin Variable Genes:** Somatic hypermutation of immunoglobulin genes occurs at a frequency that is a million times greater than mutation in other genes. Mutations are found in both variable genes and switch regions before constant genes. The molecular mechanism that introduces these mutations is intensely being studied. Hypermutation is initiated when the activation-induced cytidine deaminase (AID) protein deaminates cytosine in DNA to uracil, which causes C:G mutations. However, in B lymphocytes, substitutions of all four bases occur at similar levels, indicating that other proteins are required to generate mutations of A:T base pairs. The MSH2-MSH6 mismatch repair heterodimer and DNA polymerase eta have been implicated in the process, since mice and people deficient for these proteins have fewer mutations of A:T. Using biochemistry and genetics, we are studying how these proteins interact with DNA to generate nucleotide substitutions.

First, we showed that uracil is present on the nontranscribed strand of DNA from bacteria that express AID. Uracils were located primarily at cytosine bases, in accord with the activity of AID on single-strand DNA. This is the first demonstration of dU in cellular DNA, and is an important confirmation of the DNA deamination theory for hypermutation by AID. Second, we determined that MSH2-MSH6 binds to a U:G mismatch, and can enter the pathway at this step. MSH2-MSH6 could then recruit DNA polymerase eta, a low fidelity polymerase, since MSH2 associates with the polymerase in cells. MSH2-MSH6 stimulates the catalytic activity of polymerase eta, allowing it to move faster when it copies nucleotides, so that the polymerase can incorporate mutations at A:T pairs located downstream of the original U:G lesion. This is the first report of a functional interaction between

mismatch repair proteins and DNA polymerases. Third, we studied the roles of DNA polymerases eta and iota in mice and humans that lack the enzymes. For polymerase eta, the frequency of hypermutation and class switch recombination was normal, but the types of base changes were different. Polymerase eta-deficient clones had a decrease in mutations at A and T bases, and a concomitant rise of mutations at G and C. This finding implies that polymerase eta is an A-T mutator in hypermutation, and fills in a repair patch downstream of the dU lesion on the nontranscribed strand. For polymerase iota, variable genes were sequenced from mice with the 129 strain defect in the polymerase, and there was no effect on hypermutation or switching. To test for a dual knockout of polymerase eta and other low fidelity polymerases, we will examine mice that are deficient in both enzymes.

**Targeting of AID to Immunoglobulin Genes:** A major question in antibody diversity concerns how hypermutation is targeted to the immunoglobulin loci, and is not found elsewhere in DNA from B cells. Mutations are localized to two distinct regions: two kilobases of DNA surrounding and including the rearranged variable gene, and about two kilobases of DNA encompassing the switch region. These regions are downstream of promoters, suggesting that transcription is involved in bringing AID to the locus. To address this question, we will study how transcription proteins interact with the various enzymes involved in the hypermutation pathway. It soon may be possible to assemble all the pieces of the enigmatic hypermutation puzzle.

**Collaborator:** Roger Woodgate, National Institute of Child Health and Human Development, NIH, Bethesda, MD.

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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

**Recent Publications:**

Shahid KA, et al. *Biochemistry* 2006; 45(6): 1970-1978.

Richards S, et al. *Nucleic Acids Res* 2005; 33(17): 5382-5393.

Puri N, et al. *Biochemistry* 2004; 43(5): 1343-1351.

Majumdar A, et al. *J Biol Chem* 2003; 278(13): 11072-11077.

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

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

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

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

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

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

**Collaborators:** Dr. Paul Miller, Johns Hopkins, Baltimore, MD; Dr. Peter Glazer, Yale University, New Haven, CT; Dr. Irving Wainer, Laboratory of Clinical Investigation, NIA, NIH, MD.

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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 position at NIA in 2000. He became a tenured Senior Investigator in 2006.

**Keywords:**

helicase
genomic instability
DNA repair
replication
RecQ
Werner syndrome

**Recent Publications:**

Choudhary S, et al. *J Biol Chem* 2006; 281(9): 6000-6009.

Summers JA, et al. *Cancer Res* 2005; 65(4): 1223-1233.

Gupta R, et al. *J Biol Chem* 2005; 280(27): 25450-25460.

Sharma S, et al. *J Biol Chem* 2005; 280(30): 28072-28084.

Doherty KM, et al. *J Biol Chem* 2005; 280(33): 29494-29505.

**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.

**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.

**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. To understand the molecular functions and biological substrates that BACH1 helicase acts upon, we have systematically evaluated the ability of purified recombinant BACH1 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 BACH1 to catalytically unwind DNA structures proposed to be key intermediates of cellular DNA metabolism. The results from these unwinding studies provide a platform to investigate the molecular interactions of the BACH1/BRIP1 helicase with its protein partners in double strand break repair by homologous recombination.

**Collaborators:** Donald Jerina, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; Curt Harris, National Cancer Institute, NIH, Bethesda, MD; Sharon Cantor, Department of Cancer Biology, University of Massachusetts Medical School, Worchester, MA; Teresa Wilson, Radiation Oncology Research Laboratory, University of Maryland, Baltimore, MD; Alessandro Vindigni, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy; Ian Hickson, Cancer Research UK Laboratories, Oxford, UK; Vilhelm Bohr, 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 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:**

oxidatative DNA damage
base excision repair
structure-function relationship
susceptibility factors

**Recent Publications:**

Sokhansanj BA, et al. *Cancer Epidemiol Biomarkers Prev* 2006; 15(5): 1000-1008.

Fan J, et al. *J Biol* Chem 2006; 281(7): 3889-3898.

Harrigan JA, et al. *Nucleic Acids Res* 2006; 34(2): 745-754.

Muftuoglu M, et al. *Cancer Res* 2006; 66(1): 113-124.

Wong HK, et al. *J Cell Biochem* 2005; 95(4): 794-804.

Wilson DM III. *J Mol Biol* 2005; 345(5): 1003-1014.

**Base Excision Repair:** Base excision repair (BER) is the major pathway for correcting most spontaneous and oxidative DNA damage. The main steps of BER consist of: (1) excision of the damaged base (e.g. 8-oxoguanine), (2) incision of the DNA backbone at the abasic site product, (3) removal of the abasic terminal fragment, (4) gap-filling synthesis, and (5) ligation of the final nick. Evidence indicates that defects in BER can lead to increased cancer susceptibility, neurodegeneration, and premature aging symptoms. Our focus has been to define the biochemical and cellular contributions of several key participants in the BER process.

**Structure-Function Mechanism:** Proteins of BER are often found to interact with and/or regulate the functional capacity of other pathway participants. However, in many instances, the mechanism by which these proteins communicate is unknown. We are identifying the interfaces of certain protein-protein interactions, as well as determining the contribution of protein and DNA dynamics in pathway coordination. Moreover, we are working to elucidate the precise biochemical functions of a few central BER participants.

**The Major Human Abasic Endonuclease, Ape1:** Ape1 is the major corrective enzyme for abasic (AP) sites in DNA and initiates repair by incising the phosphodiester DNA backbone 5' to the damage. This enzyme also functions in specific strand break contexts to remove 3'-oxidative blocking termini, e.g. phosphoglycolate residues, as well as 3'-mismatched nucleotides, from DNA. Recently, we have found that Ape1 can incise at AP sites in several biologically-relevant DNA conformations, including fork and bubble structures. Our efforts are now examining whether abasic

lesions are repaired by Ape1 in a gene-specific manner, specifically in cooperation with the protein defective in the human premature aging disorder Cockayne syndrome, CSB.

**Single-Strand Break Repair Protein, XRCC1:** XRCC1 has no enzymatic activity, but has been proposed to operate as a scaffold protein in BER by binding DNA single strand breaks (specifically nicks and gaps) and recruiting certain BER proteins. We are defining the *in vitro* and *in vivo* roles of specific XRCC1 interactions, as well as searching for novel complex partners. Additional analyses include defining the cellular phenotypes of XRCC1 mutant cell lines, characterizing the activities of site-specific mutant and variant XRCC1 proteins, and examining the health consequences of an XRCC1 deficiency in an animal model.

**Susceptibility Factors:** Another area of research includes determining the impact of genetic variation on BER protein function. It has been proposed that genetic differences in the human population can give rise to proteins that are less effective at repairing DNA damage, thus rendering the individual more susceptible to a particular environmental exposure. By defining the functional capacity of variant proteins in the oxidative DNA damage response pathways, we are building a foundation on which we can better predict the relationship of genetic variation to human disease and the aging phenomenon. Additional research is currently exploring the potential to selectively regulate BER capacity in normal and/or tumor cells to improve the efficacy of anti-cancer treatment paradigms.

**Collaborators:** Kenneth Boheler, Laboratory of Cardiovascular Science, National Institute on Aging, NIH, Baltimore, MD; Vilhelm Bohr, Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD; Marit Otterlei, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; Bahrad Sokhansanj, School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA; Larry Thompson, Biosciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; Alan Tomkinson, Radiation Oncology Research Laboratory, Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD; Wei Yang, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

# Laboratory of Neurogenetics

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

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

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

## Laboratory of Neurogenetics Staff

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| | |
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| Alice Zhao | Postbac IRTA Fellow |
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| C. Paisan-Ruiz | Visiting Fellow |

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| J. Raphael Gibbs | Computer Info Scientist |
| Richard Todt | Computer Specialist |

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| Mika Shimoji | Visiting Fellow |
| Lizhen Wang | Visiting Fellow |

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

**Keywords:**

neurogenetics
Alzheimer's disease
Parkinson's disease
neurodegeneration

**Recent Publications**:

Hardy J, et al. *Ann Neurol* 2006; 60(4): 389-398.

Hardy J, et al. *Biochem Soc Trans* 2005; 33(Pt 4): 582-585.

Myers AJ, et al. *Hum Mol Genet* 2005; 14(16): 2399-2404.

Pittman AM, et al. *J Med Genet* 2005; 42(11): 837-846.

Edwards-Lee T, et al. *Neurology* 2005; 64(2): 377-379.

Singleton A, et al. *Hum Mol Genet* 2004; 13(1):R123-R126.

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

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

**Collaborators:** Andrew Lees, M.D., Martin Rossor, M.D., Huw Morris, M.D., Nick Wood, M.D., Henry Houlden, M.D., Rohan DeSilva, Ph.D., University of London; Ron Peterson, M.D., Ph.D., Mayo Clinic; Lars Lannfelt, M.D., Ph.D., Uppsala University, Sweden; Bengt Winblad, M.D., Ph.D., Karolinska Institute, Stockholm; Alison Goate, Ph.D., Washington University; Mike Owen, M.D., Ph.D., University of Wales; Dave Morgan, Ph.D., University of South Florida; Karen Duff, Ph.D., New York University.

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

**Keywords:**

neurogenetics
Parkinson's disease
parkinsonism
ataxia
dystonia
stroke
dementia

**Recent Publications**:

Singleton AB. *Trends Neurosci* 2005; 28(8): 416-421.

Nichols WC, et al. *Lancet* 2005; 365: 410-412.

Gilks WP, et al. *Lancet* 2005; 365: 415-416.

Hernandez DG, et al. *Ann Neurol* 2005; 57(3): 453-456.

Paisan-Ruiz C, et al. *Neuron* 2004; 44(4): 595-600.

Singleton AB, et al. *Science* 2003; 302(5646): 841.

In recent years, an extremely successful approach to understanding disease has arisen from the study of rare familial forms of disorders related to more common "sporadic" disease. This is a research paradigm that was successful in Alzheimer's disease (AD). The identification of the APP, PS-1 and PS-2 mutations as causal of rare forms of early-onset familial AD led to a huge increase in our knowledge of the pathogenic mechanisms underlying the common late-onset form of AD. We 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 α-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.

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

it is of paramount importance to design a viable strategy for the delineation of genetic predisposition in complex traits. We 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 are applying extremely high throughput dense SNP genotyping in large patient cohorts, this research aims to identify genes that underlie rare monogenic forms of disease and to identify common genetic variability that confers risk for disease.

**Collaborators:** Katrina Gwinn-Hardy, National Institute of Neurological Disorders and Stroke, NIH; Robert Nussbaum, National Human Genome Research Institute, NIH; 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.

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

**Keywords:**

Parkinson's disease
neurons
cell culture models

**Recent Publications**:

Greggio E, et al. *Neurobiol Dis* 2006; 23(2): 329-341.

Cookson MR, et al. *Annu Rev Biochem* 2005; 74: 29-52.

Beilina A, et al. *Proc Nat Acad Sci USA* 2005; 102(16): 5703-5708.

Canet-Aviles RM, et al. *Proc Nat Acad Sci USA* 2004; 101(24): 9103-9108.

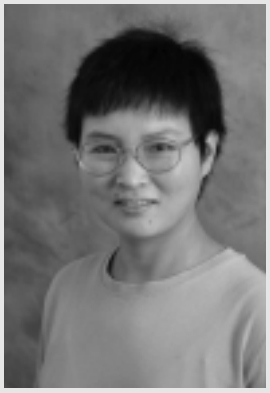
Miller DW, et al. *J Biol Chem* 2003; 278(38): 36588-36595.

In **Parkinson's 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's 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. We are actively working on understanding the biochemical functions of both DJ-1 and PINK1. We have been trying to find modifications and interactions of DJ-1 that affect its ability to protect cells against mitochondrial damage. For PINK1, which is a mitochondrial kinase, we have been identifying substrates that may play a role in cell death.

The two dominant genes, α-synuclein and LRRK2/dardarin, are linked by the observation that most cases with either mutation have PD-like symptoms but also a high frequency of Lewy bodies. This suggests that mutations convert these molecules to a neurotoxic form and that the mutations are associated with protein deposition. 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. For example, we have recently found 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; Myriam Gorospe, Laboratory of Cellular and Molecular Biology NIA, NIH; J. Timothy Greenamyre, Emory University; Gregory Petsko, Brandeis University; Andrew Singleton, Laboratory of Neurogenetics, NIA, NIH; 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**:

Hardy J, et al. *Alzheimer Dis Assoc Disord* 2006; 20(1): 60-62.

Paisan-Ruiz C, et al. *J Med Genet* 2006; 43(2): e09.

Addington AM, et al. *Mol Psychiatry* 2005; 10(6): 581-588.

Adighibe O, et al. *Neurobiol Aging* 2005; [Epub].

Hardy J, et al. *Biochem Soc Trans* 2005; 33(Pt 4): 582-585.

Myers A, et al. *Hum Mol Genet* 2005; 14(16): 2399-2404.

Pittman A, et al. *J Med Genet* 2005; 42(11): 837-846.

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, α 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 Computational Biology Section, Transgenesis Unit.

**Keywords:**

Alzheimer's disease
ALS
Parkinson's disease
neurodegenerative disease
mouse model

**Recent Publications**:

Hardy J, et al. *Ann Neurol* 2006; 60(4): 389-398.

Laird FM, et al. *J Neurosci* 2005; 25(50): 11693-11709.

Cai H, et al. *J Neurosci* 2005; 25(33): 7567-7574.

**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 Alzheimer's disease (AD), 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.

**I. The Molecular Pathogenesis of AD:** A wide variety of studies demonstrate that genetic mutations linked to Alzheimer's disease (AD) invariably increase the production and deposition of amyloid β (Aβ) peptides, strongly supporting the idea that excessive Aβ accumulation contributes to the pathogenesis of AD. Aβ peptides are derived from amyloid precursor protein (APP) by endoproteolytic cleavages of BACE1 and γ-secretase. Previously, we along with others have demonstrated that knockout of BACE1 in mice completely abolished the production of Aβ. Recently, we have crossed these BACE1 knockout mice with mutated APP and presenilin 1 (PS1) transgenic mice. We found that formation of Aβ deposition, dystrophic neurites, as well as astrogliosis and microgliosis are completely prevented and cognitive impairments are fully rescued in these BACE1 null and APP/PS1 triple transgenic animals. These results strongly argue that BACE1 is a high priority therapeutic target for AD. However, even though the BACE1 knockout mice are viable and show no major pathological abnormalities, they do display subtle deficits in explorative

activities and spatial learning and memory suggesting that BACE1 is somehow important for the normal functions of the brain. In order to learn more about the biological functions of BACE1, we propose to identify new substrates or related proteins for BACE1 other than APP family proteins by proteomics approaches. Because BACE1 protein is most abundant in neurons and we are more interested in studying the functions of BACE1 in the brain, we will define the proteins that display different expression levels in BACE1 KO neurons. Once we have validated candidates, we will examine their potential contributions to the normal functions of the nervous system and pathogenesis of AD. In addition, we are also engaged in identifying factors that regulate either the stability or β-secretase activity of BACE1.

Another issue has not been fully addressed in AD is how Aβ peptides or their aggregates affect the functions of neurons. We plan to address this question by generating a line of conditional APP transgenic mice in which the APP transgene is selectively expressed by a subset of neurons. By comparing the morphological and physiological changes of wild-type to the adjacent mutant APP expressing neurons, we will be able to determine whether intracellular Aβ acts in a cell autonomous or in a heterologous fashion to cause neuronal damages.

**II. The Molecular Pathogenesis of Mutations in ALS2 and Dynactin:** Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron diseases. ALS also presents in rare cases as a juvenile-onset disease, and in a subset of these cases is inherited through mutations in the ALS2 gene. Genetic analyses suggest that this type of juvenile ALS is associated with the loss of ALS2 function, presumably its guanine-nucleotide-exchange factor (GEF) activities. We have generated the ALS2 knockout mice to model this type of motor neuron disease to address the following questions: what are the physiological function(s) of ALS2, and, how do mutations in ALS2 affect this function? In conjunction, we have also used yeast-two hybrid and co-immunoprecipitation approaches to identify the upstream or downstream signaling pathways in which ALS2 is involved.

Recently, a point mutation in Dynactin has been linked to motor neuron diseases. Dynactin is an intrinsic component of the protein complex mediating the intracellular transport. But, how the mutation in Dynactin particularly affects the protein or vesicle transport, or other functions in motor neurons is not clear. We also have no clue about why this mutation particularly causes problems in motor neurons. We are in the middle of

developing Dynactin mutation knockin and conditional knockout mouse models to address these questions. Because defects in axonal transportation have been found in many different kinds of neurodegenerative disease, these Dynactin mouse models will also shed light on some common pathogenic pathways that lead to neuronal degeneration.

**III. The Molecular Pathogenesis of PD:** Parkinson's disease is the second most common neurodegenerative disease. Recently, two recessive mutations in DJ-1 have been identified that are linked to the Parkinson's disease. There are many functions of DJ-1 that have been reported. But, how these functions are related to the normal function of dopaminergic neurons in the basal ganglia, or why the loss of DJ-1 specifically causes the death of this small population of neurons is not clear. We have modeled this genetic deficit in mice by knocking out the DJ-1 gene. Meanwhile, we also have tried to define DJ-1 interacting proteins by yeast-two hybrid screening and other methods. These models should reveal more information on the role of DJ-1 in brain function.

**Collaborators:** Don Price, M.D., Philip Wong, Ph.D., David Borchelt, Ph.D., Jeff Rothstein, M.D., Ph.D., Rick Huganir, Ph.D., and Jeremy Nathans, M.D., Ph.D., The Johns Hopkins University School of Medicine; Mark Cookson, Ph.D. Laboratory of Neurogenetics, NIA, NIH; Julius Zhu, Ph.D., University of Virginia; Bob Nussbaum, M.D., Genetic Diseases Research Branch, National Human Genome Research Institute, NIH.

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**Biography:** Dr. Fabienne Wavrant-De Vrièze is a human geneticist whose research interests focus on the genetics of neurological disease. Dr. Wavrant-De Vrièze received her B.Sc. and Ph.D. from the University of Lille, France where she investigated a new genetic factor contributing to Alzheimer's disease. Dr. Wavrant-De Vrièze performed her postdoctoral training at the Mayo Clinic in Jacksonville, Florida, studying the genetic basis of neurological diseases such as Alzheimer and Guam's disease. In 2002, she joined the NIA as a Facility Head within the Laboratory of Neurogenetics. Dr. Wavrant-De Vrièze's group is helping to seek the genetic causes of diverse neurological diseases.

