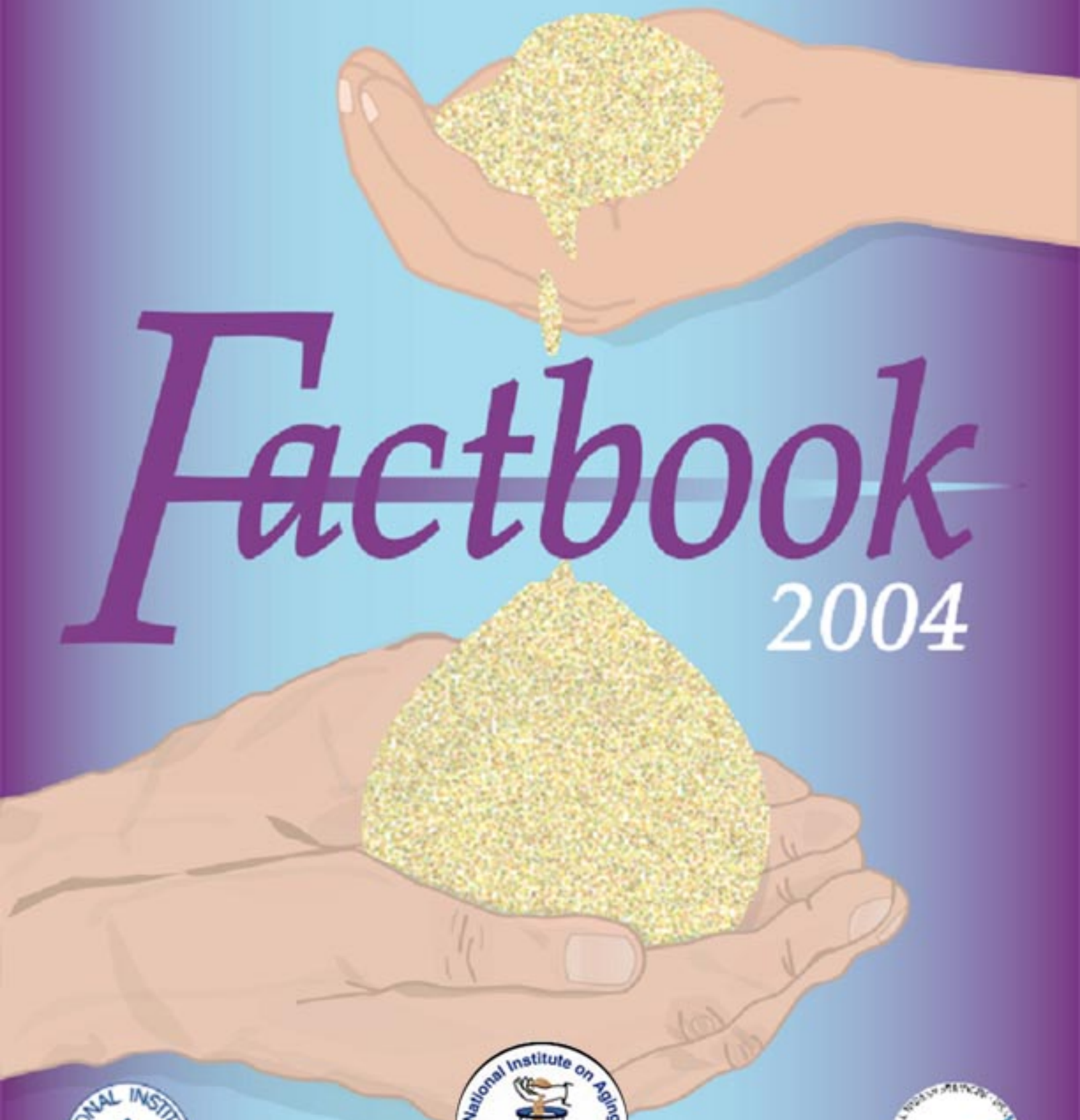
National Institute on Aging
Intramural Research Program



Factbook

2004

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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 in the Clinical Center 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 and collaboration 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.

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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 nine functional units, each headed by a tenured or senior scientist: the Cardiovascular Gene Therapy Unit, the Cardiovascular Biology Unit, the Calcium Signaling 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 Vascular structure Cardiovascular function at rest	Drugs Postural reflexes Exercise stress	Age, gender, race, socioeconomic status Disease (CAD, hypertension), risk factors, and prevention Genetics
Intact Animals **Heart Failure** **Hypertension** **Aging** **Preconditioning** **Arterial Injury**	Arterial remodeling of aging Cardiac remodeling post myocardial infarction, endogenous na/K ATPase ligands Gene expression VSMC proliferation and migration	Novel drugs	Age Growth factors Diet Thyroid status Local or systemic drug delivery Gene therapy Stem cell therapy
Isolated Heart or Cardiac Muscle	Myocardial contractile properties, excitation-contraction coupling, Ca2+ signals, action potentials	Ischemia Anoxia, hypoxia Free radicals Neuropeptides Novel drugs Stretch	Age Diet Exercise Hyperthyroid state Cardiomyopathy Heart failure
Cardiac Cells **Myocytes** **Fibroblasts**	Membrane ionic channel currents Cardiac cell contraction Cystolic Ca2+ signals Mitochondrial Ca2+ signals Sarcolemmal ion transport Sarcoplasmic Reticulum function Apoptosis	Receptor stimulated second messengers Neuropeptides Stretch Anoxia, hypoxia Free radicals Novel drugs Anesthetics Growth factors Novel endocardial factors Novel endothelial factors	Development Age Disease Heart failure Hypertension Diet Growth factors Hypoxia
Vascular Smooth Muscle and Endothelial Cells	Cystolic Ca2+ and pH regulation Proliferation and secretion Chemotaxis and invasion Matrix regulation Tubulin/microtuble dynamics Differentation regulation Angiogenesis	Shear stress Receptor agonists/antagonists Growth factors Anoxia, hypoxia Stretch Anti-microtubule agents Matrix degradation Antisense inhibition and gene overexpression	Atherosclerosis Arterial injury Aging Dedifferentation
Stem Cells	Mechanisms of pluripotency	Homing factors	Differentation into heart and vascular cells
Sub-Cell Organelles	Na/K transport systems Sarcolemmal ion channels Sarcoplasmic reticulum Ca2+ cycling Mitochondrial membrane potential regulation, ATP K+ channels	Ionic composition Adenine nucleotides Neuropeptides Ischemia, anoxia Drugs Reactive oxygen species	Age Heart failure Hypertension
Molecules	Genomics-SAGE cDNA assays Control mechanism of gene expression in heart and vascular cells, ryanodine receptors, IP 3 receptors, G proteins Expression of (1) isozymes: e.g. myosin heavy chain, Na-K ATPase, (2) proteins HSP oncogenes, ANF, pump or channels proteins (e.g. SR Ca ATPase, sarcolemmal Ca 2+ and K+ channels	Ionic transportation mechanisms Stretch mechanisms Growth factors Neuropeptides Nitric Oxide Reactive oxygen species	Age Hormones Hypertension Heart failure Genetic manipulation

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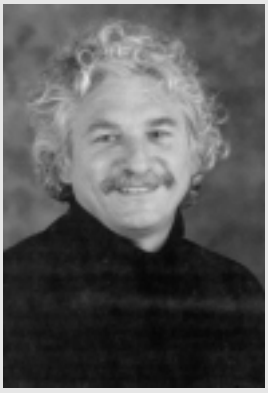

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Biography: Dr. Lakatta received his M.D., Magna cum laude, at Georgetown University School of Medicine. Following an internship and residency in Medicine at Strong Memorial Hospital, University of Rochester, Rochester, N.Y., he trained in basic research for two years at the NIH. Subsequently, he completed his cardiology fellowship at Georgetown and Johns Hopkins University Schools of Medicine. This was followed by a year of basic research training in the Department of Physiology, University College and the Cardiothoracic Institute, London England. Dr. Lakatta is the Chief of the Laboratory of Cardiovascular Science, National Institute on Aging. He also holds adjunct appointments as Professor, Department of Physiology, University of Maryland School of Medicine, and Professor, Cardiology Division, Johns Hopkins School of Medicine. Dr. Lakatta is recognized 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 Research, the Association of American Physicians. 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:

Xiao RP, et al. *Circulation* 2003; 108(13): 1633-1639.

Scuteri A, et al. *J Hypertens* 2003; 21(7): 1339-1346.

Boheler KR, et al. *Proc Natl Acad Sci* 2003; 100(5): 2754-2759.

Vinogradova TM, et al. *Circ Res* 2002; 90(1): 73-79.

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.

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

Keywords:

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

Recent Publications:

Yang D, et al. *Circ Res* 2003; 92(6): 659-667.

Zhu WZ, et al. *J Clin Invest* 2003; 111(5): 617-625.

Xiao RP, et al. *Circulation*

Pan Z, et al. *Nat Cell Biol* 2002; 4(5): 379-383.

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

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

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

Nanoscale Excitation-Contraction Coupling in Heart Cells: The central theme of our research has been on cardiac excitation-contraction coupling (ECC) mechanism at the molecular level. By visualizing the smallest packets of trigger Ca^{2+} entry, Ca^{2+} sparklets from openings of single L-type Ca^{2+} channels in the plasma membrane, we were able to determine the fidelity, kinetics, and stoichiometry of Ca^{2+} sparklets in activating the elementary release Ca^{2+} signals, Ca^{2+} sparks from clusters of ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR). We found that the sparklet-spark coupling in cardiac myocytes is governed by the classic Ca-induced Ca^{2+} release (CICR) mechanism, and follows a stochastic process of the first order kinetics. This not only delineates ECC at an unprecedented resolution, but also allows for the first real-time visualization of intermolecular communication in intact cells. We then investigated RyR interactions within their two-dimensional paracrystalline arrays in cells, and the long-standing question on the exact nature of Ca^{2+} sparks in terms of the number of RyRs involved. With the aid of Ca^{2+} sparklets as the optical standard, we measured spark Ca^{2+} fluxes and discovered the quantal substructure of Ca^{2+} sparks. The quantal analysis indicated that Ca^{2+} sparks are not all-or-none activation of the RyR arrays. Rather, a spark involves a variable number (1-8) of RyRs, which comprise only a small fraction of the array of typically 100 RyRs. Interplay of RyRs confers new regulatory mechanisms to controlling Ca^{2+} release, which was heretofore unappreciated. In particular, RyRs in multi-channel sparks are subject to negative feedback regulation and manifest a thermodynamically irreversible behavior, resulting in brief and stereotyped release duration. Furthermore, we explored possible mechanism underlying termination of ECC. Our results provided unequivocal evidence that individual Ca^{2+} release units become refractory after firing of a spark, supporting the notion on use-dependent inactivation of RyRs that we proposed in the last Board of Scientific Counselors review. These three major advancements have considerably deepened our understanding of the fundamental mechanisms concealed in the nanoscopic space of ECC machinery in the heart.

Termination of Ca^{2+}-Induced Ca^{2+} Release: In cardiac myocytes, Ca^{2+} release from RyR in the SR is activated by the Ca^{2+}-induced-Ca^{2+} release (CICR) mechanism. CICR, with its inherent positive feedback, is expected to operate in an “all-or-none” fashion. In order to generate Ca^{2+} transients of graded amplitude and robust stability, a regulatory mechanism must exist to counteract the regenerative CICR. Several mechanisms, including inactivation, adaptation, and stochastic closing of RyRs have been proposed, but no conclusive evidence has yet been documented. Our recent study has shown

that FK506-binding protein (FKBP), an immunophilin and accessory protein of RyR, constitutes a prominent regulator of CICR via shortening the duration of the elementary release events (Ca^{2+} sparks) and accelerating the desensitization of RyR to Ca^{2+}. To elucidate the primary termination mechanism of CICR, we first developed a novel fluorescent technique. By combination of a fast, linear Ca^{2+} indicator, Oregon Green BAPTA 5N, and a high concentration of Ca^{2+} chelator, EGTA, Ca^{2+} release was visualized as discrete "Ca^{2+} spikes" restricted to T tubule-SR junctions, each consisting of single or a few Ca^{2+} sparks. Increasing the open duration and promoting the reopens of Ca^{2+} channels with the Ca^{2+} channel agonists, FPL64176, did not prolong or trigger secondary Ca^{2+} spikes, even though 2/3 of the SR Ca^{2+} remained available for release by caffeine. Latency analysis revealed that Ca^{2+} spikes coincided with the first openings, but not with the reopens, of L-type Ca^{2+} channels. Furthermore, after an initial maximal release (e.g., at 0 mV), even a multi-fold increase in unitary Ca^{2+} current produced by a hyper polarization step to -120 mV, failed to trigger additional release, indicating an absolute refractoriness of RyRs. When the release was submaximal (e.g., at +30 mV), tail currents upon hyper polarization did activate additional Ca^{2+} spikes; confocal images revealed that the tail release originated from those unfired during depolarization. These results indicate that Ca^{2+} release is terminated primarily by a highly localized, use-dependent inactivation of RyRs, but not by stochastic closing and adaptation of RyRs, or depletion of SR Ca^{2+} in intact ventricular myocytes.

Collaborators: William C. Balke, University of Maryland; Wayne Chen, University of Calgary; Xiang-Dong Fu, University of California San Diego; Robert Dirksen, Rochester University; Jianjie Ma, University of Medicine and Dentistry of New Jersey; Eduardo Rios, Rush University; James Russell, National Institute of Child Health and Human Development, NIH; Arnold Schwartz, Cincinnati University; James Sham, Johns Hopkins University; Yibin Wang, University of California Los Angeles; Caihong Wu, Peking University, Beijing, China; Zhuan Zhou, Institute of Neuroscience, Shanghai, China; Edward G. Lakatta, Michael D. Stern, Rui-Ping Xiao, Kenneth Boheler, Laboratory of Cardiovascular Science, NIA.

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

Keywords:

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

Recent Publications:

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

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

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

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

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

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

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

(ROS). Recent research is focusing on the relationship between the mitochondrial electrochemical gradient, ROS production, induction of the permeability transition pore, and functional sequella, including ischemia/reperfusion and myocardial preconditioning.

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

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

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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. *Proc Natl Acad Sci USA* 2003; 100(20): 11612-11617.

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

Fedorova OV, et al. *Circulation* 2002; 105(9): 1122-1127 .

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

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

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

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 mice 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-adrenerging 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 the consensus is that similarly to β-1 AR, activation of β-2 AR during CHF is harmful. Recent research in LCS 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.

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Keywords:

cardiovascular aging
arterial stiffness
ventricular-vascular coupling
congestive heart failure

Recent Publications:

Eldadah ZA, et al. *Chest* 2002; 121(4): 1377-1378.

Research interests focus on cardiac and vascular structure and function, and include 1) characterizing their determinants to identify potential targets for interventions; 2) exploring how they interact with and influence cardiovascular aging and reserve, both in the absence and in the presence of subclinical as well as clinical diseases; and 3) evaluating how they modulate the response to standard and novel therapies for hypertension and heart failure. Research interests also include exploring non-pharmacological interventions in heart failure.

The research agenda of the Human Cardiovascular Studies Unit includes four major programs. The first program focuses on exploring the age-associated changes in vascular structure and function, including arterial wall thickness and compliance, 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 vascular 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. We have an interest in both systolic heart failure as well as the elusive diastolic heart failure, which afflicts and is particularly burdensome in the elderly. The fourth program is a translational cardiovascular research program that is actively being developed, and which, we hope, will capitalize 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 compliance, investigating how they interact with aging, lifestyle, the environment, and various disease states. We are also exploring how they impact 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 vascular 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 cardiovascular 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 address aspects of both systolic heart failure as well as diastolic heart failure, which afflicts and is particularly burdensome in the elderly. Epidemiologic data indicate that up to 40% of patients with CHF have normal ejection fractions. However, there is a paucity of studies on this distinct clinical entity. As a result, the pathophysiology of heart failure with preserved systolic function remains poorly elucidated, and effective therapies — which are badly needed — have not been developed yet.

Collaborators: Edward Lakatta, M.D., David Anderson, Ph.D., Mark Talan, M.D., Ph,D., Laboratory of Cardiovascular Science, NIA; Luigi Ferrucci, M.D., Ph.D., Wallace Johnson, M.D., Shari Ling, M.D., E. Jeffrey Metter, M.D., Clinical Research Branch, NIA; Reubin Andres, M.D., Laboratory of Clinical Investigation, NIA; Michele Evans, M.D., Laboratory of Cellular and Molecular Biology, NIA; Don Ingram, Ph.D., Laboratory of Experimental Gerontology, NIA; Dan L. Longo, M.D., Laboratory of Immunology, NIA; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA; Joseph Rifkind, Ph.D., Molecular Dynamics Section, NIA; David Schlessinger, Ph.D., Alexei Sharov, Ph.D., Laboratory of Genetics, NIA; Alan Zonderman, Ph.D., Laboratory of Personality and Cognition, NIA; Edward Kasper, 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; Steven Gottlieb, M.D., University of Maryland; Kim Sutton-Tyrrell, Ph.D., University of Pittsburgh.

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

Keywords:

calcium signals
excitation-contraction coupling
ryanodine receptors
mathematical modeling

Recent Publications:

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

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

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

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

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

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

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

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

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

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

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Keywords:

Na, K-ATPase
endogenous inhibitors
hypertension
protein kinases

Recent Publications:

Priyadarshi S, et al. *Kidney Int* 2003; 63(5): 1785-1790.

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

Fedorova OV, et al. *Hypertension* 2002; 39(2): 298-302.

Fedorova OV, et al. *Circulation* 2002; 105(9): 1122-1127.

Fridman AI, et al. *J Hypertens* 2002; 20(6): 1189-1194.

Two endogenous digitalis-like inhibitors of the Na/K-ATPase (NKA), sodium pump ligands (SPLs), endogenous ouabain (EO) and marinobufagenin (MBG), coexist in mammalian tissues, are responsive to NaCl loading and plasma volume expansion, and differ with respect to their targets (α-3 and α-1 isoforms of NKA). SPLs play an important role in the pathogenesis of NaCl sensitive hypertension. In NaCl-loaded Dahl salt sensitive rats (DS), brain EO triggers peripheral production of MBG, a natriuretic and a vasoconstrictor, via an ATII sensitive pathway. Administration of anti-MBG antibodies to hypertensive DS lowers the blood pressure.

Pharmacological antagonism of SPLs may open new possibilities in the treatment of hypertension and its complications. Thus, PKC-induced phosphorylation sensitizes α-1 NKA to MBG. An interaction of PKC-dependent phosphorylation and MBG on NKA activity underlies the synergistic vasoactive effects of MBG and other endogenous vasoconstrictors in hypertension and is a target for antihypertensive therapy of hypertension. Accordingly, chronic treatment of hypertensive DS rats with cicletanine, a compound, which directly inhibits PKC, produces a marked antihypertensive effect that is associated with desensitization of α-1 NKA to MBG.

Changes in SPLs levels and myocardial NKA isoforms accompany development of compensatory left ventricular hypertrophy (LVH) and transition to chronic heart failure (CHF) in DS. Development of LVH was accompanied by elevated MBG, an increased sensitivity of cardiac NKA to MBG, and an up-regulation of α-1 NKA protein in the myocardium. The transition to CHF was accompanied by a decrease in α-1 NKA protein, a reduction in plasma MBG, and a decrease in the sensitivity of NKA to MBG. Conversely, neonatal α-3 NKA was produced within the failing myocardium, plasma EO rose, and the sensitivity of NKA to ouabain enhanced 7-fold

compared to control. Thus, a shift in endogenous NKA ligands production is linked to a shift in myocardial NKA isoforms in DS during LVH and CHF. In patients with CHF, MBG exhibits progressive increases similar to ANP, varies with CHF severity, and correlates with LV systolic function. In CHF, the concurrent production of two natriuretic hormones, a vasorelaxant, ANP, and a vasoconstrictor, MBG, potentiate each other's natriuretic effects, but may offset their respective vasoactive actions. ANP differentially modulates the inhibitory effect of MBG on the sodium pump via PKG-induced phosphorylation/dephosphorylation of the NKA.

SPLs contribute to blood pressure elevation in preeclampsia, which complicates up to 10% of pregnancies, and is a major factor in worldwide maternal mortality. In three studies, DIGIBIND (i.e. Fab fragments of digoxin antibodies), due to its ability to bind circulating SPLs, decreased blood pressure in preeclamptic patients. Recently, we have shown that MBG (rather than EO) becomes elevated in preeclampsia. In collaboration with the University of Montreal and Tulane University (New Orleans, LA), we established that renal excretion of MBG increases in pregnant rats with experimental preeclampsia. Moreover, administration of anti-MBG antibodies to these rats was associated with a sustained decrease in blood pressure together with activation of the sodium pump in aorta. Thus, MBG is a novel factor in the pathogenesis of preeclampsia, and antibodies to MBG may be more effective than DIGIBIND in treatment of preeclampsia.

In conclusion, SPLs are important factors in the pathogenesis of and targets for intervention in NaCl-sensitive hypertension, preeclampsia, and hypertensive heart disease.

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

Keywords:

heart
development
calcium handling proteins
molecular biology

Recent Publications:

Boheler KR, et al. *Proc Natl Acad Sci USA* 2003; 100(5): 2754-2759.

Anisimov SV, et al. *Genomics* 2002; 80(2): 213-222.

Anisimov SV, et al. *Mech Dev* 2002; 117(1-2): 25-74.

Yang HT, et al. *Proc Natl Acad Sci USA* 2002; 99(14): 9225-9230.

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

Our work is focused on use of an *in vitro* differentiation model of mouse embryonic stem cells and embryonic carcinoma cells in an attempt to further understand the consequences of development and of altered gene expression on function of these proteins. Additionally, research in the laboratory has led to the development of mouse models having temporal and spatial control of gene expression. This system is currently being tested and plans are underway to actively apply this system to mouse transgenic models and to differentiating ES cells. Our recent experiments have exploited functional genomics to examine differentiation, development, aging and disease.

Signal Transduction Pathways Mediating SERCA2 Expression: We have exploited the human SERCA2 promoter to examine this gene's regulation *in vitro*. Transfection into neonatal rat cardiomyoctyes of the 2.8 kb human SERCA2 promoter constructs linked to reporter sequences indicate a lack of response with any of the adrenergic agonists, but regulation via sp1 and sp3 transcription factors has now been shown. Recent studies with Nuclear run-on assays have also indicated that transcriptional control of

SERCA2 gene expression is not the primary mechanism responsible for increased mRNA, protein and function of SERCA2 seen perinatally. Studies are underway, to elucidate the mechanisms responsible for the post-transcriptional regulation, one possibility of which may relate to an alternatively spliced isoform of SERCA2 seen in the fetal myocardium, whose expression is greatly reduced late in gestation.

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

Embryonic Stem Cells and Myocardial Development: This research area involves a model of *in vitro* differentiation of cardiomyocytes originating from embryonic stem cells (R1) and embryonic carcinoma cells (P19). The research is aimed at understanding the developmental processes involved in cardiac myocyte differentiation and development. To identify, atrial versus ventricular like cells, expression vector constructs have been made that link atrial and ventricular markers to the green fluorescence protein (GFP) and other selection markers. These constructs have been introduced into ES cells and positive transformants identified through neomycin resistance selection. From this work, we hope to use various molecular techniques to identify

and analyze various transcription factors and growth factors that promote cardiac cell division and differentiation and importantly, the sequence of their activation and inhibition. Specifically, we are examining the expressed sequences of differentiating P19 cells through a technique called serial analysis of gene expression (SAGE). This technique takes advantage of PCR and type II restriction enzymes to isolate short sequences sufficient to identify RNA products expressed at any time point. Currently, SAGE analyses have been performed on adult mouse myocardium, 3+3 day *in vitro* differentiating P19 cells and a comparative analysis is underway with 3+0.5 day *in vitro* differentiating P19 cells. Through this technique, we hope to use the information gained about the expressed sequence pattern to target and specifically identify gene products that are important to cardiac differentiation. Using targeted ES cells, we have been able to demonstrate a role of the ryanodine receptor in the control of heart rate development.

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

Collaborators: Dr. Michael Crow, Johns Hopkins University; Dr. Jennifer Van Eyk, Johns Hopkins University; Professor Antoon F.M. Moorman, University of Amsterdam, Netherlands; Dr. Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany; Dr. Edward G. Lakatta, Laboratory of Cardiovascular Science, NIA.

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Biography: Dr. Rui-Ping Xiao has been working in the Laboratory of Cardiovascular Science since February 1990. She was trained as a physiologist and molecular pharmacologist at Tong-Ji Medical University, China, and at the University of Maryland, where she received her M.D. and Ph.D., respectively.

Keywords:

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

Recent Publications:

Chakir K, et al. *Mol Pharmacol* 2003; 64(5): 1048-1058.

Xiao R-P. *Circulation* 2003; 108(13): 1633-1639.

Zhu WZ, et al. *J Clin Invest* 2003; 111(5): 617-625.

Jo SH, et al. *Circ Res* 2002; 91(1): 46-53.

Hagemann D, et al. *Trends Cardiovasc Med* 2002; 12(2): 51-56.

Liao P, et al. *Circ Res* 2002; 90(2): 190-196.

Xiao R-P. *Sci STKE* 2001; 104: RE15.

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) b-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 electrophysiologic, 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 Proliferative Diseases-related Genes: 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.

Publications-continued:

Zhu WZ, et al. *Proc Natl Acad Sci USA* 2001; 98(4): 1607-1612.

Vinogradova TM, et al. *Circ Res* 2000; 87(9): 760-767.

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

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.

Furthermore, 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 be also involved in the pathogenesis of a variety of cancers.

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 sequentially involves G_i, Gß, phosphoinositide 3-kinase (PI3K), and Akt. Furthermore, *in vivo* overexpression of $ß_1$AR, but not $ß_2$AR, induces heart muscle cell hypertrophy and heart failure in transgenic mouse models. 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 $ß_2$AR/G_i coupling is exaggerated. The enhanced G_i signaling underlies the heart failure-associated dysfunction of $ß_2$AR. Based on the dual G coupling of $ß_2$AR, we conceptualize that receptor ligands may selectively activate a subset(s) of the post-receptor signaling pathways. By screening a variety of $ß_2$AR ligands, we have identified one ligand (fenoterol) that selectively activates G_S, bypassing the G_i signaling. Strikingly, fenoterol is able to restore the markedly depressed $ß_2$AR contractile response in two experimental chronic heart failure models. Our most recent studies provide compelling evidence that stimulation of $ß_1$AR, but not $ß_2$AR, induces cardiac apoptosis. The anti-apoptotic effect of $ß_2$AR stimulation in cardiac myocytes is mediated by G_i-Gγ subunits-PI3 kinase-Akt signaling pathway. These studies not only reveal the diversity and specificity of ß-AR subtype and G protein interactions, but also provide new insights for understanding the co-existence and different functional roles of $ß_1$AR and $ß_2$AR in healthy and failing hearts.