**Keywords:**

neurogenetics
dementia
neurodevelopment
genome screen
linkage analysis

**Recent Publications**:

Seal JL, et al. *J Med Genet* 2006; 43(11): 887-892.

Grupe A, et al. *Am J Hum Genet* 2006; 78(1): 78-88.

Hernandez D, et al. *Neurosci Lett* 2005; 389(3): 137-139.

Lahiri DK, et al. *Neurobiol Aging* 2005; 26(10): 1329-1341.

Myllykangas L, et al. *J Neurol Sci* 2005; 236(1-2): 17-24.

Evans W, et al. *Neurosci Lett* 2004; 369(3): 183-185.

Neurodegenerative diseases are complex diseases with diverse forms of transmission within the same pathology. Indeed, disorders such as Alzheimer and Parkinson's diseases can present as rare familial forms as well as more common "sporadic" forms. Consequently, the study of the genetic causes of these diseases is difficult. The two most common approaches that are being used are association and linkage studies. The first is based on the investigation of candidate genes chosen according to their biological function. By studying the segregation of their genotype and the transmission of the disease, genetic variation can be implicated in the disease. The second kind of study uses genetic markers that are spread out through the whole genome. Statistical analysis allows us to identify which markers are likely to be located near a gene that is involved in the development of the disease.

The main goal of our group is to discover loci that have genes that could potentially be involved in the development of diverse pathology, and then to study their association with disease. We are studying several forms of dementia, as well as other forms of neurodegenerative diseases and neurodevelopmental disorders. We have performed 3 genome screens in collaboration with colleagues from the NIH as well as with other groups.

The last stage of the genome screen being performed on siblings affected by the late-onset form of Alzheimer's disease (AD) will soon provide us with clues about which chromosomal region to focus on, in order to identify the gene(s) that contribute to this pathology. To date, only 1 gene has been

identified as a risk factor for late-onset AD, apolipoprotein E. Currently, several genes are being studied to establish if they are linked to AD. Those genes were chosen according to their position on chromosomal loci that were suggested to be linked to the disease. Those chromosomal regions are sizeable, and they contain a large number of genes. By refining the genome screen and adding more samples to the population being studied, we hope to narrow down these genetic regions and ultimately identify the genetic variation contributing to disease.

The two other genome screens were performed on samples that present neurodevelopmental diseases. Our collaborations with Drs. Judith Rapoport, NIMH, NIH, and Maximilian Muenke, NHGRI, NIH, consists of a 10cM genome screen on families that are affected by the childhood-onset form of schizophrenia (COS) in the first case, while the pathology that we are investigating in the second case is the Holoprosencephaly (HPE).

It is believed that COS is a disease that causes abnormal maturation of certain brain structures precipitated by multiple genetic defects. Cytogenetic abnormalities, which are due to changes in autosomes and sex chromosomes, have been found to be related to COS. Although twin and adoption studies have shown that genetics has an influence on the cause of COS, the level and nature of this effect is unclear (Kendler and Diehl, 1993). Although it is unclear whether schizophrenia has a single cause or multiple underlying causes, evidence suggests that it is a pathology likely involving a genetic predisposition, a prenatal insult to the developing brain and stressful life events. The role of genetics has long been established; the risk of schizophrenia rises from 1 percent with no family history of the illness, to 10 percent if a first degree relative has it, to 50 percent if an identical twin has it. But, it has not yet been determined which specific genes cause the brain abnormalities related to COS. The analysis of this project is currently ongoing; but so far, the analysis of the genome screen has led us to discover a segmental uniparental isodisomy on 5q32-qter in a patient with childhood-onset schizophrenia.

HPE is a disorder in which the fetal brain does not grow forward and divide as it is supposed to during early pregnancy. It is a birth defect that occurs during the first few weeks of intrauterine life. Although many children with HPE have normal chromosomes, specific chromosomal abnormalities have been identified in some patients. There is evidence that in some families, HPE is inherited (autosomal dominant as well as autosomal or X-linked

recessive inheritance). Several genes have already been identified that play a role in HPE. Indeed, to date, four genes have been identified that cause HPE in some families, but changes in these genes are found in only 10-20% of patients with HPE. Thus, more progress will be needed to understand the causes of HPE in the remaining families. The genome screen has been completed for this project and candidate genes are currently being studied.

**Collaborators:** Maximilian Muenke, M.D., National Human Genome Research Institute, NIH; Judith Rapoport, Ph.D., National Institute of Mental Health, NIH; Alison Goate, Ph.D., Washington University School of Medicine; Mike Owen, Ph.D., University of Wales College of Medicine; Lahiri Debomoy, Ph.D., Indiana University School of Medicine; Pentti Tienari, Ph.D., University of Helsinki, Finland; Giancarlo De Ferrarri, Ph.D., University of Washington School of Medicine.

# 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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**Biography:** Dr. Mattson received his Ph.D. in Biology from the University of Iowa in 1986. After 3 years of postdoctoral studies in Developmental Neuroscience at Colorado State University, Dr. Mattson took a faculty position at the Sanders-Brown Research Center on Aging at the University of Kentucky Medical Center where he was promoted to Full Professor in 1997. Dr. Mattson is currently Chief of the Laboratory of Neurosciences at the National Institute on Aging, and Professor of Neuroscience at Johns Hopkins University. He is Editor-in-Chief of the Journal of Molecular Neuroscience, and a Managing or Associate Editor of the Journal of Neuroscience, Journal of Neurochemistry and Journal of Neuroscience Research. In addition, he has edited 7 volumes in the areas of mechanisms of cell death, aging and age-related neurodegenerative disorders. Dr. Mattson has received numerous awards including the Metropolitan Life Foundation Award and the Alzheimer's Association Zenith Award. He is considered a leader in the area of cellular and molecular mechanisms underlying neuronal plasticity and neurodegenerative disorders, and has made major contributions to understanding of the pathogenesis of Alzheimer's disease, and to its prevention and treatment. Dr. Mattson has published more than 300 original research articles and more than 80 review articles. He is the most highly cited neuroscientist in the world.

**Keywords:**

neurodegenerative disorders
calcium and oxyradicals
signal transduction
synaptic plasticity

**Recent Publications:**

Arumugam TV, et al. *Nat Med* 2006; 12(6): 621-623.

Cutler RG, et al. *Proc Natl Acad Sci USA* 2004; 101(7): 2070-2075.

Kruman II, et al. *Neuron* 2004; 41(4): 549-561.

Maswood N, et al. *Proc Natl Acad Sci USA* 2004; 101(52): 18171-18176.

Mattson MP. *Nature* 2004; 430(7000): 631-639.

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

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

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

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

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

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

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

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

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

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

Sphingomyelin and cholesterol are important components of the plasma membrane of neurons where it functions in cellular signal transduction and cellular responses to stress. By analyzing spinal cord and brain tissues from human patients and mouse models, investigators in this section of the LNS have shown that profound abnormalities in sphingomyelin metabolism

occur in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. The alterations, which include accumulation of long-chain ceramides and cholesterol esters, occur 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; 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; 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; D. Alan Butterfield, University of Kentucky; Don Gash, University of Kentucky; Jeff Johnson, University of Wisconsin; Mathias Jucker, University of Tubingen.

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

**Keywords:**

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

**Recent Publications:**

Chow DK, et al. *Exp Gerontol* 2006; 41(3): 252-260.

Wilson MA, et al. *Aging Cell* 2006; 5(1): 59-68.

Gami MS, et al. *Aging Cell* 2006; 5(1): 31-37.

Iser WB, et al. *Mech Ageing Dev* 2005; 126: 1090-1096.

Glenn CF, et al. *J Gerontol A Biol Sci Med Sci* 2004; 59(12) : 1251-1260.

**Genetics of Longevity in *C. elegans*:** The nematode, *C. elegans*, is quickly becoming a favorite organism for studying the genetics of longevity. Under laboratory conditions, wild-type *C. elegans* adults have a 2 to 3-week lifespan. Genetic mutations have been identified which allow worms to live up to three times longer. The molecular identification and characterization of the genes responsible for these mutant phenotypes has provided new insights into pathways controlling lifespan. In particular, we have learned that multiple pathways control longevity in *C. elegans*. Some of these lifespan pathways interact, while others function independently of the rest. In addition, *C. elegans* lifespan can be lengthened by caloric restriction, as has been documented for other species. Humans and nematodes share many of the same genes, so it is likely that human longevity will be controlled by some of the same genes that control *C. elegans* lifespan. Thus, studies of genetic control of longevity in *C. elegans* will help to reveal mechanisms that also control human longevity.

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

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

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

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

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

system function. Our strategy for contributing to this effort is to use the worm to identify genes whose function is required for successful nervous system aging.

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

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

Finally, the IMG unit will use *C. elegans* as a tool for rapidly screening compounds that can mimic longevity extension of caloric restriction. Several studies have documented the fact that *C. elegans* lifespan is extended by dietary restriction, as has been shown for other species of invertebrates and vertebrates. In addition, caloric restriction has been shown to enhance stress resistance in nematodes and other species and may therefore aid in successful aging. However, it may be difficult to convince the human population to submit to dietary restriction voluntarily. An alternative strategy is to identify chemical compounds which are non-toxic and mimic the effects of caloric restriction. In collaboration with other labs in the LNS, chemical compounds will be rapidly screened for lifespan-extending effects in *C. elegans* and lead compounds identified in such screens will be further characterized for effects on mammalian aging phenotypes.

**Summary:** The research program of the IMG unit is targeted to provide a comprehensive understanding of how aging affects the nervous system. Studies of neuronal insulin-like control of longevity will identify factors that determine longevity and help us understand how lifespan could be increased. These studies also investigate the role of stress resistance in longevity control. Nervous system aging in normal and challenged backgrounds will reveal gene products critical for successful aging.

Nematodes will also be useful for rapidly identifying chemical compounds affecting longevity that may offer therapeutic potential in humans. Together, this work will provide insight into challenges confronting the aging nervous system as well as strategies for coping with them.

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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. *Ageing Res Rev* 2006; 5(3): 332-353.

Maudsley S, et al. *J Pharmacol Exp Ther* 2005; 314(2): 485-494.

Maudsley S, et al. *Cancer Res* 2004; 64(20): 7533-7544.

Davidson L, et al. *J Biol Chem* 2004; 279(12): 11906-11916.

Davidson L, et al. *J Biol Chem* 2004; 279(3): 1980-1993.

**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.

**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.

**Control of Synaptic Transmission by Lipid Raft Microdomain Structure:** Lipid rafts and caveolae are cholesterol and sphingomyelin-rich membrane microdomains found in many tissues and are thought to be involved in lipid and protein trafficking, signal transduction, cell surface proteolysis and the organization of higher order multi-protein complexes. Caveolae are small flask-shaped membrane invaginations that contain a high density of a cholesterol-binding protein called caveolin. Lipid rafts tend to have a flat membrane structure, predominate in the central nervous system (CNS) and are mostly caveolin free. In the CNS lipid rafts are characterized by their high concentrations of a protein called flotillin. Flotillin appears to act as a scaffolding protein within the raft. Due to their high densities of cholesterol and sphingomyelin compared to the rest of the cells plasma membrane lipid rafts can induce and maintain the clustering of membrane components upon certain stimuli. This compartmentalization capacity is important for the generation of intracellular signals and the recruitment of downstream effector molecules. Thus as accessory proteins can interact with GPCR structures to create receptorsomes then multiple receptorsomes can be then organized by lipid raft microdomains.

Recent evidence has shown that alterations in lipid rafts may be associated with various diseases, *e.g.*, diabetes, certain forms of cancer, atherosclerosis and degenerative muscular dystrophies. Flotillin and caveolin levels are both observed to be altered in relation to disease processes and with aging, suggesting a concomitant alteration in the lipid raft/caveolae structure. Caveolin knock out mice also can display a considerably reduced lifespan compared to wild type animals as well as severe cardiovascular disorders and pulmonary fibrosis.

More importantly lipid rafts have also been proposed to play a role in neurodegenerative disorders such as Alzheimer's disease (AD). In AD disruptions of lipid rafts are thought to contribute to the production and aggregation of the neurotoxic amyloid $A_{\beta}$ protein. Primitive senile plaques in non-demented persons also show strong flotillin expression and in AD patients there is an elevated flotillin presence in the cortex. Studies have also shown an accumulation of flotillin in lysosomes of neurons having neurofibrillary tangles, a second hallmark of AD. Not only are flotillins involved in amyloid processing but through their capacity to cluster in lipid rafts they are also thought to possess a structural scaffolding action. Flotillins can therefore control the membrane organization of c-src tyrosine kinases, GPCRs, structural proteins and even GLUT-4 glucose transporters. Therefore not only are the physico-chemical properties of the rafts themselves important, *i.e.*, high lipid density to enforce juxtaposition of

related signaling proteins, for cell signaling but also the functional state of the intrinsic scaffolding proteins such as flotillin is important. We are investigating whether flotillin-mediated alterations in lipid rafts can change the correct protein constituency and stoichiometry of neuroprotective signaling mechanisms.

It is highly likely that lipid rafts themselves crucially control individual neurotransmission events, in aging and disease models. Lipid raft structure appears to be coupled to the dynamics of the actin-cytoskeleton, this is of specific importance for synaptic plasticity as dendritic spines are strongly influenced by the capacity of the cell to regulate its actin cytoskeleton. Lipid rafts control the location of AMPA-glutamate receptors at the post-synaptic area as well as many other crucial neuronal signaling factors, *e.g.*, phospholipase D1, adenylyl cyclase and the EGFR. This relationship between signaling factors and the raft enables efficient signal processing by promoting cross-talk between the different signaling cascades. Therefore any pathological disruption of the protein stoichiometry in the raft will result in a reduction of synaptic efficiency that may be a precursor for the loss of neuronal functions in aging and degeneration. It is therefore clear that a detailed quantitative analysis, using isotopic mass label mass spectrometry *e.g.*, iTRAQ, of the actual levels and ratios of protein components of the rafts is required to understand the role of these organizing structures in synaptic transmission in aging and disease. As with our interest in receptorsomes, it is likely that therapeutics can even be directed towards idiosyncratic receptor systems created by their presence in distinct forms of lipid raft microdomains.

**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.

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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 and are in preclinical and clinical development, and 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-ß peptide
tumor necrosis factor-α
p53 inhibitors
apoptosis
glucagon-like peptide-1
Alzheimer's disease
type 2 diabetes

**Recent Publications:**

Luo W, et al. *J Med Chem* 2006; 49(7): 2174-2185.

Sambamurti K, et al. *Curr Alzheimer Res* 2006; 3(1): 81-90.

**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).

## Alzheimer's Disease:

**Acetylcholinesterase Inhibition:** Although the neuropathological quantification of ß-amyloid plaques and neurofibrillary tangles in the AD brain is the basis for confirming disease diagnosis after death, it is the neocortical synapses rather than the plaques and tangles that correlate best with psychometric indices of cognitive performance in AD. The loss of cholinergic synaptic markers in selected brain regions remains one of the earliest events leading to AD, with the cholinergic system being the most affected of the neurotransmitters and intimately involved in memory processing. Additionally, there are numerous mechanistic-based interactions linking the cholinergic system to Aß genesis, Tau phosphorylation, apoptotic cell death and inflammatory process that form a self-propagating

**Publications-continued:**

Greig NH, et al. *Curr Alzheimer Res* 2005; 2(3): 281-290.

Luo W, et al. *J Org Chem* 2005; 70(16): 6171-6176.

Luo W, et al. *J Med Chem* 2005; 48(4): 986-994.

Engelstein R, et al. *Neurobiol Dis* 2005; 18(2): 282-285.

Greig NH, et al. *Proc Natl Acad Sci USA* 2005; 102(47): 17213-17218.

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 (in current assessment by the U.S. and British Army).

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 novel experimental drug, phenserine (licensed to Axonyx, New York, NY), a long-acting and brain-directed, selective AChE inhibitor, is now in phase 3 clinical assessment in AD patients. Thus far, it appears to be well tolerated in elderly individuals, particularly when compared to currently available prescription anticholinesterases. Specifically with regard to phenserine, multiple phase I clinical trials have now been completed (to assess single and multiple dose tolerability in the elderly, as well as bioavailability). One phase 2 study, to characterize tolerability and actions on cognition in AD, has been successfully completed, and a further phase 2 clinical trial to characterize actions on disease progression, with an emphasis on Aß levels in CSF and plasma as well as cognition, is presently ongoing, as is a phase 3 clinical trial that is focused on cognition.

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 Aß plaques and neurofibrillary tangles. The association of BChE with the AD neurotoxic peptide, ß-amyloid, has been shown to dramatically amplify the toxicity of the peptide. In addition, a mutant variant of BChE, the K form, when found together with the ApoE 4 allele, is associated with an increased susceptibility of sporadic AD. Hence, inappropriate BChE activity can increase the risk of AD and accelerate the disease process.

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 Aß peptide.

**ß-amyloid Precursor Protein (ß-APP) and Amyloid-ß (Aß ) Peptide Inhibitors:** Another of our focuses to develop therapeutics for treating AD relates to reducing the production and secretion of Aß. It is widely believed that Aß plays a central role in the progressive neurodegeneration observed in AD; diminishing the level of Aß has therefore emerged as a critical goal in AD therapy. Aß 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-, ß- and γ-), to different protein fragments, including toxic Aß 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 Aß 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 Aß toxic fragments.

In this regard, we have focused on the pharmacophore of (-)-phenserine: a tricyclic hexahydropyrrolo[2,3b]indole with a phenylcarbamate. In cell culture studies, (-)-phenserine lowered APP and Aß 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 Aß, it was found to significantly lower both. 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. 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 Aß levels. Utilizing the pharmacophore of (-)-phenserine, we have developed a novel series of compounds to optimize action against APP and Aß and to minimize anticholinesterase activity.

**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. 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 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. Additionally, PBMC 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 activity of these novel compounds in classical models of (i) neurodegeneration as well as cancer (with specific focus on angiogenesis) is the focus of current studies.

**Neurodegeneration:** Collaborative studies with Mark Mattson, Ph.D., (Chief, Laboratory of Neurosciences, NIA, NIH, Baltimore, MD) are focused on modifying the course of apoptotic cell death. Apoptosis is a major form of cell death that involves a stereotyped sequence of biochemical and morphological events. Inhibition of rate limiting biochemical steps within this cascade of events can halt and rescue cells from a variety of physiological and pharmacological insults that induce cell death via apoptosis. Studies have focused on the design, synthesis and assessment of a novel series of potent compounds that inhibit the intracellular protein, p53. These compounds protect cells of neuronal origin from toxic concentrations of a variety of insults, including the AD Aβ peptide, in tissue culture, and largely protect the brain from ischemic insults in *in vivo* rodent studies. Additional studies have demonstrated potency in a widely used model of Parkinson's disease. The focus of our studies is to test the clinical utility of p53 inhibition with emphasis on neurodegenerative diseases such as AD, Parkinson's disease and stroke. However, p53 inhibitors hold potential in protecting normal tissue from the toxicities associated with chemotherapeutic agents and radiation therapy in cancer treatment, and form a further focus of future research.

**GLP-1 Agonists, Type 2 Diabetes and Neurodegeneration:** Collaborative studies with Josephine Egan, M.D., (Diabetes Section, Laboratory of Clinical Investigation, NIA, Baltimore, MD) 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. 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. Additional research has supported the transition of Ex-4 from the laboratory and into clinical trials as an experimental therapeutic for type 2 diabetes. Studies in cell culture and rodents indicate that Ex-4 is some 13-fold more potent due to its higher GLP-1 receptor affinity, and it is considerably longer acting than GLP-1. In clinical trials Ex-4 peptide appears, thus far, to be both safe and effective in controlling blood glucose levels in subjects afflicted with type 2 diabetes. Current studies in the laboratory are focused on understanding the mechanism of action of Ex-4 and analogues, further optimizing their action and developing minimized peptides to allow the future design of peptidomimetics.

Although predominantly located on pancreatic islet cells, numerous reports now document GLP-1 receptor expression in both the rodent and human brain (for review see: Perry T and Greig NH, J Alzheimers Dis 2003 and Trends Pharmacol Sci 2003). It still remains to be established whether or not GLP-1 is produced by neural cells, but GLP-1 present in the bloodstream can enter brain; utilizing a blood-brain barrier peptide transport system. Intestinally derived peptides, 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 recent studies indicate that GLP-1 can stimulate the formation of new ß-cells in rodents (partly by enhancing ß-cell proliferation and partly by enhancing the differentiation of duct progenitor cells to mature ß-cells). This fueled our interest to assess a neurological role for GLP-1. Based on the described action of GLP-1 on islet cell differentiation, we hypothesized a neurotrophic role for GLP-1 within the nervous system. Our focus has been to evaluate the role(s) of GLP-1 and related analogues, *in vitro* and *in vivo*, to test this hypothesis with a view to developing the most promising ones as an alternative and potentially valuable novel therapeutic intervention for central and peripheral degenerative disorders, such as stroke and peripheral neuropathy associated with type 2 diabetes mellitus.

Using cell culture techniques, we have established the presence of the GLP-1 receptor (GLP-1R) on neural cell lines, such as PC12 cells as well as primary rat hippocampal cells by RT-PCR analysis of RNA and GLP-1R-induced increases in intracellular cAMP. Furthermore, GLP-1R stimulation

induced differentiation in 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 is a focus of current studies. In addition, GLP-1R agonism provided complete protection against cell death induced by glutamate neurotoxicity in cultured hippocampal neurons, as has been shown by other neurotrophic factors (e.g., NGF and BDNF), suggesting that GLP-1-like peptides may play a significant role in protecting hippocampal neurons against excitotoxic damage and potentially against other types of brain injury. Protection, likewise, was afforded against Aß (particularly $Aß_{1-42}$) as well as cellular oxidative stress and membrane lipid peroxidation induced by iron.

Studies have been undertaken to elucidate whether or not these actions in cell culture models translate to animals. Specifically, using a well established rodent model of neurodegeneration, we have shown complete amelioration of an ibotenic acid induced cholinergic brain lesion following infusion of GLP-1R agonist administration, as assessed by quantitation of the cholinergic cell marker, choline acetyltransferase. Actions on other well established rodent neurodegenerative models are also being assessed and suggest that neuroprotective effects in cell culture translate to animal studies.

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# 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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**Keywords:**

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

**Recent Publications:**

Terracciano A, et al. *J Gerontol B Psychol Sci Soc Sci* 2006; 61(2): P108-P116.

Costa PT Jr., et al. *Handbook of Personology and Psychopathology* 2005, New York, Wiley; pp. 257-270.

Weiss A, et al. *Psychol Aging* 2005; 20: 182-185.

McCrae RR, et al. *Eur J Pers* 2005; 19: 269-286.

Terracciano A, et al. *Psychol* Aging 2005; 20(3): 493-506.

Costa PT Jr, et al. *J Pers Dis* 2005; 19(5): 533-539.

*The Five Factor Model:* Personality is central to understanding the individual person, and it influences, to some degree, nearly all aspects of experience and functioning in everyday life. A major obstacle to progress in personality psychology for many decades was the inability of psychologists to agree on taxonomy of traits that would offer a comprehensive yet manageable set of trait constructs. Since 1983, this Laboratory has contributed to a worldwide consensus that the Five-Factor Model points to such taxonomy. The broad factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness appear to encompass most specific traits, and offer a framework for systematic literature reviews and research designs. 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. These traits are rooted in biology, endure in adulthood, and influence an extraordinary range of psychological outcomes. 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.

Several groups of researchers have advanced a personality typology based upon three replicable, empirical person clusters derived from measures of the Five-Factor Model. These clusters or types have been labeled Resilient (below average Neuroticism (N) scores and above average Extraversion

(E), Openness (O), Agreeableness (A) and Conscientiousness (C) scores), Overcontrolled (high in N, low in E and O), and Undercontrolled (below average in A and C). The present research attempted to replicate these types in four large and diverse adult samples: (1) the Baltimore Longitudinal Study of Aging (N=1,856), (2) the East Baltimore Epidemiologic Catchment Area study (N=486), (3) the UNC Alumni Heart Study (N=2,420), and (4) a HIV risk reduction intervention study (N=274). Ward's hierarchical cluster procedure followed by k-means centering was used to derive empirical clusters from the 5 NEO-PI-R domain scores in each sample. To assess the replicability of empirical clusters to the 3 typological clusters, kappa coefficients were computed. Based upon the kappas, a clear replication of the proposed types was found in only one sample, the ECA (kappa = .62), but several others had a greater-than-chance resemblance. Because these personality types are thought to relate to different patterns of psychopathology, e.g., externalizing problems for the Undercontrolled types, and internalizing problems for the Overcontrolled types, then type membership should be a powerful predictor of certain outcomes. Results in the ECA sample showed that the 3 types predicted measures of psychosocial functioning, depression, and personality disorders. Specifically, Resilient types were less depressed, had superior psychosocial functioning, and fewer personality disorder symptoms.

The failure of the 3 personality types to replicate in 3 of the 4 samples leads to the conclusion that they are not robust empirical entities. It should be noted that the Resilient type was empirically replicated in all 4 samples, while the Undercontrolled was replicated in none. Nevertheless, while the types do not refer to distinct homogeneous classes of persons, they do have utility as convenient labels summarizing the combinations of traits that individually and together relate to important outcomes.

**Personality Development in Adulthood and Adolescence: Towards a Life-Span Approach:** For many years, this Lab has been studying issues of personality continuity and change using a variety of personality instruments. We extended at both extremes our life-span approach by studying personality between the ages of 12-18 on the one hand, and personality in the very old, or advanced old age, 65-100 year.

With collaborators in this country and abroad, we have now begun to trace the antecedents of adult development: personality development in early adolescence. Three studies were conducted to assess mean level changes in personality traits during adolescence. Self-reports on versions of the Revised NEO Personality Inventory (NEO-PI-R) were used to assess the five major factors of adult personality. A four-year longitudinal study of intellectually

gifted students (N = 230) was supplemented by cross-sectional studies of non-selected American (N = 1,959) and Flemish (N = 789) adolescents. Neuroticism appeared to increase in girls, and Openness to Experience increased in both boys and girls; Extraversion, Agreeableness, and Conscientiousness were stable between age 12 and age 18. Detailed longitudinal research is needed to determine whether increases in Openness influence the processes of moral and ego development that occur in this age period. The results as a whole extend knowledge of the development curve of personality traits backward from adulthood and help bridge the gap with child temperament studies.

**Molecular Genetics of Personality Structure:** New opportunities have recently arisen for pursuing basic research on the biology of personality. Behavioral genetics studies have established that genes play a role in determining levels of personality traits. Recent behavior genetic studies suggest that the phenotypic Five-Factor Model is largely the product of genetic influences. LPC is committed to searching for the molecular genetic basis of personality trait structure.

SardiNIA: Investigators in the Laboratory of Personality and Cognition are participating with other intramural scientists in the Laboratory of Cardiovascular Science and the Laboratory of Genetics, to examine the degree to which genetic factors underlie normal personality traits, as well as indicators of arterial stiffness and other risk factors for heart disease in the Sardinian population. The next phase of the project will involve full genome scanning of the entire population to identify chromosomal regions or loci for as many of the broad and narrow personality traits, as well as the personality profiles or configurations associated with the DSM-IV personality disorders, referred to earlier.

Currently, we are examining the 10K chip SNP data from 4,500 individuals to identify chromosomal regions or loci for the 30 facets and the five broad domains of the FFM. Subsequent phases of the project will involve analysis of 500K genotype scan

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

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

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

**Towards a Dimensional Model for Axis II of the DSM-V:** Axis II of the DSM-IV is used for the diagnosis of personality disorders, which are defined as inflexible and maladaptive personality traits. Over 50 studies linking normal personal dimensions and personality disorders have led to a fundamental reconceptualization of the field of personality and psychopathology: Personality disorders do not correspond to discrete psychiatric entities, rather they are better construed as a systematic collection of problems in living associated with different dimensions of personality. A crucial step in the diagnosis of personality disorders (PDs), Axis II of the Diagnostic and Statistical Manual of Mental Disorders (DSM), involves the determination of clinical severity of problems or impairments in functioning that are related to personality and not life circumstances. To date, this has been problematic because of the lack of a systematic guide to assessing problems and dysfunctions.

In previous work, we have outlined an alternative approach to PD assessment that is based on the Five-Factor Model of Personality (FFM). Our approach advocates four steps: (1) personality assessment using an FFM-based instrument, (2) identification of problems in living that are likely associated with a given personality profile, (3) assessment of problem severity, (4) comparison of the observed personality profile to recognized patterns associated with specific conditions (e.g., DSM PDs).

Recently, comparison and categorization of problem content of five leading instruments according to their association with different personality factors was performed. Of the 608 items classified from the five instruments, all but 72 or 12% were previously classified confirming the comprehensiveness of the previous five-factor model based catalog. The analyses also resulted in a small but significant number of additions to the earlier catalogue of personality-related problems in living.

The four-step process provides a new approach to PD diagnosis that is rooted in the FFM, a comprehensive and universal model of personality with a known developmental course and strong links to hereditary factors. The updated list of personality-related problems helps to streamline clinical assessments because it allows clinicians to focus their questions towards areas in which clients are most likely to experience problems. Ultimately, this new approach may be incorporated in a future version of the DSM.

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

**Keywords:**

personality structure
longitudinal studies
openness to experience
cross-cultural research

**Recent Publications:**

McCrae RR, et al. *J Pers Soc Psychol* 2005; 88(3): 547-561.

Terracciano A, et al. *Science* 2005; 310(5745): 96-100.

McCrae RR, et al. *J Pers Soc Psychol* 2005; 89(3): 407-425.

Personality traits are dimensions of individual differences in the tendencies to show consistent patterns of thoughts, feelings, and actions. Traits are important because their influence is pervasive: They affect personal interactions and social support, health habits and somatic complaints, attitudes and values, ways of coping, occupational and recreational interests, and much more. For the past 20 years, research in this laboratory has utilized a particular version of trait structure, the Five-Factor Model, and an instrument developed to assess 30 specific traits that define the five factors, the Revised NEO Personality Inventory (NEO-PI-R). Work in the past year has emphasized basic research on the generalizability of the model and its development in adulthood across cultures.

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

Since the publication of the NEO-PI-R in 1992, researchers outside the U.S. have translated the instrument into over 40 different languages, and many have collected data for their own research purposes. In collaboration with these investigators, we have conducted cross-cultural studies of personality structure. In the first of these we reported an analysis of personality structure in Hong Kong Chinese and Japanese samples. Using statistical methods

developed in part in this Laboratory, we showed that the Five-Factor Model is well replicated in both these non-Indo-European languages. Subsequent research has extended this finding to several other languages-in fact, to date no study using an authorized translation, adequate sample size, and appropriate analysis has failed to replicate the five-factor structure of the NEO-PI-R. These data suggested that the Five-Factor Model might be a human universal.

More recently, we turned from personality self-reports to observer ratings. With collaborators in 51 cultures, we asked college students to describe a person they knew well who was a native-born citizen of their country. Ratings were obtained for college-age and adult men and women. With these data we showed that the structure of observer-rated personality replicates that of self-reported personality; that it is found in all cultures examined; and that gender differences in personality traits found in Americans are replicated around the world.

In the first half of this century, anthropologists attempted to assess the modal personality of various groups and relate personality to features of culture. In an updating of this endeavor, recent analyses have examined the mean levels of personality traits across cultures. Analyses of self-report data showed that personality profiles obtained in different languages or versions are comparable to the original, that subgroups (men and women, students and adults) from the same culture have similar personality profiles, and that culture-level analyses of personality traits show the same Five-Factor structure seen in analyses at the individual level. These profiles are geographically organized: adjacent countries such as Spain and Portugal or the U.S. and Canada typically show similar profiles.

We replicated most of these findings in the observer-rating study. The most robust finding was that European cultures (and the related cultures of America, Australia, and South Africa) are more extraverted than Asian and African cultures. These intercultural differences in the mean level of personality traits are generally modest in magnitude; individual differences within each culture are more pronounced than group differences between cultures.

Although there are reliable differences between cultures in the mean levels of personality traits, additional analyses showed that they are not related to commonly-held national character stereotypes. For example, both Americans and Canadians believe that Americans are much more assertive than Canadians, but in fact there is little difference in measured assertiveness.

**Personality Development:** American studies of adult personality development can be summarized by saying that three of the factors (Neuroticism, Extraversion, and Openness) decrease, whereas the other two (Agreeableness and Conscientiousness) increase with age; most of the change occurs between age 18 and age 30. These cross-sectional differences might reflect cohort effects attributable to the historical experience of different generations of Americans. But other nations have had very different histories during the same period, and if age differences are due to cohort effects, it is unlikely that the same kinds of age differences would emerge in cross-sectional studies in those countries. However, reanalysis of data provided by collaborators in twelve countries (including Portugal, Russia, Turkey, Croatia, and South Korea) show very similar patterns of age differences, suggesting that these may perhaps best be interpreted as effects of intrinsic maturation. When data from the observer-rating study were examined, the same age trends were found for Extraversion, Openness, and Conscientiousness across a wide range of cultures. However, effects for Neuroticism and Agreeableness were very weak. It remains to be seen whether self-reports or observer ratings provide the more accurate account of age differences.

Using data from the Baltimore Longitudinal Study of Aging, we examined longitudinal changes in personality traits over 15 years using Hierarchical Linear Modeling (HLM). In general, these results paralleled the cross-sectional results seen around the world. However, some subtle differences were detected. In particular, Neuroticism appears to increase very slightly after age 80, and Conscientiousness decreases after age 70. Individual facets within each of the five factors showed distinctive patterns. For example, the Excitement Seeking facet of Extraversion declined early in adulthood, whereas the Activity facet was stable until after age 50.

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

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

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**Biography:** Dr. Zonderman earned his undergraduate degree in Behavior Genetics from the University of Massachusetts and his doctorate in Psychology from the University of Colorado. After a postdoctoral fellowship in multivariate statistics at the University of California, Berkeley, and academic positions at University of California, Davis and The Johns Hopkins University, he joined NIA as a Senior Staff Fellow in the Stress and Coping Section. Since 1997, he has been Chief of the Cognition Section in the Laboratory of Personality and Cognition. His research interests include individual differences in cognition and personality and their relationship with adult morbidity and mortality, predicting the onset of cognitive impairments and Alzheimer's disease, and the role of genetics in cognitive declines and personality.

**Keywords:**

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

**Recent Publications:**

Nguyen HT, et al. *Psychol Aging* 2006; 21(1): 119-126.

Zonderman AB. *J Geriatr Psychiatry Neurol* 2005; 18(4): 192-195.

Waldstein SR, et al. *Hypertension* 2005; 45(3): 374-379.

Dal Forno G, et al. *Ann Neurol* 2005; 57(3): 381-387.

**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). Cognitive tests have been administered to participants in the BLSA since 1960. Some individuals presently in the study have as many as seven repeated assessments beginning in the 1960's.

The cognitive tests administered to participants in the BLSA reflect our primary interest in pathological cognitive impairments, especially Alzheimer's disease (AD). The cognitive testing program is divided into two batteries, one for longitudinal prediction and another for cognitive and neuropsychological outcomes. The longitudinal repetitions of these tests distinguish typical changes in performance associated with aging from changes in performance which may be associated with disease when combined with neurological and neuropsychological outcomes and clinical diagnoses of AD.

An increasingly important area of research in the Cognition Section focuses on factors that reduce the risk of cognitive declines. An example of this focus is the finding that nonsteroidal anti-inflammatory drugs reduce the risk of Alzheimer's disease. Another example of this focus is based on recent findings that estrogen replacement therapy reduces the risk for both AD and

cognitive declines in post-menopausal women. In an intervention study testing the effects of hormone replacement on cognition, we are examining the effects of estrogen and testosterone in older women and men in conjunction with structural and functional neuroimages.

**Cognitive Declines in Aging Subjects Free of Dementing Diseases:** In people with no signs of dementia, some cognitive abilities resist decline while other abilities show characteristic age-related changes beginning in the 50's or 60's. Research by investigators in the Cognition Section has shown that vocabulary scores generally resist declines, and may increase slowly over time until there are small decreases after the eighth or ninth decades. Immediate visual memory shows a much different pattern of change. We found that errors in immediate visual recall increased exponentially with increased age in both cross-sectional and longitudinal analyses.

We also found that there were different rates of change in separate types of errors over time. Distortions, rotations, perseverations and mislocations were the most frequent errors across all ages. Although older participants made significantly greater errors regardless of error type, the greatest age differences were found for distortions and omissions. Men and women showed similar patterns of age-associated increases in errors, but there was a significant interaction between gender and error type indicating that women across all ages made more omissions and rotations, not other types of errors. Longitudinal analyses showed that distortions, omissions and rotations increased with age. Although women made more omission errors, men showed steeper increases with age.

**Long-Term Predictions of Cognitive Impairment and Dementia:** The onset of cognitive impairment is either a discrete event or a gradual process that manifests over time. We asked whether changes in previous test performance predict evidence of cognitive impairment assessed by the Mini-Mental Status Examination (MMSE) over relatively long intervals. We hypothesized that visual memory administered prior to the MMSE would significantly account for cognitive impairment after controlling for age at mental status exam and vocabulary score (a measure highly related to general intelligence). The correlations between visual memory and MMSE over 6-8 and 9-15 years were .36 and .34 ($p < .05$). These results provide preliminary evidence that mental status can be predicted, at least in part, by earlier performance on cognitive tests. Although the present findings are limited to only these cognitive tests, they provide important evidence that early signs of dementia may be detectable as many as 6-15 years prior to noticeable decline on mental status tests.

Six-year changes in immediate visual memory predicted Alzheimer's disease (AD) prior to its onset. Individuals with diagnoses of AD had larger changes in immediate memory performance over the six-year interval prior to the estimated onset of their disease than subjects without AD. Six-year longitudinal change in immediate visual memory performance also predicted subsequent cognitive performance 6-15 and 16-22 years later, even after adjusting for the influences of age, general ability, and initial immediate memory. These results provide evidence that change in immediate visual memory performance has long-term prognostic significance. These results further suggest that change in recent memory performance may be an important precursor of the development of the disease.

Analyses comparing BLSA participants who developed dementing illnesses with nondemented participants also showed that particular errors in visual memory may be more sensitive markers of impairment than others. More than 5 years before the onset of illness, demented individuals made more distortion errors than participants who did not develop dementing illnesses. In addition, individuals with signs of dementia had significantly greater rates of change in perseverations, rotations, and size errors compared with nondemented participants. These findings suggest that immediate visual memory is an important test for distinguishing normal from pathological cognitive decline and that specific types of errors in short-term memory may be important early markers of dementia.

**Risks and Protective Factors for Cognitive Decline:** If cognitive decline is an important predictor of pathological cognitive aging then it seems reasonable to investigate factors that decrease or increase the risk of cognitive decline. Estrogen replacement therapy (ERT) is increasingly recommended for postmenopausal women due to its potential beneficial effects on physical health in older women. The possibility of a protective effect on cognitive function has also been suggested. In the BLSA, women receiving hormone treatment at the time of testing made significantly fewer errors in immediate visual recall than women who were not on hormone therapy. Less memory change was found in women who started hormone therapy between examinations than women who never received hormone therapy. These findings support the notion that estrogen has a beneficial role on cognitive functioning in aging women.