Modulation of Cardiac Excitation-contraction Coupling 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_2terminal 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.

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 Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)-mediated positive feedback regulation of the L-type Ca^{2+} current ($I_{Ca,L}$). In freshly dissociated rabbit single SA node cells, specific CaMKII inhibitors, a peptide CaMKII inhibitor or KN-93 (0.1 - 3.0 μM), but not its inactive analog KN-92, depressed the rate and amplitude of spontaneous action potentials (APs) in a dose-dependent manner. Strikingly, 3 μM KN-93 or 10 μM CaMKII peptide inhibitor completely arrested SA node cells, which indicates that basal CaMKII activation is obligatory to the genesis of pacemaker AP via modulating properties of ICa,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 heart rate- or stimulation frequency-dependent augmentation of cardiac contractility and acceleration of relaxation. 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.

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. Edward G. Lakatta, and Heping Cheng, Laboratory of Cardiovascular Science, NIA.

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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.

Keywords:

blood pressure
breathing
hypertension
sodium chloride
sodium pump inhibitors

Recent Publications:

Scuteri A, et al. *J Hypertens* 2003; 21(7): 1339-1346.

Anderson DE, et al. *Int J Behav Med* 2002; 9(3): 216-227.

Anderson DE, et al. *Am J Hypertens* 2001; 14(8 Pt 1): 761-767.

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

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.

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The **Laboratory of Cellular and Molecular Biology (LCMB)** is currently comprised of five independent research programs headed by either a tenure track scientist or a senior investigator. These programs include the Gene Regulation Section, the Cancer Molecular Genetics Unit, the DNA Repair Unit, the RNA Regulation Unit, and the T Lymphocyte Signaling Unit.

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

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

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Biography: Dr. Sen received his 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 Brandies 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:

gene regulation
locus activation
chromatin structure
lymphocyte activation
NF-κB

Recent Publications:

Hesslein DG, et al. *Genes Dev* 2003; 17(1): 37-42.

Chowdhury D, et al. *Immunity* 2003; 18(2): 229-241.

Calame K, et al. *Immunoglobulin Genes* 2003; In press.

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 till Cm. V_H genes are inactive at this stage, which ensures that D_H to J_H recombination takes place first. Our analysis suggests that DJ_H recombination activates V_H genes that lie closest to the D_H/Cμ regions. Other parts of the V_H locus are activated independently: the 5'V_H J558 family requiring IL-7 and the intermediate V_H10 genes responding to tyrosine kinase signals.

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

1A. The D_H/Cμ Locus - Within this 90 kb lies the first tissue-specific transcriptional enhancer identified, the μ enhancer (μE). 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 μE 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.

Analysis of μE - 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 μE 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. Current studies of the μ enhancer aim to address such mechanistic issues in the context of transcription and recombination. A second major theme is to study the enhancer as a modulator of chromatin structure, since this property very likely directly impacts its function as a recombination enhancer. Two examples of ongoing studies are described below.

a. Transcriptional Synergy between E47, Ets-1 and TFE3 To circumvent the complexity of the full enhancer (and its 18 associated proteins), we have taken the approach of functionally dissecting smaller domains of the enhancer. One such domain comprises the motifs μE2, μA and μE3 that bind the proteins E47, Ets-1 and TFE3, respectively. Several lines of evidence indicate that these motifs work together. To address this we are reconstituting Ets-1 dependent synergy between E47 and TFE3 using purified factors and looking for additional proteins biochemically and genetically. The motivation for such studies comes not only for their relevance to μ enhancer function, but also to understand the molecular basis of *combinatorial control*.

b. μ Enhancer and Chromatin Structure The goals are to determine the effects of individual, or combinations of proteins, on chromatin structure. Towards this end, enhancer-containing plasmids are assembled into chromatin *in vitro* using a fully reconstituted system (in collaboration with Mike Pazin, Massachusetts General Hospital), in the presence or absence of purified enhancer binding proteins. We use structural assays such as nuclease digestion, nucleosome positioning and restriction enzyme accessibility, and functional assays such as *in vitro* transcription and RAG cleavage (in collaboration with David Schatz, Yale). Recent results show that TFE3 alone can find its site and bind to nucleosome assembled plasmids. This results in nucleosome positioning and induction of a nuclease hypersensitive site. Our immediate objectives are to understand the contribution ETS-domain proteins and E47 in this context, and to identify *chromatin-remodeling activities* in B cell extracts that are required for structural alterations.

Other Sequences that Activate D_H-Cμ - We recently identified a possible regulatory site close to DQ52. We are currently characterizing the region for transcriptional and recombination enhancer activity, as well as identifying proteins that mediate the effects. In collaboration with Gene Oltz (Vanderbilt), we will analyze the chromatin structure of alleles deleted for this sequence, or a combined deletion of this sequence and the μ enhancer, to determine their contribution to the overall structure of the D_H/Cμ locus. The double deletion will also indicate whether these sequences are sufficient to activate the 90kb domain, or whether additional sequences are likely to contribute. In parallel, we are continuing to map nuclease hypersensitive sites in the remaining 30kb that we have not yet analyzed. If additional sites are identified, we will evaluate their role in locus activation by deleting them, and testing their function in artificial substrates.

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

Implications for Allelic Exclusion of V_H Genes - 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_HJ558 genes, according to our model loss of IL-7 signals results in allelic exclusion of this family. We are currently testing several predictions of this model as well as investigating the mechanism of V_H gene inactivation.

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

A second interesting aspect of these studies is the relationship of the enhancer to MAP kinase signals. Activation of MAP kinases further increases TCRβ enhancer activity. Interestingly, under these conditions the intervening sequence motifs are no longer necessary for enhancer activity; that is, enhancer activity is only dependent on the ETS and CBF elements. Because ETS and CBF proteins are expressed in multipotent hematopoeitic precursors, this leads to a model for the *initiation* and *maintenance* of enhancer activity. We propose that an early thymocyte may receive a MAP kinase-activating signal from the thymic microenvironment, which turns on the TCRβ enhancer using pre-existing ETS and CBF proteins in these cells. Once the MAP kinase signal has terminated, TCRβ enhancer activity may be maintained by newly expressed βE5 binding proteins. This model provides a pathway by which gene expression mediated by transient differentiation signals may be maintained at subsequent stages.

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.

3A. Subcellular Dynamics of NF-κB Proteins - We recently showed that NF-κB/IκBα complexes in cells are in constant flux due to entry and exit from the nucleus. This is because nuclear localization signals (NLS) in NF-κB (particularly the p50 component) are not well hidden by association with IκBα. This permits the complex to enter the nucleus. Once in the nucleus, a strong nuclear export sequence (NES) in IκBα takes over and directs the complex back to the cytoplasm. We have proposed that the nuclear export function of IκBα ensures against spurious NF-κB-dependent gene activation that may occur due to inappropriate nuclear entry of NF-κB. IκBα-dependent export may also participate in terminating an NF-κB response when the stimulus that activated it is no longer present.

This dynamic state is cell-type specific. We have found that c-Rel is associated with IκBα only in B cells, but not in pre-B cells or T cells. This means that c-Rel constitutively transits in and out of the nucleus only in B cells. Yet, at the end of a TNFα signal in T cells, c-Rel transiently associates with IκBα presumably to be retrieved from the nucleus. This IκBα associated c-Rel must be ultimately transferred to IκBβ to re-establish the resting state of the cell; how this is brought about remains unclear. Interestingly, IκBβ and IκBε do not have export potential; instead they hide the NLS of Rel proteins more effectively, thereby preventing nuclear entry. Ongoing effort is directed at understanding how IκBα synthesis and post-translation modifications help to time the end of NF-κB-dependent transcription, and the functional consequences thereof. We are also investigating the functional significance of the B cell-specific c-Rel/IκBα complex.

3B. Functional Differences Between p65 and c-Rel - p65 and c-Rel are two closely related, but functionally different, Rel family members. One of the striking differences pertinent to immune function is that T cells in c-Rel$^{-/-}$ mice do not make IL-2 in response to TCR signals. However, IL-2 dependent proliferation is normal when these cells are provided with exogenous IL-2, as is p65 induction in response to TCR. The question is where is the crucial difference between the two proteins that allows only one to activate IL-2; conversely, where is the c-Rel responsive element in IL-2 that does not respond to p65, and what is the basis for the difference? (The DNA binding specificity of the two proteins is quite similar, suggesting that target specificity is unlikely to be a major factor in their distinctive properties). This problem also has some practical appeal because the part of c-Rel that distinguishes it from p65 is a potential target for *immunosuppressive drugs* that will block T cell activation (by blocking IL-2) without affecting p65-dependent transcription.

Collaborators: Eugene Oltz, Ph.D., Vanderbilt; David Schatz, Ph.D., Yale; Mike Pazin, Ph.D., Massachusets General Hospital; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA; Stephen Smale, Ph.D., University of California, Los Angeles; Satyajit Rath, M.D., Ph.D. , N.I.I., India; Joan Press, Ph.D., Brandeis; Robert Woodland, Ph.D., University of Massachusetts; Rachel Gerstein, Ph.D., University of Massachusetts; Janet Stavnezer, Ph.D., University of Massachusetts.

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Recent Publications:

Mazan-Mamczarz K, et al. *Proc Natl Acad Sci USA* 2003; 100(14): 8354-8359.

Galban S, et al. *Mol Cell Biol* 2003; 23(7): 2316-2328.

Fan J, et al. *Proc Natl Acad Sci USA* 2002; 99(16): 10611-10616.

Wang W, et al. *Mol Cell Biol* 2002; 22(10): 3425-3436.

Research Summary: Aging is characterized by a general decline in the ability of individuals to adequately respond to different stresses, either environmental or endogenously generated. Changes in the expression of many stress-regulated genes is believed to play an important role in determining cell fate. While the transcriptional events serving to regulate the expression of these genes have been extensively studied, it is becoming increasingly clear that post-transcriptional regulatory mechanisms also play a critical role regulating gene expression during stress. These post-transcriptional processes, still poorly understood, rely primarily on changes in the stability of the mRNA, but are likewise linked to events such as nuclear export of mRNA and mRNA translation, which are linked to mRNA turnover. Our long-term efforts are three-fold: 1) to search for RNA-binding proteins and target mRNA regions involved in regulating the export, stability and translation of labile mRNAs; 2) to elucidate the signaling events that regulate such post-transcriptional modifications; and 3) to study the implications of post-transcriptional gene regulation on physiological and pathological processes.

Regulation of Stress-Response Genes Through Altered mRNA Turnover: We and others have shown that the expression of specific stress-response genes (p21, cyclin D1, etc.) is highly influenced by changes in the stability of the encoding mRNAs. Major research efforts underway in the laboratory seek to carry out global assessments of changes in mRNA turnover using cDNA arrays. We previously developed a new method based on the use of cDNA arrays to investigate the global contribution of transcription and mRNA turnover and applied it to the study of stress-

regulated gene expression patterns. This novel method compares large-scale hybridization patterns generated with steady-state mRNA with those generated using newly transcribed RNA, generated and isolated using the nuclear run-on technology. By comparing the values from each analysis we can ascertain the extent to which stimulus-regulated modifications in gene expression are due to transcriptional changes and to post-transcriptional changes. In the stress study, changes in mRNA stability were found to account for approximately 50% of all gene expression changes. We are actively applying this methodology to the large-scale investigation of mRNA turnover changes during other cellular responses.

Signaling Events Regulating mRNA Turnover in Response to Stress: Several recent studies have provided increasing support for the notion that mRNA stability is regulated through mechanisms akin to those controlling gene transcription, i.e., signal transduction pathways involving phosphorylation events. While transport of the RNA-binding protein HuR from the nucleus to the cytoplasm is emerging as a key step in the activation of HuR function, the mechanisms underlying this process remain poorly understood. We recently identified the AMP-activated kinase (AMPK), an enzyme involved in responding to metabolic stresses, as a potent regulator of the levels of cytoplasmic HuR. Inhibition of AMPK increased HuR presence in the cytoplasm, enhanced binding of HuR to mRNAs encoding p21, cyclin B1 and cyclin A, and elevated their expression and half-life. Conversely, AMPK activation resulted in reduced cytoplasmic HuR, decreased levels and half-life of mRNAs encoding p21, cyclin A and cyclin B1, and diminished HuR association with the corresponding transcripts. We thus propose a novel function for AMPK as a regulator of cytoplasmic HuR levels, which in turn influences the mRNA-stabilizing function of HuR and the expression of HuR target transcripts. Among the specific target molecules that appear to mediate the transport of HuR through the nuclear pore are several *import* proteins, including importin α1, importin β1 and importin β2. Ongoing studies are aimed at elucidating the precise mechanisms whereby AMPK regulates HuR import through the various importins. Additional signal transduction pathways involved in regulating mRNA turnover are the subject of ongoing investigation.

Role of HuR on Protein Translation: HuR is a ubiquitously expressed member of the Hu family of RNA-binding proteins, which also comprises the primarily neuronal proteins HuB, HuC, and HuD. Hu proteins possess three RNA-recognition motifs through which they bind with high affinity and specificity to target mRNAs containing regions rich in adenines and uracils (AU-rich elements or AREs), and to regulate their stability,

translation, or both. Our recent findings provide evidence in support of an additional level of post-transcriptional gene regulation by HuR in response to genotoxins. Exposure to UVC strongly induced expression of the tumor suppressor p53 without elevating p53 mRNA levels or the cytoplasmic export of the p53 mRNA. Instead, UVC irradiation strongly promoted p53 translation. HuR was found to associate with the 3'-untranslated region (UTR) of the p53 mRNA in a UVC-dependent manner *in vitro* and *in vivo* and to enhance p53 translation after UVC. Our current efforts are focused on elucidating whether other HuR target mRNAs might also be subject to altered translation, and testing whether HuR may jointly increase the stability and translation of target mRNAs during specific cellular responses.

Regulation of mRNA Turnover During Cellular Senescence: Cellular aging is accompanied by alterations in gene expression patterns. Using three models of replicative senescence, we recently described the influence of HuR in coordinately regulating the expression of cyclin A, cyclin B1 and c-fos, whose expression decreases during senescence. We demonstrated that HuR levels, HuR binding to target mRNAs encoding cyclin A, cyclin B1 and c-fos, and the half-lives of such mRNAs, were lower in senescent cells. We further showed that HuR levels directly influenced the senescent phenotype and that mRNA turnover played a critical role during the process of replicative senescence. Given that the cytoplasmic presence of HuR (believed to be required for HuR function) was recently shown to be inhibited by AMPK, we have been examining the function of this kinase during cellular senescence. We recently found that AMP:ATP ratios are higher in senescent cells, and AMPK activity was accordingly elevated in senescent cells. Current work in the laboratory is investigating the involvement of AMPK and HuR in the process of *in vitro* senescence. Experimental approaches based on the use of chemical regulators of AMPK activity as well as adenoviral vectors expressing either constitutively active or dominant negative isoforms of the kinase, were found to directly influence the implementation of the senescent phenotype. The bulk of our results indicate that AMPK activation can cause premature fibroblast senescence, since interventions that induced AMPK activity cause premature senescence in IDH4 and IMR-90 cells, while reductions in AMPK activity cause delays in the onset of cellular senescence. We further propose that AMPK-triggered cellular senescence is implemented, at least in part, through an AMPK-triggered reduction in HuR function.

Although the link between *in vitro* cellular senescence and human aging remains controversial, a diminution in proliferative capacity is also a hallmark of *in vivo* aging. Therefore, knowledge of the mechanisms

regulating gene expression during *in vitro* senescence is likely to aid in our understanding of *in vivo* aging, as well as contribute to our comprehension of age-related diseases such as cancer and hyperplasia, where control of proliferation is lost. Our findings further suggest that orchestrated gene expression during senescence may be regulated by proteins such as HuR that coordinately regulate the stability of mRNAs encoding critical proliferation- and senescence-associated proteins. The study of additional senescence-associated labile mRNAs and RNA-binding proteins has become an area of great interest within the Unit.

Influence of the von Hippel-Lindau (VHL) Tumor Suppressor on Gene Expression: The precise mechanisms whereby the VHL gene product exerts its tumor suppressor function have not been fully elucidated. Based on evidence that the von Hippel-Lindau (VHL) tumor suppressor protein is associated with polysomes and interacts with translation regulatory factors, we recently set out to investigate the potential influence of pVHL on protein translation. To this end, renal cell carcinoma (RCC) cells that either lacked or expressed VHL through stable transfection were used to prepare RNA from cytosolic and polysome-bound fractions. Hybridization of cDNA arrays using RNA from each fraction revealed an influence of pVHL on protein translation: a subset of transcripts preferentially associated with polysomes in VHL-deficient cells (revealing genes that were subject to pVHL-repressed translation) and a subset of transcripts preferentially associated with polysomes in VHL-expressing cells (genes subject to pVHL-induced translation). Among the transcripts that were preferentially associated with polysomes in VHL-deficient cells was that encoding the tumor necrosis factor (TNF)α, an observation that further shown to be mediated through the TNFα 3' UTR. Among the transcripts that were preferentially associated with polysomes in VHL-expressing cells was the mRNA encoding the tumor suppressor p53. Additional assays revealed that the RNA-binding protein HuR was capable of binding the 3'UTR of the p53 mRNA preferentially in VHL-expressing cells. Use of siRNA effectively reduced HuR expression and markedly decreased p53 translation and p53 abundance. Our ongoing efforts demonstrate a novel function for the pVHL tumor suppressor as regulator of protein translation. Given the well established links of TNFα and p53 on tumor development, our findings further suggest that VHL-mediated changes in protein translation may contribute to pVHL's tumor suppressive functions in RCC.

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senescence

Recent Publications:

Mambo E, et al. *Cancer Res* 2002; 62(5): 1349-1355.

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

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

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

Breast Cancer and DNA Repair: Breast cancer accounts for 15-18% of all deaths among women every year, with about 180,000 new cases being diagnosed every year. Even though the causes of breast cancer remain unknown, several lines of evidence suggest that accumulation of DNA damage coupled with defects in DNA repair play an important role in breast cancer. It has been speculated that DNA base damage may lead to mutations that subsequently can be carcinogenic. Of primary importance are the base lesions caused by reactive oxygen species (ROS). Cellular DNA is exposed to ROS either endogenously by cellular metabolism or through exogenous exposure to environmental mutagens. ROS induce a wide range of DNA

lesions. Thymine glycol (Tg) and 8-hydroxyguanine (8-oxoG) are some of the most deleterious oxidative base lesions. Thymine glycol is a toxic lesion that blocks DNA replication and transcription, causing cell death. 8-oxoG is a premutagenic lesion. In order to avoid the harmful effects of 8-oxoG, organisms have developed mechanisms for repairing this damage. 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 damages in the etiology of breast cancer. It has been shown that 8-oxoG is repaired via the base excision repair (BER) pathway. To date, there are no reports on the removal of 8-oxoG or other oxidative DNA base lesions in breast cancer cells. Therefore, it remains to be established whether BER of oxidative lesions is altered during breast carcinogenesis. We therefore, hypothesized that the transformation from normal to malignant breast tissue may result from defects in oxidative DNA damage repair, consequently leading to mutations in important genes. Such a defect may occur in the nuclear and/or the mitochondrial genome. Mitochondrial DNA (mtDNA) encodes 13 proteins that are involved in oxidative phosphorylation. Oxidatively induced mutations in the mtDNA can lead to dysfunctional mitochondria, and have been implicated in degenerative diseases, cancer and aging. Therefore, effective oxidative damage repair processes are essential in order for the cell to maintain the integrity of the mitochondrial genome. We examined the ability of nuclear and mitochondrial extracts from a non-neoplastic mammary epithelial cell line and breast cancer MCF-7 and MDA-MB-468 cell lines to incise 8-oxoG and Tg lesions from duplex oligonucleotides. We have reported three important findings in this study: first, 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. Second, nuclear extracts from both breast cancer cell lines removed 8-oxoG more rapidly and efficiently than mitochondrial extracts. Third, nuclear extracts were shown to remove Tg more rapidly than 8-oxoG. We have shown 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. The genetic status of mtDNA from these breast cancer cells remains to be determined through sequence analyses. Therefore, we conclude that repair of 8-oxoG in the mitochondrial genome may be crucial in the development of breast cancer. Our studies may provide a basis for novel molecular interventions of breast cancer. We further propose that other forms of cancer

may be defective in oxidative DNA damage repair. We have also hypothesized that mitochondrial DNA of these cells may have excessive oxidative damage caused by defective oxidative repair. To address this hypothesis, mitochondrial and genomic DNA from these and other breast cancer cell lines will be analyzed by LC/GC mass spectrophotometry to determine the basal level oxidative damage. We will also assess induction of oxidative DNA damage by treating cells with specific oxidative damaging agents (e.g., Menadione, gamma irradiation, or hydrogen peroxide), for analysis of rates of lesion formation via LC/GC mass spectrophotometry.

In our most recent work, we have begun to evaluate the role of the BRCA 1 gene in oxidative damage repair. We are using two cell lines (CRL2336 and CRL2337) that are either homozygous or heterozygous for BRCA-1 mutation. The wt control for this project is the AG10009 lymphoblast cell line. Preliminary data suggests that nuclear repair of oxidative lesions, 8-oxoG, thymine glycol and 5-hydroxycytosine is reduced in cells homozygous for the BRCA-1 mutation relative to wild-type cells. Mitochondrial repair of oxidative lesions in this mutant cell line is comparable to that of wild-type cells. Once we have confirmed the repair phenotype of the BRCA1 mutant cell lines, further investigation will be directed to examining whether the specific repair enzymes involved in oxidative lesion repair (e.g., human endonuclease III (hNTH1) for thymine glycol) complexes with BRCA1 and other members of the BASC complex (Brca1-associated genome surveillance complex) as defined Wang et al. (BRCA1, ATM, NBS1, BLM, MRE-11, RAD50, MSH2, MLH1, MSH6). It is possible that the BRCA1 gene may play an important role in oxidative DNA repair in mammary tissue possibly partially explaining one of its roles in breast tumorigenesis.

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

Prostate Cancer and DNA Repair: Prostate cancer is the most prevalent cancer among American men and is classified as the second leading cause of their cancer mortality. In the United States, there will be 220,900 new cancer cases in 2003 making prostate cancer one of the cancers with the fastest rising incidence in this country as well as in Western Europe. While certain dietary, genetic, lifestyle and environmental factors are implicated in prostate cancer risk, the molecular mechanisms underlying the etiology of the disease are largely unknown.

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. Several research groups have begun to identify genes associated with heritable forms of prostate cancer and genes, in which somatic mutations or other somatic alterations may set the stage for the development and/or progression of the disease. To this end, it has been shown by several groups that hypermethylation of the π-class glutathione S-transferase gene (GSTP1) promoter region inhibits transcription of the gene and is associated with prostate cancer development. The function of GSTP1 has been proposed as a gene that defends genomic DNA in prostate cells from environmental or endogenous DNA-damaging agents. Environmental carcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons that result from cooking meat at high temperatures may play a role as it has been shown that 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine can induce prostate cancer in rats.

Reactive oxygen species (ROS), most notably the hydroxyl radical, generated endogenously by cellular metabolism are known to cause oxidative DNA damage that has been implicated in prostate carcinogenesis. Research on the development of prostate cancer suggests that symptomatic and asymptomatic chronic and acute inflammation occurs in the prostate over the life span and acts in synergy with environmental, genetic, and dietary factors to cause injury to prostatic epithelium. In response to this injury, cellular proliferation has been shown to occur. This proliferation is accompanied by oxidative stress that is related to the ongoing inflammatory process that in turn may result in high rates of oxidative damage to DNA. Other findings that implicate a role for oxidative DNA damage in prostate carcinogenesis include work by Bostwick et al. showing that SOD1, SOD2 and catalase levels are lower in prostate intraepithelial neoplasia and prostate cancer relative to benign prostate epithelium. 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. Further evidence supporting the hypothesis that defective repair of oxidative DNA damage may be pivotal in prostate carcinogenesis has been provided by work on genetic polymorphisms in the base excision repair (BER) gene OGG1. Taken together these data suggest that reactive oxygen species and oxidative DNA damage may be critical in the development of prostate cancer.

Using LC/MS and GC/MS, we show 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. Concomitantly, the levels of NEIL1 and NEIL2, enzymes that incise these lesions, are reduced in both cancer cell lines. 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.

Interaction of Human 8-oxoguanine-DNA Glycosylase with Proliferating Cell Nuclear Antigen: Strand Discrimination as a Mutation Avoidance Strategy: Human 8-oxoguanine-DNA glycosylase (OGG1) is the major enzyme for repairing 7-8, dihydro-8-oxoguanine (8-oxoG), a pre-mutagenic guanine base lesion produced by reactive oxygen species (ROS). The mutagenicity of 8-oxoguanine lies in its propensity to mispair with adenine during DNA replication. The importance of 8-oxoguanine and its repair by OGG1 are underscored by the frequent absence of the OGG1 allele in human lung tumors and the increased incidence of lung tumors in mice lacking a functional OGG1. 8-oxoguanine can occur in DNA by the oxidation of guanine in a G:C pair and by the incorporation of 8-oxoG into the newly synthesized nascent strand opposite cytosine or adenine during DNA replication or repair synthesis. Mispairings of 8-oxoG, when repaired by OGG1, could fix mutations if 8-oxoG in the parental strand is removed from a mispair with adenine. Accordingly, OGG1 should act only to remove 8-oxoG formed in DNA in situ and newly incorporated 8-oxoG in the nascent strand. If 8-oxoG in the parental strand becomes mispaired during DNA replication and is subsequently removed by

OGG1, a G to T transversion mutation could result. Using co-immunoprecipitation, we identified an interaction between OGG1 and proliferating cell nuclear antigen (PCNA). PCNA is a multi-functional protein involved in DNA replication, repair synthesis and cell cycle regulation. The interaction of OGG1 with PCNA is of particular interest because known PCNA-binding proteins, such as DNA polymerases and components of the mismatch repair system, perform their functions on newly synthesized DNA are directed to the nascent strand via a directional interaction with PCNA. Using an *in vitro* binding assay and mutant OGG1 proteins, we have identified a functional consensus PCNA binding motif in the C-terminus of OGG1. Additionally, using immunofluorescence, we have shown that OGG1 and PCNA co-localize at sites of DNA synthesis *in vivo*. The association of OGG1 and PCNA suggests a bimodal mechanism of OGG1-mediated repair of 8-oxoguanine. In non-dividing cells, OGG1 and perhaps other DNA repair proteins may indiscriminately remove 8-oxoguanine as it occurs in DNA. During replication however, the OGG1-PCNA interaction may serve to direct OGG1 to the nascent strand in order to prevent fixation of mutations in the parental strand. The functional consequences of the interaction of OGG1 with PCNA, which are likely to be highly significant *in vivo*, are currently being investigated.