We continue to extend our present studies on the risks and protective factors for cognitive declines and dementias. In particular, as we gather additional repeat data on which to base reliable measures of cognitive trajectories, we will relate apoE and other genotypic and genomic measures to determine whether there are critical periods of decline. In addition, we will examine the role of modulators of cognitive decline such as hypertension and hormone replacement therapy, particularly in conjunction with MRI anatomical and PET functional assessments. We will also examine chronicity of hypertension, adequacy of blood pressure control, and differential effects and interactions with other known risks such as apoE genotype.

**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. Although we expect data collection to begin in 2004, pilot studies have demonstrated the utility of using mobile medical research vehicles in the community.

**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:**

Resnick SM, et al. *J Clin Endocrinol Metab* 2006; 91(5): 1802-1810.

Moffat SD, et al. *Neurobiol Aging* 2006; 27(7): 965-972.

Tosun D, et al. *Neuroimage* 2006; 29(3): 838-852.

Beason-Held LL, et al. *Neurobiol Aging* 2005; 26(2): 237-250.

**Brain Changes as Predictors of Cognitive and Memory Decline:** The goal of our research program is to identify brain changes which may predict declines in memory and other cognitive functions in older individuals. We use magnetic resonance imaging (MRI) to measure the structure of the brain and positron emission tomography (PET) to measure changes in regional cerebral blood flow (rCBF) during the performance of memory tasks and over time. A variety of risk and protective factors for cognitive impairment and dementia are examined.

**Early Markers of Alzheimer's Disease - Brain Changes in the Baltimore Longitudinal Study of Aging (BLSA)**: We are performing a longitudinal neuroimaging study involving annual MRI and PET scans and neuropsychological evaluations in selected BLSA participants aged 55 and older. This longitudinal design provides a sensitive 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.

The specific goals of this study are: to determine the rate of brain changes with age, including increases in brain atrophy and ischemic/demyelinating white matter abnormalities; to determine the association between trajectories of memory and cognitive change and changes in brain structure and function; and to determine whether risk and protective factors, such as genetic susceptibility factors, hormone replacement therapy, use of non-steroidal anti-inflammatory agents, and vitamins, modulate these relationships. An understanding of the associations between brain and neuropsychological changes, as well as early detection of these changes, will be critical in identifying individuals likely to benefit from new interventions in preventing and treating Alzheimer's disease and other memory problems in the elderly.

MRI data from the first 5 years of our longitudinal brain imaging study have been published. A great deal of effort in our laboratory has focused on the development and validation of an image processing approach that provides sufficient accuracy for longitudinal studies. Quantitative analysis of MRI volumes, including separate estimates of gray and white tissue volumes and cerebrospinal fluid (CSF), revealed cross-sectional age and sex differences in brain and ventricular volumes. The cross-sectional findings from the Year 1 MRI scans indicate less gray and white matter volume and more ventricular CSF in older compared with younger participants; the magnitude of these findings is different across frontal, parietal, temporal and occipital brain regions. There were no detectable changes in lobar brain volumes over a one-year period, but there was a small but significant increase in the volume of the ventricles. In contrast to findings over one-year, four-year follow-up data revealed significant tissue loss in both gray and white matter volumes, even in a subgroup of very healthy elderly. Annual rates of tissue loss were $5.4 \pm 0.3$, $2.4 \pm 0.4$, and $3.1 \pm 0.4$ $cm^3$ per year for total brain, gray, and white volumes, respectively, and ventricles increased by $1.4 \pm 0.1$ $cm^3$ per year (3.7, 1.3, 2.4, and 1.2 $cm^3$ in very healthy). Investigation of age effects on tissue characteristics was performed through quantification of changes in MR signal intensities. We found a significant negative association between age and gray-white contrast at initial evaluation ($r = -.49$, $p < 0.0001$) and longitudinal decline in gray-white contrast over the four-year interval. These longitudinal changes in tissue contrast are unrelated to changes in gray and white matter volumes, indicating that each provides unique information. We will investigate whether these measures of qualitative changes in tissue characteristics enhance our ability to detect cognitive impairment.

We have also examined the effect of Apolipoprotein E genotype on hippocampal volumes and rates of longitudinal hippocampal volume loss. Neuroimaging study participants without dementia who carry the e4 allele (e4+) did not differ from those negative for the e4 allele (e4-) at initial evaluation. In contrast, e4+ individuals showed a faster rate of hippocampal volume loss than age, sex and education matched e4- individuals. Because both the presence of the e4 allele and hippocampal volume loss are risk factors for Alzheimer's disease (AD), our findings suggest one mechanism by which e4 genotype may confer an increased risk for AD.

In addition to morphologic predictors of cognitive impairment and AD, we are investigating the utility of early blood flow changes as predictors of cognitive and memory change. PET-rCBF studies are performed annually as part of our BLSA neuroimaging study. These scans are obtained under three conditions: during rest and the performance of verbal and figural delayed recognition tasks. This procedure is conceptualized as a cognitive stress test to examine age-associated changes in rCBF during increased demand. Our memory tasks produce robust patterns of CBF activation, with increased blood flow in prefrontal cortex (right > left), bilateral insula and visual association areas during memory recall. In addition, voxel-based maps of the associations between age and resting rCBF (normalized for global CBF) demonstrate significant negative correlations between age and CBF in the insular and superior temporal regions, and in visual association cortex (Areas 18 and 19) bilaterally for both men and women. To our knowledge, this sample represents the largest study of associations between age and regional CBF studied with PET and provides a detailed map of age differences in blood flow during a period of accelerating cognitive and memory decline. Complementary to our PET studies, we perform cross-sectional studies of age differences in brain activation using functional MR (fMR) and behavioral probes targeted to specific brain regions. Because our volumetric fMR studies and behavioral studies suggest specific vulnerability of orbital frontal cortex and mesial temporal regions to age changes, we have conducted fMR studies of aging using a delayed-match-sample paradigm to investigate orbital frontal regions and a virtual navigation task to investigate age effects on parahippocampal activation.

**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 replacement therapy on risk for Alzheimer's disease and cognitive and memory decline in older women. We have shown that women in the BLSA

who had ever used estrogen replacement therapy had a reduced risk of developing Alzheimer's disease in comparison with women who had never used hormone therapy. We have also shown that nondemented women in the BLSA who were using estrogen replacement therapy performed better on a test of short-term memory for designs compared with never-users. In a small subgroup of women with memory assessments prior to and following initiation of hormone treatment, the estrogen therapy appeared to protect against age-associated decline in memory. We have also compared ERT users and nonusers who participate in our longitudinal imaging study. ERT users and nonusers showed significant differences in the patterns of brain activation during the performance of memory tasks. Most recently, we reported that ERT users compared with nonusers showed greater relative increases over a 2 year period in CBF in the hippocampus, entorhinal cortex, posterior parahippocampal gyrus, and portions of the temporal lobe. Interestingly, these regions overlap substantially with those showing physiologic abnormalities in early AD and in individuals at increased genetic risk for AD.

These findings, suggesting possible beneficial effects of hormone therapy 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 hormone treatment 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), examines the effects of hormone treatment (combination estrogen plus progestin in women with a uterus and estrogen only in women without a uterus) on longitudinal change in memory and other cognitive functions within the context of the large randomized intervention trial.

**DHEA and Cognition:** Dehydroepiandrosterone (DHEA) is a widely available hormone marketed as an anti-aging dietary supplement beneficial for physical and cognitive health. We have examined the associations of plasma concentrations of DHEA sulfate (DHEAS) and longitudinal changes in DHEAS with cognitive changes in older men in the BLSA. In this large sample, there were no associations between DHEAS concentrations or longitudinal changes in DHEAS and multiple measures of cognitive change. These data offer no support for the hypothesized relationship between endogenous DHEA levels and cognitive health.

**Testosterone and Cognition:** In contrast to the lack of associations between endogenous DHEA concentrations 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. Interestingly, these associations with specific aspects of cognition 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; Randy McIntosh, Ph.D, Rotman Institute, University of Toronto; Sally Shumaker, Ph.D., Steve Rapp, Ph.D., Mark Espeland, Ph.D., Wake Forest University.

# 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 NIH. The Section's goals are to develop original animal and *in vitro* models to understand and quantify dynamic aspects of *in vivo* 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 with positron emission tomography (PET).

**Why Study *In Vivo* Brain Lipid Metabolism?** Phospholipids and their component fatty acids 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. 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 animals and humans could help to elucidate and localize active roles of lipids in normal and diseased brain.

**Methods Employed:** Awake normal and genetically altered (knockout and transgenic) rodent models, tracer kinetics, mathematical modeling, molecular biology, enzyme chemistry, analytical chemistry (HPLC, GC), quantitative autoradiography, PET (humans).

**Summary of Recent Research Findings:** (I) We used kinetic rate data for different lipid metabolic pathways to estimate that brain lipid metabolism consumes up to 25% of net brain ATP utilization, attesting to the active participation of lipids in brain functional activity. (II) We experimentally confirmed our hypothesis that the measured incorporation rates of plasma arachidonic acid (AA) and docosahexaenoic acid (DHA) into brain phospholipids equal their respective rates of metabolic loss within brain, and that AA is lost mainly *via* cyclooxygenase-2 following acute drug-induced neuroreceptor-mediated phospholipase A2 activation. (III) In awake rodents,

we used acute and chronically administered drugs to elucidate and image brain phospholipase A2 activation coupled to different G-protein and ionotropic receptors. (IV) We produced extensive experimental support for our hypothesis that drugs effective against bipolar mania commonly downregulate the brain AA cascade: AA turnover in phospholipids, enzymes that regulate AA turnover, and transcription factors and kinases that influence the expression of the targeted enzymes. We also showed that chronic lithium's selective effects on receptor-initiated signaling *via* AA were consistent with it normalizing the suggested neurotransmission imbalance of bipolar mania. (V) We characterized a rat model of dietary n-3 polyunsaturated fatty acid deprivation, more clinically relevant than the extreme 3-generational deprivation models generally studied. These deprived rats demonstrated bipolar disorder-like behaviors, and changes in brain lipids, enzymes (phospholipase $A_2$ and cyclooxygenases) and neurotrophic factors (e.g. brain derived neurotrophic factor) opposite to those produced by antimanic drugs. (VI) We characterized and imaged upregulated brain AA metabolism in our rat model of chronic lipopolysaccharide-induced neuroinflammation. (VII) We quantified changes in AA kinetics and phospholipase A2 activity in the sympathetically denervated heart of awake rats, as well as in the liver. (VIII) We used PET to image baseline brain AA incorporation in young human volunteers, and showed that incorporation corrected for atrophy did not differ between old and young subjects. We are finding increased brain AA incorporation in Alzheimer disease patients, suggesting that increased incorporation can be used as a marker for neuroinflammation, and are able to measure incorporation in response to visual activation and drugs. We also calculated the human brain's daily rates of consumption of DHA and AA, in relation to current dietary recommendations. (IX) We characterized differential brain molecular and neurochemical responses to neurotoxic and neuroinflammatory stressors in mice in which cyclooxygenase-1 and cyclooxygenase-2 were knocked out.

## Brain Physiology and Metabolism Section Staff

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**Biography:** Dr. Rapoport received his M.D. from Harvard Medical School, interned in Medicine at Bellevue Hospital, New York, and received post-doctoral training at the Department of Physiology, University of Uppsala, Sweden, and at the Laboratory of Neurophysiology, National Institute of Mental Health (NIMH). He was appointed as a tenured scientist at NIMH in 1968, and in 1978 as 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.

**Keywords:**

arachidonic
lithium
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brain
phospholipid metabolism
positron emission
tomography (PET)
diet
docosahexaenoic
phospholipase

**Recent Publications:**

DeMar JC, et al. *J Lipid Res* 2006; 47(1): 172-180.

Basselin M, et al. *J Neurochem* 2006; 96(3): 669-679.

Rao JS, et al. *Neuropsychopharmacology* 2005; 30(11): 2006-2013.

DeMar JC, et al. *J Neurochem* 2004; 91(5): 1125-1137.

Giovacchini G, et al. *J Nucl Med* 2004; 45(9): 1471-1479.

Dr. Rapoport's research focus is the study of brain phospholipid and polyunsaturated fatty acid metabolism in awake unanesthetized rodents and to extend these findings for studies in humans. He has developed unique methods to examine the kinetics of metabolism in appropriate genetic animal models, during signal transduction initiated by centrally acting drugs, following short-term dietary deprivation of n-3 nutritionally essential polyunsaturated fatty acids, and in relation to neuroinflammation; and to relate changes in kinetics to changes in specific enzymes that regulate the kinetics, such as phospholipases A2 and cyclooxygenases. One important contribution is his demonstration that each of three agents that are effective in treating the mania of bipolar disorder – lithium, valproic acid and carbamazepine – when administered chronically to rats at clinically relevant doses, downregulate brain arachidonic acid (AA) metabolism – AA turnover in phospholipids, formation of prostaglandin E2 – and the enzymes that control this metabolism – cytosolic phospholipase A2 and cyclooxygenase 2. These common effects suggest that arachidonic acid metabolism is disturbed in bipolar mania, and they provide a basis for new therapeutic approaches to the disease and for examining lipid metabolism targeting by antidepressants as well. Dr. Rapoport also has shown how short-term dietary n-3 polyunsaturated fatty acid deprivation markedly alters both the brain arachidonic and docosahexaenoic acid cascades, as well as expression of neurotrophic factors such as brain-derived growth factor. Finally, he has extended his imaging methods to examine brain arachidonic and docosahexaenoic acid metabolism in human subjects in relation to health and Alzheimer disease, using appropriate radiotracers and positron emission tomography (PET).

**Collaborators:** Dennis Murphy, National Institute of Mental Health, NIH; Joseph Deutsch, School of Pharmacy, Hebrew University, Jerusalem, Israel; Richard Carson, Department of PET Imaging, Yale University, New Haven, Connecticut; Margaret Weis, School of Pharmacy, Texas Tech University, Amarillo, Texas.

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**Biography:** Dr. Bosetti received her Pharm.D., *magna cum laude*, from the University of Pisa in 1996, and her Ph.D. in molecular and experimental medicine in 2000 from Sant'Anna School, Department of Medicine and Surgery, Pisa, Italy. She joined the National Institute on Aging, Brain Physiology and Metabolism Section, in 1999 and became a tenure-track investigator in April 2004.

**Keywords:**

brain
cyclooxygenase
arachidonic acid
prostaglandin
excitotoxicity
neuroinflammation

**Recent Publications:**

Choi SH, et al. *J Neurochem* 2006; 98(3): 801-811.

Weerasinghe GR, et al. *Brain Res Bull* 2006; 69(6): 614-621.

Rao JS, et al. *Brain Res Mol Brain Res* 2005; 139(2): 217-224.

Bosetti F, et al. *J Neurochem* 2004; 91(6): 1389-1397.

**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 psychiatric diseases. The AA cascade involves the hydrolysis of AA from the stereospecifically numbered (*sn*)-2 position of phospholipids by a phospholipase $A_2$ ($PLA_2$), conversion *via* cyclooxygenase (COX-1 and COX-2), lipoxygenases and cytochrome P450 epoxygenase of some of the unesterified AA into bioactive eicosanoids.

The expression of COX, the enzyme that metabolizes AA to bioactive prostaglandins, is tightly regulated by glutamate neurotransmission, inflammatory mediators, and oxidative stress, factors that are increased in acute and chronic neurological diseases, as well as aging. Thus, in aging as well as in chronic neurodegenerative diseases, cumulative activation of COX over time may lead to downstream cellular changes that impact negatively on neuronal survival and/or synaptic function. While the exact sequence of events that culminate in neuronal death are unknown, an understanding of the genetic characteristics and molecular mechanisms that trigger excitotoxic cell death may offer therapeutic strategies for such disorders. My research goal is to elucidate the role of COX-1 and COX-2 and their products in the mechanism of neurodegeneration in animal models for excitotoxicity, neuroinflammation and neurodegeneration using knockout and transgenic mice models.

**I. Changes in the Arachidonic Acid Cascade During Normal Aging:** Aging is associated with an increased vulnerability of neurons to degeneration and with CNS-related functional deficits. Only few and discrepant data are available about COX expression and activity as well as prostaglandin profile during aging. We have previously demonstrated that $cPLA_2$ and COX-2 protein levels decrease as a function of age, possibly as a consequence of synaptic dysfunction. $cPLA_2$ and COX-2 enzymes have

been shown to be localized at post-synaptic sites in neurons, where they are involved in the stimulatory activity of excitatory neurons. Therefore, their lower levels in the older animals may reflect synaptic changes and remodeling that occurs during brain maturation, and particularly, the loss or dysfunction of dendritic spines occurring in aging. To further test how the brain AA cascade is altered during normal aging, we examined the $PLA_2$/ COX prostaglandin biosynthetic pathway in hippocampus and cerebral cortex of young and aged rats. Our findings indicate that AA metabolism is not altered in the cerebral cortex but is specifically altered during aging in the hippocampus, which is critical for age-related memory deficits.

**II. Involvement of Cyclooygenase in the Mechanism of Brain Excitotoxicity:** The susceptibility of COX deficient mice to neuronal damage has been studied previously by examining the response of these animals to neurotoxic compounds such as MPTP and NMDA. Since it has been hypothesized that excitotoxicity may play a role in neurodegenerative diseases, we are using COX-1 and COX-2 deficient mice to examine the role of these enzymes in mediating excitotoxic neuronal damage. Our data indicate that COX-2 deficient mice are more vulnerable to kainate-induced seizures and neuronal damage and that their increased susceptibility may involve changes in the expression of genes influencing neuronal excitability, as well as differences in brain COX-1 versus COX-2 -derived prostanoid profile.

**III. Role of COX-1 and COX-2 in Neuroinflammation:** Neuroinflammation is a key component in the progression of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. It remains to be elucidated whether inflammation is either the cause or the effect of the neuropathological changes associated with the disease. Microglia play 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 enzymes play a central role in the inflammatory cascade by converting AA to bioactive prostanoids. COX-2 has emerged as a critical pathogenic factor in brain diseases associated with activation of N-methyl-D-aspartate (NMDA) receptors, including stroke and neurodegenerative diseases. Neurons and, to a greater extent, glia also express COX-1, which could also be as detrimental as COX-2 in the process of neuroinflammation. Therefore,

it is important to determine the involvement of each isoenzyme in the mechanisms of neuroinflammation and neurodegeneration. To do so, we are examining the response of COX-1 or COX-2 deficient mice to the intracerebroventricular (icv) injection of lipopolysaccharide (LPS), which is a model of directly activated innate immunity in brain, a potent inducer of AA-mediated inflammatory cascade, and an activator of microglia. Elucidating the role of each COX isozyme in neuroinflammation will help to develop better therapeutic approaches for those neurodegenerative diseases with a marked inflammatory component.

**IV. Microarray Analysis of Gene Expression in Brain of COX-1 or COX-2 Knockout Mice:** COX-1 or COX-2 may, in turn, regulate the expression of genes involved in other biological processes in the brain. To further elucidate the specific function and interactions of COX-1 and COX-2 in the brain and possibly identify other potential targets of COX inhibitors, we used microarray analysis to determine the effect of either COX-1 or COX-2 deficiency on global gene expression in mouse hippocampus and cerebral cortex. We found changes in genes involved in GABA signaling and homocysteine metabolism, that gave some insights into the potential mechanism of increased susceptibility of COX-2 deficient mice to kainate-induced excitotoxicity. Alterations in the expression of genes implicated in fatty acid biosynthesis, cholesterol metabolism, JAK/STAT signaling, glutathione metabolism, and citrate cycle reflect the wide ranging interactions of COX in normal brain physiology.