The Role of Uracil DNA Glycosylase in Base Excision Repair: Uracil is a normal base in RNA but a miscoding lesion in DNA. Incorporation of uracil in the genome can occur through deamination of cytosine and also through occasional use of dUTP instead of TTP during DNA replication resulting in premutagenic U:G or U:A base pairs. Unrepaired uracil in DNA mispairs with adenine resulting in mutagenic phenotype that is potentially carcinogenic.

To avoid the mutagenesis associated with unrepaired or insufficient repair of uracil, most organisms harbor uracil DNA glycosylase (UDG) in their cells. This enzyme is encoded by the ung gene in the nuclear genome but a splice variant is translocated to the mitochondria of higher organisms. $Ung^{-/-}$ mice have a modest increase in spontaneous mutation frequency (Nielsen et al., Mol. Cell, 2000, 5: 1059-1065). Quite recently, Nilsen and co-workers showed that ung deficient mice have increased incidence of B-cell lymphomas at old age (Nilsen et al., Oncogene; 2003, 22: 5381-5386). These data provide the first evidence of cancer development due to a deficiency in a DNA glycosylase in mice model in addition to a possible role of ung in the immune system. Furthermore, the data by Nilsen and co-workers also provide a link between carcinogenesis, DNA repair and the aging process.

The UDG is a highly ubiquitous DNA repair enzyme that initiates the repair of uracil through the versatile DNA repair pathway known as base excision repair (BER). The major steps in BER include: scission of the bond between the inappropriate base and the sugar (glycosylic activity) by a DNA glycosylase leaving an apurinic/apyrimidinic (AP) site, phosphodiester bond cleavage by AP lyase activity of the same enzyme or by an AP endonuclease (AP lyase activity), addition of the correct nucleotide by DNA polymerases (polymerization) and ligation by DNA ligases. The polymerization step has been shown to diverge into two sub pathways: the short- and the long patch. In the short-patch BER sub pathway, only one nucleotide is incorporated after the glycosylic step, whereas, in the long-patch sub pathway, more than one nucleotide is incorporated. To date, the *in vivo* significance and regulation of the two BER sub pathways in the repair of uracil remains unclear. We have hypothesized that uracil repair in the mitochondria is accomplished via the short-patch mechanism and that the sub pathways of BER are largely determined by the nature of DNA glycosylases involved. Our preliminary results obtained using mitochondrial extracts of human lymphoblastoid origin suggest that uracil repair in the human system is accomplished exclusively via the short-patch BER sub pathway.

In order to understand the mechanism of uracil BER in the mammalian mitochondria, we have engaged in a collaborative study with Dr. Samuel H. Wilson's Laboratory of Structural Biology at the National Institute of Environmental Health Sciences, NIH. In this study, we are using wild type and UDG knockout mouse fibroblasts, which were established by Dr. Wilson's group and oligonucleotides containing a single uracil at specific location. Using this model system, we are assessing glycosylic activities of mitochondrial and nuclear isoforms of UDG. In addition, we are examining the mechanism of nucleotide incorporation (repair synthesis) in uracil BER. We are also studying the size (nucleotides) of the repair patch generated in the course of uracil repair. Furthermore, we are using this system to determine the nature of protein-protein interactions involved during the repair of uracil in mouse system. Since UDG is a pure DNA glycosylase without an associated AP lyase activity, it must perform the glycosylic bond scission and then hand over the resulting AP site to an AP endonuclease for further processing in order to complete the repair process. This notion would be consistent with the "passing the button" model proposed some years ago by Dr. Wilson. To ascertain if this is the case, we intend to perform repair synthesis reactions using cell-free extracts from mouse UDG-knockout and wild type cells in the presence of purified AP endonuclease. Proficient repair synthesis is expected in the presence of both UDG and AP

endonuclease if the proposed model is true. However, the lack of UDG in the knockout cells may not support proficient repair synthesis even if AP endonuclease is present. This project may also allow us to determine if the mouse system harbors back-up DNA repair pathways for uracil.

Collaborators: Adabalayma Balajee, Ph.D., Columbia University School of Medicine; Myriam Gorospe, Ph.D., Laboratory of Cellular and Molecular Biology, NIA; Charles Egwuagu, Ph.D., M.P.H., National Eye Institute, NIH; Nikki Holbrook, Ph.D., Yale University; Miral Dizdaroglu, National Institute of Standards and Technology; Pawel Jaruga, National Institute of Standards and Technology; Samuel Wilson, National Institute of Environmental Health Sciences, NIH; Robert Sobel, University of Pittsburgh.

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Keywords:

ovarian cancer
gene expression profiling
drug resistance
SAGE

Recent Publications:

Sherman-Baust CA, et al. *Cancer Cell* 12003; 3(4): 377-386.

Rangel LB, et al. *Oncogene* 2003; 22(46): 7225-7232.

Morin PJ. *Drug Resist Updat* 2003; 6(4): 169-172.

Hough CD, et al. *Cancer Res* 2000; 60(22): 6281-6287.

Research Summary: Ovarian cancer is the fifth most common cause of cancer deaths among women in the U.S., yet very little is known about the molecular mechanisms involved in the development of this disease. In order to address this problem, we have completed a large-scale serial analysis of gene expression (SAGE) study in epithelial ovarian cancer. The analysis was aimed at two major problems in ovarian cancer: difficulty of detection and drug resistance. A better understanding of gene expression and pathways in ovarian cancer, as well as the changes in expression associated with drug resistance 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, which have been found consistently elevated in ovarian tumors. The identification and characterization of these pathways will provide a better understanding of ovarian cancer at the molecular level.

Identification of Ovarian Cancer-specific Biomarkers: In the past few years, we have examined gene expression in ovarian cancer and normal ovarian tissues using serial analysis of gene expression (SAGE). 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 created ovarian cancer cell lines that are resistant to cisplatin. Using this model, we have utilized SAGE 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.

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

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Biography: Dr. Wange received his Ph.D. from the Department of Pharmacology at Vanderbilt University in 1991. He received his postdoctoral training at the Cell Biology and Metabolism Branch of the National Institute of Child Health and Human Development (NICHD) before becoming an Investigator in the Laboratory of Cellular and Molecular Biology in 1997. His research focuses on the roles of lipid and protein kinases and phosphatases in the regulation of T lymphocyte activation, growth and proliferation.

Keywords:

T cell antigen receptor (TCR)
T lymphocyte activation
signal transduction
protein kinases
lipid phosphatases

Recent Publications:

Seminaro MC, et al. *Oncogene* 2003; 22(50): 8195-8204.

Seminaro MC, et al. *Semin Immunol* 2002; 14(1): 27-36.

Shan X, et al. *Mol Cell Biol* 2001; 21(21): 7137-7149.

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

Aging and T Lymphocyte Activation: The long term goal of the lab is to gain a better understanding of the mechanisms whereby immunosenescence arises in aging animals. Immunosenescence is characterized by a deterioration of both cellular and humoral immunity, and has been proposed to have its roots in declining T-cell function as a consequence of changes in the ability of the T cells in aged animals to respond to mitogenic stimuli. Studies have found little difference between young and old animals with respect to the expression level of the T-cell antigen receptor (TCR) or other cell surface receptors involved in responding to mitogenic stimuli. Therefore, it is hypothesized that the decline in responsiveness to mitogens may reflect changes in intracellular signaling pathways. Indeed, many differences have been observed in some of the early TCR-initiated signaling events in T-cells isolated from young animals compared to old. However, none of these changes seem to sufficiently account for the age-associated decline in T-cell function. Effective investigation of the signaling defects that give rise to declining T-cell function with age is hampered by the lack of a complete understanding of the signaling pathways involved in normal (i.e. young) T-cell activation. This being so, we are currently attempting to uncover new portions of the signaling pathway that are downstream of engagement of the T-cell antigen receptor.

Tyrosine Kinases in T-Cell Receptor Signaling: One area of interest of the laboratory is the study of the tyrosine kinases (TKs) that act very early in the process of TCR signaling. Three families of TKs have been implicated in TCR signaling, these include the Src-family kinases Lck and Fyn, the Syk-family kinases ZAP-70 and Syk, and the Tec-family kinases, Itk, Tec and Rlk. The laboratory has a long-standing interest particularly in the study of ZAP-70 (Zeta-chain Associated Protein) and Itk (Inducible T cell Kinase). In normal T cell activation in response to a fully competent

activation signal, ZAP-70 is required for all distal TCR signaling events. However, under conditions where a suboptimal TCR partial agonist or antagonist binds to the TCR, intracellular signals are generated independently of ZAP-70 activation. Using T cell model systems that differ in their ability to express ZAP-70, we are investigating the nature of these ZAP-70-independent TCR signaling pathways. These pathways are likely to be important in committing non-productively stimulated T cells to a non-responsive (anergic) state, which is phenotypically similar to the state of immunosenescent T cells.

Itk, unlike ZAP-70 is much less pervasive in its effects on signaling pathways downstream of TCR engagement. Nonetheless, it plays an important role in calcium mobilization, by phosphorylating and modulating the activity of phospholipase C, and in "inside-out" signaling from the TCR to adhesion factors. Itk also plays a critical role in modulating changes in the cytoskeleton that are initiated upon TCR stimulation. Our lab has a keen interest in understanding both how Itk activation is regulated, as well as in understanding how it signals to its downstream effectors. We recently found that ZAP-70 regulates Itk activation by controlling the ability of Itk to interact with other signaling molecules (Lat and SLP-76) that are required for the assembly of multi-molecular signaling complexes that are important in Itk activation and other signals downstream of TCR signaling. Current efforts are focusing on discerning exactly how it is that the mulit-molecular signaling complexes assembled by Lat and SLP-76 result in Itk activation. In another approach, we have also been investigating the role of lipid kinases and phosphatases in regulating Itk localization and activation, and have found that the expression level of the lipid phosphatase PTEN plays an important role in regulating Itk localization to the plasma membrane, an important step in Itk activation.

Lipid Phosphatases in Regulating TCR Signaling: The regulation of initiation of cellular growth, cell cycle entry and protection from apoptosis are critically important events downstream of productive engagement of the T cell antigen receptor. A critical regulator of these events is the lipid phosphatase PTEN. This enzyme is a tumor suppressor, and catalyzes the reversal of the reaction catalyzed by the lipid kinase, phosphoinositide (PI)-3 kinase, which is activated following TCR signal. The laboratory has an interest in understanding what role PTEN plays in regulating the proximal events of TCR signaling, as well as distal events involving the activation of various transcription factors and changes in gene expression. These studies

are being pursued in the Jurkat T cell line, which has the advantage of being naturally PTEN-null. For these studies, we have generated stable cell lines which can be induced to express PTEN. Confirmation of the findings from the Jurkat cell lines is being carried out in normal human T cells as well as normal and genetically modified mouse T cells.

Role of PTEN in Growth Regulation in T cells and T Cell Leukemias: As an extension of our interest in the role of PTEN in regulating TCR signaling pathways and T cell activation, we also have an interest in a broader understanding of the role of PTEN in regulating cellular growth and proliferation in T cells and leukemias/lymphomas derived from T cells. Our recent discovery that the commonly studied acute lymphocytic T leukemia line Jurkat does not express functional PTEN, has allowed us to develop this cell line as an ideal model system to study the effects of restored PTEN expression on growth, proliferation and apoptosis. Indeed, using this model system we have revealed an unusual mode of action of PTEN in regulating cellular proliferation. In most cell lines studied to date, the restoration of PTEN to PTEN-null tumor cell liens results in a combination of G1 cell cycle arrest and induction of apoptosis. However, in the Jurkat T cell line, when PTEN is restored, the cells show diminished proliferation, not as a consequence of cell cycle arrest or increased apoptosis, but rather as a consequence of uniformly delayed progression through all stages of the cell cycle. The laboratory is working to further characterize the pathway involved in this unusual response, and attempting to determine whether this is indicative of a general difference in the mechanism by which PTEN acts as a tumor suppressor in hematopoietic tumors as opposed to solid tumors.

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

Laboratory of Clinical Investigation

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The **Laboratory of Clinical Investigation (LCI)** is comprised of 4 Sections and 2 Units with 8 principal investigators. These are as follows: Bioanalytical Chemistry and Drug Discovery Section; Diabetes Section; Hematology/Oncology Section; Cancer Immunology/Immunotherapy Unit; Molecular and Clinical Pharmacology Section; and Nuclear Magnetic Resonance Unit. 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 and Unit 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 β_2-adrenergic receptor.

Diabetes Section (DS): (PIs Drs. Josephine Egan and Michel Bernier) 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 has made this therapeutic target and drug attractive to the private sector, where it is now in later stages of clinical development. 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.

Hematology/Oncology Section (PI Dr. Eric Westin) and **Cancer Immunology/Immunotherapy Unit:** (PI Dr. Igor Espinoza-Delgado) Development of combination therapies that exploit the immunomodulating properties of bryostatin-1 to enhance responses to rituximab or IL-2 are a major drug development theme in the Section and Unit. Dr. Espinoza-Delgado's laboratory research is focused on developing the rationale for immunomodulation in the treatment of non-Hodgkin's lymphoma. This is translated directly into phase I clinical study in his patient-oriented research program. It benefits from the opportunity to collaborate with Dr. Wainer's Bioanalytical Chemistry and Drug Discovery Section in the development of methods for biological fluid and tissue analysis of the various drugs that have previously been difficult or impossible to measure at clinically relevant concentrations. In addition Dr. Espinoza-Delgado has begun to collaborate with Dr. Richard Spencer and the *in vivo* Nuclear Magnetic Resonance Unit to trace distribution of immunologically altered cells injected into the experimental animal and to phenotypically characterize cancer animal models.

Molecular and Clinical Pharmacology Section (MCPS): (PIs Drs. Nikolai Soldatov and Darrell Abernethy) 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. 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.

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

Keywords:

calcium
calcium antagonists
hypertension
pharmacodynamics

Recent Publications:

Abernethy DR, et al, *Clin Pharmacol Ther* 2002; 71: 186-195.

Tanus-Santos JE, et al, *Pharmacogenetics* 2002; 12: 407-413.

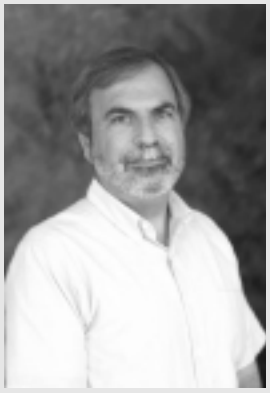
Kobrinsky E, et al, *J Biol Chem* 2003; 278: 5021-5028.

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

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

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

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



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

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

Keywords:

cancer cachexia
drug metabolism
immobilized receptors
high throughput screens

Recent Publications:

Jozwiak K, et al. *Anal Chem* 2002; 74: 4618-4624.

Bartolini M, et al. *J Chromatogr A* 2003; 987: 331-340.

Rodriguez-Rosas ME, et al. *J Chromatogr B* 2003; 794: 99-108.

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

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

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

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

Immobilized Receptors, Transporters and Enzymes: We have developed liquid chromatographic stationary phases containing immobilized receptors, enzymes and transporters as an on-line, flow system for use in new drug discovery and in the characterization of lead drug candidates. These columns can range in size from standard lc columns to micro-columns, can be used to screen complex chemical mixtures, to characterize single compounds and to screen phage libraries. The columns can be used with characterized targets - e.g. nicotinic, GABA, NMDA, estrogen receptors, P-glycoprotein and other ABC transporters, cytochrome P450 and other enzymes including phenylethanolamine N-methyltransferase and dopamine 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. Current research involves the development of other ABC transporter columns, the creation of an opioid receptor column, and development of a β_2-adrenergic receptor column.

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

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

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

Keywords:

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

Recent Publications:

Egan JM, et al. *J Clin Endocrinol Metab* 2002; 87(3): 1282-1290.

Egan JM, et al. *J Clin Endocrinol Metab* 2002; 87(8): 3768-3773.

Egan JM, et al. *Am J Physiol Endocrinol Metab* 2003; 284(6): E1072-E1079.

Doyle ME, et al. *Regul Pept* 2003; 114(2-3): 153-158.

Aging and Type 2 Diabetes: Our section studies insulin secretion and beta cell function because it is a challenging and relevant scientific area of investigation. Diabetes is a very contemporary health problem that affects 16 million Americans. Ten percent of these individuals suffer from an absolute deficiency of insulin (type 1 diabetes), and the rest are diabetic because their beta cells do not provide an adequate amount of circulating insulin (type 2 diabetes). Patients with diabetes encounter a number of life threatening illnesses, so effective treatment of this disorder is a highly desired goal. As the prevalence of type 2 diabetes continues to increase with increasing age, understanding the pathogenesis of this disease is also highly relevant to the mission of our Institute. An in-depth understanding of what controls beta cell function and mass might reveal new therapeutic possibilities to prevent diabetes and/or treat patients with type 2 and type 1 diabetes.

Design of Drugs for Treating Type 2 Diabetes: Insulinotropic peptides are synthesized in enteroendocrine cells of the gut. When food is eaten, they are secreted into the blood stream, resulting in their increased concentrations in plasma. The two best described insulinotropic peptides are GLP-1 and GIP, sometimes collectively referred to as incretins. Often binding of incretins to their specific receptors on beta cells increases insulin release. GLP-1 maintains insulinotropic activity in type 2 diabetes and analogs of GLP-1 as well as agonists of the GLP-1 receptor are under development as treatments for diabetes. One such agonist, xendin-4, was given for 31 days to type 2 diabetic patients in a study by the NIA. Most recently we have been involved in studies of GIP physiology. Clinical work by other groups have shown that GIP is not an effective insulinotropic agent in type 2 diabetes. We have developed a more potent and longer-lasting GIP analog,

which we are currently testing in a randomized double-blind clinical trial, again sponsored by the NIA, in type 2 diabetic patients. In parallel basic studies, we have been attempting to unravel the mechanism by which the enteroendocrine cells sense glucose and macronutrients, and the downstream signaling which ultimately results in exocytosis. We have uncovered a novel signaling pathway for hormone - secreting cells analogous to chemosensation in lingual cells. This pathway provides the mechanism by which the enteroendocrine cells release their peptide products into the blood stream in proportion to the calories eaten. If we can unravel this pathway, we might be able to develop non-nutrient based secretagogues of these hormones which would be useful to treat type 2 diabetes and obesity.

Regulation of Beta Cell Function and Mass: For a decade, our laboratory has investigated the molecular mechanisms responsible for the decline in beta cell function with aging and type 2 diabetes. We uncovered the fact that the gut peptide, GLP-1, plays a role in beta cell replication and differentiation. Other investigators have shown it to be an anti-apoptotic factor in beta cells. Recently we also showed that GLP-1 and exendin-4 upregulate pdx-1, a critical transcription factor for beta cells. Also recent work in our laboratory has shown that the Notch receptors and ligands are present and functional in beta cells. These play critical roles in determining cell fate choices related to proliferation, differentiation and apoptosis, in a context dependent manner. We have found that in unstimulated beta cells, Notch is mainly cytoplasmic, but with glucose stimulation, and even more so with exendin-4/GLP-1, it is translocated to the nucleus. Moreover, we have shown that activation of Notch leads to increases in IRS2 protein. Disruption of the gene for IRS2 in mice has already been shown to lead to beta cell failure. We are presently attempting to understand how Notch activation leads to increases in IRS2 protein as IRS2 plays an important role in islet proliferation, differentiation and survival.

Collaborators: Dr. Nigel Greig, Laboratory of Neurosciences, NIA; Dr. Andrew Young, Amylin Pharmaceuticals, San Diego; Dr. Grady Meneilly, University of British Columbia; Dr. Robert Margolskee, Mt. Sinai Medical Center.

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

Keywords:

insulin
receptors
signal transduction
protein-protein interactions
filamin A
phospholipase C-γ1

Recent Publications:

Kwon Y-K, et al. *J Cell Biol* 2003; 163(2): 375-384.

He H-J, et al. *J Biol Chem* 2003; 278(29): 27096-27104.

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

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

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

The central theme of the research in my laboratory involves the use of molecular biology and biochemistry to study the mechanism whereby the signal generated by the insulin binding to its cell surface receptor is transmitted intracellularly to various enzymes and proteins. Our objectives are to dissect the signaling networks, specific functional complexes, and molecular mechanisms that underlie the regulation of mitogenesis and metabolism by insulin. Specifically, the laboratory is actively engaged in (a) understanding structure/function relationships of the insulin receptor and two newly-described interacting proteins, namely the actin-binding protein, filamin A, and phospholipase C-gamma1 (PLCγ1); (b) assessing the contributions of these interactions to insulin signaling in insulin-responsive cells (e.g., hepatocytes, adipocytes); and (c) applying the gained knowledge to devise strategies to interfere with aberrant mechanisms in insulin signal transduction seen in patients with obesity, type II diabetes and aging.

We have recently identifed filamin A and PLCγ1 as genuine partners of the insulin receptor in cultured cell lines and in a primary culture of rat hepatocytes, which reflects the potential for physiological significance. Filamin A and PLCγ1 possess discrete domains that allow their binding with the insulin receptor. A mechanistic understanding of the regulation of these specific functional complexes could ultimately be used for the design of novel approaches to control the activity and/or intracellular redistribution of these signaling molecules in various insulin resistance states. In addition, using mutational techniques, we are mapping a functional domain of the insulin receptor involved in receptor-mediated PLCγ1 interaction and in

coupling of the activated receptor to downstream signal transduction molecules. Finally, we are studying the role of PLCγ1 and filamin A in adipocyte differentiation and as modulators of insulin-stimulated metabolic responses in hepatocytes and adipocytes.

Current studies are using multiple methods (antibody arrays, DNA arrays, proteomic technologies) to identify candidate genes that are up- or down-regulated in cells expressing filamin A or PLCγ1 constructs versus control hepatocytes or adipocytes following exposure to insulin. The pathways regulating these candidate genes will then be studied in detail. Obesity-linked insulin resistance and changes associated with aging have been linked to proinflammatory cytokine signaling. New efforts within the laboratory include research activities to dissect related signaling mechanisms in the control of proinflammatory responses in freshly isolated human T-lymphocytes. We have recently been involved in a collaborative effort to elucidate unexplored mechanisms in the regulation of cytokine signaling by short-lived farnesylated proteins. This information may provide perspectives for new strategies to improve insulin resistance.

Collaborators: Dr. Jonathan S. Bogan, Yale University School of Medicine; Dr. Michael T. Crow, The Johns Hopkins University School of Medicine; Dr. Banabihari Giri, Laboratory of Immunology, NIA.

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

Keywords:

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

Recent Publications:

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

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

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

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

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

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

With the near completion of the draft human genome sequence earlier this year, previous sequence data has led to isolation of a candidate tumor suppressor gene termed mrr1. Using ests for analysis, a partial candidate cDNA has been isolated and based on sequence analysis of this and other available sequence, the mrr1 protein analyzed.

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

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

Keywords:

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

Recent Publications:

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

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

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

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

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

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

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

Collaborators: Dr. Maria Carla Bosco, Giannina Gaslini Institute, Genova, Italy; Dr. Richard Childs, National Cancer Institute, NIH; Dr. Adrian Senderowicz, National Institute of Dental and Craniofacial Research, NIH.

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Biography: Dr. Nikolai Soldatov received his Ph.D. degree in bioorganic chemistry in 1981 from Shemyakin Institute of Bioorganic Chemistry of the USSR Academy of Sciences, Moscow. In 1983, while on postdoctoral training in Shemyakin Institute, he initiated research on calcium channels. In 1986 he joined the Institute of Medical Biotechnology led by cosmonaut Prof. B. Egorov and studied the relationship between calcium channels and primary and secondary messengers of human fibroblasts proliferation and memory, learning and nootropic effects in the brain. In 1990, he joined the HHMI laboratory of Dr. G. Blobel at the Rockefeller University, New York. He cloned the human L-type calcium channel and investigated its genomic structure. In 1993-96, he worked at the Department of Pharmacology of the University of Bern, Switzerland. He constructed a representative panel of human calcium channel splice variants and investigated, in collaboration with Prof. H. Reuter, their pharmacological and electrophysiological properties. In 1996 he moved to Georgetown University, Washington, D.C., where he worked as an Assistant Professor of the Department of Pharmacology. He studied mechanisms of voltage- and calcium-induced inactivation, and the role of C-terminal tail of the channel in calcium signaling. In July 1999, Dr. Soldatov 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*.

Keywords:

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

Recent Publications:

Soldatov NM, et al. *Trends Pharmacol Sci* 2003; 24(4): 167-171.

Kobrinsky E, et al. *J Biol Chem* 2003; 278(7): 5021-5028.

Shi C, et al. *J Biol Chem* 2002; 277(9): 6813-6821.

Functional Architecture and Regulation of Human L-type Ca^{2+} Channel: 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 the functional state of the Ca^{2+} channel (resting, open, inactivated) is stabilized by voltage clamp. This idea of differential voltage-gated FRET was successfully implemented by combining FRET microscopy with a patch clamp to study molecular dynamics of the human vascular L-type Ca^{2+} channel in real time and in the live cell. The Ca^{2+} channel α_{1C} pore-forming subunit was genetically fused with N-terminal EYFP and C-terminal ECFP and the labeled channel was functionally expressed in COS1 cells. This revealed voltage-gated mobility of the cytoplasmic tails of the $Ca_v1.2$ channel and its essential regulatory role in intracellular signaling.

Anchoring of the C-terminal tail to the plasma membrane caused an inhibition of its state-dependent mobility, channel inactivation, and CREB-mediated transcription. Release of the tail restored these functions suggesting a direct role for voltage-gated mobility of the C-terminal tail in Ca^{2+} signaling. Future investigation of the functional architecture of this and other ion channels using voltage-gated differential FRET microscopy of various functional parts and subunits may complement crystallographic studies by providing characterization of the dynamic molecular changes associated with distinct functional states of ion channels in live cell.

Molecular Determinant of the Voltage-dependent Inactivation: Currents through the Ca^{2+} channel inactivate by fast and slow mechanisms. We previously described the naturally occurring A752T mutation at the cytoplasmic pore region of the human channel (Soldatov, *Proc Natl Acad Sci USA*, 1992). This mutation prevented a large (~25%) fraction of the current from inactivation. Incorporation of similar mutations in the analogous positions of the four repeats of the α_{1C} subunit completely inhibited both Ca^{2+}-dependent and slow inactivation. The mechanisms of functional targeting of the outlined annular determinant of slow inactivation by C-terminal Ca^{2+} sensors of inactivation and regulatory β-subunit are the subjects of ongoing investigation using electrophysiology, immunochemistry, FRET, and transgenic animal models.

Calcium Sensors of Calcium Channel: The voltage-gated L-type Ca^{2+} channel is inhibited by permeating Ca^{2+} but not Ba^{2+} ions. This Ca^{2+}-induced inactivation serves as an important feedback mechanism in Ca^{2+} signal transduction that generates great variety of cellular responses. The C-terminal tail of the channel is crucial for Ca^{2+}-induced inactivation. Two C-terminal motifs, LA and K, were identified. LA serves as a Ca^{2+} sensor site that binds calmodulin (CaM) at low resting free Ca^{2+} concentration, and K as the binding site for the Ca^{2+}-loaded CaM. A Ca^{2+}-dependent transfer of CaM from LA to the K-motif removes CaM from the inner mouth of the pore and thus eliminates slow inactivation by facilitating the constriction of the pore.The mobile C-tail then shuttles the Ca^{2+}/CaM complex with the K-motif to a downstream target of the Ca^{2+} signaling cascade, where Ca^{2+} is released as an activating stimulus. Apo-CaM rebinds to LA and returns to the pore region for a new cycle of Ca^{2+}-signal transduction. This model predicts strong modulation of the Ca^{2+} channel open probability and the C-tail-mediated signal transduction by intracellular Ca^{2+} release because of the inhibition of the dissociation of Ca^{2+} from the complex of CaM with locus

K. In addition, cross-linking by CaM_{LA}, similar to that which occurs in the pore, might have a role in regulation by the channel C-tail of downstream targets such as ryanodine receptor. These factors might have important physiological implications for the relationship between Ca^{2+}-induced inactivation and Ca^{2+}signal transduction in complex systems such as cardiac muscle.

Collaborators: Darrell R. Abernethy, M.D., Ph.D., Evgeny Kobrinsky, Ph.D., Jo Beth Harry, Ph.D., Swasti Tiwari, Ph.D., Cheng Zhang Shi, Laboratory of Clinical Investigation, NIA; Christoph Romanin, Ph.D., University of Linz, Austria; Gregory Harms, Ph.D., University of Würtzburg, Germany; Martin Morad, Ph.D., Georgetown University, Washington, D.C.

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Keywords:

magnetic resonance imaging and spectroscopy
heart
cartilage
muscle

Recent Publications:

Chen CT, et al. *Arthritis Rheum* 2003; 48(4): 1047-1056.

Galban CJ, et al. *J Magn Reson* 2003; 161(2): 148-153.

Petersen EF, et al. *J Magn Reson Imaging* 2003; 17(5): 603-608.

McConville P, et al. *Circulation* 2003; 107(16): 2146-2152.

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

A Bioreactor System for Magnetic Resonance Microimaging and Spectroscopy of Chondrocytes and 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.

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.

Collaborators: Shari M. Ling, M.D., Clinical Research Branch, NIA; Edward Lakatta, M.D., Laboratory of Cardiovascular Science, NIA; Nancy P. 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.

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

diseases, disability and mortality. The Biometry Section (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 IRP, NIA, other NIH Institutes, other government agencies, and the private sector. LEDB research interests use data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE); the Women's Health and Aging Study (WHAS); the Honolulu-Asia Aging Study (HAAS); the Health, Aging and Body Composition (Health ABC) Study; Age, Gene/Environment Susceptibility (AGES) Study Reykjavik, Iceland; the MacArthur Studies of Successful Aging; the InChianti Study; and a new study of the effects of vascular factors on cognitive function in the Action to Control Cardiovascular Risk in Diabetes (ACCORD-MIND), as well as other epidemiologic studies.

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

Keywords:

epidemiology
chronic diseases
hypertension
physical activity
longevity
vascular stiffness

Recent Publications:

Havlik R, et al. *J Aging Phys Act* 2003; 11: 156-166.

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

Sutton-Tyrrell K, et al. *Hypertension* 2001; 38: 429-433.

Longevity: Gaining a better understanding of the factors influencing longevity is a research challenge. In particular, the assessment of the impact of chronic diseases versus the effects of aging processes has been difficult. Investigative opportunities to address these questions exist in a number of LEDB studies including Health ABC, HAAS, and AGES Study Reykjavik, Iceland. A unique opportunity to assess the life-course events in families and possible relationships with longevity presented itself in Trieste, Italy. The city has a comprehensive vital statistics system as well as a unique health care system, where a high proportion of deaths are autopsied. Previous reports from other populations have suggested that siblings of long-lived persons survive longer than expected, but the mechanism is unclear. We assessed whether survival and causes of death in siblings of persons dying at ages 95 years and older differed from those among siblings of individuals who died between 65 and 75 years of age, although being born at about the same time early in the century. Siblings of the long-lived showed a 1.8- and a 3.2 fold increase in the probability of reaching ages 85-89 and 90-94, respectively, while for younger ages the difference between the two groups was not different. Interestingly, about a 2-fold excess of dying of perinatal conditions was found among siblings of the short lived. This result was mainly due to an increased risk of dying of prematurity. This increase in risk may be an early indicator of genetic and/or environmental factors affecting longevity. This finding requires confirmation, so additional families are being collected. In addition, possible mechanistic studies involving the review of stored pathological material as well as the assessment of possible candidate genes in families are being considered.

Vascular Stiffness and Physical Activity: Cardiovascular disease provides an example of the possibilities of collaborative research where individuals with similar interests but different types of expertise can come together to initiate and complete studies. As an example, the Laboratory of Cardiovascular Science has developed an indirect measure of vascular stiffness called Aortic Pulse Wave Velocity (APWV). This measurement had been used in the Baltimore Longitudinal Study of Aging and some correlates, such as systolic blood pressure and physical activity, were identified. The measurement of APWV was initiated in the Health ABC Study in order to test some of the previously identified correlates and to identify new ones. For example, an association between visceral fat and APWV has been identified. Moderate or greater physical activity, exercise, and fitness variables were independently associated with less vascular stiffness, even after inclusion of other correlates of APWV. Physical activity's association with APWV was particularly strong when levels of physical activity were quite low, suggesting that a minimal amount of activity might be sufficient to reduce arterial stiffness in older adults. Another opportunity for population level assessment of this method occurred with the inception of the Activity Counseling Trial (ACT) by the National Heart, Lung, and Blood Institute. This was a randomized intervention trial of various educational strategies to boost physical activity over a 2-year period. The recommendation of adding the APWV measurement to ACT has provided an opportunity to investigate cross-sectional relationships as well as the effects of initiated exercise on vascular stiffness. An inverse relationship between high density lipoprotein cholesterol and APWV was identified at baseline. After 2 years, the various treatment arms did not have a significant effect on APWV. However, in a secondary analysis increased walking time over 2 years was predictive of reduced vascular stiffness. Women showed more of an effect than men. Modest physical activity may have a beneficial effect on large vessel structure, consistent with other outcome studies. Additional analyses are planned to address a number of unanswered questions, including quantification of risk from vascular stiffness, reasons for male-female differences, and effects of varying levels of physical activity.

Genetic Epidemiology: During the last few years, the potential of using unique populations to generate information on associations with candidate genes as well as for gene-searching activities has become quite evident. The Hawaii Family Diabetes Study (HFDS) utilized the family structure found in the HAAS. A number of siblings have been part of the HAAS cohort, and a subset of the siblings manifested considerable evidence of glucose dysregulation. On this basis, a supplement to HAAS was developed to investigate this aspect further. The objective of the study was to evaluate candidate regions for potential replication of previous results suggestive of

linkage at specific sites in Japanese Americans. The data included 529 siblings from 175 families, and diabetes or impaired glucose homeostasis was used as the phenotype for the analysis. Evidence of decreased proportion of sharing 2 alleles by siblings was observed at the previously identified marker D14S297 in 107 discordant sib-pairs. A test of association for this marker was also significant. The results suggest additional support for the hypothesis that a susceptibility gene for Type 2 diabetes may reside near this marker on Chromosome 14. Further analyses are being initiated to identify a possible candidate gene in this area. In the future there will be other studies that will have the potential to contribute toward the understanding of genetic influences on, and genetic-environmental interaction with, age-associated diseases and conditions. Besides the LEDB-sponsored AGES Study Reykjavik, Iceland, LEDB is collaborating with laboratories at the GRC to implement a gene-searching study in Sardinia.

Collaborators: Dr. Edward Lakatta, Laboratory of Cardiovascular Science, NIA; Dr. Dennis Taub, Laboratory of Immunology, NIA; Dr. David Schlessinger, Laboratory of Genetics, NIA; Dr. Josephine Egan, Laboratory of Clinical Investigation, NIA; Dr. John Hardy; Laboratory of Neurogenetics, NIA, Dr. Andrew Singleton, Laboratory of Neurogenetics, NIA; Dr. Ilija Kovac, National Human Genome Research Institute, NIH; Dr. Alexander Wilson, National Human Genome Research Institute, NIH; Dr. Kamal Masaki, University of Hawaii; Dr. Beatriz Rodriguez, University of Hawaii; Dr. Giorgio Stanta, University of Trieste; and Dr. Kim Sutton-Tyrrell, University of Pittsburgh.

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

Keywords:

epidemiology
chronic diseases
disability
functional status

Recent Publications:

Lunney J, et al. *JAMA* 2003; 289: 2387-2392.

Volpato S, et al. *Diabetes Care* 2003; 26: 70-75.

Rantanen T, et al. *J Am Geriatr Soc* 2003; 51: 636-641.

Lamb S, et al. *Stroke* 2003; 34: 494-501.

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 Honolulu-Asia Aging Study and the InChianti Study.

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

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

Assessment Methods: A number of research activities are directed at improving our ability to evaluate older persons in epidemiologic studies, including objective measures of physical performance, measures of exercise tolerance, and measures of muscle mass. Previous research that demonstrated that performance measures of functioning predict incident disability in previously non-disabled subjects has been replicated in several EPESE sites. Predictive equations developed from this work give risk estimates for disability onset so that sample size calculations for clinical trials of disability prevention may be made. 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. Research into the use of the SPPB in the hospital setting is now 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. Recent research 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 early life markers of low income and education and how they relate to functional status in middle age.

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

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

Collaborators: Dr. Luigi Ferrucci, Longitudinal Studies Section, Clinical Research Branch, NIA; Drs. Linda Fried, Judy Kasper, Karen Bandeen-Roche, Johns Hopkins Medical Institutions; Dr. Mary McDermott, Northwestern University School of Medicine; Drs. Marco Pahor, Brenda Penninx, Graziano Onder, Wake Forest University School of Medicine; Dr. Ann Shumway-Cook, University of Washington, Seattle; Drs. David Reuben and Teresa Seeman, University of California, Los Angeles; Dr. Stephanie Studenski, University of Pittsburgh; Drs. David Curb and

Kamal Masaki, University of Hawaii; Dr. Suzanne Leveille, Research and Training Institute of the Hebrew Rehabilitation Center for Aged and Harvard Medical School; 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. Dorit Carmelli, Stanford Research Institute; Dr. Chiara Corti, University of Padua, Padua, Italy; Drs. Howard Bergman and Francois Beland, McGill University, Montreal, Canada; Dr. David Melzer, University of Cambridge, Cambridge, England; 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 received her M.D. degree from Albert Einstein College of Medicine, New York, New York in 1978. She trained in internal medicine at Montefiore Hospital, Bronx, New York and in geriatric medicine at Harvard University, Division on Aging, where she was a Kaiser Fellow in Geriatric Medicine. She obtained a M.S. in Epidemiology from Harvard School of Public Health and also has a M.S. in Human Nutrition from Columbia University College of Physician's and Surgeons. From Harvard, she joined the Office of Analysis and Epidemiology at the National Center for Health Statistics. Dr. Harris moved to the National Institute on Aging in 1991, where she is Chief of the Geriatric Epidemiology Section. Dr. Harris has developed the Geriatric Epidemiology Section to cover a broad range of topics ranging from molecular and genetic epidemiology and body composition to health disparities. The goal of this research is to identify new risk factors for disease and disability amenable to intervention.

Keywords:

molecular and genetic epidemiology
bioimaging
chronic disease
aging

Recent Publications:

de Rekeneire N, et al. *Diabetes Care* 2003; 26(7): 1986-1992.

Tylavsky FA, et al. *Am J Clin Nutr* 2003; 77(2): 356-363.

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

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

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 (see website: www.nih.gov/nia/research/intramural/edb/healthabc). We have established a large repository of specimens and continue to seek innovative ideas and collaborators for the use of these samples.

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

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

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

Collaborators: Lenore Launer, Ph.D., M.P.H, Neuroepidemiology Unit, NIA; Dennis Taub, Ph.D., Laboratory of Immunology, NIA; Eleanor Simonsick, Ph.D., Laboratory of Clinical Investigation, NIA; Luigi Ferrucci, M.D., Ph.D., Longitudinal Studies Section, Clinical Research

Branch, NIA; 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., Dr.P.H., Jane Cauley, Ph.D., Bret Goodpaster, Ph.D., University of Pittsburgh; Stephan Kritchevsky, Ph.D., Fran Tylavsky, Ph.D., Ron Shorr, M.D., University of Tennessee, Memphis; Steven Cummings, M.D., M.P.H., Michael Nevitt, Ph.D., Susan Rubin, M.S., Susan Averbach, M.S., Emily Kenyon, Ph.D., Thomas Lang, Ph.D., Thomas Fuerst, Ph.D., Charles Peterfy, M.D., University of California, San Francisco; Russell Tracy, Ph.D., University of Vermont; Marjolein Visser, Ph.D., Free University, Amsterdam, Netherlands; Stefania Maggi, M.D., M.P.H., University of Padua, Padua, Italy; Mauro Zamboni, M.D., University of Verona, Verona, Italy; Dennis Taaffe, Ph.D., University of Brisbane, Australia; 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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Keywords:

longitudinal studies
mathematical modeling
sleep
driving

Recent Publications:

Foley D, et al. *Neurology* 2003; 60: 709-711.

Lunny J, et al. *JAMA* 2003; 289: 2387-2392.

Foley D, et al. *Sleep* 2003; 26: 596-599.

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 Unit, LEDB, NIA; Drs. Jack Guralnik and June Lunney, Epidemiology and Demography Section, LEDB, NIA; Dr. Susan Redline, Division of Clinical Epidemiology, Case Western Reserve University, Cleveland; Dr. Andrew Monjan, Neuroscience and Neurospsychology of Aging Program, NIA; 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.

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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.

Keywords:

epidemiology
neurologic diseases
genetic and
environmental risk factors

Recent Publications:

Schmidt R, et al. *Ann Neurol* 2002; 52: 168-174.

Launer L, et al. *Ageing Res Rev* 2002; 1: 61-77.

Peila R, et al. *Diabetes* 2002; 51: 1256-1262.

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

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

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

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

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

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

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

Collaborators: Tamara Harris, M.D., M.S., Jack Guralnik, M.D., Ph.D., Laboratory of Epidemiology, Demography, and Biometry, NIA; 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; 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.

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 the Nutritional and Molecular Physiology Unit (NMPU). One of the major projects of the NMPU is the longitudinal study of the potential beneficial effects of calorie restriction on aging in nonhuman primates. A second major area of investigation for this unit involves *in vivo* rodent models and *in vitro* cellular models to identify protective mechanisms invoked by calorie restriction. Within the BNS, a third 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 this research, investigations are directed toward preclinical development of pharmacological, genetic, and nutritional interventions that improve behavioral function.

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Biography: Dr. Ingram was trained in psychology and gerontology at the University of Georgia where he received his Ph.D. in 1978. From 1978-79 he served as a National Institute of Mental Health-supported postdoctoral fellow in behavior genetics at the Jackson Laboratory. He came to the NIA in 1980 as a Staff Fellow in the Laboratory of Behavioral Sciences and then moved to the Laboratory of Cellular and Molecular Biology in a tenured position in 1985. He was appointed as Chief of the Behavioral Neuroscience Section in 2000 when he joined the Laboratory of Neurosciences. In 2002 he was appointed Acting Chief of the newly created Laboratory of Experimental Gerontology (LEG). The LEG conducts basic research in experimental models focused on interventions that retard aging processes. A major project is a longitudinal study of the potential beneficial effects of calorie restriction on aging in nonhuman primates. A second major area of investigation involves *in vivo* rodent models and *in vitro* cellular models to identify protective mechanisms invoked by calorie restriction. A third major project involves a standardized research 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 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 this research, investigations are directed toward preclinical development of pharmacological, hormonal, genetic, and nutritional interventions that improve behavioral function. To identify age-related structural changes in the brain and alterations produced through various interventions, morphometric analysis using unbiased stereology is applied. Dr. Ingram serves on the editorial boards of several journals, including the *Neurobiology of Aging*, *Experimental Aging Research*, *Journal of Anti-Aging Medicine*, and *CNS Drug Reviews*, and he is an editor for *Gerontology*. He has also served in numerous positions within the Biology Section of the Gerontological Society of America, and he is a past president of the American Aging Association.

Keywords:

brain aging
behavioral performance
memory
neurotransmitters

Recent Publications:

Anson RM, et al. *Proc Natl Acad Sci USA* 2003; 100: 6216-6220.

Mattison JA, et al. *Exp Gerontol* 2003; 38: 35-46.

Luo Y-Q, et al. *J Neurochem* 2002; 80: 354-361.

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

Regarding age-related decline in memory performance, we have focused on the cholinergic and glutamatergic systems and their interaction. For cholinergic interventions, we have collaborated with Dr. Nigel Greig of the Laboratory of Neurosciences to develop a novel class of cholinesterase inhibitors, that are long-acting, highly specific for acetylcholinesterase, with a wide range of therapeutic efficacy and low toxicity within this range. For glutamatergic interventions, we are examining manipulations of the glycine

Publications-continued:

Mouton PR, et al. *Brain Res* 2002; 956(1): 30-35.

Roth GS, et al. *Science* 2002; 297: 811.

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

and polyamine sites on the N-methyl-D-aspartate (NMDA) glutamate receptor as well as generators of nitric oxide (NO) that is activated through the NMDA receptor. We have found that combinations of glycine agonists and polyamine agonists can act synergistically to improve learning performance. NO donors are also being assessed to overcome age-related learning impairments. Collaborating with Dr. Peter Mouton, we are examining the role of estrogen in preserving memory and reducing glia-mediated inflammation in a mouse model of Alzheimer's disease. In addition to the behavioral analysis, the latter project is part of a larger collaboration with Drs. Peter Mouton and Mathias Jucker that involves quantitative morphometrics using unbiased stereology in a variety of mouse models. Specifically, we are assessing age-related changes in the numbers of neurons, synapses, and glia, in the hippocampus of mice from different genders and strains including transgenics and knock-outs. The objective is to relate specific neuromorphometric parameters to age or treatment-induced changes in cognitive performance. In collaboration with Drs. Nan-Ping Weng and Dan Longo of the Laboratory of Immunology, we are using microarray technology to identify genes involved in memory formation and possible age-related changes in gene expression. Several candidate genes have been identified that show little expression in the hippocampus of learning-impaired rats compared to higher levels of expression in young rats.

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

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

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

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

Laboratory of Genetics

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The **Laboratory of Genetics (LG)** includes a Human Genetics Section, directed by David Schlessinger, a Human Genetics and Integrative Medicine Section, headed by Clair Francomano, a Transcription Remodeling and Regulation Unit directed by Weidong Wang, the Developmental Genomics Section under the direction of Minoru S.H. Ko, 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. Seven major types of study are in progress:

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

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

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

4. Nuclear organelles that determine large-scale chromatin remodeling events. Such events are involved in chromosome dynamics related to large-scale control of gene expression and DNA repair. The Transcription Remodeling and Regulation Unit 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.

5. 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 an increased rate of follicula atresia during fetal life, and skin appendage formation and regeneration.

6. The genetics of aging-related complex conditions is being approached by interactive studies with the "founder" population in Sardinia. Initial phenotypes to be studied along with epidemiological factors include arterial stiffness, selected psychiatric/psychological traits. For this project investigators from 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 (Alexei Sharov and Timur Nedorezov) are working with Antonio Cao and Giuseppe Pilia, human geneticists at the University of Cagliari, Sardinia, and Goncalo Abecasis, a statistical geneticist at the University of Michigan.

7. 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. Currently, the group is continuing to develop relevant tools, applying them to determine the spatial distribution of differentially expressed gene products in pre-implantation mouse embryos, and to screen for mutants in *C. elegans*.

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, and chromatin analysis techniques. Among the specific projects of the Unit is the detailed mapping and sequencing of the mouse t-complex, a region important for embryonic development and developmental genetics, and to study long-range control of genes with regulatory elements at a great distance from the transcription unit.

Among specific technological improvements that are being developed are techniques for the recovery of complete genes and YACs in circular, autonomously replicating clones (in the Gene Recovery Unit), and protocols to make and analyze high-quality cDNA libraries from very few cells from subregions of embryos (in the Developmental Genomics and Aging Section) and in collaborating with the Microarray Laboratory run by Kevin Becker (see Research Resources Branch) and Agilent Technologies to develop gene expression profiling with microarrays 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 has served as President of the American Society for Microbiology in 1995, and as the Director of the Human Genome Center at Washington University from 1987-97. During his tenure as Center director, he oversaw the development of the X chromosome map and of much related technology, with the concomitant finding of a number of disease genes. He is currently a councilor of the Human Genome Organization (HUGO) International, and President, HUGO Americas.

Keywords:

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

Recent Publications:

Galaviz-Hernandez C, et al. *Gene* 2003; 309: 81-89.

Cui CY, et al. *Hum Mol Genet* 2003; 12(22): 2931-2940.

Schlessinger D, et al. *Am J Med Genet* 2002; 111: 328-333.

Cui CY, et al. *Hum Mol Genet* 2002; 11: 1763-1773.

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

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. Studies at the level of gene regulation in chromatin. Projects are designed to understand tissue- and developmentally-restricted expression of two genes, one that when mutated causes inherited premature ovarian failure (see below), and another that is placental-specific (PLAC1) and possibly involved in fetal well-being. Promoter and enhancer element functions are being analyzed in those instances. The regulatory processes involve features of chromatin; analyses of open and closed chromatin are projected for the genes recovered in chromatin form in artificial chromosomes in the Gene Recovery and Analysis Unit, headed by Ramaiah Nagaraja.

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

Premature ovarian failure. A subset of women with premature ovarian failure (POF) have a defect that is also associated with eyelid dysplasia (BPES, the blepharophimosis-ptosis-epicanthus inversus syndrome). We have identified a “winged helix” transcription factor, FOXL2, that is

mutated to cause both the eyelid and ovarian follicle defects. In correlated developmental work, a mouse knockout model has been developed that recapitulates features of BPES, and systematic studies are under way of gene cohorts specifically expressed during the development of ovarian follicles, including the target genes controlled by FOXL2.

Skin appendage formation. The gene mutated in X-linked anhidrotic ectodermal dysplasia (EDA) provides an entree to an embryonic branch point that leads to teeth, hair follicles, and sebaceous and sweat glands. The Tabby mouse has been shown to be an experimental model for the human condition, and interacting genes can be found both by genomic approaches and by genetic studies of some of the other 175 inherited ectodermal dysplasias. Transgenic Tabby animals containing various isoforms of the EDA protein are revealing both the capacity of isoforms to initiate or maintain the restoration of some skin appendages, and to sustain the hyperproliferation of others.

The projected work will depend on the Gene Recovery and Analysis Unit, directed by Ramaiah Nagaraja, and collaborating groups. Related efforts are studying the genetic potential in the embryo-regulatory t-complex region of the mouse; and more directly relevant to human conditions, an extensive 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.

Another group adapting new technology, the Image Informatics and Computational Biology Unit headed by Ilya Goldberg is helping to complete the range of necessary technology for functional genomics by developing 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 image informatics - the collection, maintenance, and analysis of biological images and associated data. The aim of this project is to standardize how image information is stored, extracted and transported between different software applications. In pilot work, current versions of OME have addressed applications of high-resolution imaging to track intracellular particles in real time, and screening applications involving hundreds of thousands of images. Currently, the group is continuing to develop relevant computational tools and information systems, and to apply them to two specific biological areas: determining the spatial distribution of differentially expressed gene products in pre-implantation mouse embryos, and visual screening of populations of the nematode *C. elegans* in which one gene is inactivated at a time across the genome.

Collaborators: Professor J.M. Cantu, University of Guadalajara Medical School; Dr. Michele D'Urso, International Institute of Genetics and Biophysics, Naples; Professor Raj Thakker, M.D., Oxford University; Professor Antonino Forabosco, University of Modena; Dr. Giuseppe Pilia, Italian Research Council, Cagliari; Dr. Juha Kere, Karolinska Institute; Dr. Anand Srivastava, Greenwood Genetics Center; Dr. Peter Sorger, Massachusetts Institute of Technology; Dr. Jason Swedlow, The University of Dundee; Dr. John Schimenti, The Jackson Laboratories; Dr. Bruce Roe, University of Oklahoma; Dr. Kuniya Abe, RIKEN Institute (Tokyo).

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

Keywords:

skeleton
connective tissue
genomics
musculoskeletal pain

Recent Publications:

Liberfarb RM, et al. *Genet Med* 2003; 5: 21-27.

Ho NC, et al. *Radiology* 2002; 223: 767-771.

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

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

The **Heritable Disorders of Connective Tissue** are a heterogeneous group of conditions affecting multiple organ systems, including skeleton, skin and vasculature. While rapid advances have been made in recent years toward understanding the genes underlying many of these disorders, genes responsible for many others remain to be found. In addition, much remains to be done if we are to understand the pathogenesis of those disorders for which genes are already known, the relationship between genotype and phenotype, and additional genes that act to modify the phenotype of known mutations. Moreover, the availability of molecular markers for many of these conditions facilitates prenatal or presymptomatic diagnosis, raising multiple ethical and social issues that merit exploration. The relationships between specific mutations and phenotype are being explored in the type II collagenopathies and Marfan syndrome, a connective tissue disorder caused by mutations in the fibrillin gene on chromosome 15.

Specific **Skeletal Dysplasias** under investigation in the laboratory include Cartilage-Hair Hypoplasia, and disorders caused by mutations in the fibroblast growth factor receptor 3 gene.

A major effort is underway to characterize mutations in the gene encoding **Fibroblast Growth Factor Receptor 3** (FGFR3) and to understand the biochemical pathways leading to specific FGFR3-related phenotypes, including achondroplasia, hypochondroplasia and thanatophoric dysplasia. Recent studies have found highly specific FGFR3 mutations in a previously unrecognized phenotype of severe skeletal dysplasia with acanthosis nigricans and mental retardation.