**Collaborators:** Robert Langenbach, National Institute of Environmental Health Sciences, NIH; Jean Harry, 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; Mark Mattson, Laboratory of Neurosciences, National Institute on Aging, NIH; Stefano Vicini, Georgetown University School of Medicine; Lawrence Marnett, Vanderbilt University School of Medicine; Mary Ann Ottinger, University of Maryland.

# 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**:

Nagababu E, et al. *Nitric Oxide* 2006; 15(1): 20-29.

Ravi LB, et al. *Neurobiol Dis* 2005; 19(1-2): 28-37.

Nagababu E, et al. *Antioxid Redox Signal* 2004; 6(6): 967-978.

Jia Y, et al. *Biochem J* 2004; 384(Pt 2): 367-375.

**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; Darrell Abernethy, M.D., Ph.D., Laboratory of Clinical Investigation, NIA, NIH; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA, NIH; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA, NIH; E. Jeffrey Metter, M.D., Clinical Research Branch, Longitudinal Studies Section, NIA, NIH; Jerome Fleg, M.D., National Heart, Lung, and Blood Institute, NIH; Donald Ingram, Ph.D., Pennington Biomedical Research Center, Baton Rouge, LA.

# Clinical Research Branch

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The **Clinical Research Branch (CRB)** is organized into the Office of the Clinical Director and five sections (Longitudinal Studies Section, Health Disparities Research Section, Translational Research and Medical Services Section, Clinical Support Services Section and the Clinical Information and Data Management Section).

The overall goals of the CRB are: 1) the conduct of major longitudinal studies of aging including the Baltimore Longitudinal Study on Aging (BLSA) and the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) studies; 2) to support and carry out translational research in the major areas of clinical research focus of NIA Intramural Research Program laboratories including longitudinal studies and interventional trials with a focus on cardiology, neurology, endocrinology and oncology disease areas. In the latter, the branch: 1) provides the infrastructure needed to promote high quality clinical research and to ensure patient safety including: protocol review, clinic infrastructure, nursing and physician support, clinical informatics, data and safety management; 2) monitors and maintains quality assurance of the intramural clinical research program; 3) develops and implements clinical program priorities, allocates clinical resources; 4) integrates the established research themes and projects with clinical relevance from various IRP laboratories and branches; 5) evaluates program effectiveness and represents the IRP in management and scientific decision-making meetings within the Institute; 6) coordinates the credentialing of health care providers within the Institute; 7) coordinates and provides clinical research training for NIA staff and fellows; and 8) develops novel approaches for carrying out translational research in an efficient and cost-effective manner.

Ongoing research projects within the branch include: two large longitudinal studies, the BLSA and HANDLS; studies of factors predisposing patients to osteoarthritis and evaluation of muscular changes contributing to disability from this disease and studies of neuromuscular/strength changes with aging. The NIA IRP Cytapheresis Unit is also a part of CRB. This unit conducts cytapheresis on BLSA participants and other normal volunteers providing important clinical research materials (T-cells, B-cells) to program investigators examining immunosenescence, the role of telomeres in human aging and other age related research. In addition, the CRB supports all other clinical studies conducted within the NIA IRP through provision of Protocol Support, Pharmacy Support and Clinical Core Laboratory Support under the Office of the Clinical Director and Nursing Support under the Clinical Support Section of the Branch.

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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 650 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 will also employ novel research tools, mobile medical research vehicles to improve participation rates and retention among non-traditional research participants. Wave 1 of HANDLS pilot phase was successful in addressing its primary goal, assessing the feasibility of conducting a community-based study using a mobile medical research vehicle. The first wave of the pilot allowed refinement of the logistical requirements for the conduct of clinical research focused on several scientific and clinical domains among a low socioeconomic sample. Wave 2 of the pilot phase, currently being conducted in the same West Baltimore neighborhood, will permit further logistical assessments of the mobile medical research vehicle (MRV I) and the newly procured mobile medical research vehicle II (MRV II), evaluation of retention strategies for non-traditional research participants, conduct a 3-year interim follow-up on participants to verify and expand on findings from wave 1 of the pilot, and evaluation of new questionnaires and physical assessments to be used in the upcoming epidemiological study. The epidemiologic phase of the study will commence in early 2004 once the research and development contract for the household listing/population sampling and recruitment is awarded.

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, an initiative has begun to implement 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. In addition, with internal audit functions, access control and security, it will provide an information capture framework that is compliant with increasingly restrictive and complex FDA, Privacy Act, HIPAA and other requirements for generation of research data consistent with Good Clinical Practice guidelines and protection of identifiable health information.

The **Translational Research and Medical Services Section** will support Clinical Investigators on-site at Harbor Hospital. Shari Ling, M.D. (Rheumatology/Geriatrics), Edgar Miller, Ph.D., M.D. (Hypertension/Renal Disease), William B. Ershler, M.D. (Hematology/Oncology), Chee Chia, M.D. (Endocrinology), Samer Najjar, M.D. (Cardiology), and Nazli McDonnell, M.D. (Genetics) are current members of the section. They lead a variety of studies and participate jointly in multidisciplinary studies that cut across subspecialties. A neurologist is being recruited. 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. 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 and the development of age-associated frailty.

**Keywords:**

epidemiology
disability
frailty
inflammation

**Recent Publications:**

Cesari M, et al. *Am J Clin Nutr* 2006; 83(5): 1142-1148.

Ferrucci L, et al. *J Gerontol A Biol Sci Med Sci* 2005; 60(11): 1414-1419.

Ferrucci L, et al. *Am J Med* 2005; 118(11): 1288.

Ferrucci L, et al. *Blood* 2005; 105(6): 2294-2299.

**Research Interests:** Aging is accompanied by a global susceptibility for a number of different diseases and functional decline that cannot be readily assessed by the currently available approaches. However, the mechanism that leads to such a susceptibility to disease and disability in the elderly 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. It is widely recognized that physical and cognitive function are strong predictors of mortality, independent of other traditional medical markers of poor health status. Recent data suggest 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 global susceptibility to disease that specific individuals develop over the aging process. In other words, 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." In studying frailty, we took a different approach. We conceptualized frailty as a dynamic process that becomes evident earlier in life, when specific interventions are more likely to be effective. We also hypothesized 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. 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.

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 will be 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.

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**Keywords:**

aging
longitudinal studies
neuromuscular
cerebrovascular
prostate

**Recent Publications:**

Martel GF, et al. *Exp Physiol* 2006; 91(2): 457-464.

Conwit RA, et al. *BMC Physiol* 2005; 5: 15.

Parsons JK, et al. *Cancer Epidemiol Biomarkers Prev* 2005; 14(9): 2257-2260.

Metter EJ, et al. *J Gerontol A Biol Sci Med Sci* 2005; 60(7): 840-846.

Hougaku H, et al. *Am J Cardiol* 2005; 95(8): 1006-1010.

**Prostate Aging and Disease:** The Baltimore Longitudinal Study of Aging (BLSA) is characterizing normal aging in the prostate and identifying transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition, the research is using information about structure and function of the prostate to improve early detection of prostate disease. Clinical evaluations of prostate growth and function have been made in over 800 men with and without prostate disease and the availability of stored sera and genetic material. Prospectively, BLSA men aged from 30 to 79 have physiological assessment, clinical examination and imaging of their prostate. To date, the major accomplishments have come from analyses of prostate specific antigen (PSA) which show that PSA increases more over a period of years in men who develop BPH than in those who do not. The rate of change in PSA is even greater in men who develop prostate cancer, and the increases go up exponentially 5-7 years before diagnosis. Furthermore, the ratio of free to total PSA is able to distinguish men who develop prostate cancer from those who do not about 10 years before 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 before 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 before 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 was performed to estimate prostate volume as well as the percentage of epithelial and stromal tissue. Longitudinal evaluation of

the prostate size found it 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 3 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 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 increasing muscle strength and power and how they change over time are long-term predictors of increased 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 low muscle strength, muscle power, muscle mass, and slow movement speed are each independent predictors of mortality, arguing that both muscle and nervous system contributions to movement are important for survival.

**2. Exploratory Studies for Alternative Approaches to Exercise:** We have been interested in finding alternative strategies that can be used to maintain muscle integrity and strength. Resistive and aerobic training can help to improve performance in the elderly. However, these are typically supervised activities that require attendance at specific sites or venues. Such activities, while beneficial, are not ideal for all individuals. Therefore, alternative approaches are needed in order to offer an array of opportunities for exercising. We have focused on examining subjects with osteoarthritis of the knee. This group of individuals tends to be sedentary and do very little physical activity. In a pilot study, we examined two approaches to encourage increased activity. First, we examined the use of a pedometer to act as a motivator for walking. We found improvements in how much walking subjects did on a routine basis over a 12 week period. The second approach was the use of a home-based, self-administered electromyostimulation of the thigh muscle in order to strengthen the muscle. The protocol generated up to 40% of the maximal force that subjects were able to generate in extending their knee. This level of stimulation is well tolerated and subjects continued to use the equipment at home over the course of the study. Over a 12 week period, we found significant improvements in muscle strength. At present, we are examining the use of the pedometer in the National Guard for individuals who fail their physical fitness test, and are planning further studies to explore the use of electromyostimulation.

**3. Examination of the Motor Unit and Its Relationship to Muscle Strength and Exercise Response:** A clinical protocol has been developed that explores motor unit function at different levels of muscle exertion in the quadriceps. The goal of this project is to understand the changes that occur in motor units with aging, and the effects of these changes on muscle strength and how these changes affect the exercise response. Over the past 20 years *in vivo* techniques allow for the direct examination of the motor units in humans. Most studies that have examined age-related changes in motor units have focused on old versus young rather than examining the entire adult lifespan. They do not allow for an assessment of where during the lifespan 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 resistance training, smaller units are able to generate fixed forces in the absence of improved strength to a nontraining task. We are now examining changes with age in the BLSA.

**Age-Associated Race and Gender Differences in the Carotid and Intracerebral Arteries:** This project examined intracerebral blood flow velocity and resistance, carotid blood flow velocity, and carotid wall characteristics using doppler ultrasonographic techniques in BLSA participants. The goal is to determine whether differences in either carotid or intracerebral parameters may explain racial and gender differences in stroke and coronary heart disease, and whether changes in arterial characteristics are associated with fitness and frailty. We have found that intimal-media thickness of the common carotid artery increases with age concomitant with dilitation. Greater carotid wall thickness is associated with increasing risk for the development of both overt and silent coronary heart disease after adjusting for age, and common carotid wall thickness is thicker in the presence of asymptomatic coronary disease. Carotid doppler ultrasonography is commonly used during evaluation of cerebrovascular disease. Our findings suggest that examining the carotid wall thickness can increase the suspicion for coronary artery disease. In a related analysis, we found that women who use estrogen replacement postmenopausally show less arterial stiffness than women who are not on replacement. We have also observed that age change in flow velocities in the carotid artery is poorly correlated with the flow velocities in the middle cerebral arteries. We have compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease. Recently, we examined the impact of alcohol use, and serum androgen levels on arterial stiffness. We found that both moderate alcohol usage and higher serum testosterone have a positive impact with lower arterial stiffness.

**Collaborators:** Jerome Fleg, M.D., National Heart, Lung, and Blood Institute, NIH; Robin Conwit, M.D., William Brown, M.D., Tufts University; Daniel Stashuk, Ph.D., University of Waterloo, Ontario, Canada; Benjamin Hurley, Ph.D., University of Maryland, College Park; Laura Talbot, R.N., C.S., Ed.D., Ph.D., Uniformed Services University of the Health Sciences; William Palosky, Ph.D., NASA; S. Mitchell Harman, M.D., Ph.D., Kronos Research Foundation, Phoenix, Arizona.

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**Biography:** Dr. B. Gwen Windham received her medical degree in 1996 from the University of Mississippi, where she also completed an internal medicine internship and residency. Dr. Windham completed fellowship training in geriatric medicine at Johns Hopkins Bayview Medical Center, a Masters of Health Science in the Epidemiology of Aging and a Certificate in Gerontology at Johns Hopkins Bloomberg School of Public Health in 2003 while supported by the Epidemiology and Biostatistics of Aging training grant (NIH T32 AG00247). She was a co-investigator on the Women's Health and Aging Study II, where she was primarily responsible for supervising vision and hearing assessments, medical adjudication of cardiovascular disease, and updating the medications database. She joined the National Institute on Aging, Clinical Research Branch in 2003 as a staff physician and a co-investigator for the Baltimore Longitudinal Study of Aging. Since joining the NIA, she has worked closely with Dr. Luigi Ferrucci and Dr. Shari Ling to create the current standardized physical examination used in the Baltimore Longitudinal Study of Aging. In addition, she implemented new protocols to assess autonomic function and visual fields in participants of the Baltimore Longitudinal Study of Aging.

**Keywords:**

vision
frailty
inflammation
leptin
heart rate variability

**Recent Publications:**

Ble A, et al. *J Gerontol A Biol Sci Med Sci* 2006; 61(3): 278-283.

Ble A, et al. *Arch Intern Med* 2005; 165(19): 2222-2227.

Ble A, et al. *Am J Cardiol* 2005; 96(7): 991-995.

Windham BG, et al. *J Am Geriatr Soc* 2005; 53(7): 1179-1190.

Ferrucci L, et al. *Genus* 2004; LXI(1): 39-53.

**1. Autonomic Nervous System and Aging:** The current paradigm being used for the new design of the Baltimore Longitudinal Study of Aging (BLSA) includes the longitudinal assessment of the physiologic subsystems that are important for mobility. This is consistent with one of the goals of the BLSA, which is to learn how multiple impairments interact with age in causing disability. The neurological system is hypothesized to be one of the critical subsystems of interest in this process. In particular, the autonomic nervous system continuously regulates the internal environment of the body to maintain homeostasis by monitoring and controlling blood flow, blood pressure, extracellular fluid volume and distribution, energy expenditure, and visceral smooth muscle and glands including the liver, pancreas, and adrenals. The autonomic nervous system (ANS) also regulates the immune system at regional, local, and systemic levels. Regionally, sympathetic nerves innervate immune organs, including the thymus, spleen and lymph nodes, releasing neurotransmitters such as norepinephrine. Neurotransmitters then act systemically on immune cells (e.g. lymphocytes); both norepinephrine and epinephrine inhibit proinflammatory cytokines, (IL-12, TNF-$\alpha$, and interferon $\gamma$) and stimulate anti-inflammatory cytokines (IL-10 and transforming growth factor $\beta$). The current design of the BLSA will allow the study of the interactions among the multiple systems regulated by the ANS.

The current accepted non-invasive measure of autonomic function is heart rate variability (HRV), which is a measure of the variability in the time between normal sinus beats rather than variability in the actual heart rate. In the BLSA, HRV is being assessed via 24-hour Holter monitors. We hypothesize that autonomic function will be an important factor in maintaining mobility and other components of physical function and metabolic functions such as glucose metabolism and inflammation. We will examine the potential mediating and modifying roles of inflammation, a strong independent predictor of physical function decline, on autonomic function and how this relates to the development of physical and cognitive decline. The connection between autonomic function and inflammation is also being investigated in a collaborative study established to investigate associations between autonomic function and rheumatoid arthritis, a prevalent and debilitating inflammatory disease of the joints.

Preliminary results from the BLSA demonstrate that lower HRV is associated with higher glucose levels in the latter part of the oral glucose tolerance test in persons without diabetes. Future research will explore whether autonomic function predicts persons at risk of developing glucose intolerance and will attempt to identify characteristics that contribute to maintenance of a healthy autonomic nervous system.

Other developing or ongoing collaborative placebo-controlled studies are evaluating whether the drug glucophage, a biguanide oral hypoglycemic medication used to treat diabetes, affects autonomic function in non-diabetics with metabolic syndrome and whether omega-3 fatty acid supplementation improves autonomic function in persons with kidney disease.

**2. Renal Function in Aging:** Early studies from the BLSA demonstrated that, on average, renal function declines approximately 1ml/min in men after the age of 50. Such studies did not inlcude women. In addition, numerous formulas are now available to estimate the glomerular filtration rate (GFR). However, these formulas are notoriously variable and inaccurate in older adults. Serum creatinine, common to most of these formulas, performs poorly in persons as an indicator of renal function, especially when muscle mass, the major source of creatinine production, is low. This is often the case in frail, older adults. In the InCHIANTI study, grip strength, a surrogate of muscle mass, explained variance of calculated creatinine clearance from timed urine collections above that of the Cockcroft-Gault equation, although the additional clinical value of grip strength in estimating

renal function is questionable. Cystatin C is a cysteine protease inhibitor produced by all human nucleated cells, filtered by the kidney, and degraded by the proximal tubule. It is therefore considered by many to be a reliable indicator of GFR and can be measured in blood. To better understand aging-associated changes in renal function, we are investigating cystatin C as a marker of renal function and its role in the aging process independent of kidney function. Ongoing analyses are investigating longitudinal changes in renal function in women with plans to include assessments of cystatin C in stored samples of men and women participants. This will allow comparisons of cystatin C, creatinine clearance from timed urine collections, estimating equations, and in the near future, a true measure of GFR using iohexol clearance.

Cystatin C has also been shown to be predictive of cardiovascular events, all-cause mortality, cardiovascular-related mortality, incident myocardial infarctions and strokes in persons 65 years and older independent of renal function, and predicted incident peripheral arterial disease better than creatinine or the MDRD equation. It has been demonstrated that higher levels of inflammation are associated with downregulation of cystatin C production by macrophages. There is also some evidence that cystatin C may be a negative biomarker of inflammation. However, cystatin C has been associated with higher levels of C-reactive protein and fibrinogen in the Cardiovascular Health Study, a community-dwelling population of older adults. These reports suggest that the utility of cystatin C extends beyond assessing renal function and perhaps is indicative of other underlying pathophysiologic processes. Additional studies that allow a more complete examination of these relationships is warranted, especially in older persons. Using data collected in the BLSA, we will explore the role of cystatin C in understanding renal function changes with aging, how cystatin C can be used clinically as a measure of renal function in older persons, and attempt to clarify the role of cystatin C in the development of cardiovascular and inflammatory-related diseases.

**3. Vision and Aging:** Contrast sensitivity, visual acuity, visual fields, and to a lesser extent, stereopsis, have all been shown to be related to various mobility measures and activities that contribute to independent living. Longitudinal data are currently available on contrast sensitivity, visual acuity, and stereopsis in the BLSA. Visual field screening tests were implemented in 2006 in the BLSA and are ongoing. We will examine the contributions of these vision domains to mobility and gait function in the BLSA, how these relationships change with age, and how vision interacts with other systems that are important for maintaining mobility.

**Collaborators:** Stefano Fumagalli, M.D., Ph.D., Department of Critical Care Medicine and Surgery, Unit of Gerontology and Geriatric Medicine, University of Florence; John Sollers, III, Ph.D. and Julian Thayer, Ph.D., Ohio State University; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA, NIH; Shari M. Ling, M.D., Clinical Research Branch, NIA, NIH; Shirin E. Hassan, Ph.D., Johns Hopkins University School of Medicine, Wilmer Eye Institute; Edgar (Pete) Miller, M.D., Ph.D., Clinical Research Branch, NIA, NIH; Joan Bathon, M.D. and Jon Giles, M.D., Johns Hopkins University, Division of Rheumatology; Dan Wichterle, M.D., Charles University, Prague; Ravi Varadhan, Ph.D., Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine; Michael Shlipak, M.D., M.P.H., General Internal Medicine Section, Veterans Affairs Medical Center, San Francisco, CA.

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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 at Johns Hopkins University School of Medicine. She joined the Translational Research and Medical Services Section at the National Institute on Aging in September 2004.