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

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

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

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

Keywords:

chromatin-remodeling
SWI/SNF
helicase
genome instability
cancer
Fanconi anemia
Bloom syndrome

Recent Publications:

Meetei AR, et al. *Nature Genet* 2003; 35: 165-170.

Meetei AR, et al. *Mol Cell Biol* 2003; 23(10): 3417-3426.

Nie Z, et al. *Mol Cell Biol* 2003; 23(8): 2942-2952.

Xue Y, et al. *Proc Natl Acad Sci USA* 2003; 100(19): 10635-10640.

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

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

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

1. Mammalian SWI/SNF-Related Chromatin-Remodeling Complexes: The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. In mammals, the SWI/SNF-related complexes 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 KB cells, BAF and PBAF. We have recently isolated a novel complex containing a chromosomal translocation fusion partner for mixed lineage leukemia protein (MLL). We are continuing characterization of these complexes and investigate their mechanism of action.

4. 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 also 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 WRN, the Helicase Involved in Werner's Premature Aging Disease: Many human helicases discovered to date are related to DNA repair diseases, including Werner Syndrome (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, and Bloom's Syndrome. Many of the gene products have only been identified recently and their mechanisms of action are not known. We recently found that the gene product encoded by WRN is present in a high molecular

weight complex in HeLa cells. We have now purified this complex and identified all of its subunits by microsequencing. We are now studying the functions of the WRN complex. Hopefully, this will lead to better understanding of the human aging process.

2. Purification of a Complex Containing BLM, the Helicase Involved in Bloom Syndrome: This disease resembles Werner syndrome in genomic instability and cancer predisposition; but the patients do not display premature aging conditions. The gene defective in this disease belongs to the same family of RecQ helicase as WRN. We have purified three distinct BLM-containing complexes from HeLa cells. Interestingly, one of the complexes, termed BRAFT, also contains five of the Fanconi anemia (FA) complementation group proteins (see below). FA resembles BS in genomic instability and cancer predisposition, but most of its gene products have no known biochemical activity and the molecular pathogenesis of the disease is poorly understood. BRAFT displays a DNA-unwinding activity, which requires the presence of BLM because complexes isolated from BLM-deficient cells lack such an activity. The complex also contains topoisomerase IIIa and replication protein A, proteins that are known to interact with BLM and could facilitate unwinding of DNA. We show that BLM complexes isolated from a FA cell line have a lower molecular mass. Our study suggests a connection between the BLM and FA pathways of genomic maintenance. The findings that FA proteins are part of a DNA-unwinding complex consistent with a function for FA proteins in DNA repair. Currently, we are investigating the role of a component of the BRAFT complex, BLAP75, in BLM function.

3. 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. Genetic studies have identified 8 complementation groups for the disease. Among them, 6 genes have been cloned. However, these gene products show no sequence homology to known proteins in the database and they have not been reported to have any biochemical activity. We have isolated an FA core complex to a significant level of purity. We found that this complex has five known FA proteins and four new components (they are named FAAPs for FA-Associated Proteins). We identified all these components by mass spectrometry. One new component, PHF9, was found to contain a ubiquitin ligase motif as well as the corresponding activity. By three different approaches—small interfering RNA (siRNA) knockdown, knockout mice, and identification of an FA patient carrying a PHF9 mutation—we show that PHF9 represents a new FA complementation group and is required for FANCD2 monoubiquitylation *in vivo*. Our data suggest that PHF9 plays a crucial role

in the FA/BRCA pathway as the catalytic subunit required for FANCD2 monoubiquitylation. We are continuing to investigate whether other components of the FA core complex are novel FA genes. Several of these new components were found to have DNA-interacting domains. We are investigating whether the FA core complex may have corresponding activities, which should help to understand how the FA core complex is involved in the pathophysiology of this disease.

4. Purification of a Complex Involved in Rothmund-Thompson Syndrome: This disease also is characterized by genome instability and higher risk of cancer. The gene mutated in the disease belongs to the same RecQ helicase family as WRN and BLM. We have now purified the RecQ4 complex from HeLa cells and have identified all its components. The components in this complex are completely different from those in WRN or BLM complexes, and the functional studies are now underway.

Collaborators: Drs. Hans Joenje, Johan de Winter, Annette Medhurst, Quinten Waisfisz, Henri vad de Vrugt, Anneke Oostra, Free University, Netherlands; Drs. Michael Wallisch and Maureen Hoatlin, Oregon Health and Sciences University; Drs. Richard Gibbons and Doug Higgs, Oxford University; Dr. Jacques Cote, Laval University Cancer Research Center; Drs. Jiemin Wong, Jun Qin, and Colin Bishop, Baylor College of Medicine; Drs. Everett Chen and Michael Cleary, Stanford University; Dr. Trevor Archer, National Institute of Environmental Health Sciences, NIH; Dr. Bernard Weissman, University of North Carolina.

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Biography: Dr. Ko received his M.D. degree in 1986 and his Ph.D. in 1991 from Keio University School of Medicine in Tokyo. He held positions as Researcher from 1988 to 1991 and as Group Leader from 1991 to 1992 at the Furusawa MorphoGene Project, ERATO, JST, Japan. In 1992, he moved to the United States as Assistant Professor at the Center for Molecular Medicine and Genetics, Wayne State University in Detroit, Michigan, where he was promoted to Associate Professor and received tenure in 1997. He joined the NIA in Fall of 1998 to establish the Developmental Genomics and Aging Section within the Laboratory of Genetics. In one earlier study, using a steroid hormone inducible gene, he demonstrated a stochastic component in the regulation of expression of individual genes at a single cell level. He has also developed three methods that aid in profiling systematic gene expression in specific cell types. These are: 1) PCR-based amplification of a complex mixture of cDNAs, which allows the analyses of a cohort of genes expressed in the small number of cells; 2) a way to construct a normalized cDNA library in which the abundance of individual cDNA species is equalized; and 3) an efficient PCR-based method for localizing mouse cDNAs or ESTs on the genetic map. His group has recently established 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 ~22,000 gene features. These resources have been provided to the research community and also facilitate some of the approaches in our research group.

Keywords:

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

Recent Publications:

Suemizu H, et al. *Dev Biol* 2003; 253: 36-53.

Carter MG, et al. *Genome Res* 2003; 13: 1011-1021.

Kimber WL, et al. *Reprod Biomed Online* 2003; 6: 318-322.

Tanaka TS, et al. *Genome Res* 2002; 12: 1921-1928.

VanBuren V, et al. *Genome Res* 2002; 12: 1999-2003.

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

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

proteomics. Primary means of achieving this goal have been expressed sequence tag (EST) projects, which essentially comprise single-pass sequencing of randomly picked cDNA clones. One major difficulty to construct a cDNA library from early embryonic materials is the scarcity of the starting materials. We have recently developed a novel design of linker-primer that allows one to amplify differentially long tracts (average 3.0 kb with size ranges of 1 - 7 kb) or short DNAs (average 1.5 kb with size ranges of 0.5 - 3 kb) from a complex mixture. The method allows one to generate cDNA libraries enriched for long transcripts without size selection of insert DNAs. All our recent cDNA libraries have been made by this new method, and thus, a significant fraction of these cDNA clones contain complete open reading frame ("full-length"). We have thus far generated ~140,000 ESTs from early mouse embryos and mouse stem cells (http://lgsun.grc.nia.nih.gov/cDNA/cDNA.html).

Previously we assembled and released two non-overlapping mouse gene sets: NIA Mouse 15K cDNA Clone Set, containing ~12,000 unique cDNA clones, and NIA Mouse 7.4K cDNA Clone Set, containing ~7,400 unique cDNA clones. These clone sets have been freely distributed to the research community. During this period, we have continued to generate cDNA libraries and obtain cDNA sequences. Furthermore, we have developed a glass-slide microarray platform containing in situ-synthesized 60-mer oligonucleotide probes representing approximately 22,000 unique mouse transcripts, assembled primarily from sequences of stem cell and embryo cDNA libraries. This microarray has been made commercially available from Agilent Technologies. We have optimized RNA labeling protocols and experimental designs to use as little as 2 ng total RNA reliably and reproducibly. At least 98% of the probes contained in the microarray correspond to clones in our publicly-available collections, making cDNAs readily available for further experimentation on genes of interest. Future plans include the expansion of the set of unique genes by sequencing more cDNA clones from various embryonic collections, and the preparation and use of cDNA microarrays from the expanded set of unique genes.

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

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

In our previous work, we identified many genes that show stage-specific expression patterns during preimplantation mouse development. However, these genes have been identified by EST frequency, which is a relatively inaccurate and far from ideal way to do gene expression profiling. The cDNA microarray-based gene expression profiling will provide more reliable information. To this end, we have been working on a large-scale gene expression profiling of each stage of preimplantation mouse development. To start to examine whether these genes identified here might be involved in the immortal/mortal transition process, we are currently analyzing the nature of these genes in greater detail. We are also testing various antisense oligonucleotides and siRNA to inhibit the function of specific gene in preimplantation mouse embryos. Finally, we are also developing a high throughput whole-mount in situ hybridization technique on preimplantation embryos.

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

As a first step, large-scale gene expression profiling was performed on embryo-derived stem cell lines to identify molecular signatures of pluripotency and lineage specificity. Analysis of pluripotent embryonic stem (ES) cells, extraembryonic-restricted trophoblast stem (TS) cells, and terminally-differentiated mouse embryo fibroblast (MEF) cells identified expression profiles unique to each cell type, as well as genes common only to ES and TS cells. Whereas most of the MEF-specific genes had previously been characterized, the majority (67%) of the ES-specific genes was novel and did not include known differentiated cell markers. We suggest that pluripotency requires a set of genes not expressed in other cell types, while lineage-restricted stem cells, like TS cells, express genes predictive of their differentiated lineage. The identification of genes that are specifically expressed in ES cells has provided an important first step for understanding the pluripotency of stem cells. These genes can be used as markers for pluripotent stem cells. Furthermore, the manipulation of these genes in ES cells and other cell types can further elucidate their function and provide possible means to harness the cellular pluripotency. To extend this work and extract the common features of stem cells, we have thus far profiled the following stem cells: (1) Differentiation and lineage commitment of pluripotent mouse embryonic stem (ES) cells. Expression profiling of mouse ES cells at six time points during the course of their differentiation has been performed. (2) Mesenchymal stem cells and derivative osteoblast cells. (3) Neural stem cells and neuron/glia cells. We are currently working on data analysis and independent validation of results. We also plan to do expression profiling on embryonic germ (EG) cells, on ES cells with the altered expression of Stat3, and on ES cells with altered expression of Oct-3/4.

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

Laboratory of Immunology

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The goals of the **Laboratory of Immunology (LI)** research program are aimed at uncovering information leading to a better understanding of fundamental cellular, genetic, and molecular mechanisms that contribute to changes in the immune system during the aging process and to diseases that are age-associated (e.g., increasing incidence with advancing age). There are six major areas of concentration and long-term development within LI: 1) the molecular examination of telomere length and telomerase activity in lymphocyte populations; 2) the molecular analysis of differentially-regulated genes involved in lymphoid cell and organ development, differentiation, trafficking, and activation; 3) molecular mechanisms of memory lymphocyte formation and maintenance; 4) the study and use of biological response modifiers to optimize and control leukocyte trafficking, activation, and organ engraftment in normal and aging hosts; 5) induction of antigen-specific tolerance and use in transplantation and autoimmunity; and 6) the cellular and molecular dynamics involved in thymic involution and regeneration. The Clinical Immunology Section (CIS) focuses on several important project areas including the role of inflammation and cytokines in neurodegeneration and Alzheimer's disease, the role of lipid rafts and cholesterol in the maintenance of chemokine signaling and cellular activation in the aged host and the immunoregulatory effects of pituitary and metabolic hormones in mononuclear cell activity. In addition, CIS is using techniques of high throughput gene expression profiling to unravel pathways involved in the metastasis of melanoma, a highly immune-modulated cancer. Especially important are those pathways associated with chemokine signaling, and pathways reflective of melanocyte development that go awry in metastasis (e.g., Wnt5a). The Lymphocyte Differentiation Unit is currently investigating the influence of age on telomere length and telomerase expression in peripheral blood T and B-lymphocytes, regulation and function of telomerase in lymphocytes, and the mechanisms of generation and maintenance of memory T lymphocytes. The Lymphocyte Cell Biology Unit's recent work has focused on understanding the cell biology of lymphomas, tumor-induced immunosuppression, the roles of PTEN and caveolin in lymphocyte

activation and function, and defining the role of CD28-mediated costimulatory signal in immune responses particularly in cancer and autoimmune disease.

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

Keywords:

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

Recent Publications:

Lillard JW Jr, et al. *Blood* 2003; 101(3): 807-814.

Dixit VD, et al. *Endocrinology* 2003; 144(4): 1496-1505.

Baek JY, et al. *Biochem J* 2003; 373(Pt 1): 191-200.

Nguyen DH, et al. *Exp Cell Res* 2003; 285(2): 268-277.

Nguyen DH, et al. *J Immunol* 2002; 168(8): 4121-4126.

Nguyen DH, et al. *Blood* 2002; 99(12): 4298-4306.

Chemokines, Aging, and Immune Responses: The recruitment of lymphocytes into inflammatory sites requires several activation events including endothelial cell activation by inflammatory cytokines, the expression of adhesion molecules, cellular adhesion, diapedesis, and migration via established chemotactic gradients. Over the past 10 years, members of the *chemokine* super family have been shown to induce adhesion, chemotaxis, activation, and degranulation of human and rodent leukocytes and lymphocytes both *in vitro* and *in vivo*. We are currently examining a role for chemokines in lymphocyte activation and as immunodjuvants in vaccine-based studies with hapten-carrier protein complexes. In addition, the laboratory is also examining the ability of various chemokines and other G-protein receptor ligands to modulate other T, B, and NK cell effector functions as well as antigen-presenting cell activities. Furthermore, studies examining the differential expression of various cytokines, chemokines and their cell surface receptors, post cellular activation via mitogens, hormones, lipids, and stress factors are also under investigation. As no cytokines or chemokines are ever alone within an inflammatory site, it is critical to determine how these various growth factors influence each others' signals and functions. Using purified rodent, primate, and human immune cell subsets, we have observed a significant dampening of aged lymphocyte and mononuclear cell migration, adhesion, and chemokine receptor signaling in response to ligand stimulation compared to younger control populations. The age-related changes that appear to play a role in this chemokine hyporesponsiveness include signaling defects through cell surface receptors, differences in cell surface receptor expression after cellular activation, and preferential expression or lack of expression of certain chemokine receptors on circulating immune subsets within an aged host. 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.

Cholesterol and Lipid Rafts in T Lymphocyte Signaling and Trafficking: Relevance to Aging and Inflammatory Disease:
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. 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 describing membrane microdomains enriched in cholesterol, sphingolipids, glycosylphosphatidylinositol-anchored proteins, and acylated signaling molecules on the plasma membrane of immune and non-immune cells. These rafts are believed to be important signaling platforms as well as sites of assembly for the TCR signaling complex. One of the most crucial components in maintaining the higher lipid order of rafts is cholesterol, and extraction of cholesterol by cyclodextrins (circular multimeric sugars), disrupts lipid rafts and increases overall membrane fluidity. The removal of cholesterol has profound effects on the ability of several GPCRs to bind their ligand. In addition, direct oxidation of cholesterol within the plasma membrane or treatment of cells with oxidized cholesterols called “oxysterols” also inhibits chemokine binding and activity as well as HIV-1 infectivity. Moreover, the cholesterol balance appears to be quite strict as the continued addition of cholesterol to the T cell membranes also inhibits chemokine receptor function and T cell activation. Finally, we have explored the ability of TCR or CD4 engagement to mediate adhesion molecule, chemokine receptor, and cholesterol colocalization and found that colocalization is dependent on the presence of cellular cholesterol, cytoskeletal reorganization, and lck signaling. These cell surface rearrangements that result in the capping of chemokine receptors, adhesion molecules, and lipid rafts to the site of CD4 contact may serve as a mechanism for HIV propagation and pathogenesis. Our current findings provide insight into the role of cholesterol and oxidized cholesterols in chemokine receptor structure and function. 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 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. We believe that a greater understanding of the various signaling and cell surface proteins associated with lipid rafts may provide great insight into age-related alterations in cell signaling and migration.

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 lipid rafts, exerts both specific and potent inhibitory effects on the TCR- and leptin-mediated expression of the proinflammatory cytokines via functional GHS-R and possible a novel GHS receptor on the surface of human mononuclear and T cells. Moreover, ghrelin administration into endotoxin challenged mice significantly inhibits inflammatory cytokine mRNA expression in the spleen, liver and lungs as well as serum cytokine levels. Furthermore, the expression of ghrelin, leptin and their receptors as well as GH appear to be significantly diminished with age within specific immune subsets and lymphoid organs, including the thymus. Administration of ghrelin and leptin to aged mice using implanted osmotic pumps resulted in a reversal of thymic involution and restored thymic GH expression. Our laboratory and others have demonstrated that the hormones, prolactin, GH and IGF1 potentiate human and rodent lymphocyte activation and proliferation in response to various antigens and stimuli both *in vitro* and *in vivo*. These hormones have also been shown to modulate a variety of leukocyte functions including potentiating lymphocyte activation and thymic engraftment and regeneration. Together, these data support the existence of a functional immunoregulatory network playing a significant role in cytokine regulation, cellular activation and survival. These data also support the potential therapeutic use of ghrelin and GHS-R agonists in the management of wasting associated with chronic inflammation and cancer and in restoration of thymic function in immunocompromised individuals.

Molecular and Biological Mechanisms of Age-associated Thymic Involution: One of the consequences of an aging immune system is the process of thymic involution. The thymus undergoes a progressive reduction in size due to profound changes in its architecture associated with thymic epithelia atrophy and decreased thymopoiesis. This decline is systemically followed by decreased numbers of circulating naive T cells and cell-mediated immune responses which may play a role in the increased tumorigenesis, autoimmunity, and infectious diseases observed within an aging host. Despite the extensive study of the pathophysiology of the aging thymus, the precise molecular mechanism involved in the involution process remains unclear. 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 progressively aging thymi and spleens, microarray analysis was performed using three distinct custom-made cDNA microarrays developed within our laboratory as well as 26K oligonucleotide murine arrays. The success of this project relies upon the reliability of the molecular profiling of 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. Subsequently, we plan to conduct serial analysis of gene expression (SAGE) in the thymi and spleens of mice of varying ages, H-2 and genetic backgrounds, and known involution mouse models. Our current data would suggest that thymic involution may be strain dependent and may in part be associated with distinct genetic factors rather than simply aging. We are currently analyzing the data obtained from the gene profiles of aged spleens, thymi, bone marrow, B cells, T cells and thymocytes from mice of various ages as well as from aged mice infused with GH, ghrelin or leptin. It is unclear whether certain lymphoid organs or cellular components play a critical role in longevity and life span. 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 an age-dependent manner in these different organ systems.

Role for Homocysteine in Immunoregulation and Disease Pathology: Homocysteine (Hcy) is the immediate precursor of the amino acid, methionine. In humans, blood concentrations of Hcy may become elevated as a result of deficiency in folate, vitamin B6 or vitamin B12 and has recently been identified as a putative risk factor for a number of age-associated disease states including arteriosclerosis, myocardial infarction, arterial occlusive disease, Alzheimer's disease and neural tube defects. A specific role for Hcy or any of its metabolites, such as S-adenosyl Hcy

(SAH) or Hcy thiolactone, in these conditions has not yet been firmly established. Particularly absent is a description of the effects of elevated Hcy levels on immune function. Several studies have examined the effects of Hcy on monocyte, neutrophil and B cell function, inflammation and chemokine production; however, little is known about the Hcy effects on T lymphocytes. Our initial studies revealed that treatment of resting human T cells with Hcy resulted in a dose-dependent increase in apoptotic cell death. D,L Hcy was more potent than Hcy thiolactone in this respect while SAH was found to be significantly less active or inactive in many cases. We also found that the pro-apoptotic effects of Hcy were abrogated with the addition of pan-caspase PARP inhibitors to the cell cultures. These results suggest that D,L-Hcy, like other apoptotic stressors, leads to the activation of the caspase cascade and eventually to the cleavage of the key cellular proteins, like PARP, eventually leading to the typical morphological changes observed in cells undergoing apoptosis. We have also found that Hcy-mediated apoptosis is inhibited by the phosphatase and protein synthesis inhibitors, calcium chelators, Bcl-2, and Bcl-xl. Moreover, we have also found that Hcy appears to potentiate cellular death induced by a number of other established apoptotic signals including activation-induced cell death (AICD), heat shock, and Fas ligand- and HIV-mediated T cell death. In addition to the pro-apoptotic effects of Hcy, stimulation of mononuclear cells or isolated T cells with immobilized anti-CD3 mAb in the presence of Hcy or thiolactone but not SAH resulted in a significant increase in cell division and expression of several type 1 cytokines. More detailed examination of the Hcy effects on T cell activation revealed that this type 1 cytokine production profile is mediated, in part, through the production of IL-18 and possibly IL-12. The precise mechanism involved in the generation of these cytokines is currently under investigation but we believe the Hcy effect is being mediated, in part, by specific stress-associated signals resulting from Hcy treatment. Overall, Hcy appears to exert a number of differential effects on immune cells, which may alter immune function in the circulation and tissue microenvironment with age and disease pathology. A greater understanding of the potential modulatory effects of Hcy and its metabolites on immune function may result in the development of potential therapeutic strategies to control and optimize immune responses with age and in various age-associated disease states.

Collaborators: Nicholas Lukacs, Ph.D., University of Michigan; Francis Ruscetti, Ph.D., National Cancer Institute, NIH; William Murphy, Ph.D., University of Nevada; James Lillard, Ph.D., University of Alabama at Birmingham; Gunnar Nilsson, Ph.D., Uppsala University, Sweden; Dan L. Longo, M.D., Laboratory of Immunology, NIA.

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Keywords:

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Yano S, et al. *J Immunol* 2003; 171(5): 2510-2516.

Ghosh P, et al. *Blood* 2002; 99(12): 4517-4524.

Hu L, et al. *J Immunol* 2002; 168(3): 1273-1280.

Mi QS, et al. *Eur J Immunol* 2002; 32(4): 1139-1146.

Murphy WJ, et al. *Immunol Rev* 2001; 181: 279-289.

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

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

Shaping the Pre-immune B Cell Repertoire: We have developed a number of transgenic mice and mice with targeted mutations as tools for understanding the mechanisms (receptor dependent and independent) that shape the B cell repertoire. These strains and their response to the antigen phosphocholine (PC) have been used to define the relationships between antigen recognition and protective, ineffective and detrimental immune responses and to understand the impact that an aging immune repertoire has on immune response. The immune response to PC is important because in mice it has been shown to confer a high degree of protection against infection by Streptococcus pneumoniae (SPn.), a pathogen that poses a significant risk to elderly, very young and immunocompromised individuals. Recently, we have shown that the mouse VH1 gene is essential for immune response to PC and PC-mediated protection against infection by SPn. Furthermore, by examining the associations between the VH1 gene and various light chains in PC-specific B cells, we have identified IgL chain structural determinants that may explain differences in the relative affinity/avidity of VH1/VL chain combinations for different PC containing antigens. Analysis of these models should provide insight into the complimentary contribution of interactions between VH and VL genes to protective versus ineffective immune responses to common determinants expressed on different pathogens.

We have also observed that a small subset of normal B cells disobey the dogma of allelic exclusion by expressing more than one type of immunoglobulin. These dual isotype expressing B cells express receptors that recognize antigens that are both autoreactive and essential for protection against infectious pathogens. We had suggested that coexpression of a second antigen receptor may provide a mechanism termed “receptor dilution” 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. This may be a general mechanism for shaping the B cell repertoire. Our current observations in wild type C57BL/6 mice have demonstrated that dual receptor expressing B cells are a part of the normal wild type B cell repertoire. Interestingly, analogous to the VH and VK genes used in the PC-transgenic model, the VH and VK genes expressed by this small population of B cells in wild type C57BL/6 mice have inferred specificities for both autoreactive antigens as well as antigens expressed on pathogens.

In addition, we have shown that TdT (terminal deoxynucleotidyl transferase) plays an important role in shaping the immune repertoire. Specifically, we have demonstrated that the generation of the dominant M603id response to immunization with Proteus morganii is dependent upon TdT expression. Subsequently, we have demonstrated that TdT expression affects the size of a population of autoreactive, PtC-specific B1 B cells as well as the expressed VH gene repertoire in this 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 Mutant p53 in TGF-β-mediated Growth Suppression in B Cell Lymphoma Cells: The tumor suppressor p53 is well known for its ability to inhibit the growth of cells that have suffered genetic damage or stress induced by their environment. It is able to inhibit cell growth by a number of mechanisms including induction of apoptosis and senescence. The importance of p53 as a tumor suppressor is further documented by the facts that p53 null mice have dramatically higher rates of tumor formation than their wild type counterparts and p53 gene is mutated in approximately half of all human cancers. Although many of the biochemical functions of wild type p53 have been elucidated, the "gains-of-function" associated with p53 mutations are still, for the most part, poorly understood. However, evidence suggests that these functions may promote enhanced cell growth and tumor cell metastasis. While mutant p53 is a target for cancer drug development (mainly antisense strategies), little is known about the regulation of its expression. We have reported that TGF-β treatment resulted in growth suppression associated with a decrease in expression of mutant p53 of a B cell lymphoma cell line. The goal of this study is to understand the mechanism underlying TGF-β-mediated down regulation of mutant p53 and subsequent growth arrest, and analyze the gain-of-function properties for different mutant p53s.

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Keywords:

immunological memory
memory T cells
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immune senescence
aging

Recent Publications:

Son NH, et al. *Mech Ageing Dev* 2003; 124(4): 427-432.

Ning Y, et al. *Hum Mol Genet* 2003; 12(11): 1329-1336.

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Hodes RJ, et al. *Nat Rev Immunol* 2002; 2(9): 699-706.

Weng N-P, et al. *Ann NY Acad Sci* 2002; 975: 46-56.

Research: The research interests of this laboratory are focused on two areas: (1) molecular and cellular mechanisms of memory T cell generation and maintenance and (2) roles of telomere and telomerase in lymphocyte lifespan and aging. In order to study the molecular features of memory T cells, we have developed lymphocyte-specific human and mouse cDNA microarray gene filters and analyzed general gene expression profiles of naïve, effector, and memory T cells in both human and mouse. These analyses lead to the identification of genes that are differentially expressed in naïve, effector, and memory T cells as well the kinetic changes in gene expression during naïve CD4 T cell activation. Our findings provide a starting point for elucidating the contribution of various differentially expressed genes in the process of memory T cell formation and maintenance. Telomeres and telomerase are the key components that regulate cellular replicative lifespan. Recently, we have analyzed telomere length in naïve and memory B cells with age, and the expression of natural telomeric genes in human fibroblasts and T cells. Our results demonstrate that there is no simple correlation between telomere length and expression of telomeric genes. Currently, we are characterizing the functions of differentially expressed genes in memory T cells, and determining the mechanisms of telomerase regulation and function in lymphocytes.

Molecular and Cellular Mechanisms of Memory T Cell Generation and Maintenance:

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 of the recent progresses in characterizing the functions of lymphocytes, the mechanisms underlying the generation and maintenance of memory lymphocytes remain largely unknown.

The gene expression profile of T helper (Th) cells as they transition from naïve to effector to memory states reveals limited differences among the resting Th populations

Generation of memory lymphocytes is an essential process for adaptive immunity, yet the many steps in the progression from naïve to memory T cells are only partially defined. We have identified commonly and differentially expressed genes in naïve, effector, rested effector, and memory cells of Th1 and Th2 lineages. The overall gene expression pattern is dramatically different between effector and naïve cells and also between effector cells and rested effector cells. Fewer differences are seen between rested effector and memory cells, suggesting effector expressed genes are associated with the activated state of the effector cells, and rested effector cells have acquired many, if not all, characteristics of memory cells. Comparable subsets of Th1 and Th2 lineage showed very limited differences. Together, we have presented a general analysis of gene expression and identified differentially expressed genes in $CD4^+$ T cells during differentiation. The identification of differentially expressed genes in naïve, effector, and memory $CD4^+$ T cells of both Th1 and Th2 lineages provides a starting point for elucidating how the processes of memory T cell formation and maintenance are programmed. A better understanding of these processes is essential for the rational design of vaccines and for the development of strategies for clinical intervention to control autoimmune diseases.

Kinetic assessment of general gene expression changes during human naïve $CD4^+$ T cell activation

The consequence of naïve $CD4^+$ T cell activation is the differentiation and generation of effector cells. How the engagement of T cell receptors and co-stimulatory receptors leads to profound differential changes is not fully understood. To assess the transcription changes during T cell activation, we developed human T cell specific cDNA microarray gene filters and examined the gene expression profiles in human naïve $CD4^+$ T cells for 10 continuous time points during the first 24 hours after anti-CD3 plus anti-CD28 (anti-CD3/CD28) stimulation. We report here a global and kinetic

analysis of gene expression changes during naïve $CD4^+$ T cell activation, and identification of 201 genes having expression levels that significantly changed after activation. Based on the temporal change, there are 15 genes that changed between 0-1 hr (early), 26 genes between 2-8 hr (middle), and 160 genes between 16-24 hr (late) after stimulation. Functional analyses of these genes show their roles in maintenance of resting status, activation, adhesion/migration, cell cycle progression, cytokine production, and apoptosis. Significantly, the majority of these genes were novel to T cells and their functions cover a broad range from signaling to transcription to cytokine production to structural changes during T cell activation. These results present a general kinetic view of the molecular changes of naïve $CD4^+$ T cells in response to T cell receptor-mediated activation for the first time, and provide a molecular basis in understanding how the complex network of gene expression regulation is programmed during $CD4^+$ T cell activation.

Regulation of Telomere Length and Telomerase Gene Expression in Human Lymphocyte Lifespan and Aging:

Telomere, the terminal structure of chromosomes, has captivated considerable attention recently for its newly discovered function involving the regulation of cellular replicative lifespan. Every telomere consists of an array of tandem hexamer repeats, $(TTAGGG)_n$ and the binding proteins. The inability of DNA polymerase to completely replicate the ends of chromosomes results in a loss of 50-200 base pair telomere repeats with cell division in normal human somatic cells. It has thus been proposed that a minimal length of telomeres is essential for cellular replication and telomere reduction is a mechanism for limiting replicative lifespan in normal somatic cells. In contrast, germline and malignant cells have infinite lifespan and maintain telomere length due to expression of telomerase. Telomerase is a unique reverse transcriptase consisting of two essential components, telomerase RNA template (hTER) and telomerase reverse transcriptase (hTERT), and functions to synthesize telomere repeats, which serve to protect integrity of chromosomes and to prolong replicative lifespan of cells. The selective presence of telomerase in the germline and malignant cells but not in most normal human somatic cells has been hypothesized as a basis for the immortality of the germline and of malignant cells. Our goals are to understand the regulation and function of telomere and telomerase in lymphocyte function, lifespan, and aging.

Stable telomere length and telomerase expression from naïve to memory B lymphocyte differentiation

Telomere length and telomerase activity play important roles in regulating replicative lifespan of cells. The length of telomeres also serves as a marker for the replicative history and for the remaining replicative potential of cells. Differential telomere length has been reported in human naïve and memory T cells but not in naïve versus memory B-lymphocytes. We report here an analysis of telomere length and induced telomerase expression in naïve ($CD27^-$) and memory ($CD27^+$) B cells from normal adults. Although both naïve and memory B cells lose telomere repeats with age, there is no consistent difference in telomere length between these two B cell subsets. Furthermore, both naïve and memory B cells are capable of inducing telomerase activity at similar levels after *in vitro* stimulation independent of donor's age. Finally, there is a slow increase of memory B cells in peripheral blood with age. These findings suggest that B cells are capable of maintaining telomere length during differentiation from naïve to memory B cells and this ability is maintained through age. These results demonstrate that T- and B-lymphocytes, although closely related in immune function, nevertheless employ quantitatively or qualitatively different mechanisms for the regulation of telomere length and homeostasis.

Telomere length and the expression of natural telomeric genes in human fibroblasts

Progressive telomere shortening occurs with division of normal human cells, and eventually leads to replicative senescence. The mechanism by which the shortened telomeres cause growth arrest is largely unknown. Transcriptional silencing of genes adjacent to telomeres, also called telomere position effect, has been hypothesized as a possible mechanism of telomere-mediated senescence. However, there is no report regarding telomere position effect on natural telomeric genes in human cells. To address whether the expression of natural telomeric genes is regulated by telomere length, we combined quantitative RT-PCR with quantitative fluorescence in situ hybridization to comparatively analyze the expression of 34 telomeric genes and telomere length of their 24 corresponding chromosome ends in young and senescent human fibroblasts. We demonstrated here that telomere length alone is not sufficient to determine the expression status of natural telomeric genes. An extended analysis of a tandem of eight telomeric genes on a single chromosome end revealed a discontinuous pattern of changed expression during telomere shortening and some of the changes are senescence-specific rather than non-dividing related.

This study has led to the identification of a total of eighteen telomeric genes with differential expression in young versus senescent cells. While the functions of most of these differentially expressed genes are unknown, CDK10, previously referred to as PISSLRE, has been reported as a member of the CDC2-related kinase that play a role in regulating the G2/M phase of cell cycle, and GAS11 has been implicated in cell growth arrest. Furthermore, it is worth noting that four telomeric genes on the long arm of chromosome 16, where a frequent loss of heterozygosity has been observed in breast cancer, exhibited increased expression in senescent cells. If replicative senescence is one of the protective mechanisms against tumor formation, it is conceivable that senescence-associated genes may play significant roles in tumor suppression. Further studies will be necessary to elucidate the biological function of the telomeric genes that are differentially expressed in senescent cells. These findings suggest that there is no simple correlation between telomere length and expression of telomeric genes. The role of telomeric genes in cellular senescence requires further study.

Collaborators: Richard J. Hodes, M.D., National Cancer Institute and National Institute on Aging, NIH; Yi Ning, M.D, Ph.D., University of Maryland School of Medicine; Peter Lansdorp, M.D, Ph.D., Terry Fox Laboratory, BC Cancer Agency; Susan L. Swain, Ph.D., Trudeau Institute; Pierre Henkart, Ph.D., National Cancer Institute, NIH; Kevin Becker, Ph.D., Gene Expression and Genomics Unit, Research Resources Branch, NIA.

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Keywords:

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

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Sen J, et al. *Trends Immunol* 2001; 22(6): 297-298.

Sen J, et al. *Cell Mol Biol* 2001; 47(1): 197-215.

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, NF−κB 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.

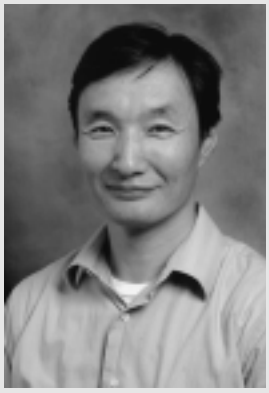
Wnt-β-Catenin Pathway in the Development and Function of Immune Cells: Because the Wnt pathway-associated transcription factors, TCF-1 and LEF-1, have been shown to be critical for T cell maturation, we hypothesized that Wnt signals may be important in the thymus. We have determined that Wnt-1, -3A, -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 alters 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 (ΔCat-Tg) in thymocytes. ΔCat-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 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, Harvard University; Jill McMahon, Harvard University; Thomas J. Hudson, McGill University, Canada; Walter Birchmeier, Max Delbrueck Center, Berlin, Germany.



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Biography: Dr. Arya Biragyn (Bira Arya) received his Ph.D. from the Institute of Molecular Biology at Engelgardt, Academy of Sciences of Russia, Moscow, in 1991. He obtained postdoctoral training from the University of Illinois at Urbana from 1991-1992 and the National Cancer Institute from 1992-1996. From 1996-2000 he was a scientist at the Science International Applications Corp. in Frederick, MD, where he worked on the development of new generation therapeutic vaccines for B cell lymphomas. In 2000 he moved to the Experimental Transplantation and Immunology Branch, National Cancer Institute as a Staff Scientist, where he continued his cancer vaccine studies. In 2003 he became an Investigator in the tenure-track program at the Laboratory of Immunology, National Institute on Aging.

Keywords:

chemokine
defensin
APT targeting
cancer
immunotherapy
DNA vaccines

Recent Publications:

Biragyn A, et al. *Science* 2002; 298(5595): 1025-1029.

Biragyn A, et al. *Blood* 2002; 100(4): 1153-1159.

Yang D, et al. *Trends Immunol* 2002; 23(6): 291-296.

Oppenheim JJ, et al. *Arthritis Res* 2002; 4(Suppl 3): S183-S188.

Ruffini PA, et al. *Haematologica* 2002; 87(9): 989-1001.

Vaccine Development: The lab works on the development of simpler and more potent vaccines for cancer and AIDS utilizing a chemo-attractant-based antigen delivery strategy. Immune responses to vaccines can be modulated towards either humoral or cellular responses at will depending on the chemokine used. Vaccine efficacy is tested in several murine tumor models, such as MC38/Muc1 breast cancer, Ras mutant tumors, and particularly B cell malignancies 38C13, A20, BCL1 and MOPC315, the most representative models for human B cell malignancies due to their non-immunogenic or weakly immunogenic features in syngeneic mice. Furthermore, vaccine efficacy is being improved further by expressing target antigens as self-assembled particles, which display chemokine and defensin moieties on their surface to target/deliver tumor and other antigens via receptors differentially expressed on iDC and other professional APCs. Simplicity of vaccines is being achieved by utilizing naked DNA immunizations. However, delivery of DNA *in vivo* is further improved and simplified by using attenuated and self-distractive Listeria or other vehicles, such as mammalian DNA incorporated in empty HBsAg particles expressing chemo-attractants.

Cytokinesas Vaccine Adjuvants: The vaccine strategy developed in the lab also works well for other clinically relevant diseases, such as AIDS. DNA vaccines expressing weakly immunogenic Env of HIV-1 fused with pro-inflammatory chemokines generated broadly neutralizing antibodies and elicited antigen-specific systemic and mucosal immune responses. Currently, he is working to establish a strategy for development of

multivalent vaccines for AIDS by using mixture of fusion proteins with various chemokines and defensins, which elicit both humoral and cellular responses to number of HIV antigens.

Enhancement of Antigen Presentation by Defensins: APCs, particularly immature DCs, can be activated with endogenous antimicrobial peptides, such as murine β-defensin 2. He demonstrated that murine β-defensin 2 acted via TLR-4 to up regulate expression of co-stimulatory molecules and production of inflammatory Th1 cytokines by iDCs. The work is being expanded to study molecular mechanisms and 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.

Collaborators: Julia R. Dorin, Western General Hospital, Edinburgh, UK; Yukio Koide, M.D., Hamamatsu University School of Medicine, Japan; Larry W. Kwak, M.D., Ph.D., National Cancer Institute, NIH; Joost J. Oppenheim, M.D., National Cancer Institute, NIH; Marjorie Robert-Guroff, Ph.D., National Cancer Institute, NIH.

Laboratory of Molecular Gerontology

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

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

The area of oxidative DNA damage and its processing is of particular interest to us. Repair of oxidative DNA base lesions is investigated in whole cells, in mitochondria and in cancer cells. We are 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 is studying DNA helicases, ATPases and exonucleases, such as the Werner syndrome, Bloom syndrome and Cockayne syndrome proteins. These enzymes are also essential in maintaining genomic instability and we are investigating their function at a biochemical level and their interactions with other proteins. A major goal is to understand the role of these proteins in important DNA metabolic processes and to clarify their role in important pathways. We are interested in understanding the role of these proteins in the normal aging process.

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

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

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

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

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

Keywords:

DNA repair
oxidative damage
Cockayne syndrome
Werner syndrome
mitochondria

Recent Publications:

Kyng KJ, et al. *Proc Natl Acad Sci USA* 2003; 100(21): 12259-12264.

Harrigan JA, et al. *J Biol Chem* 2003; 278(25): 22686-22695.

Tuo J, et al. *FASEB J* 2003; 17(6): 668-674.

Kyng KJ, et al. *Oncogene* 2003; 22(8): 1135-1149.

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

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

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

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

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

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

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

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

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

The gene defective in WS, the WRN gene, is a member of the RecQ helicase family. Helicases play roles in a number of DNA 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 endjoining, the pathway in which Ku exerts its main function. WRN also interacts with p53, possibly in the pathway of apoptosis, since WS cells have attenuated apoptosis. Further, we have recently discovered that WRN protein interacts functionally and physically with Flap endonuclease 1 (FEN-1), a protein involved in DNA

replication and DNA base excision repair. This suggests that WRN protein plays a role in one or both of those processes. Recently, we have found more protein partners of WRN, further supporting that it has a role in DNA repair and recombination.

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

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

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

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

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

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

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

We have generated stable human cell lines with functional domain knockout of different regions of the CSB gene. Mutations are introduced by site-directed mutagenesis, 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. These results demonstrate that the CSB protein plays a role in base excision repair of oxidative DNA damage. Thus, this protein has several roles in DNA metabolism, it is involved in transcription, DNA repair, apoptosis and chromatin assembly. Studies are now aimed at further structure/function analysis of CSB protein and aimed at further clarification of its function in these pathways.

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

Collaborators: J. Hoeijmakers, Erasmus University Rotterdam, Rotterdam, Netherlands; C.C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, NIH; K.H. Kraemer, National Cancer Institute, NIH; A.P. Grollman, State University of New York at Stony Brook; I. Hickson, Inst. Molecular Medicine, Oxford, United Kingdom; George Martin, University of Washington, Seattle, Washington; Erling Seeberg, University of Oslo, Norway.

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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 her present position at the NIA.

Keywords:

immunoglobulin
somatic hypermutation
DNA polymerases
mismatch repair

Recent Publications:

Winter DB, et al. *J Immunol* 2003; 170(11): 5558-5562.

Gearhart PJ, et al. *Oncogene* 2003; 22(35): 5379-5380.

McDonald JP, et al. *J Exp Med* 2003; 198(4): 635-643.

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

Polymerase eta is defective in people with xeroderma pigmentosum variant disease. We sequenced variable genes from three patients and found that their frequency of hypermutation was normal, but the types of base changes were different. Polymerase eta-deficient clones had a three-fold decrease in the proportion of mutations at A and T with a concomitant rise of mutations at G and C. It is notable that this shift in mutation pattern is consistent with the specificity of the polymerase when copying non-damaged DNA *in vitro*. This finding implies that polymerase eta is an A-T mutator in hypermutation, and another polymerase acts at G and C nucleotides. We are currently trying to identify proteins that interact with polymerase eta during hypermutation.

In collaboration with R. Woodgate, we have studied the specificity of polymerase iota on DNA substrates that might be formed during hypermutation. The overall fidelities of the polymerase are 10-fold lower when it fills a template at a DNA terminus compared to when it fills a longer template. However, the frequency and pattern of hypermutation in mice that are deficient for polymerase iota were similar to wildtype mice, suggesting that its participation in hypermutation is subtle, and other polmerases can compensate in its absence.

Collaborators: 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:

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

Puri N, et al. *Biochemistry* 2002; 41(24): 7716-7724.

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

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. Eventually this approach will be used to modulate genomic sequences with targeted gene knockout as a specific application.

Collaborators: Dr. Paul Miller, Johns Hopkins; Dr. Peter Glazer, Yale University; Dr. Irving Wainer, Laboratory of Clinical Investigation, NIA.

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Keywords:

helicase
genomic instability
Werner syndrome
DNA repair
replication

Recent Publications:

Sharma S, et al. *J Biol Chem* 2003; 278(26): 23487-23496.

Driscoll HC, et al. *J Biol Chem* 2003; 278(42): 41126-41135.

Sharma S, et al. *Mol Biol Cell* 2004; 15(2): 734-750.

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

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

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

Collaborators: Dr. Vilhelm Bohr, Laboratory of Molecular Gerontology, NIA; Dr. Michael Seidman, Laboratory of Molecular Gerontology, NIA; Dr. Ian Hickson, University of Oxford; Dr. Mark Kenny, Albert Einstein Cancer Center; Dr. Curt Harris, National Cancer Institute, NIH; Dr. Dmitry Gordenin, National Institute of Environmental Health Sciences, NIH; Dr. Robert Bambara, University of Rochester; Dr. Donald Jerina, National Institute of Diabetes and Digestive and Kidney Diseases, NIH.

David M. Wilson, III, Ph.D., Investigator
Unit of Structure and Function in Base Excision Repair

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Biography: Dr. David Wilson received his Ph.D. in Molecular Biology from Loyola University of Chicago, Stritch School of Medicine, in 1993. He performed his postdoctoral research training at Harvard University School of Public Health. In 1997 he became a Senior Biomedical Scientist at Lawrence Livermore National Laboratory in the Biology and Biotechnology Research Program. While at Livermore, he was also an adjunct faculty member 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:

Kelley MR, et al. *Cancer Res* 2003; 63(3): 549-554.

Lowry DF, et al. *J Mol Biol* 2003; 329(2): 311-322.

Wilson DM, et al. *Front Biosci* 2003; 8: d963-d981.

Mohrenweiser HW, et al. *Mutat Res* 2003; 526(1-2): 93-125.

Wilson DM, et al. *J Mol Biol* 2003; 330(5): 1027-1037.

Base Excision Repair: Base excision repair (BER) is the major pathway for correcting most spontaneous and oxidative DNA damage. In brief, 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. Our focus has been to understand the molecular mechanisms of repair of abasic sites and oxidative DNA single strand breaks, and to define the coordinated effort between the proteins of BER. Towards this end, we have isolated several BER proteins and have developed biochemical assays to assess their individual and cooperative structure-function relationships.

The Major Human Abasic Endonuclease, Ape1: Ape1 is the major corrective enzyme for abasic sites in DNA, initiating repair of this common lesion by incising the phosphodiester backbone 5' to the damage site. This enzyme also functions in specific strand break contexts to remove 3'-oxidative blocking termini, e.g. phosphate and phosphoglycolate residues, from DNA. Recently, the 3' to 5' exonuclease activity of Ape1 was found to contribute to the excision of certain 3'-mismatched nucleotides, a function likely important in maintaining genetic integrity. Our efforts continue to define the biochemical and mechanistic properties of this essential and multi-functional mammalian DNA repair enzyme.

Single-Strand Break Repair Protein, XRCC1: XRCC1 has been proposed to operate as a critical scaffolding factor in BER by binding DNA single strand breaks (specifically nicks and gaps) and recruiting other BER proteins, such as DNA polymerase β. We are defining the *in vitro* and

in vivo functional consequences of specific XRCC1 interactions, as well as searching for novel complex partners. Additional analysis includes defining the cellular phenotypes of XRCC1 mutant cell lines, and characterizing the activities of site-specific mutant and variant XRCC1 proteins.

Structure-Function Mechanism: Proteins of BER are often found to complex with or to regulate the functional capacity of other pathway participants. However, in many instances, the mechanism by which these proteins interact or communicate is unknown. Through collaborative efforts employing sophisticated biophysical techniques, most notably nuclear magnetic resonance (NMR) spectroscopy, we are working to define the precise protein-protein contact interfaces and to determine the contribution of protein- and DNA-dynamics to pathway coordination.

Susceptibility Factors: A related area of research includes determining the impact of genetic variation on DNA repair function. It has been proposed that certain genetic differences found in the human population will result in proteins that are less effective at repairing DNA damage, thus rendering the affected individual more susceptible to environmental or food agent exposures that create DNA lesions. By defining the functional capacity of variants in the oxidative DNA damage response pathways, we are building a foundation on which we can better predict the relationship of variation to human disease and the aging process. Additional analysis includes evaluating the effect of environmentally-relevant metal ions on DNA repair efficiency, with the belief that certain metal compounds will inhibit specific DNA repair responses.

Collaborators: Vilhelm A. Bohr, M.D., Ph.D., Laboratory of Molecular Gerontology, NIA; Mark R. Kelley, Ph.D., Department of Pediatrics, Indiana School of Medicine; David Lowry, Ph.D., WR Wiley Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory; Marit Otterlei, Ph.D., Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology; Larry Thompson, Ph.D., Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory; Alan Tomkinson, Ph.D., Institute of Biotechnology, University of Texas Health Science Center at San Antonio; Christine Walter, Ph.D., Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio; Teresa Wilson, Ph.D., Department of Radiation Oncology, University of Maryland School of Medicine.

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 pathogeneses of the disease and for developing treatments for these devastating disorders.

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Biography: Dr. John Hardy is a human geneticist and molecular biologist whose research interests focus on neurological disease. Dr. Hardy received his B.Sc. (Hons) degree from the University of Leeds, UK (1976) and his Ph.D. from Imperial College, London, UK where he studied dopamine and amino acid neuropharmacology. 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:

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

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

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

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

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

Collaborators: Andrew Lees, M.D., Martin Rossor, M.D., Huw Morris, M.D., Nick Wood, M.D., Henry Houlden, M.D., Rohan DeSilva, Ph.D., University of London; Ron Peterson, M.D., Ph.D., 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, dysautonomia, stroke and Parkinson's disease. In 2001 he joined the NIA as an Investigator within the newly created Laboratory of Neurogenetics. Dr. Singleton's group investigates the genetic and cellular mechanisms underlying simple-Mendelian and complex neurological diseases.

Keywords:

neurogenetics
X-linked dystonia
Parkinson's disease
parkinsonism
ataxia
stroke
dementia
hyperhidrosis

Recent Publications:

Payami H, et al. *Mov Disord* 2003; 18(4): 425-429.

Hardy J, et al. *Lancet Neurol* 2003; 2(4): 221-228.

Hardy J, et al. *Science* 2003; 300(5620): 739-740.

Hernandez D, et al. *Parkinsonism Relat Disord* 2003; 9(6): 317-320.

Hague S, et al. *Ann Neurol* 2003; 54(2): 271-274.

Eerola J, et al. *Neurology* 2003; 61(7): 1000-1002.

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 are applying this approach to a number of disorders. Lubag or X-linked recessive dystonia parkinsonism (XDP) is a rare inherited movement disorder; however, given the clinical phenotype associated with this disorder, delineation of the disease process in XDP will be informative for Parkinson's disease, dystonia and related movement disorders. We are currently involved in a positional cloning project aimed at identifying this gene defect.

Our group, in collaboration with that of Dr. Gwinn-Hardy's of NINDS, employs two clinical research coordinators who recruit and expand families. In addition to XDP, we have actively recruited >300 families with a history of various neurological diseases, including but not limited to parkinsonism, dystonia, stroke, diffuse Lewy body disease, ataxia, essential tremor and hyperhidrosis. Once again the aim of this family collection is to aid in the identification of genes important in the pathogenesis of disease. A direct result of this work was the recent identification of a genetic triplication of the α-synuclein gene causing Parkinson's disease (see Singleton, et al. selected publications).

With the beginning of the new millennium, we are entering the post genome era. Now the vast majority of human genes have been sequenced and their sequences will be available on the web. In the last 10 years, the application of molecular genetics has led to the unraveling of the etiologies of many of the single gene disorders that lead to neurodegenerative disease, but has barely begun to allow the dissection of the more complex genetics of most neurodegenerative disease which do not show simple patterns of inheritance. The only genes that have been unambiguously identified as risk factors for non-mendelian disorders are the prion gene in iatrogenic and idiopathic Creuzfeldt Jakob disease (Collinge, et al. 1991: Palmer, et al. 1991), the apolipoprotein E gene in Alzheimer's disease (Corder, et al. 1993) and the tau gene in progressive supranuclear palsy (Baker, et al. 1999); in the cases of both the prion and tau genes, there were good genetic or pathologic reasons for suspecting their involvement in disease etiology (Hsiao, et al. 1989, Flament, et al. 1991); thus, apolipoprotein E is the only neurodegenerative risk-factor gene found, in part, through positional genetic analysis (Pericak-Vance, et al. 1990).

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

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

general genetic community include sib-pair analysis, candidate gene association studies, whole genome association studies and linkage disequilibrium mapping. We employ some aspect of all of these techniques in our sample series and it seems clear that rather than using one approach, a complimentary battery of techniques is likely to yield success. Furthermore, as the contribution of an individual genetic defect to disease decreases, geneticists will have to rely increasingly on biology rather than statistics to prove pathogenicity.

Collaborators: Don Cleveland, Ph.D., University of California, San Diego; Matthew Farrer, Ph.D., Mayo Clinic, Jacksonville; Karen Parko, M.D., Shiprock Medical Center; Virgilio Evidente, M.D., Mayo Clinic, Scotsdale; Sub Subramony, M.D., University of Mississippi Medical Center; Horacio Kaufmann, Mount Sinai School of Medicine; Katrina Gwinn-Hardy, M.D., National Institute of Neurological Disorders and Stroke, NIH; Robert Nussbaum, M.D., National Human Genome Research Institute, NIH; Henry Houlden, M.D., University College Medical School, London, UK; Andrew Lees, University College Medical School, London, UK.

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

Keywords:

Parkinson's disease
neurons
cell culture models

Recent Publications:

Cookson MR, et al. *Hum Mol Genet* 2003; 12(22): 2957-2965.