**Keywords:**

growth hormone
thymic involution
enteroendocrine hormones
neuroendocrine hormones
glucagon-like peptide-1
glucose-dependent insulinotropic polypeptide
type 2 diabetes mellitus

**Recent Publications:**

Maggio M, et al. *J Endocrinol Invest* 2006; 28(Suppl 2): 15-19.

Chia CW, et al. *Drug Dis Today* 2005; 2(3): 295-301.

**Research Interests:** Dr. Chia's research interests focus on 1) endocrinology of aging, more specifically on how the age-related decline in immune and metabolic functions is associated with changes in endocrine hormones and functions, and whether hormonal interventions can reverse these changes; 2) the physiology of various enteroendocrine hormones such as glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), ghrelin, etc., in relation to aging as well as different metabolic states; 3) translational research in the development of GLP-1 and GIP analogs for treatment of type 2 diabetes mellitus.

Thymic involution begins early in life, as young as one year of age, and continues throughout life. There has been considerable interest in developing means to either restore T cell production in the involuted thymus or delay its rate of decline. The link between the immune and endocrine systems was first proposed 75 years ago when thymic atrophy was observed in rats after hypophysectomies. A number of studies have supported a role for growth hormone (GH) on immune function and thymic growth. Early studies by Dr. Dan L. Longo and colleagues have shown GH to be a potential thymopoietic factor in Snell-Bagg (DW/J) mice by inducing thymic hyperplasia. GH has been shown to stimulate thymopoiesis in aged rodents by increasing both thymic size and cellularity. While the precise mechanism(s) involved in the thymotrophic effects of GH remains to be defined, several theories have suggested that GH increases the number of thymic progenitors recruited from the bone marrow to the thymus, hence promoting thymopoiesis. Despite a number of studies focusing on the effects of GH on thymic activity in rodents, little to no data currently exists on its effects in humans. We are interested in studying whether recombinant human GH (rhGH) can in fact stimulate thymic and immune functions in human. If so, the potential implication of using GH in treating people with diminished immune capacity such as the elderly is substantial.

It is well established that the pathogenesis of type 2 diabetes mellitus (DM) involves insulin resistance and impaired insulin secretion. During the progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to DM, insulin levels are first elevated to compensate for insulin resistance but are reduced when frank diabetes develops due to b-cell failure. However, it is still unclear how these abnormalities arise. Many factors influence insulin action and secretion from b-cells besides glucose. These factors may be derived from the brain, liver, muscle, fat, gut, and other still unknown sources. Enteroendocrine peptides such as GLP-1, GIP, and ghrelin as well as adipokines such as leptin and adiponectin are just a few examples. We are interested in studying the underlying physiology of various enteroendocrine hormones such as GLP-1, GIP, and ghrelin in relation to aging as well as different metabolic states so that we can better understand the progression from NGT to DM and learn more about why insulin resistance develops and insulin secretion decompensates.

As stated before, type 2 diabetes is characterized by insulin resistance and insulin deficiency. After a meal, b-cells of the pancreas sense the rise in plasma glucose and secrete insulin to metabolize the glucose and to reset the plasma glucose level back to the pre-fed state. Insulin is also regulated by two enteroendocrine hormones, GLP-1 and GIP, both of which have very potent insulinotropic effect. GLP-1 is synthesized by the enteroendocrine L-cells in the gut which sense glucose and fat levels in digested food. GIP is synthesized by the enteroendocrine K-cells in the gut and also modulates glucose-dependent insulin release. In human, both GLP-1 and GIP are rapidly inactivated by an enzyme dipeptidyl peptide (DPP IV). In collaboration with Dr. Egan from the Laboratory of Clinical Investigation, we are conducting clinical trials on GIP and GLP-1 analogs which have longer half-lives for potential treatment of Type 2 diabetes.

**Collaborators:** Josephine M. Egan, M.D., Laboratory of Clinical Investigation, NIA, NIH; Dan L. Longo, M.D., Laboratory of Immunology, NIA, NIH; Dennis D. Taub, Ph.D., Laboratory of Immunology, NIA, NIH; Luigi Ferrucci, M.D., Ph.D., Longitudinal Studies Section, Clinical Research Branch, NIA, NIH; Shari M. Ling, M.D., Clinical Research Branch, NIA, NIH; Tamara B. Harris, M.D., M.S., Laboratory of Epidemiology, Demography, and Biometry, NIA, NIH.

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**Biography:** Dr. Ling received her bachelor's degree from the University of Santa Clara, and her master's degree in Direct Service from the Ethel Percy Andrus School of Gerontology at the University of Southern California. She received her M.D. degree from Georgetown University, where she was elected into the AOA honor society. She completed her internship and residency in Internal Medicine at Georgetown University Medical Center, and served a year as Chief Resident in the Department of Medicine at Georgetown University. She subsequently completed a fellowship in Rheumatology at Georgetown University, and a second fellowship in Geriatric Medicine at Johns Hopkins University. She joined the Laboratory of Clinical Investigation in 2000, and transferred to the Clinical Research Branch in 2003.

**Keywords:**

osteoarthritis
mobility function
muscle
biomarkers
inflammation

**Recent Publications:**

Ling SM, et al. *Arthritis Rheum* 2006; 55(2): 256-263.

Maggio M, et al. *Am J Cardiol* 2006; 97(10): 1525-1529.

Maggio M, et al. *J Clin Endocrinol Metab* 2006; 91(1): 345-347.

Maggio M, et al. *J Endocrinol Invest* 2005; 28(11): 116-119.

Research interests include osteoarthritis, mobility function, inflammation and aging.

**Osteoarthritis (OA)** is a painful, chronic, progressive illness for which available therapies remain palliative. OA has been thought of as a disease limited to cartilage degeneration that progressed slowly, resulting in functional limitations late. Our research objectives are to identify early indicators of arthritis development, progression and arthritis-associated functional limitations as a prelude to the development of interventions capable of effectively modifying the disease and preserving mobility function.

**Musculoskeletal Aging, Arthritis and Functional Limitations:** The Baltimore Longitudinal Study of Aging (BLSA) represents a study of normative aging through which knees, hands, bone quality and body composition have been assessed periodically in some participants over the years using conventional radiography, single then dual photon xray absorptiometry respectively. In close collaboration with the investigators in the Longitudinal Studies Section (LSS) of the Clinical Research Branch, we have integrated validated and clinically relevant standardized questionnaires, physical examination and state-of-the-art imaging techniques that will be applied to all eligible BLSA participants. These efforts will allow us to document the process of musculoskeletal aging and delineate the transition from normal aging to the development of common musculoskeletal conditions (i.e. arthritis and osteoporosis) in healthy elderly, but also progression of disease.

Functional limitations are a known consequence of OA, but had been thought of as a late complication. In close collaboration with LSS investigators, we will be able to uniquely delineate the effects of musculoskeletal aging (muscles, bones, and joints) and diseases on mobility biomechanics and function, in the context of other aging body systems and diseases. In collaboration with colleagues at the Johns Hopkins University investigators of the Women's Health and Aging Study, we previously demonstrated that even well-functioning women with OA are more likely to report functional limitations that women without OA. We also observed that functional limitations were associated with lower knee extensor strength, higher body weight, and greater pain severity, but were previously not able to determine which came first. We recently reported the results of the longitudinal data acquired from the Women's Health and Aging Study II (ages 70-79 years) who initially reported no lower extremity limitation (e.g., difficulty walking one-quarter mile) or difficulty in activities of daily living (ADL; e.g., transferring) and were observed over 72 months for transitions to categories of greater functional limitations. Lower extremity OA, higher body mass index, and lower knee extensor strength independently increased the risk of transition to combined lower extremity and ADL difficulty first over 72 months. Two modifiable factors, higher relative weight and lower knee extensor strength, substantially affected these transitions, and therefore warrant increased attention in the management of lower extremity OA.

**Inflammation:** Aging is accompanied by a pro-inflammatory state expressed by the increasing levels of inflammatory cytokines, including interleukin-6 (IL- 6), tumor necrosis factor alpha (TNF-$\alpha$) and interleukin-1beta (IL-1$\beta$). At the same time, aging is associated with a decrease in serum testosterone (T) levels. In close collaboration with LSS investigators of the BLSA, systematic evaluation of these and other inflammatory markers has been integrated into the parent study. These markers will allow us to investigate their relevance to musculoskeletal aging and age-associated disease states.

**Arthritis and Inflammation:** Inflammation is known to contribute to the painful symptoms of arthritis, but vary in its severity depending on the kind of arthritis. We are studying three different forms of arthritis. As mentioned above, osteoarthritis is the most common form of arthritis. Because obesity and excess weight are known risk factors for OA development and are also associated with high inflammatory markers, we are collaborating with members of the Johns Hopkins Arthritis Center to determine the extent to which inflammation changes as overweight or obese adults with arthritis

lose weight, and also whether inflammatory markers might predict the success of weight-reduction efforts. We are also interested in rheumatoid arthritis – a systemic disease that causes progressive destruction of all joints as well as progressive muscle wasting, osteoporosis and cardiovascular disease. Since inflammation may be attenuated by the autonomic nervous system, we have initiated a study of a cohort of patients with rheumatoid arthritis (RA) to determine the associations between inflammatory factors, hormonal homeostasis and autonomic nervous system function as measured by heart rate variability in collaboration with our colleagues at the Johns Hopkins Arthritis Center. Finally, we have initiated a study of polymyalgia rheumatica (PMR) – an intensely inflammatory condition that preferentially affects older adults with the sudden development of pain and stiffness of the shoulder and hip joints although clinically distinct from OA and RA. We are using PMR as a natural model of the effects of acute and systemic inflammation on drug metabolism and anemia.

**Arthritis and Muscle Malfunction:** We have initiated several studies that test the hypothesis that inflammation contributes to OA development, progression and disability. We initiated a clinical study to determine whether muscle strength, mass and function are important determinants of mobility function in adults with knee OA, and if these characteristics are associated with local inflammatory factors and circulating biomarkers. We are recruiting adults 50 years and older with and without osteoarthritis of the knee of comparable age, body weight and physical activity level. Muscle strength, muscle mass and mobility function will be correlated with biomarkers, and compared between the two groups. We are striving to complete our enrollment of 88 participants by the end of next year. Since knee OA risk is substantially increased following surgical removal of the knee meniscus, we will also be studying the inflammatory response and its contribution to muscle malfunction in patients who undergo arthroscopic removal of damaged meniscus.

**Summary:** These observational studies comprise a critical step towards the development of specific interventions capable of directly and effectively counteracting the putative factors responsible for the deleterious consequences of aging on the musculoskeletal system and age-associated diseases.

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**Keywords:**

nutrition
antioxidants
kidney disease
hypertension

**Recent Publications:**

Thornley-Brown D, et al. *Arch Intern Med* 2006; 166(7): 797-805.

Appel LJ, et al. *JAMA* 2005; 294(19): 2455-2464.

Jee SH, et al. *Arch Intern Med* 2005; 165(19): 2299-2304.

Miller ER III, et al. *Ann Intern Med* 2005; 142(1): 37-46.

**Research Interests:** Three inter-related areas of cardiovascular and kidney disease research: hypertension, oxidative damage, and kidney disease prevention.

**Hypertension:** My main research emphasis has been on the effects of diet and lifestyle therapy on blood pressure. There are four feeding studies where I have served as principal investigator, or co-investigator that have improved our knowledge of the effects of macronutrients on cardiovascular disease risk factors including blood pressure and lipids. The Dietary Approaches to Stop Hypertension (DASH study) and DASH-Sodium Trial were major National Heart, Lung, and Blood Institute (NHLBI)-sponsored initiatives intended to provide the scientific rationale for national policy pertaining to nutritional recommendations for the prevention and treatment of hypertension. Following these trials was the 'Diet, Exercise, and Weight Loss - Intervention Trial' (DEW-IT) that documented the effects of comprehensive lifestyle modification (adoption of the DASH diet, regular aerobic exercise, sodium reduction and weight loss) in controlling blood pressure. Recently, the OMNI-Heart Trial (Optimal macronutrient intake for the prevention of heart disease) demonstrated the effects of macronutrient composition of three different isocaloric diets on blood pressure.

Work on mechanistic studies of blood pressure reduction has led to the publication of three meta-analyses of fish-oil, magnesium, or vitamin E supplementation, respectively on blood pressure and mortality. I remain interested in measurements issues. For example there is a large risk of misclassification of hypertension in the elderly due to oscillometric

measurements of blood pressure - following removal of the mercury sphygmomanometer from clinics. This is likely a largely under-recognized problem in the elderly with 'stiff arteries,' as this results in accurate measurements, and is a problem which needs to be addressed. Finally, I have an interest in translation research of this nature is an important extension of earlier work, which demonstrated the efficacy of diet and lifestyle therapy for blood pressure control, and is one of my important research goals.

**Oxidative Stress:** Concurrent with my role in the DASH-Sodium Trial, I have been involved with mechanistic studies on the determinants of the role of oxidative stress markers and in the association with cardiovascular disease risk factors. The infrastructure and design of the DASH and DASH-sodium trial was an ideal setting to determine the effects of dietary patterns on biological markers of oxidative stress. The goal of this research is to determine how weight loss achieved by diet and exercise alters oxidative stress/antioxidant status. We have published several papers on the effects of vitamin supplementation on markers of oxidative stress and on traditional CVD risk factors in the elderly.

**Kidney Disease:** I have great interest in studies of kidney disease progression both in observational study designs and in the setting of a clinical trial. I served as principal investigator of the African-American Study of Kidney Disease and Hypertension (AASK). This National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored trial was designed to determine the impact of three medications and two levels of blood pressure control on progression of kidney disease in patients with hypertensive kidney disease. I also served on a consensus panel of the National Kidney Foundation K/DOQI Advisory Group that published national guidelines for blood pressure management in chronic kidney disease. Translation of clinical research into clinical recommendations or guidelines is an ultimate goal of each project.

**Collaborators:** Lawrence J. Appel, M.D., Eliseo Guallar, M.D., Cheryl Anderson, Ph.D., Brad Astor, Ph.D., and Thomas Erlinger, M.D., M.P.H., Johns Hopkins University Bloomberg School of Public Health; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA, NIH; B. Gwen Windham, M.D., M.H.S., and Luigi Ferrucci, M.D., Ph.D., Clinical Research Branch, NIA, NIH.

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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.

**Research Interests:** 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 the development of the HANDLS study. Specifically, HANDLS is investigating the longitudinal effects of socioeconomic status and race on the development of cerebovascular 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. The study is 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 psychophysiological 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.

# Research Resources Branch

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The **Research Resources Branch (RRB)** provides centralized research resources and research support services essential to the productive conduct of biomedical research by the Intramural Research Program. Personnel in the Research Resources Branch represent a wide variety of talents, skills, and expertise for supporting Intramural investigators.

The Branch is divided into seven sections that focus on particular specialties or types of service. The Sections are Central Laboratory Services; Comparative Medicine; Instrumentation, Design and Fabrication; Library and Information Services; Networks, Computing, and Telephony; Photography and Arts; and Statistical and Experimental Design.

Central Laboratory Services is subdivided into Bioinformatics, Confocal Microscopy, Flow Cytometry, Gene Expression and Genomics, and Proteomics and Mass Spectrometry.

The Comparative Medicine Section includes animal husbandry for a variety of species, producing transgenic and knockout rodents, and the breeding, weaning, and mating of rodents consistent with the genetic model from which they derived.

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 (BU)** 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.

(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 support and training spanning the entire microarray process, from sample preparation through data analysis. Several GRC arrays are available for use within this Unit including the GRC Human 15K cDNA array and the Laboratory of Genetics 26K cDNA murine embroyonic array. This Unit also provides support in the production of custom arrays based on investigator specifications and provides cDNA templates for spotting. New state-of-the-art instruments and software have greatly expanded available services and capabilities of this facility.

(5) The **Proteomics and Mass Spectrometry Unit (MSU)** was formed in 2000 in response to a demand for high-sensitivity amino acid sequencing of purified and blotted proteins. The scope of this service Unit has been expanded to include amino acid sequencing, MALDI-TOF mass spectrometry, and the phosphopeptide mapping of proteins from various cellular populations.

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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:**

gene therapy
adenovirus
proliferation specific antigens
bone marrow progenitors
flow cytometry
cell cycle

**Recent Publications:**

Liu D, et al. *Neuromolecular Med* 2006; 8(3): 389-414.

Yuan B, et al. *Clin Cancer Res* 2006; 12(2): 405-410.

Martomo SA, et al. *Proc Natl Acad Sci USA* 2005; 102(24): 8656-8661.

Wiese C, et al. *Cell Mol Life Sci* 2004; 61(19-20): 2510-2522.

Kruman II, et al. *Neuron* 2004; 41(4): 549-561.

**Cell Cycle Progression and Aging:** The effects of aging on T-cell cycle progression and arrest is the subject of an on-going investigation utilizing multiparameter flow cytometry. Age-related cell cycle properties of human T cells are assessed using simultaneous measurements of DNA content and KI-67 protein expression following co-stimulation with immobilized CD3 antibody and soluble CD28. In T-cells from elderly individuals, there is increased $G_0$ cell cycle arrest that cannot be overcome following subsequent exposure to IL-2. Based on mitotic blocking, the delayed cell cycle entry in T-cells from older donors appears to be independent of early activation events.

**Adenovirus-Based Gene Therapy:** Based on the tropism of wild-type adenovirus (Ad) for the respiratory epithelia and its ability to infect nonreplicating cells, replication-defective Ad vectors were thought to be the ideal approach by gene therapy to correct the physiological defects in the airways of individuals having the inherited human disease cystic fibrosis (CF). Culminating in human clinical trials, Ad vectors have become the prototype for other gene therapy protocols targeting cancers, inherited metabolic deficiencies, and cardiovascular disease. First-generation Ad vectors that had been rendered replication defective by removal of the E1 region of the viral genome (ΔE1)or lacking the Ad E3 region in addition to E1 sequences (ΔE1E3) induce G2 cell cycle arrest and inhibit traverse across the G1/S boundary in primary and immortalized human bronchial epithelial cells, independent of the cDNA contained in the expression

cassette. Arrest is associated with the inappropriate expression and increase in cyclin A, cyclin B1, cyclin D, and cyclin-dependent kinase p34cdc2 protein levels. In some instances, infection with ΔE1 or ΔE1E3 Ad vectors produces aneuploid DNA histogram patterns and induces polyploidization resulting from successive rounds of cell division without mitosis. Cell cycle arrest was absent in cells infected with a second-generation ΔE1 Ad vector in which the entire early region E4 was deleted except for the sixth open reading frame. Current research focuses on the individual proteins encoded by the open reading frames in the E4 viral gene region and their interactions with cellular regulators of proliferation (signal transduction, transcription factors, oncogenes).

**Bone Marrow Progenitor Identification:** Gene transfer to hematopoietic stem cells (HSCs) has been hampered by their low frequency, the lack of positive selection markers, and the reduced potential for self-renewal and multi-lineage differentiation following *ex vivo* retroviral gene therapy. In mammalian bone marrow stained with the dye Hoechst 33342, bivariate flow cytometric analysis of blue and red fluorescence identifies a small cell population, termed SP cells, that constitute primitive HSCs via a mechanism thought to involve *mdr* P-glycoprotein. Using unfractionated non-human primate and murine bone marrow, SP cell staining was found to be an energy-dependent process involving dye efflux, consistent with the hypothesis that this phenomena is mediated by a member of the ATP Binding Cassette family of transporters. However, dye efflux was specifically inhibited by probencid or sulfinpyrazone, implicating involvement of other multi-drug resistance associated proteins or membrane transporters. Cells having the identical staining characteristics and responses as those of bone marrow SP cells are present in cultures of the HL-60 promyleocytic cell line and exhibited a dependence on $G_{0/1}$ entry. SP cells are therefore not unique to bone marrow, but reflect multidrug resistance protein (MRP) functional expression that is present in a small fraction of quiescent cells. Understanding the basis for Hoechst 33342 staining and subsequent discrimination of SP cells from other blood elements provides insights into the functional characteristics of primitive multipotent hematopoietic that may be advantageous for future primate gene transfer protocols.

**Collaborators:** Donna Armentano, Ph.D., Genzyme Corporation; Eugene Rosenthal, Ph.D., Office of the Director, NIH; Edward Gabrielson, M.D., Johns Hopkins; Robert Donahue, D.V.M., National Heart, Lung, and Blood Institute, NIH; Tony Eissa, M.D., Baylor College of Medicine.

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**Biography:** Dr. 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:**

cDNA microarray
bioinformatics
autoimmunity
gene expression
genetic association

**Recent Publications:**

Barnes KC, et al. *J Allergy Clin Immunol* 2006; 118(1): 70-77.

Zhang Z, et al. *Osteoarthritis Cartilage* 2006; 14(5): 449-459.

Ghorbel MT, et al. *Physiol Genomics* 2006; 24(2): 163-172.

Pie JE, et al. *J Nutr Biochem* 2006; 17(3): 157-164.

Cheadle C, et al. *Ann NY Acad Sci* 2005; 1058: 196-204.

Lee PR, et al. *Ann NY Acad Sci* 2005; 1048: 259-271.

Kyng KJ, et al. *Oncogene* 2005; 24(32): 5026-5042.

The **Gene Expression and Genomics Unit** is involved in the design, assembly, 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, Werner's syndrome; among others.

Bioinformatic development and applications include the development of BBID-relational database of biological pathways (http://bbid.grc.nia.nih.gov), the Genetic Association Database (http://geneticassociationdb.nih.gov), a literature mining tool, PubMatrix (http://pubmatrix.grc.nia.nih.gov), as well as the development of an Alzheimer's brain gene expression database.

**Collaborators:** Dr. Jim Eberwine, University of Pennsylvania; Dr. Kathleen Barnes, Johns Hopkins Medical Institutions; Dr. Paul Drew, University of Arkansas; Dr. William Freed, National Institute on Drug Abuse, NIH.

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**Biography:** Dr. Saxena received his Ph.D. degree from the All India Institute of Medical Sciences, New Delhi, India in 1986. After his postdoctoral training in Dr. Ruedi Aebersold's laboratory at the University of British Columbia in 1993, he assumed a faculty position at the University of Manitoba, Canada. In 1999, he moved to Albuquerque, New Mexico to join the Lovelace Respiratory Research Institute as a Scientist. He was also appointed as an adjunct Associate Professor in the College of Pharmacy at the University of New Mexico. In July 2003, Dr. Saxena joined NIA as Head of the Proteomics and Mass Spectrometry Unit.

**Keywords:**

proteomics
bioinformatics
mass spectrometry
liquid chromatography

**Recent Publications:**

He HJ, et al. *J Biol Chem* 2006; 281(42): 31369-31379.

Kumar S, et al. *Mol Pharmacol* 2003; 63(2): 276-282.

Chauhan D, et al. *Blood* 2003; 102(9): 3379-3386.

**Research Interests:** Dr. Saxena's research addresses the biological complexity encoded by the genome by focusing on protein products. He is building on and complements the knowledge gained from genomics. His research strategy is holistic, tightly connecting all aspects of protein analysis and biological context. He focuses on both the expression proteomics (the study of proteins in comparative biological samples) and functional proteomics (the study of how proteins interact with other cellular components in order to determine protein function). To understand the functional characteristics of proteins and their activity, he mainly focuses on cellular localization, tissue distribution, posttranslational modification state, and protein-protein interactions. His studies will help in understanding how dysregulation of signaling and regulatory pathways may lead to human disease. This approach may lead to the discovery and development of drugs through identification and characterization of defective cell signaling pathways.

The **Proteomics and Mass Spectrometry Unit (PMSU)** provides and disseminates state-of-the-art scientific and technical knowledge in proteomics that contribute to the research programs of investigators at the National Institute on Aging. In addition, this facility serves as a resource for consultation, education and training in current proteomics technology and tries to better educate graduate students for success in the increasingly competitive biotechnology sector. Currently, PMSU is capable of moderate to high throughput separation and identification of proteins using a combination of chromatographic and 2-D gel separation techniques. This facility provides three primary services: i) expression proteomics, identifying

the set of proteins expressed in a tissue, cell type, or subcellular fraction; ii) protein-protein interactions, identifying the proteins or complexes of proteins that interact with a given protein; and iii) posttranslational modifications of proteins. The key to our services is mass spectrometry-based partial sequence analysis coupled with computer-assisted searching of genomic and protein sequence databases. These tools allow us to identify unknown proteins, study the modification and turnover of proteins, and characterize protein-protein interactions.

**Collaborators:** Dr. Cathy Wu, Georgetown University; Dr. Don Kufe, Dana Farber Cancer Center; Dr. Surender Kharbanda, Dana Farber Cancer Center; Dr. Mahendra Rao, Invitrogen.

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**Biography:** Dr. Poosala received his undergraduate degree in veterinary science and Masters in Veterinary Microbiology from AP Agricultural University, India. Dr. Poosala received his Ph.D. in Veterinary Pathology from Iowa State University, Ames in 1994. He did his postdoctoral fellowship at the St. Jude Children's Hospital, Memphis and his Laboratory Animal Medicine Residency at the University of Maryland. He was the attending veterinarian and veterinary pathologist at UMD during his residency program. He started his job as veterinary pathologist and research fellow at NIA in 2001.

**Keywords:**

caloric restriction
wound healing
wound repair
tissue microarray
mouse models

**Recent Publications:**

Xu X, et al. *Cell* 2006; In press.

Baur JA, et al. *Nature* 2006; 444(7117): 337-342.

Arumugam TV, et al. *Nat Med* 2006; 12(6): 621-623.

Ravi LB, et al. *Neurobiol Dis* 2005; 19(1-2): 28-37.

**Research Interests:** Dr. Poosala's current research interests include the AGEMAP (Atlas of Gene Expression in Mouse Aging) project, wound healing in different strains of transgenic mice, dietary influences on aging, and topical applicants that promote wound healing in mice.

The **Comparative Medicine Section (CMS)** caters to the NIA Intramural Research Program investigator's laboratory animal experimentation needs. The CMS along with the Institute's Animal Care and Use Committee (ACUC) ensures the humane and safe care and use of laboratory animals. This includes high standards in animal husbandry, technical procedures, regulatory compliance and training. The AAALAC-accredited animal facility features over 30,000 square feet of housing and procedure space in three locations. The CMS staff strives to be true scientific partners and to assist investigators in all phases of research.

CMS has developed a rigorous health surveillance program in the past three years which ensures that research is not compromised by disease outbreaks.The overall health of the animal colony is maintained by a dedicated animal care and veterinary staff, efficient environmental controls and state-of-the-art housing and husbandry equipment.

Also, in the past three years, we have rederived 105 strains of mice, created a truly clean 11,000 mouse barrier facility, eradicated an MHV outbreak and streamlined a rodent import-export process. In addition, the development of a Progeny Animal Database will allow access to a virtual representation of each area of the vivarium and will help ensure accurate tracking of animal numbers. The Institute's ACUC maintains oversight of the animal program

and all ongoing animal activities. To facilitate the submission and review of Animal Study Proposals, the ACUC utilizes a web-based tracking system, the Internet Animal Study Proposal (IASP) module. CMS has also instituted an effective animal user training program that ensures that proper procedures are followed including animal handling, equipment use and facility access.

By working as a team with the research community, the CMS is able to provide consistent, reliable care and support to the NIA animal research program. The new vivarium in the NIH Biomedical Research Center opening in 2007 provides a state-of-the-art housing facility for rodents and CMS is poised to expand its range of services. The Comparative Medicine Section is currently in a position to provide the most comprehensive animal care services to the NIA Intramural Research Program community.

**Collaborators:** Dr. Kevin Becker, Gene Expression and Genomics Unit, Research Resources Branch, NIA, NIH; Dr. Mark Mattson, Laboratory of Neurosciences, NIA, NIH; Dr. Mark Talan, Laboratory of Cardiovascular Science, NIA, NIH; and Dr. Rafael de Cabo, Laboratory of Experimental Gerontology, NIA, NIH.

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**Biography:** Dr. Larry J. Brant received his B.S in Mathematics in 1968 from Frostburg State College, Frostburg, Maryland. He received his M.A. in 1972 in Mathematics from the Pennsylvania State University, University Park, Pennsylvania, and his Ph.D. in 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. *J Magn Reson Imaging* 2006; 23(2): 99-107.

Brant LJ, et al. *J Clin Epidemiol* 2005; 58(7): 701-707.

Ruttmann E, et al. *Circulation* 2005; 112(14): 2130-2137.

Nagae-Poetscher LM, et al. *J Magn Reson Imaging* 2004; 19(1): 27-33.

**Research Interests:** Development of Statistical Methods (in particular, multiple comparisons), Development of Models for Biological Processes, Longitudinal Studies, Aging, Health Screening, Epidemiology of Circumpolar Health, and Combinatorics.

The **Statistical and Experimental Design Section** is responsible for providing statistical and experimental design expertise appropriate to studies of aging and gerontology. Statistical methodology, including the use of Bayesian, maximum likelihood, and numerical computing methods, is applied and developed for longitudinal studies and other studies of aging. A major emphasis is on the development and application of methods that provide cogent, yet easily understood results.

The research and development of the Section currently focuses on several types of statistical models. These include 1) longitudinal multi-level models, which use empirical Bayesian methods to analyze the repeated measurements for all individuals in the study population as a function of the between- and within-subject variance estimates, 2) mixture models for describing and identifying high risk or preclinical disease groups of patients based on the distribution of changes in biological markers over time, 3) survival analysis techniques for studying risk factors in follow-up studies, 4) multiple comparisons for addressing the issue of multiplicity in the testing of group differences in experimental or observational designs, and 5) issues of power, sample size, and other experimental design issues.

Recent efforts in longitudinal data analysis include the development of an imputation method using estimates from a linear mixed-effects model to correct for measurement error bias in traditional risk factor analyses in both logistic regression and proportional hazards regression models. Also, methods for the prediction or classification of preclinical disease states are being developed using longitudinal measurements of biological markers and

multilevel models. Methods developed by the Section have been applied in studies of prostate cancer, pulmonary function, cardiovascular science, long-term caloric restriction in rats, and genome-wide mapping in mice.

**Collaborators:** Dr. Harry A. Guess, Dr. Jay D. Pearson, Epidemiology Department, Merck Research Laboratories; Dr. Emmanuel Lesaffre, Dr. Geert N. Verbeke, Biostatistical Center for Clinical Trials, Catholieke Universiteit, Belgium; Dr. Alena Horska, Department of Radiology, Johns Hopkins University School of Medicine; Dr. H. Ballentine Carter, Dr. Patrick C. Walsh, Department of Urology, Johns Hopkins University School of Medicine.

# 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. Darryl Murray:

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
5600 Nathan Shock Drive
Baltimore, MD 21224-6825

Direct Questions to:

Darryl M. Murray, Ph.D.
NIA IRP Postdoctoral Recruitment Specialist
Phone: 410-558-8470
E mail: murrayda@grc.nia.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:

Ms. Arlene P. Jackson
NIA IRP Predoctoral Recruitment Specialist
Phone 410-558-8121
E mail jacksona@mail.nih.gov

# 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*."

### 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.

# Board of Scientific Counselors
## National Institute on Aging
## Intramural Research Program

**Chairperson**

Lisa Berkman, Ph.D. (06/30/08)
Department of Health and Social Behavior
Harvard School of Public Health
677 Huntington Avenue, Room 709
Boston, MA 02115

**Members**

Arlan Richardson, Ph.D. (08/10/07)
Director, Barshop Institute for Longevity and Aging Studies and Professor, Department of Cellular and Structural Biology
University of Texas Health Science Center
San Antonio, TX 78245-3207

Rudolph E. Tanzi, Ph.D. (06/30/07)
Mass General Hospital-East
Genetics and Aging Unit
Bldg. 114, 16th Street
Charlestown, MA 02129-4404

Barbara E. Bierer, M.D. (06/30/08)
Senior Vice President for Research
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115

Ron Peterson, M.D., Ph.D. (06/30/08)
Department of Neurology
Mayo Clinic
200 First Street, S.W.
Rochester, MN 55905

Karen H. Ashe, Ph.D., M.D. (06/30/09)
Departments of Neurology and Neuroscience
University of Minnesota
420 Delaware Street, S.E.
Minneapolis, MN 55455

David S. Siscovick, M.D. (06/30/10)
University of Washington
UW Cardiovascular Health Research Unit
1730 Minor Avenue, Suite 1360
Seattle, WA 98101

Ellen M. Wijsman, Ph.D. (06/30/10)
Division of Medical Genetics and
Department of Biostatistics
University of Washington
Seattle, WA 98195-7720

Rafael A. Lantigua, M.D. (06/30/10)
Columbia University
622 West 168th Street
New York, NY 10032

Keith Whitfield, Ph.D. (06/30/11)
Department of Psychology and Neuroscience
235 Sociology/Psychology Bldg.
Duke University
Durham, NC 27708

Avery August, Ph.D. (06/30/11)
Associate Professor of Immunology
Center for Molecular Immunology and Infectious Disease
Pennsylvania State University
115 Henning Building
University Park, PA 16802

Gordon J. Lithgow, Ph.D. (06/30/11)
Associate Professor
The Buck Institute
8001 Redwood Blvd.
Novato, CA 94945

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

### JANUARY

Satyajit Rath, National Institute of Immunology, New Delhi, India. "The HIV Nef Protein and Pathways for Immune Evasion."

Chuxia Deng, Ph.D., GDD National Institute of Diabetes and Digestive and Kidney Diseases, NIH. "BRCA1 in Cell Cycle, Genetic Stability and Tumorigenesis."

Melissa Carpenter, Ph.D., Robarts Research Institute, London, Ontario, Canada. "Human ESC Biology."

Debasish Sinha, Ph.D., Department of Ophthalmology, Johns Hopkins University. "NUC1: A Spontaneous Mutant Rat with Blinding Ocular Pathologies."

Cristiana Stellato, M.D., Ph.D., Division of Allergy and Clinical Immunology, Johns Hopkins Asthma and Allergy Center. "Understanding Chemokines: Multi-tasking Molecules in Allergic Inflammation."

Professor James E. Haber, Professor of Biology, Rosenstiel Research Center and Department of Biology, Brandeis University. "Checkpoint Responses and Repair of a Broken Chromosome."

Kouji Matsushima and Takuya Tamatani, University of Tokyo. "A Chemokine, MIP1 alpha as an Anti-cancer Immunotherapeutic Agent."

Alberto Avolio, University of New South Wales, Sydney, Australia. "Blood Pressure and Interdependent Effects of Aging and Arterial Structure and Function."

### FEBRUARY

Fulton T. Crews, University of North Carolina at Chapel Hill. "Neural Degeneration and Regeneration in the Adult Brain: What Can We Learn from Alcohol?"

Yang Hong, Howard Hughes Medical Institute. "Molecular Control of Epithelial Polarity and Cellular Morphogensis in Drosophila."

Liya Shen, Laboratory of Carcinogenesis and Tumor Promotion, National Cancer Institute, NIH. "Complex System-level Integration in Aging and Chronic Disease Susceptibility - Insight from a Large Scale Life Course Epidemiology Study on Gdnf/Gfra1 Mutant Cohort."

Rachel Gerstein, Ph.D., Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School. "Developmental Regulation of the V(D)J Recombinase."

### MARCH

Garth M. Beach, Ph.D., Assitant Professor, Johns Hopkins University School of Medicine. "Imaging Integrated Physiology in Aging Research Methods for the Neuro, Cardiac, and Musculoskeletal Systems."

James L. Riley, Ph.D., University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. "Signal Transduction Pathways Initiated by Members of the CD28 Family."

Sandeep N. Gupta, Ph.D., Senior Scientist, GE Healthcare, Baltimore, Maryland. "MRI Advanced Applications: Examples and Experiences."

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

Yun-Bo Shi, Laboratory of Gene Regulation and Development, National Institute of Child Health and Human Development, NIH. "Cofactor Recruitment and Alteration of Histone Acetylation in Gene Regulation by Thyroid Hormone Receptor During Development."

Tom Misteli, Ph.D., National Cancer Institute, NIH. "Nuclear Architecture in Cancer and Aging."

Ling-Gang Wu, M.D., Ph.D., Synaptic Transmission Unit, National Institute of Neurological Disorders and Stroke, NIH. "Regulation of the Kinetics of Endocytosis at a Central Synapse."

Thomas Hitchcock, Department of Genetics and Biochemistry, Clemson University. "The Mutagenicity and Repair of Deoxyoxanosine."

Brooke Miller, Department of Neurobiology and Physiology, Northwestern University. "Sex and the Sleepy Mouse: Reproductive Defects in a Circadian Mutant."

Al Singer, National Cancer Institute, NIH. "CD4/CD8 Lineage Choice in the Thymus: Timing is Everything."

**APRIL**

Alessandro Vindigni, Ph.D., International Centre for Genetic Engineering and Biotechnology (ICGEB) in Trieste, Italy. "Biochemical and Structural Characterization of the Human RECQ1 Helicase."

Woong Kim, Ph.D., Pennsylvania State University. "Mechanisms that Underlie ER Associated Protein Degradation."

Nader Binesh, Ph.D., Department of Radiological Sciences, David Geffen School of Medicine at UCLA, Los Angeles, California. "Single Voxel 2D MR Spectroscopy."

Kenneth A. Kraft, Ph.D., Department of Radiology, Virginia Commonwealth University, Richmond, Virginia. "Rapid Measures of Aortic Stiffness Using Magnetic Resonance."

Ferenc Livak, M.D., Department of Microbiology and Immunology Graduate Program in Molecular and Cell Biology, University of Maryland School of Medicine. "Molecular and Cellular Diversification of the Immune System."

Bingyun Sun, Department of Chemistry, University of Washington. "Spatially and Temporally Resolved Delivery of Stimuli to Single Cells Using Nanocapsules and Laser Manipulation."

Thomas Johnson, Institute of Behavioral Genetics, University of Colorado. "Genes, Environment and Chance: Coordinately to Specify Length of Life."

Amanda Karsten Damjanovic, Ph.D., Clemson University. "Sympathetic Modulation of Mammalian Prostate Cancer."

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

Shang Tee, Ph.D., Engineering Sciences Lab 225, Cambridge, Massachusetts. "Velocity Fluctuation in Sedimentation."

Aric N. Rogers, Ph.D., Veterinary and Animal Sciences, University of Massachusetts. "Gamma-Delta T Cell Varies with the Expressed WC1 Co-receptor."

**MAY**

Zhongseng Peng, M.D., Ph.D., Department of Comparative Medicine, Johns Hopkins School of Medicine. "A New Hepatic Metastatic Model of Colon Cancer, the Effect of NSAIDs on Tumor and Tumor Metastic Mechanisms."

Xiao Hong Sun, Ph.D., Oklahoma Medical Research Foundation. "Critical Roles of E2A and HEB Proteins in T Cell Development."

Ralf Steinmeyer, Rudolf-Virchow-Zentrum, University of Wurzburg. "FRET, Anisotropy and Motion of Membrane Bound Proteins."

Rohinton Kamakaka, Ph.D., Unit of Chromatin and Transcription, National Institute of Child Health and Human Development, NIH. "The Boundary of the Silenced HMR Domain."

**JUNE**

Jie Shen, Ph.D., Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard University. "Genetic Approaches to Alzheimer's and Parkinson's Disease."

Phillippe Lory, Ph.D., Director of Research, Institute of Functional Genomics, Department of Physiology, University of Montpellier, France. "T-type Calcium Channels Are Exciting Channels for Cells: A Molecular Point of View."