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

Baptista MJ, et al. *J Neurochem* 2003; 85(4): 957-968.

Petrucelli L, et al. *Neuron* 2002; 36(6): 1007-1019.

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

In **Parkinson's disease (PD)**, there is a striking, although not entirely selective, loss of dopaminergic neurons in the substantia nigra. There are several reported linkages to PD in different families, with two causal genes identified. The routes by which dominant mutations in α-synuclein produce cell loss are being explored. The "revealing illusion of selective vulnerability," where different neuronal groups are lost at different rates, is also of specific interest. In PD, we are concerned with the selectivity of dopaminergic cells to synuclein-mediated damage. The specific relationship between cell damage induced by dominant mutations in α-synuclein and the function of the recessive gene product, Parkin, are also being investigated. Identification of substrates for Parkin (an E3 ligase linked to the ubiquitin-proteasome system) might lead us to better understand the downstream pathway leading to cell death. We are currently trying to understand the role(s) of DJ-1, a recently identified recessive gene that causes PD, in light of our current understanding of α-synuclein and parkin mechanisms.

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

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

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

Collaborators: Dr. Christopher Eckman, Mayo Clinic, Jacksonville; Dr. Matthew J. Farrer, Mayo Clinic, Jacksonville; Dr. J. Timothy Greenamyre, Emory University; Dr. Mark Mattson, Laboratory of Neurosciences, NIA; Dr. Diane D. Murphy, Dr. Craig Blackstone, National Institute of Neurological Disorders and Stroke, NIH; Dr. Leonardo Petrucelli, Mayo Clinic, Jacksonville; Dr. Benjamin Wolozin, Loyola 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

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 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 **polymorphism**.

In addition to Bioinformatics Support, our group has also been developing tools and interfaces to help the laboratory digitalizing 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 amount 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 through 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: Dr. Pankaj Agarwal, Computational Sciences and Biological Pathways, GlaxoSmithKline; Dr. Karen Kabnick, GlaxoSmithKline; Dr. Vineet Bafna, University of California San Diego; colleagues connected to 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, Transgenics Unit.

Keywords:

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

Recent Publications:

Wong PC, et al. *Nat Neurosci* 2002; 5(7): 633-639.

Wong PC, et al. *Science* 2001; 293(5534): 1434.

Cai H, et al. *Nat Neurosci* 2001; 4(3): 233-234.

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 knock 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 identified 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 the problems of 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 lead to the 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 DJ-1 gene. Meanwhile, we also have tried to define DJ-1 interacting proteins by yeast-two hybrid screening and other methods.

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; 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 the discovery of 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 2003, she joined the NIA as a Facility Head within the newly created 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:

Edland SD, et al. *Neurosci Lett* 2003; 345(1): 21-24.

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 disorder. Currently, we are performing 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, the 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 inked to 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 are being 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 which 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's 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.

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 holoprosencephaly. 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.

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Keywords:

neurodegenerative disorders
calcium and oxyradicals
signal transduction
synaptic plasticity

Recent Publications:

Duan W, et al. *Proc Natl Acad Sci USA* 2003; 100(5): 2911-2916.

Anson RM, et al. *Proc Natl Acad Sci USA* 2003; 100(10): 6216-6220.

Cheng A, et al. *Dev Biol* 2003; 258(2): 319-333.

Cutler RG, et al. *Ann Neurol* 2002; 52(4): 448-457.

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

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

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

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

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

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

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

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

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

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

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

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

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

Keywords:

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

Recent Publications:

Wolkow C, et al. *J Biol Chem* 2002; 277(51): 49591-49597.

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

Genetics of Longevity in *C. elegans*: The nematode, *C. elegans*, is quickly becoming a favorite organism for studying the genetics of longevity. Under laboratory conditions, wild-type *C. elegans* adults have a 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 *DAF-16* targets expression 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.

Collaborators: Eric Bachman, M.D., Ph.D. and Bradford B. Lowell, M.D., Ph.D., Beth Israel Deaconess Medical Center, Division of Endocrinology; Frank Rothman, Ph.D., Brown University; Angelo Russo, M.D., Ph.D., National Cancer Institute, Center for Cancer Research, NIH; Daemyung Kim, Ph.D., Changjn University, Department of Genetic Engineering; Gary Ruvkun, Ph.D., Massachusetts General Hospital, Department of Molecular Biology and Harvard University, Department of Genetics.

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

Keywords:

stem cells
aging
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ES cells
neurons
strocytes
oligodendrocytes

Recent Publications:

Lee J, et al. *Dev Biol* 2003; 253: 84-98.

Luo Y, et al. *Stem Cells* 2003; 21: 575-587.

Cheng A, et al. *Dev Biol* 2003; 258(2): 319-333.

Pevny L, et al. *Trends Neurosci* 2003; 26(7): 351-359.

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

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

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

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

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

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

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

In order to critically evaluate the roles of specific genes in the regulation of neural stem cell proliferation, differentiation and/or survival, we are initiating a major effort in which ES cell lines are derived from mice lacking expression of individual genes. For example, we are studying the role of the transcription factor NF-κB in neural stem cell fate determination by analyzing ES cells isolated from mice lacking the p65 subunit of NF-κB. Identifying factors that regulate neural precursor cell differentiation is important because there are many prominent neurological disorders for which such knowledge may provide new avenues for prevention and

treatment. Identifying instructive and trophic molecules and their stage-specific roles in regulating normal development will provide important information both in diagnosing neurological disorders and in suggesting possible therapeutic strategies. In a more general sense, we hope that the principles of differentiation we elucidate will be applicable to other developmental stages and locations where phenotypic restriction occurs.

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

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

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

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

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

Keywords:

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

Recent Publications:

Hasegawa T, et al. *J Neurochem* 2003; 87(2): 470-475.

Furukawa K, et al. *J Neurochem* 2003; 87(2): 427-436.

Yao PJ, et al. *Neurosci* 2003; 121(1): 25-37.

Wang Y, et al. *J Neurochem* 2002; 82(4): 945-952.

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

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

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

Modulation of Ion Channels by Cell Death Cascades and Neuroprotective Signal Transduction Pathways: LNS investigators have recently shown that biochemical cascades that mediate a form of programmed cell death called apoptosis can be activated locally in synaptic terminals. Interestingly, we have identified several ion channel subunits as substrates for caspases, proteases that play a pivotal role in apoptosis. For

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

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

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

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

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

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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-β peptide
tumor necrosis factor-α
p53 inhibitors
apoptosis
glucagon-like peptide-1
Alzheimer's disease
type 2 diabetes

Recent Publications:

Zhu X, et al. *J Med Chem* 2003; 46(24): 5222-5229.

Perry T, et al. *Trends Pharmacol Sci* 2003; 24(7): 377-383.

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:

Lahiri DK, et al. *J Alzheimers Dis* 2003; 5(2): 81-90.

Perry T, et al. *J Pharmacol Exp Ther* 2002; 302(3): 881-888.

Perry T, et al. *J Pharmacol Exp Ther* 2002; 300(3): 958-966.

Duan W, et al. *Ann Neurol* 2002; 52(5): 597-606.

Zhu X, et al. *J Med Chem* 2002; 45(23): 5090-5097.

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. (Collaborators: Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Medical University of South Carolina; Jack Rogers, Ph.D., Harvard, Boston, MA; Judith Flippen-Anderson, Ph.D., Naval Research Center, Washington D.C.; Tony Giordano, Louisiana State University, Shreveport, LA; Mohammad Kamal, Ph.D., University of Sydney, Australia; Axonyx Inc., New York, NY; Donald Ingram, Ph.D., Laboratory of Experimental Gerontology, NIA).

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. (Collaborators: Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Medical University of South Carolina; Jack Rogers, Ph.D., Harvard, Boston, MA; Judith Flippen-Anderson, Ph.D., Naval Research Center, Washington D.C.; Tony Giordano, Louisiana State University, Shreveport, LA; Mohammad Kamal, Ph.D., University of Sydney, Australia; Donald Ingram, Ph.D., Laboratory of Experimental Gerontology, NIA).

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. (Collaborators: Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Medical University of South Carolina; Jack Rogers, Ph.D., Harvard, Boston, MA; Judith Flippen-Anderson, Ph.D., Naval Research Center, Washington D.C.; Tony Giordano, Louisiana State University, Shreveport, LA; Donald Ingram, Ph.D., Laboratory of Experimental Gerontology, NIA).

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. (Collaborators: Prof. Tony Giordano, Ph.D., Louisiana State University, Shreveport, LA; William Douglas Figg, Ph.D., NCI, NIH, Bethesda, MD., and Prof. Debomoy Lahiri, Ph.D., Indiana University School of Medicine, Indianapolis, IN).

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. (Collaborators: Mark Mattson, Ph.D. LNS, NIA; Debomoy Lahiri, Ph.D., University of Indiana; Robert Rosenthal, M.D., University of Maryland).

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. (Collaborators: Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Medical University of South Carolina; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA; Josephine Egan, M.D., Diabetes Section, Laboratory of Clinical Investigation, NIA).

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

Keywords:

personality assessment
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five-factor model
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Recent Publications:

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

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

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

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

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

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

Basic Research in Personality - The Five-Factor Model: One focus of research has been a comparison of the NEO-PI-R system with alternative operationalizations of the Five-Factor Model and alternative taxonomies. A popular psychobiological model has been proposed by C. Robert Cloninger and colleagues who assert that there are independent temperament dimensions corresponding to chemically-coded neural networks or brain systems: dopaminergic neurons regulate the dimension of novelty seeking, serotonergic neurons regulate harm avoidance, and norepinephrinergic neurons regulate reward dependence. At the biological level, they argue that the temperament traits are associated with neurochemical substrates that have a genetic basis. One implication of this theory is that genes associated with neurotransmitters should be related to the hypothesized temperament traits. Another implication is that traits hypothesized to have a shared genetic basis should covary at the phenotypic level. According to Cloninger and colleagues, the psychobiological model, as measured by the Temperament and Character Inventory (TCI), accounts for the genetic basis of the personality phenotype, whereas alternative models of personality like the five-factor model comprise genetically and environmentally heterogeneous factors. In a study of 946 male and female participants in the BLSA to whom the TCI was administered, 587 were genotyped for a polymorphism in the dopamine D4 receptor (D4DR) and 425 were genotyped for a

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

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

Applied Research: Stress, Coping, and Psychopathology: Personality traits are important determinants of the ways in which people deal with stress. For example, Extraversion is associated with forms of coping that involve humor, talking about feelings, and seeking support; Agreeableness is associated with stoic and compliant attitudes in the face of stress. Our perspective integrates stress-and-coping research into the broader field of psychology, linked to normal adaptation, psychopathology, and the personality dimensions that affect all these. Traditionally, normal and abnormal psychology were held to be distinct and qualitatively different. Our research has shown that in many respects they are closely related, and thus that knowledge from one field is relevant to the other. For example, some of our research has focused on depression. We have shown that

depressive symptoms are related to the normal personality disposition Neuroticism, can be predicted years in advance from personality traits, and can themselves predict psychiatric diagnoses noted in hospitalization records. Perhaps most important, we have also shown that depressive symptoms and the personality traits that predispose people to depression do not increase as a normal consequence of aging. Most older people are not depressed, and those that are should receive appropriate treatment.

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

Axis II of the DSM-IV is used for the diagnosis of personality disorders, which are defined as inflexible and maladaptive personality traits. It is reasonable to ask whether these traits are the same as or different from those encountered in non-psychiatric populations. Several recent studies on this question have concurred in finding strong and replicable links between scales measuring personality disorders and the five factors in both normal and clinical populations. The potential of the five-factor model of personality to describe and differentiate personality disorders was suggested by research in North American samples of patients and psychiatrically

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

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

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Keywords:

personality structure
longitudinal studies
openness to experience
cross-cultural research

Recent Publications:

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

Personality traits are dimensions of individual differences in the tendencies to show consistent patterns of thoughts, feelings, and actions. Traits are important because their influence is pervasive: They affect personal interactions and social support, health habits and somatic complaints, attitudes and values, ways of coping, occupational and recreational interests, and much more. For the past 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 recently conducted cross-cultural studies of personality structure and development. In the first of these we reported an analysis of personality structure in Hong Kong Chinese and Japanese

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

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

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

The Origins of Personality - Behavior Genetics: According to Five-Factor Theory, personality traits are endogenous basic tendencies. Genetic factors are expected to play a major role in their origin and development, whereas environmental factors like culture should play a minor role. In collaboration with Swedish researchers, we published one of the first studies on the heritability of Openness to Experience, and we collaborated with John Loehlin and Oliver John to reanalyze the classic National Merit Twin

Study data for all five factors. A collaboration with behavior geneticists in Canada and Germany suggests that the five factors are strongly heritable in both these two cultures. In addition, that study demonstrates that more narrow and specific facet-level traits are also substantially heritable. Thus, it appears that there is a genetic basis for many of the details of personality, as well as the broad outlines.

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

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

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

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

Keywords:

heart period variability
spectral analysis
anxiety

Recent Publications:

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

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

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

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

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

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

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Keywords:

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

Recent Publications:

Resnick SM, et al. *J Neurosci* 2003; 23(8): 3295-3301.

Lamar M, et al. *Neurology* 2003; 60(1): 82-86.

Kawas CH, et al. *Neurology* 2003; 60(7): 1089-1093.

Moffat SD, et al. *J Clin Endocrinol Metab* 2002; 87(11): 5001-5007.

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.

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Keywords:

memory aging
Magnetic Resonance Imaging
Positron Emission Tomography
estrogen and cognition

Recent Publications:

Resnick SM, et al. *J Neurosci* 2003; 23(8): 3295-3301.

Kawas CH, et al. *Neurology* 2003; 60(7): 1089-1093.

Lamar M, et al. *Neurology* 2003; 60(1): 82-86.

Resnick SM, et al. *JAMA* 2002; 288(17): 2170-2172.

Moffat SD, et al. *J Clin Endocrinol Metab* 2002; 87(11): 5001-5007.

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.

Publications-continued:

Moffat SD, et al. *Behav Neurosci* 2002; 116(5): 851-859.

Davatzikos C, et al. *Cereb Cortex* 2002; 12(7): 767-771.

Lamar M, et al. *Neuropsychology* 2002; 16(2): 156-162.

Shen D, et al. *Neuroimage* 2002; 15(2): 422-434.

Resnick SM, et al. *Ann NY Acad Sci* 2001; 949: 203-214.

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 ± 0.3, 2.4 ± 0.4, and 3.1 ± 0.4 cm^3 per year for total brain, gray, and white volumes, respectively, and ventricles increased by 1.4 ± 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.

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The **Brain Physiology and Metabolism Section (BPMS)** studies brain phospholipid metabolism in intact animals and humans, as well as synaptic integrity and function in aging and Alzheimer's disease. Methods involve *in vivo* tracer studies, chemical analytical techniques, quantitative autoradiography, and positron emission tomography (PET). Studies are related to neuroplasticity and signal transduction, central action of drugs, and nutritional regulation of brain fatty acid metabolism.

(1) Brain Phospholipid Metabolism in Signal Transduction and Neuroplasticity: Radiolabeled long chain fatty acids are injected intravenously into awake rodents. By mathematical modeling, rates of incorporation into brain phospholipids, recycling and half lives are determined. Short half-lives (minutes to hours) and high turnover rates within brain phospholipids reflect their active participation in signal transduction and membrane modeling. Brain incorporation from plasma of labeled arachidonic acid, an important second messenger, is increased in response to cholinergic and dopaminergic agonists in rat models of Alzheimer's disease (chronic unilateral lesion of nucleus basalis) and Parkinson disease (chronic unilateral lesion of substantia nigra), respectively, reflecting upregulation of phospholipase A_2 mediated signal transduction. Upregulated signaling may be imaged in the human brain using positron emission tomography (PET) and [C^{11}]arachidonic, and may help in the early diagnosis and understanding of disease mechanisms of neurodegenerative disorders.

The fatty acid model can elucidate targets for centrally acting drugs with indeterminate modes of action. For example, the model has shown that lithium, used to treat manic depressive (bipolar) disorder reduces turnover of arachidonate within brain phospholipids by 80%, by downregulating gene expression (mRNA level) and enzyme activity of an arachidonic-specific phospholipase A_2. Other antibipolar disorder drugs, valproic acid and carbamazepine, have effects like lithium on brain arachidonic acid metabolism, suggesting that this metabolism is a common target for such

drugs. With this information, we may design drugs less toxic and with a wider therapeutic window than lithium for treating bipolar disorder. The model has demonstrated that the brain responds to nutritional deficiency of the polyunsaturated essential fatty acid, docosahexaenoic acid, by reducing its turnover and metabolism within brain phospholipids, thus helping to retain it.

(2) Imaging Signal Transduction in the Human Brain: *In vivo* imaging methods involving positron emission tomography (PET) were developed to examine brain blood flow and arachidonic and docosahexaenoic acid metabolism at rest and during activation in healthy subjects in relation to age, and in patients with Alzheimer's disease or chronic alcoholism. An activation, or stress test, was shown to quantify changes in signal transduction in relation to dementia progression in Alzheimer's disease, and to be effective in studying activation involving the fatty acids.

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Keywords:

phospholipid metabolism
arachidonate
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lithium
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fatty acids
Alzheimer's
synapses

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Contreras MA, et al. *Curr Opin Lipidol* 2002; 13: 267-272.

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Rapoport SI, et al. *Arch Gen Psychiatry* 2002; 59: 592-596.

Brain Phospholipid Metabolism in Relation to Signal Transduction and Neuroplasticity: Phospholipids are major constituents of cell membranes and participate in neuroplastic remodeling and signal transduction. We developed in rats an *in vivo* method and model to localize and quantify brain phospholipid metabolism and turnover of fatty acids within specific sites of brain phospholipids. A radiolabeled long chain fatty acid (unsaturated arachidonate or docosahexaenoate, saturated palmitate) is injected intravenously and its rate of incorporation into brain is measured using quantitative autoradiography and chemical analysis. With this model, we showed that lithium, used clinically to treat manic depressive disorder, reduces arachidonate turnover by some 80% without affecting turnover of docosahexaenoate and palmitate, and thus likely acts on phospholipase A_2. Additionally, C^{11}-labeled fatty acids were synthesized, in collaboration with the PET Department at NIH, and are used to image phospholipid metabolism of monkey brain with PET (tracer uptake was independent of blood flow) and to initiate a clinical PET protocol on healthy controls at rest and during activation. We plan to extend this protocol and related animal protocols to image phospholipase A_2-mediated signal transduction involving the brain, cholinergic, serotoninergic and dopaminergic systems.

Synaptic Dysfunction in Aging and Alzheimer's Disease: *In vivo* imaging methods involving positron emission tomography (PET) were developed to examine brain blood flow and metabolism at rest and during activation in patients with Alzheimer's disease and in healthy control subjects. The

activation, or stress paradigm, was found to quantify synaptic integrity. Synaptic integrity was shown to decline with dementia progression in Alzheimer's disease in two stages, the first potentially reversible and sensitive to synaptic enhancing drugs (e.g. physostigmine), the second irreversible and associated with mitochondrial and synaptic dropout.

Collaborators: Dr. William Eckelman, PET Department, Clinical Center, NIH; Dr. Norman Salem, Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, NIH; Dr. Joseph Deutsch, School of Pharmacy, Hebrew University, Jerusalem, Israel; Dr. Pietro Pietrini, University of Pisa, Italy; Dr. Harald Hampel, University of Munich, Germany; Dr. Kimmo Hatanpaa, Yale University, New Haven; Dr. Gene Alexander, University of Arizona, Phoenix.

Molecular Dynamics Section

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The **Molecular Dynamics Section (MDS)** focuses on the interplay between structure and dynamics and how these influence biological function. The section is presently involved in studying the structural and dynamic factors in hemoglobin which regulate the binding of oxygen, the uptake and release of nitric oxide as well as autoxidation with its associated release of superoxide. The finding that autoxidation of hemoglobin is appreciably enhanced at reduced oxygen pressures, has lead to the proposal of a novel method for producing oxyradicals under hypoxic conditions. Studies are being performed on erythrocytes, interaction of erythrocytes with other tissues and with whole animals to determine to what extent this mechanism contributes to the pathophysiology of aging.

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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. *J Biol Chem* 2003; 278(46): 46349-46356.

Jayakumar R, et al. *Biochim Biophys Acta* 2003; 1622(1): 20-28.

Nagababu E, et al. *Biochim Biophys Acta* 2003; 1620(1-3): 211-217.

Ajmani RS, et al. *Clin Hemorheol Microcirc* 2003; 28(1): 29-40.

Murali J, et al. *Biochem Cell Biol* 2003; 81(1): 51-59.

Nagababu E, et al. *Biochemistry* 2002; 41(23): 7407-7415.

Demehin AA, et al. *Biochemistry* 2002; 41(27): 8630-8637.

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; Mark Mattson, Ph.D., Laboratory of Neurosciences, NIA; Donald Ingram, Ph.D., Laboratory of Experimental Gerontology, NIA; Samer Najjar, M.D., Laboratory of Cardiovascular Science, NIA; Jerome Fleg, M.D., National Heart, Lung, and Blood Institute, NIH; Jeffrey Metter, M.D., Clinical Research Branch, Longitudinal Studies Section, NIA.

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 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 26 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 off 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 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 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 Support Section** will support Clinical Investigators on-site at Harbor Hospital. Dr. Shari Ling (Rheumatology/Geriatrics) is the only member of this section at the present time. NIA is currently recruiting talented young clinical investigators in Cardiology, Genetics, Endocrinology and Oncology to support ongoing and developing research programs within these areas of speciality in the NIA Intramural Research Program. It is anticipated that investigators within this section will 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 the Chief of the Longitudinal Studies Section at NIA and the Director of the Baltimore Longitudinal Study on Aging. Dr. Ferrucci received a Medical Degree and Board Certification in 1980, a Board Certification in Geriatrics in 1982 and Ph.D. in Biology and Pathophysiology of Aging in 1998 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 Institute of Aging. During the 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 is currently refining 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:

Ferrucci L, et al. *Am J Med* 2003; 115(6): 501-502.

Russo CR, et al. *Osteoporos Int* 2003; 14(7): 531-538.

Lauretani F, et al. *J Appl Physiol* 2003; 95(5): 1851-1860.

Ferrucci L, et al. *J Am Geriatr Soc* 2002; 50(12): 1947-1954.

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, independently 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 increases 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 terms, 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 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 that many complex interaction 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 plan to 1) study how the various physiological subsystems that are important for mobility interact with age in causing disability; 2) develop reference values for the integrity and functionality of the different physiologic subsystems that are implicated in mobility, to be used in clinical practice; 3) look at risk factors for the development of “soft” neurological impairments in the absence of neurological disease that is already clinically evident; 4) identify risk factors for accelerated sarcopenia and osteoporosis, including biomarkers of chronic inflammation, genetic polymorphisms and circulating levels of specific vitamins and hormones; 5) study 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 quantity of energy compatible with the 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.

Collaborators: Linda P. Fried, Jeremy Walston, Paulo Chaves, Johns Hopkins University School of Medicine; Karen Bandeen Roche, Johns Hopkins University, School of Hygiene and Public Health; Jay Magaziner, University of Maryland School of Medicine; Marco Pahor, Brenda W. Penninx, Matteo Cesari, Sticht Center on Aging, Wake Forest University School of Medicine; Stephanie Studenski, University of Pittsburgh; Mary M. McDermott, Feinberg School of Medicine, Northwestern University; Heikkinen E. Finnish, University of Jyvaskyla, Finland; Katherine L. Tucker, Jean Mayer, USDA Human Nutrition Research Center on Aging, Tufts University; Stefania Bandinelli, Benedetta Bartali, Fulvio Lauretani, Annamaria Corsi, Italian National Institute on Aging, Florence, Italy; Niccolo Marchionni, Mauro Di Bari, Stefano Fumagalli, Institute of Geriatrics and Gerontology, University of Florence, Italy; Antonio Cherubini, Umberto Senin, Institute of Geriatric Medicine, University of Perugia; Stefano Volpato, Dipartimento di Medicina Clinica e Sperimentale, Università di Ferrara, Italy; Giorgio Valenti, Marcello Maggio, Gianpaolo Ceda, Geriatrics University of Parma; Maria Luisa Brandi, University of Florence, Italy; Giuseppe Paolisso, Michelangela Barbieri, Angela Abbatecola, Department of Geriatrics and Metabolism, University of Napoli.

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

Keywords:

aging
longitudinal studies
neuromuscular
cerebrovascular
prostate

Recent Publications:

Roth SM, et al. *Exp Biol Med* 2003; 228(6): 706-709.

Talbot LA, et al. *J Rheumatol* 2003; 30(7): 1571-1578.

Conwit RA, et al. *Clin Neurophysiol* 2003; 55: 217-228.

Fang J, et al. *Urology* 2002; 59(6): 889-893.

Metter EJ, et al. *J Gerontol A Biol Sci Med Sci* 2002; 57(10): B359-B365.

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

prostate volume as well as the percentage of epithelial and stromal tissue. Longitudinal evaluation of the change in prostate size was found to increase into the fifties and the rate of change declines in older age decades. Current research is examining the natural history of 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 has 4 main components:

1. Characterization of Longitudinal Strength Changes in the BLSA: This consists of two parts. From 1960 to 1985, strength and power were measured in BLSA participants using an in-house constructed equipment that measured isometric strength and power in the upper extremities. The purpose is to determine long-term longitudinal changes (up to 25 years) in strength and power, and to relate these changes to changes in muscle mass, peripheral nerve function, daily and physical activity, and aerobic fitness. Starting in 1992, strength has been measured using a state of the art isokinetic dynamometer (Kin-Com). This equipment allows for the measurement of both concentric and eccentric strength at multiple velocities in both the upper and lower extremities. The specific purposes are to determine age-associated maximal force production of the upper and lower body musculature during the concentric and eccentric phases of exertion, at fast, slow and zero speed, and determine the angle of greatest force; determine relationships between changes in strength with age and changes in lean body mass, fat mass, bone mineral density, glucose homeostasis, functional abilities, physical activity and nutritional state. We are also interested in the contribution of muscle strength to functional performance and the development of disability, balance problems, and falls. We have shown that the age-associated declines are explained in part by change in muscle mass. However, other factors are also important including changes in nerve function and hormonal levels (e.g. testosterone). Age-asssociated changes in strength are related to functional performance as demonstrated by an association with walking speed. However, in healthy individuals, a strength level is reached where no association is observed. This level implies the presence of excessive strength potential that acts as a reserve for walking performance. In addition, there is a complex relationship between muscle strength, muscle power, muscle mass and physical activity on mortality. We found that muscle strength and power and how they change over time are long-term predictors of longevity, independent of how much muscle is present and how active you are. We believe and are currently

looking for evidence that age-associated changes in the central nervous system control of movement is a key contributor to the relationships between strength, power and longevity.