Rong Gao, Ph.D., Department of Chemistry, University of Virginia. "Analysis of the Mechanism of DNA Topoisomerase."

Kirk Brown, Ph.D., RNA Technologies, Dharmacon, Inc. "Recent Developments in siRNA-Based RNA Interference."

Richard Boyd, Ph.D., Monash University, Victoria, Australia. "Restoration of Thymopoiesis and Lymphoid Function in Immunodeficiency States."

Ernest Niggli, Professor at the Department of Physiology, University of Bern and Visiting Professor. "Termination of Calcium-Induced Calcium Release Quested by Two-Photon Microscopy."

Ana Maria Cuervo, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine. "Autophagy in Aging, Oxidative Stress, and Neurodegeneration."

Ronald Fiscus, Ph.D., Department of Physiology, Center for Gerontology and Geriatrics, The Chinese University of Hong Kong. "Anti-apoptotic Effects of the NO/cGMP/Protein Kinase G Signaling Pathway in Neural Cells, Ovarian Cells and Pancreatic Islets - Potential Downstream Involvement of BAD, IAPs and p53."

Paul Wassarman, Ph.D., Chair, Brookdale Department of Molecular, Cell and Development Biology, Mount Sinai Medical School. "Egg Zona Pellucida Glycoproteins and Mammalian Fertilization."

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

**JULY**

Giovanni Paternostro, M.D., Ph.D., Assistant Professor, The Burnham Institute, La Jolla, California. "Clinical Metabolomics."

Jean Hsieh, Ph.D., Department of Mental Health, Johns Hopkins Bloomberg School of Public Health. "Validation of a Performance-based IADL Summary Measure to Identify Difficulties in Complex Activities of Daily Living and Its Association with Cognitive Function: Results from the Women's Health and Aging Study II (WHAS II)."

Timothy J. Kamp, M.D., Ph.D., Associate Professor of Medicine and Physiology, Section of Cardiovascular Medicine, University of Wisconsin Medical School, Madison, Wisconsin. "Understanding the Diversity of L-type $Ca^{2+}$ Channels in the Heart: Beta Subunits and Caveolae."

Sean Parsons, Department of Physiology and Cell Biology, University of Nevada-Reno, School of Medicine. "Elementary Calcium Events in Knurled Myocytes" and "The ORCC in ICC: A PKA Activated Chloride Channel in Gut Pacemaker Cells."

**AUGUST**

J. Christopher Corton, Senior Research Biologist, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. "Role of PGC-1 and Regulated Nuclear Receptors in Caloric Restriction."

William Ramos, Biology Deparment, University of Texas at San Antonio. "Calorie Restriction and Non-Homologous End-Joining in the Aging Brain."

Julissa Villarreal, Biology Department, University of Texas at San Antonio. "The Effects of Age on ERK and CREB Protein in Learning and Memory."

Keji Zhao, Ph.D., Laboratory of Molecular Immunology, National Heart, Lung and Blood Institute, NIH. "Toward Defining a Human Epigenome."

Jun Haginaka, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan. "Uniformly Sized Molecularly Imprinted Polymers for Environmental and Bioanalytical Applications."

Roman Kaliszan, Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdansk, Gdansk, Poland. "pH-Gradient HPLC and its Applications in Analytical and Medicinal Chemistry."

Rafael Rosales, Department of Physics and Mathematics, Universidad Sao Paolo, Brazil. "Hidden Markov Methods for Estimation of Gating Schemes of Ryanodine Receptors and Other Channels."

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

**SEPTEMBER**

Ronald Glaser, Molecular Virology, Immunology and Medical Genetics, The Ohio State University Medical Center, Columbus, Ohio. "Chronic Stress, Aging and Immune Response: Implication for Health."

Juan Carlos Zuniga-Plucker, Department of Immunology, University of Toronto Sunnybrook and Women's Research Institute, Toronto, Ontario, Canada. "From Stem Cells to T Cells, All in a Dish."

Adam Riker, M.D., F.A.C.S., H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida. "Surgical Procurement of Human Melanoma Specimens: Implications for the Genetic Interrogation of Melanoma Behavior."

Yuri Ushkaryov, Ph.D., Department of Biological Sciences, Division of Cell and Molecular Biology, Imperial College of London, South Kensington Campus, London. "Split Personality Receptors: Fragments Stay Apart, Signal Together."

Frank Slack, Ph.D., Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut. "*C. elegans* Lifespan Regulation by a microRNA and It's Target."

Sergei Nedospasov, Ph.D., National Cancer Institute, Frederick, NIH. "Physiological Functions of TNF and Lymphotoxin Revealed by Knockout and Transgenic Mice."

Robert Casero, Ph.D., Professor of Oncology, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins School of Medicine. "Polyamine Catabolism Generated $H_2O_2$: A Therapeutic Target, A Pathologic Problem, or Both."

Masuo Ohno, Ph.D., Department of Physiology, Northwestern University, Feinberg School of Medicine. "Functional Consequences of BACE1 Deletion in Alzheimer's Mouse Models: Genetic Basis for Therapeutic Inhibition of B-Secretase."

**OCTOBER**

Zheng Li, Ph.D., The Picower Center for Learning and Memory, Massachusetts Institute of Technology. "The Double-Edged Sword: Mitochondria and Caspases in Synaptic Plasticity."

Charles D. Smith, M.D., Department of Neurology, Chandler Medical Center, University of Kentucky. "A Study of Brain Morphometry in Aging."

Dean Tantin, Ph.D., Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology. "The POU-Domain Family-Transcriptional Control and Cellular Stress."

Lawrence Samelson, M.D., Chief, Laboratory of Cellular and Molecular Biology, National Cancer Institute, NIH. "Signaling at the T Cell Antigen."

# Invited Speaker Seminars - 2005
## NIA-Intramural Research Program

J. Julius Zhu, Ph.D., University of Virginia School of Medicine. "Oncogenic Ras Signaling at Synapses."

Michael G. Agadjanyan, Ph.D., Professor, The Institute for Molecular Medicine, Huntington Beach, California. "Prototype Alzheimer's Disease Vaccine."

**NOVEMBER**

Michelle Harvie, Ph.D., Breast Cancer Prevention Team, South Manchester University Hospitat, United Kingdom. "Energy Restriction Strategies for Breast Cancer Prevention."

Adrian Clive Hayday, Ph.D., Guy's, King's and St. Thomas' Medical School, King's College, London University, Great Britain. "Mechanisms and Molecules that Integrate the Cellular Immune Response."

William J. Murphy, Ph.D., Professor, Department of Microbiology and Immunology, University of Nevada School of Medicine, Reno, Nevada. "T Regulatory Cell Suppression of NK Cells: Linkage of Adaptive and Innate Immune Responses."

Eugenie C. Scott, Executive Director, National Center for Science Education Inc., Oakland, California. "The 'Science' of Creationism and Public Science Literacy."

Michelle Harvie, Ph.D., Breast Cancer Prevention Team, South Manchester University Hospitatl, UK. "Energy Restriction Strategies for Breast Cancer Prevention."

**DECEMBER**

James N. Weiss, M.D., Kawata Professor of Medicine and Physiology, Chief Division of Cardiology, Director Cardiovascular Research Laboratory, David Geffen School of Medicine at UCLA, Los Angeles, California. "Ventricular Fibrillation: Molecule to Man."

David Sinclair, Associate Professor and Director, The Paul F. Glenn Labs for the Biological Mechanisms of Aging, Harvard Medical School, Department of Pathology. "Genes and Small Molecules that Regulate Lifespan."

# Invited Speaker Seminars - 2006
## NIA-Intramural Research Program

**JANUARY**

Li-Ping He, Research Assistant, Department of Physiology, University of Maryland, Baltimore School of Medicine. "Molecular Determinants of cAMP-mediated Regulation of the $Na^+$-$Ca^{2+}$ Exchanger Expressed Human Cell Lines."

Steven J. Burakoff, M.D., Director of the NYU Cancer Institute, Director of the Skirball Institute of Biomedical Medicine, New York. "HPK1 is a Negative Regulator of T Cell Activation."

David Le Couteur, Centre for Education and Research on Ageing, Concord RG Hospital, Australia. "Aging and the Liver Implications for Vascular Disease."

Richard Lu, University of Texas Southwestern Medical Center, Dallas, Texas. "Cortical Glial Development Regulated by Olig Genes."

Avery August, Ph.D., Associate Professor of Immunology, Center for Molecular Immunology and Infectious Disease, Department of Veterinary and Biomedical Sciences, The Pennsylvania State University. "Regulation of Airway Immune Responses by Tec Family Kinase ITK."

Jahar Bhattacharya, Professor, Physiology and Biophysics, Lung Biology Laboratory, Columbia University and Dr. Rainer Kieffmann, Lunch Biology Laboratory, Columbia University. "Hypoxia Induces Paracrine Signaling by Red Blood Cells in Lung Capillaries."

Gerald W. Zamponi, Ph.D., Professor, Department of Physiology and Biophysics, University of Calgary, Canada. "G Protein Regulation of Presynaptic Calcium Channels."

Robert Edelman, M.D., Marcela F. Pasetti, Ph.D., Jan Cerny, M.D., Ph.D., Marcelo B. Sztein, M.D., University of Maryland. "Mechanisms of Human Immunosenescence: Immune Responses of Healthy Elderly Adults Vaccinated with Recombivax-HB, a Licensed, Recominant Hepatitis B Surface Antigen Vaccine."

David Browe, Postdoctoral Associate, Department of Physiology, Virginia Commonwealth University, Richmond, Virginia. "Integrin Stretch Activates IC1, Swell via AT1 Receptors, PTK, and NADPH Oxidase in Ventricular Myocytes."

Jiong Shi, M.D., Ph.D., Faculty Physician, Department of Neurology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center. "Diagnosis and Management of NPH."

**FEBRUARY**

George Heine, The Ohio State University, Department of Plant Cellular and Molecular Biology, Columbus, Ohio. "The REDOX Control of R2R3 MYB Domains."

Mitchell B. Max, M.D., Pain and Neurosensory Mechanisms Branch, National Institute of Dental and Craniofacial Research, NIH. "Dissecting Chronic Pain Syndromes as Complex Genetic Disorders."

Mary Lou Voytko, Neurobiology and Anatomy Department, Wake Forest University. "Primate Menopause: How Estrogen Affects Cognition and Neurobiology in Monkeys."

Michael May, Ph.D., Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania. "A Novel Signaling and Adapter Protein Associated with the I-Kappa B-Kinase Complex in T Cells."

# Invited Speaker Seminars - 2006
## NIA-Intramural Research Program

Erich D. Jarvis, Ph.D., NIH Director's Pioneer Award Winner, Principal Investigator, Department of Neurobiology, Duke University. "The Neurobiology of Vocal Learning."

**APRIL**

Antonia Cao, Director of Instituto di Neurogenetica e Neurofarmacologia, Cagliari, Italy. "Genetic Disorders in Sardinia."

Yunfei Huang, Ph.D., Research Fellow, Department of Neuroscience, Johns Hopkins University School of Medicine. "Molecular Mechanisms Underlying Neuronal Activity-Dependent Neurite Growth and Synaptic Modification."

Larry Sherman, Ph.D., Associate Professor, Division of Neuroscience, Oregon National Primate Research Center, Oregon Health Sciences University. "Hyaluronan Inhibits Neural Progenitor Cell Differentiation During Neurodegeneration."

Anuradha Lohia, Ph.D., Bose Institute, Calcutta. "Genome Content and Microtubular Assembly is Regulated by a Novel Variant of the Mitotic Kinesin Eg5 in Entamoeba Histolytica."

Philipp von Landenberg, Institute for Clinical Chemistry and Laboratory Medicine of the Johannes Gutenberg University of Mainz. "Infection-associated Antiphospholipid Antibodies: A Clue for Understanding the Pathogenesis?"

Ronald Germain, Deputy Chief, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH. "Understanding T Cell Adaptive Immunity: From Molecules to Models to Movies."

**MAY**

Yunfei Huang, Ph.D., Research Fellow, Department of Neuroscience, Johns Hopkins University School of Medicine. "Epigenetic Regulation of the Early Embryo Specific Gene Oct-3/4."

Einar M. Sigurdsson, Ph.D., Assistant Professor of Psychiatry and Pathology, New York University School of Medicine. "Immunotherapy for Alzheimer's and Prion Diseases."

Henriette van Praag, Ph.D., Staff Scientist, Laboratory of Genetics, Salk Institute for Biological Studies. "Regulation and Function of Neurogenesis in the Adult Hippocampus."

Anindya Dutta, M.D., Ph.D., Harry F. Byrd Professor in Biochemistry and Molecular Genetics, Professor in Pathology, Mellon Prostate Cancer Research Institute, University of Virginia Medical School. "Regulation of DNA Replication in Human Cancer Cells."

Steven R. Houser, Laura H. Carnell Professor of Physiology, Director, Cardiovascular Research Center, Temple University School of Medicine, Philadelphia, Pennsylvania. "Should We Repair or Replace Failing Cardiac Myocytes."

Arne Akbar, Ph.D., Immunology and Molecular Pathology, University College, London. "The Regulation of Telomerase Activity by Co-Stimulatory Signals During Memory CD8+ T Cell Differentiation."

Carl Wu, Ph.D., Chief, Laboratory of Molecular Cell Biology, National Cancer Institute, NIH. "Controlling Gene Expression by ATP-Dependent Chromatin Remolding Complexes."

# Invited Speaker Seminars - 2006
## NIA-Intramural Research Program

Katrina Gwinn-Hardy, M.D., Program Director, Extramural Research Program, National Institute of Neurological Disorders and Stoke, NIH. "What Can Be Done at an Intramural Clinical Center (that isn't easily done anywhere else?)."

William Bara-Jiminez, M.D., Staff Clinician, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, NIH. "Levodopa-Induced Motor Complications in Parkinson's Disease."

Anna Kenney, Ph.D., Memorial Sloan-Kettering Cancer Center, New York, NY. "Sonic Hedgehog Mitogenic Signaling in Brain Development and Brain Tumors."

**JUNE**

Ronald E. Gress, M.D., Chief, Medical Oncology Clinical Research Unit, National Cancer Institute, NIH. "Immune Reconstitution and the Interface of Cancer Chemotherapy and Immunotherapy."

Xiang-Dong Fu, Ph.D., Professor with Tenure, Department of Cellular and Molecular Medicine, University of California, San Diego. "Novel Genomic Technologies that Shape Up Our Understanding of Regulated Gene Expression in Mammalian Cells."

Jennifer L. Fiori, Ph.D., University of Pennsylvania School of Medicine, Philadelphia, PA. "Bone Morphogenetic Signaling Pathways in Fibrodysplasia Ossificans Progressiva."

Kathleen Saumer, Department of Molecular Biology, Princeton University. "Recruitment and Activation of the Saccharomyces Telomerase Complex."

Martin Morad, Professor of Pharmacology and Medicine, Georgetown University School of Medicine, Washington, D.C. "40 Years of Calcium Signaling... and Finally Diversity."

Jennifer Van Eyk, Ph.D., Director, Johns Hopkins NHLBI, Bayview Proteomics Center. "Stem Cells and Proteomic Insights."

Vincenzo Casolaro, M.D., Ph.D., Assistant Professor of Medicine, The Johns Hopkins University School of Medicine. "Regulation of Human Th1 and Th2 Cells: Some Second Thoughts."

Justin Blethrow, B.A., Department of Cellular and Chemical Biology. "Rapid Parallel Identification of Protein Kinase Substrates."

**JULY**

Michael O'Connell, University Orthopaedics, Southampton, UK. "Heparan Sulfate Proteoglycans in BMP Signaling in Fibrodysplasia Progressive."

**AUGUST**

Madhav A. Thambisetty, Institute of Psychiatry, London, UK. "Novel Approaches to Peripheral Biomarkers in Alzheimer's Disease."

Giulio Maria Pasinetti, M.D., Ph.D., Mount Sinai School of Medicine, Department of Psychiatry. "Neuronal SIRT1 Activation as a Novel Mechanism Underlying the Prevention of Alzheimer's Disease Amyloid Neuropathology by Calorie Restriction."

# Invited Speaker Seminars - 2006
## NIA-Intramural Research Program

**SEPTEMBER**

Cara J. Gottardi, Ph.D., Department of Medicine, Division of Pulmonary and Critical Care, Feinberg School of Medicine, Northwestern University. "Coordination of Cadherin-based Adhesion and Signaling by β Catenin."

Barbara Osborne, Ph.D., University of Massachusetts, Department of Veterinary and Animal Science. "Notch as a Regulator of T cell Function."

Bradley Willcox, M.D., University of Hawaii, Pacific Health Research Institute. "Novel Predictors of Healthy Aging: The Hawaii Lifespan Study."

**OCTOBER**

Malene Hansen, Ph.D., University of California, San Francisco, Department of Biochemistry and Biophysics. "Discovery of New Longevity Genes in the Nematode *C.elegans*."

Rafael Casellas, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH. "New Roles for ATM and Tip60 in DNA Repair and B Cell Ig Gene Recombination."

Ravi Sirdeshmukh, Centre for Cellular and Molecular Biology, India. "Proteomics and Biomarkers for Cancer."

Jorg Goronzy, Lowance Center for Human Immunology, Emory University School of Medicine. "Life Transitions of Memory T Cells."

Calvin Harley, Geron Corporation. "Telomerase and hESCs: Science, Translational Research, and Product Development at Geron Corporation."

Vernon Sondak, M.D., H. Lee Moffitt Cancer Center and Research Institute. "Melanoma and Age: What is the Relationship?"

Andrew McLachlan, Ph.D., Center for Education and Research on Aging, Concord RG Hospital, Sydney, Australia. "Understanding Herb-Drug Interactions."

Lawrence A. Loeb, M.D., Ph.D., Department of Pathology, University of Washington. "Random Mutations in Human Cancers: Origin and Consequences."

**NOVEMBER**

Ursula Storb, M.D., Department of Molecular Genetics and Cell Biology, University of Chicago. "How Does the Deoxy Cytosine Deaminase, AID, Create Mutations in Immunoglobulin Genes?"

Andres Buonanno, Ph.D., National Institute of Child Health and Human Development, NIH. "The Neuregulin-ErbB Signaling Pathway Regulates Synaptic Plasticity in the CNS: Possible Implications in Schizophrenia."

Matthew D. Stone, Ph.D., University of Minnesota, Department of Biochemistry, Molecular Biology and Biophysics. "Generation of Novel Anti-Coagulant Proteins Through Chemical Modification of Factor Villa."

# Research In Progress Seminars
## NIA-Intramural Research Program

### 2005

Ramaiah Nagaraja, Ph.D., Laboratory of Genetics. "Gene Content and Candidates for Embryonic Lethal Mutants in the Giant Growth Regulatory T-complex Region on Mouse Chromosome 17."

Arsun Bektas, Ph.D., Laboratory of Genetics. "Klotho Genotype Associations with Complex Disorders in Aging."

Vishwa-Deep Dixit, Ph.D., Laboratory of Immunology. "Ghrelin: A Metabolic Key to Immunity."

Didier Brochet, Ph.D., Laboratory of Cardiovascular Science. "Calcium Blinks in the Heart."

Ranjan Sen, Ph.D., Laboratory of Cellular and Molecular Biology. "Functional Consequences of NF-κB/IκB Shuttling."

Alexander Weiss, Ph.D., Laboratory of Personality and Cognition. "Personality Trait Structure and Development in Primates."

### 2006

Chang-Yi Cui, M.D., Ph.D., Laboratory of Genetics. "Ectodyplasin Regulates Lymphotoxin, Wnt, BMP, SHH Pathways for Hair Follicle Development."

## Index of Principal Investigators

## Index of Keywords

### Symbol



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### B



### C

**U**



**V**



**W**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**Public Health Service**
**National Institutes of Health**
**National Institute on Aging**