2. Comparison of Exercise Response to Resistive Strength Training in Young and Old Subjects: This project was completed under contract with the University of Maryland, College Park, Dr. Ben Hurley, principal investigator. The specific purposes are: (1) Determine the relationship between changes in lean body mass or muscle mass and changes in glucose regulation with age and strength training. (2) To determine if changes in strength or muscle mass can predict changes in total or regional bone mineral density. (3) To determine what factors best explain strength losses associated with aging and detraining and strength gains associated with strength training. We have found that young and elderly women and men respond relatively similar to resistive training. In all groups, strength increased 25-35%, with evidence of muscle hypertrophy. What was most striking was that the strength gains achieved over 9 weeks of training persisted for at least 6 months without further training. In examining, muscle samples from these subjects, we have found that the cellular production of myostatin, a factor that is produced by muscle cells as they atrophy, is suppressed by resistive training suggesting that this may represent a useful target for pharmacological intervention.

3. 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 tend 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 used 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.

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

Age-Associated Race and Gender Differences in the Carotid and Intracerebral Arteries: This project is studying intracerebral blood flow velocity and resistance, carotid blood flow velocity, and carotid wall characteristics using doppler 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 that the common carotid wall thickness is thicker in the presence of asymptomatic coronary disease. Carotid doppler ultrasonography is commonly used during evaluation of cerebrovascular disease. Our findings suggest that examining the carotid wall thickness can increase the suspicion for coronary artery disease. In a related analysis, we found that women who use estrogen replacement postmenopausally show less arterial stiffness than women who are not on replacement. Improved arterial function may be another result of hormone replacement therapy that

contributes to lower rates of heart disease. We have also observed that age change in flow velocities in the carotid artery is poorly correlated with the flow velocities in the middle cerebral arteries. We have compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease. Currently we are examining the impact of alcohol use, and serum androgen levels on 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.

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, Network, Computing, and Telephony, Photography and Arts, and Statistical and Experimental Design.

The Central Laboratory Services is subdivided into Bioinformatics, Confocal Microscopy, Flow Cytometry, Gene Expression and Genomics, and Mass Spectrometry.

The Comparative Medicine Section includes animal husbandry for a variety of species, producing transgenic and knockout rodents, and the breeding, weaning, and mating of rodents consistent with the genetic model from which they derived.

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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 15K cDNA murine embroyonic array. This Unit also provides support in the production of custom arrays based on investigator specifications and provides cDNA templates for spotting. New state-of-the-art instruments and software have greatly expanded available services and capabilities of this facility.

(5) The **Mass Spectrometry Unit (MSU)** was formed in 2000 in response to a demand for high-sensitivity amino acid sequencing of purified and blotted proteins. The scope of this service Unit has been expanded to include amino acid sequencing, MALDI-TOF mass spectrometry, and the phosphopeptide mapping of proteins from various cellular populations.

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Flow Cytometry Unit

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Biography: Dr. Robert Wersto received his Ph.D. from the Department of Biochemistry and Biophysics, Loyola University of Chicago in 1982. Dr. Wersto did his postdoctoral work in the Departments of Pathology and Hematology at the University of Rochester using the first commercially available flow cytometers and sorters. From 1985 until 1989, he was Assistant Professor of Pathology, Albert Einstein College of Medicine in the Bronx and Head of Flow Cytometry and Analytical Cytology. After a brief stay in industrial biotechnology, Dr. Wersto joined the Pulmonary Branch, National Heart, Lung, and Blood Institute (NHLBI) and played a seminal role in the first human gene therapy trial for cystic fibrosis. Most recently, he headed the flow cytometry laboratory in the non-human primate gene transfer program within the Hematology Branch, NHLBI. In mid 1999, he moved to the Flow Cytometry Unit, Research Resources Branch at the National Institute on Aging.

Keywords:

gene therapy
adenovirus
proliferation specific antigens
bone marrow progenitors
flow cytometry
cell cycle

Recent Publications:

Yoon DS, et al. *Am J Pathol* 2002; 161(2): 391-397.

Wersto RP, et al. *Cytometry* 2001; 46(5): 296-306.

Donahue RE, et al. *Mol Ther* 2001; 3(3): 359-367.

Donahue RE, et al. *Blood* 2000; 95(2): 445-452.

Cell Cycle Progression and Aging: The effects of aging on T-cell cycle progression and arrest is the subject of an on-going investigation utilizing multiparameter flow cytometry. Age-related cell cycle properties of human T cells are assessed using simultaneous measurements of DNA content and KI-67 protein expression following co-stimulation with immobilized CD3 antibody and soluble CD28. In T-cells from elderly individuals, there is increased G_0 cell cycle arrest that cannot be overcome following subsequent exposure to IL-2. Based on mitotic blocking, the delayed cell cycle entry in T-cells from older donors appears to be independent of early activation events.

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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Gene Expression and Genomics Unit

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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:

Ghorbel MT, et al. *J Biol Chem* 2003; 278(21): 19280-19285.

Cho-Chung YS, et al. *Nat Biotechnol* 2003; 21(5): 492.

Cheadle C, et al. *J Mol Diagn* 2003; 5(2): 73-81.

Kyng KJ, et al. *Oncogene* 2003; 22(8): 1135-1149.

Cho YS, et al. *Proc Natl Acad Sci USA* 2002; 99(24): 15626-15631.

Vawter MP, et al. *Schizophr Res* 2002; 58(1): 11-20.

The **Gene Expression and Genomics Unit** is involved in the design, assembly, application, and analysis of cDNA arrays and related gene expression systems. Three main areas of research include; a) applications in gene expression; b) technology development in array based assays; and c) genomic bioinformatic applications that integrate genetic and gene expression studies.

This year, gene expression studies using cDNA arrays have included Cockayne syndrome, cocaine abuse, T cell induction, schizophrenia, and caloric restriction, among others.

Efforts in technology development of cDNA arrays include projects in large scale development of high-density nylon membrane/radioactive based cDNA arrays in multiple species including mouse, human, among others, as well as development of analytical tools in the analysis of gene expression data.

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) as well as a literature mining tool, PubMatrix (http://pubmatrix.grc.nia.nih.gov).

Collaborators: Dr. Jim Eberwine, University of Pennsylvania; Dr. Kathleen Barnes, Johns Hopkins Medical Institutions; Dr. Ted Dawson, Johns Hopkins Medical Institutions; Dr. Yoon Cho-Chung, National Cancer Institute, NIH; Dr. Mark Vawter, University of California; Dr. William Freed, National Institute on Drug Abuse, 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:

Brant LJ, et al. *J Roy Statist Soc A* 2003; 166: 51-62.

Morrell CH, et al. *Commun Stat* 2003; 32: 437-459.

Horska A, et al. *J Magn Reson Imaging* 2002; 15: 137-143.

Wu AW, et al. *J Am Geriatr Soc* 2002; 50(2): 230-237.

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 in the
National Institute on Aging
Intramural Research 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.

Predoctoral IRTA Fellowship. 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 Fellowships. 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.

How to Apply:

Please send the following items to the Laboratory:

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
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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.

How to Apply:

Please send the following items to the Laboratory:

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

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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.

How to Apply:

Students can apply for the program electronically at the NIH Research and Training web site http://www.training.nih.gov/student/internship/internship.asp.

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.

How to Apply:

Students can apply for the program electronically at the NIH Research and Training web site http://www.training.nih.gov/student/srfp/index.asp.

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.

How to Apply:

Students can apply for the MARC program electronically at the NIH Research and Training web site http://www.training.nih.gov/.

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Nathan W. Shock Memorial Lecture

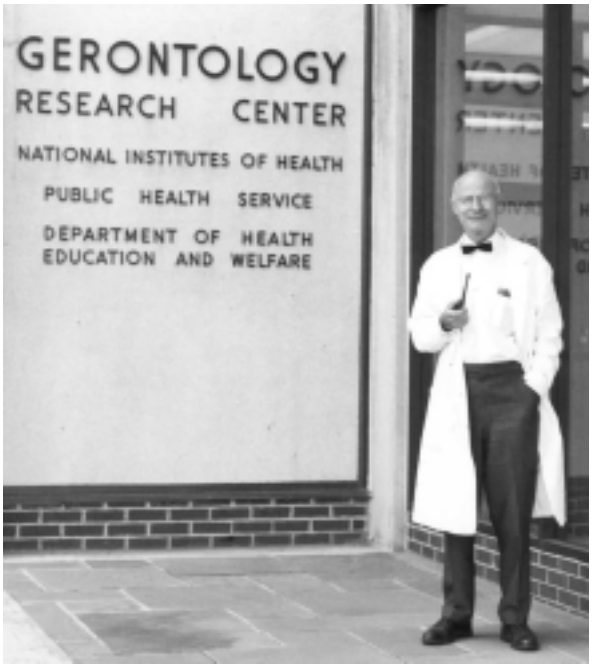
The National Institute on Aging initiated the Nathan W. Shock Annual Lecture in 1990 in honor of Nathan W. Shock, former NIA Scientific Director and NIH Scientist Emeritus, to pay tribute to his pioneering efforts in the field of gerontology. This award provides an opportunity to recognize a scientist, who has made significant contributions to our understanding of the basic mechanisms of aging.

In 2003, the annual lecture was expanded to a one-day scientific symposium featuring presentations by innovative and distinguished leaders in the field of aging. The 1st Annual Nathan W. Shock Symposium "Advances in Aging Research - 2003," was held on September 17, 2003 at the Sheppard Pratt Conference Center in Towson, Maryland. The Nathan W. Shock Lecture was presented by Nir Barzilai, M.D., Associate Professor of Medicine, Albert Einstein College of Medicine, Yeshiva University, "*New Insights on the Biology of Longevity*."
Dr. Barzilai is internationally recognized for his study of both endocrinology and geriatrics. His research focuses on identifying genetic and other factors that contribute to longevity.

Symposium speakers:

Carol Greider, Ph.D., Associate Professor, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, "*Telomerase and the Consequences of Telomere Dysfunction."*

Elizabeth Barrett-Connor, M.D., Professor, Family and Preventive Medicine, Cancer Prevention and Control Program, University of California, San Diego, "*Hormone Replacement Therapy."*



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.

Caleb Finch, Ph.D., Professor of Biological Sciences and Neurology, Director, Alzheimer Disease Research Center, University of Southern California, "*The Nexus of Inflammation and Nutrition in Aging."*

John Hardy, Ph.D., Chief, Laboratory of Neurogenetics, National Institute of Aging, NIH, "*Current Status of the Amyloid Hypotheses of Alzheimer's Disease."*

Mark A. Smith, Ph.D., Professor, Department of Pathology, Case Western Reserve University, "*Amyloid-β: All BARK and No Bite."*

Nathan W. Shock Memorial Lecture

Past 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."*

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."*

Index of Principal Investigators

Index of Keywords

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Invited Speaker Seminars - 2003

JANUARY

Ephraim Yavin, Ph.D., Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcholism, NIH. "Phospholipid Profile Alterations Modulate Oxidative Stress Induced Kinase Activation and Apoptotic Cell Death."

Gadi Beck, Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel. "Evolutionary and Functional Analyses of an Invertebrate Trk Receptor."

Zonglin Hu, Ph.D., University of Kansas Medical Center. "Dynamic Oscillation of the Min Proteins in E. Coli, How and Why?"

Pierre Alfred Henkart, Ph.D., Chief, Lymphocyte Cytotoxicity Section, Experimental Immunology Branch, National Cancer Institute, NIH. "T Lymphocyte Granule Exocytosis and Suicide Protection."

Charlotte A. Peterson, Ph.D., Associate Professor of Geriatrics, University of Arkansas for Medical Sciences, Center on Aging. "Changes in Myogenic Progenitor Potential with Age."

Jinzhe Mao, Department of Biology, Georgia State University. "Molecular Mechanisms Underlying Modulation of G-protein Coupled Inward Rectifier K+ Channels."

Robert Tycko, Ph.D., Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, NIH. "Molecular and Supramolecular Structure of Amyloid Fibrils."

France Carrier, Ph.D., Assistant Professor, School of Medicine, Biochemistry and Molecular Biology Department, University of Maryland. "Roles of Nucleolin and Nucleophosmin in the Genotoxic Stress-Response."

FEBRUARY

Rodney L. Levine, Ph.D., Chief, Section of Protein Function in Disease, National Heart, Lung, and Blood Institute, NIH. "Regulation of Protein Turnover by Oxidation."

Susan S. Wallace, Ph.D., Professor and Chair, Department of Microbiology and Molecular Genetics, The Markey Center for Molecular Genetics, University of Vermont, Burlington, Vermont. "Processing of Oxidative DNA Base Damages."

Leroy Worth, Jr., Ph.D., Laboratory of Genetics, Division of Intramural Research, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC. "Mechanisms of DNA Mismatch Repair Initiation."

Didier Brochet, Ph.D., Department of Physiology, School of Medical Sciences, University of Bristol, United Kingdom. "The Role of the Na, K ATPase in Regulating Excitability of Mammalian Arterial Smooth Muscle: Mechanism of Relaxation to Altered Potassium Concentrations."

Joshua M. Hare, M.D., Associate Professor of Medicine, Johns Hopkins University School of Medicine. "Nitric Oxide and Hypertrophic Cardiomyopathy of the Elderly."

Dalane Kitzman, M.D., Associate Professor, Internal Medicine-Cardiology, Wake-Forest University, Winston-Salem, NC. "Diastolic Heart Failure in the Elderly."

Marc M. Greenberg, Ph.D., Department of Chemistry, Johns Hopkins University. "Using Organic Chemistry to Study DNA Damage and Repair."

MARCH

Geraldine Seydoux, Ph.D., Associate Professor, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine. "Control of Embryonic Polarity and Germ Cell Fate in C. elegans."

Rodney L. Levine, Ph.D., Chief, Section of Protein Function in Disease, National Heart, Lung, and Blood Institute, NIH. "Regulation of Protein Turnover by Oxidation."

Professor Stefan Herzig, M.D., Department of Pharmacology, University of Cologne, Germany. "Altered L-type Calcium Channel Gating in Human Heart Failure - Molecular Mechanisms."

Nikita Orlov, Ph.D., Consultant to ADE Corporation, Westwood, MA. "Algorithms and Models for Wafer Defect Analysis."

Ormond A. MacDougald, Ph.D., Associate Professor, University of Michigan Medical School. "Role of Wnt Signaling in Adipose Tissue Development."

Shih-Jen Hwang, Ph.D., University of Texas, M.D. Anderson Cancer Center. "Germline p53 Mutation in a Sarcoma Cohort Sex Difference and Smoking Exposure in Cancer Risk."

Sankar Mitra, Ph.D., Department of Human Biological Chemistry and Genetics, Sealy Center for Molecular Science, University of Texas Medical Branch. "Covalent Modification of Early Proteins in DNA Base Excision Repair."

William S. Agnew, Ph.D., Professor and Director, Department of Physiology, Johns Hopkins University School of Medicine. "Regulated Recombination of Molecular Domains in the Single Gene Transcriptome of a Voltage-Sensitive Calcium Channel."

APRIL

Joanna Klapacz, Department of Chemistry, Wayne State University. "Study of Transcription-Dependent Increases in Base Substitution Mutations in E. Coli."

Ulus Atasoy, M.D., Duke University Medical Center, Department of Molecular Genetics, Durham, NC. "HuR, RNAi and Ribonomics: Towards a Better Understanding of Post-transcriptional Gene Regulation."

Gokhan Hotamisligil, M.D., Ph.D., Associate Professor of Nutrition, Harvard School of Public Health. "Stress, Inflammation, and Metabolic Disease."

Zheng Li, Department of Neurobiology and Anatomy, West Virginia University. "Mechanisms of Ethanol-Induced Damage to the Developing Cerebellum: Effects on the Cerebellar Granule Cells."

Seminar Speakers--continued

MAY

Philipp E. Scherer, Ph.D., Associate Professor, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY. "The Role of Adipocyte-derived Factors on Carbohydrate and Lipid Metabolism."

Rajendra Prasad, Ph.D., Research Associate, Laboratory of Structural Biology, National Institute of Enviromental Health Sciences, NIH, Research Triangle Plaza, North Carolina. "Human DNA Polymerase B-dependant Short and Long Base Excision Repair in Mammalian Cells."

Leon H.F. Mullenders, Ph.D., Department of Toxicogenetics, Leiden University Medical Center, The Netherlands. "Transcription Inhibition and Transcription Coupled Repair: Key Players in Different Mechanisms. Which are the Mechanisms, Which are the Players?"

Poloko Leotlela, Ph.D., Molecular Endocrinology Group, University of Oxford, Botnar Research Centre. "Molecular Genetics of Multiple Endocrine Neoplasia Type 1."

Julie A. Johnson, PharmD., Professor of Pharmacy Practice, Pharmaceutics, and Medicine, University of Florida. "B-blocker Pharmacogenetics."

Yingpei Zhang, Ph.D., Assistant Professor-Research, Department of Cellular and Structural Biology, The University of Texas Health Science at San Antonio. "Apoptosis and Aging."

Andreas Sarris, M.D., Ph.D., Hematology and Oncology, Hygeia Hospital, Athens, Greece. "Biologic Features of Hodgkin's Disease: A Window into Prognosis and a Guide for Rational Experimental Therapy. Results from the International Hodgkin Study Group."

JUNE

Majed N. Al-Jamali, Oklahoma State University, Stillwater, Oklahoma. "Utilizing Genomic Tools to Understand the Tick Host Interactome."

Dr. Jianyuan Luo, Institute for Cancer Genetics, Columbia University. "Negative Control of Tumor Suppressor p53 by Mammalian Sir2."

Dr. Sige Zou, Howard Hughes Medical Institute, University of California, San Francisco. "Genomic and Genetic Approaches Toward Understanding Aging in Drosophila Melanogaster."

Bronwen Martin, MRC Human Reproduction Sciences Unit, University of Edinburgh Chancellor's Building. "Endocrine Disruption and Pituitary Re-programming."

Mark Cannell, Ph.D., Professor and Chair, Department of Physiology, University of Auckland, New Zealand. "Making Light Work in the Lens and Cardiac Muscle - From Molecular Biology to Function and Back."

JULY

Leway Chen, M.D., Director, Cardiac Transplantation, Department of Medicine, Strong Memorial Hospital, Rochester, NY. "Diastolic Dysfunction."

Seminar Speakers--continued

Adam Antebi, Ph.D., Max-Planck-Institute for Molecular Genetics. "Endocrine Regulation of C. elegans Development and Aging."

Benjamin Sredni, Ph.D., Chief Scientist, Bar Ilan University, Israel. "Arresting IL-10 -Induced Associate Pathology by AS101: Role of P21waf Upregulation."

David Borchelt, Ph.D., Department of Neuroscience, The Johns Hopkins School of Medicine. "Protein Aggregation in Neurodegenerative Diseases."

Hans R. Scholer, Ph.D., Professor of Reproductive Medicine, School of Veterinary Medicine, University of Pennsylvania. "From Embryonic Stem Cells to Oocytes: A Stepwise Approach to Nuclear Transfer Therapy."

Lynn Heltemes Harris, Ph.D., Columbia, Maryland. "BCR Surface Expression Level: Influence on Development and Tolerance."

Frank Rothman, Ph.D., Brown University. "Microarray Studies of Hutchinson-Progeria Fibroblasts."

AUGUST

Kevin Doherty, Ph.D., Department of Biochemistry, City University of New York. "Cloning and Characterization of the BLM3 Gene of Saccharomyces Cerevisiae."

Yiting Liu, Department of Biochemistry, Dartmouth Medical School. "Redundancy and Trafficking of SNARE Proteins in ER to Golgi Transport in Saccharomyces Cerevisiae."

Alan Daugherty, Ph.D., Gill Heart Institute, University of Kentucky. "Angiotensin II Induced Vascular Pathologies - Common or Divergent Mechanisms."

Carlo Ventura, M.D., Ph.D., University of Sassari, Italy. "Autocrine and Intracrine Signaling for an Endorphinergic System in Embryonic Stem Cell Cardiogenesis."

SEPTEMBER

Robert J. Mark, Ph.D., Wyeth Research, Neuroscience Discovery Research. "WAVE-Pancortin Interaction: A Novel Proapoptotic Pathway in Neurons."

Richard N. Jones, Sc.D., Senior Research Associate, Research and Training Institute, Hebrew Rehabilitation Center for Aged, Boston, Massachusetts. "Modern Measurement and the Assessment of Adult Cognitive Function."

Jay M. Edelberg, M.D., Ph.D., Assistant Professor, Weill Medical College of Cornell University, New York City. "Young Stem Cells for the Aging Heart."

Husseini K. Manji, M.D., Chief, Laboratory of Molecular Pathophysiology, National Institute of Mental Health, NIH. "Neuroplasticity and Cellular Resilience in Mood Disorders."

OCTOBER

Scott M. Thompson, Ph.D., Department of Physiology, University of Maryland. "Changing Your Mind: Pre- and Postsynaptic Plasticity in the Injured Hippocampus."

Jane Freedman, M.D., Associate Professor of Medicine and Pharmacology and Experimental Therapeutics, Boston University School of Medicine. "Bridging Thrombosis and Inflammation."

Dr. Martin Schroder, Senior Research Associate, Department of Biological Chemistry, University of Michigan Medical Center. "Regulation of Starvation and Differentiation Responses by the Unfolded Protein Response."

Bai Lu, Ph.D., Laboratory of Cellular and Synaptic Neurophysiology, National Institute of Child Health and Human Development, NIH. "BDNF and Activity-dependent Synaptic Plasticity."

Dr. Istvan Merchenthaler, Research Scientist, Wyeth Research. "Neuroprotection by Estrogen in Animal Models of Focal and Global Ischemia."

Dr. Sebastian Fugman, Section of Immunobiology, Yale School of Medicine. "Molecular Mechanisms of DNA Rearrangements During Lymphocyte Development."

David Ginty, Ph.D., Department of Neuroscience, Johns Hopkins University School of Medicine. "Growth Factor Signaling and the Control of PNS Development."

Dr. Michael Pazin, Department of Pathology, Harvard Medical School. "Chromatin Remodeling Regulates Gene Expression in a T Cell Line."

Professor Dennis Wray, School of Biomedical Sciences, University of Leeds, Leeds, UK. "The Role of Intracellular Regions in the Activation of Potassium Channels."

NOVEMBER

Dr. Gap Lee, Yale University, New Haven, CT. "Regulation of Th2 Cytokine Locus During Th2 Cell Differentiation."

Dolores Ciufo, Technology Specialist, Invitrogen Corporation. "Tools for RNAi Analysis: New Tools for Blocking the Expression of a Specific Gene."

Dr. Richard Baird, Central Institute for the Deaf, St. Louis, MO. "Hair Cell Repair and Regeneration in the Inner Ear."

Athanase Benetos, M.D., Ph.D., Professor of Internal Medicine and Geriatrics, University of Nancy, France. "Telomere Length - A Marker of Arterial Aging."

Chistiane Richter Landsberg, Ph.D., Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine. "Stress Responses in Oligodendrocytes: Implications for Neurodegenerative Diseases."

Mark Haigney, M.D., Associate Professor of Medicine, Uniformed Services University of Health Sciences, Bethesda, MD. "Autonomic Modulation of the Cardiac Na-Ca Exchanger: What I've Learned from Failure."

DECEMBER

William J. Juskso, Ph.D., Professor of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo. “Pharmacodynamics and Pharmacogenomics of Corticosteroids.”

Lenore Launer, Ph.D., Investigator, and Tamara Harris, M.D., Senior Investigator, Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, NIH. “The Age/Gene Environment Susceptibility Study- Progress and Opportunities for Collaboration.”

Edward J. Calabrese, Ph.D., Environmental Health Sciences, University of Massachusetts. “Re-examining the Nature of the Dose Response in the Low Dose Zone: The Case for Hormesis.”

Research In Progress Seminars - 2003

Tobi Limke, Ph.D., PRAT Fellow, Laboratory of Neurosciences. “Regulation of Neural Stem Cell Proliferation and Differentiation.”

Wenzhen Duan, Ph.D., IRTA Fellow, Laboratory of Neurosciences. “Insulin and BDNF Signaling Pathway: Involvement in Neuronal Survival and Energy Metabolism Regulation.”

Dzung Nguyen, Ph.D., IRTA Fellow, Laboratory of Immunology. “Cholesterol and Chemokine Receptor Function.”

Carl Sasaki, Ph.D., Research Fellow, Laboratory of Immunology. “Two-Dimensional Gel Electrophoresis Analysis on NF-Kappa B Signaling.”

Kirill Tarasov, M.D., Ph.D., Visiting Fellow, Laboratory of Cardiovascular Science. “Post-SAGE Analysis of Differentiation- Responsive Genes in Embryonic Stem Cells.”

Heping Cheng, Ph.D., Investigator, Laboratory of Cardiovascular Science. “Mitochondrial Ryanodine Receptor: A Transducer for Excitation-Metabolism.”

Wenzhen Duan, Ph.D., IRTA Fellow, Laboratory of Neurosciences. “Regulation of Energy Metabolism by Brain Neurotrophin Signaling: Roles in Calorie Restriction and Aging.”

Min Zhu, Ph.D., IRTA Fellow, Laboratory of Experimental Gerontology. “Caloric Restriction and It’s Insulin Sensitizing Signaling: New Insights with Remaining Questions.”

TracyAnn Perry, Ph.D., Visiting Fellow, Laboratory of Neurosciences. “Neuroendocrine Targets: The “Magic Bullet” for Type II Diabetes, Obesity, and Diseases of Aging.”

Rafa deCabo, Ph.D., IRTA Fellow, Laboratory of Experimental Gerontology. “New Insights on Mechanisms of Calorie Restriction: An *In Vitro* Model and the Plasma Membrane Redox System.”

Myriam Gorospe, Ph.D., Senior Investigator, Laboratory of Cellular and Molecular Biology. “Post-transcriptional Regulation of Gene Expression During Cellular Proliferation, Stress, and Senescence.”

Ti Lin, Ph.D., Biologist, Laboratory of Genetics. “Tissue-Specific Mouse Models for Thanatophoric Dysplasia Type II.”

Satya Saxena, Ph.D., Staff Scientist, Facility Head, Mass Spectometry Unit, Research Resources Branch. “Novel Proteomics Approaches to Study Drug Resistance in Cancer Cells.”

Xiangru Xu, Ph.D., Visiting Fellow, Laboratory of Neurosciences. “The Expression, Regulation, and Functional Assignment of CAPRI, Ca2+ - promoted Ras Inactivator, in Central Nervous System.”

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