



SUNY
DOWNSTATE
Medical Center

CENTER FOR CARDIOVASCULAR AND MUSCLE RESEARCH

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Dr. Robert Furchgott, Distinguished Professor Emeritus at SUNY Downstate and member of the Executive Committee of the Center for Cardiovascular and Muscle Research was awarded the Nobel Prize in Physiology and Medicine in 1998 for his remarkable discovery of an endothelial derived relaxing factor which he later identified as nitric oxide. Its role as a neurotransmitter/neuromodulator has had a profound effect in many areas of biomedicine particularly in its role as a regulator of vascular function.



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PRESIDENT'S STATEMENT

The Center for Cardiovascular and Muscle Research (CCMR) is a innovative and very productive unit of the SUNY Downstate Medical Center. Since its inception in 1993, it has provided a focus for the application of molecular genetics to the understanding of cardiac and muscle cellular physiology and pathophysiology. In the not too distant future, that understanding will lead to a clearer understanding of cardiovascular disease mechanisms. This in turn, will allow us to refine diagnostic techniques and treatments of these diseases, by incorporating findings from studies of molecular genetics.

The Center has worked hard from it's inception to forge collaborative links between basic scientists and clinicians, and to facilitate the transfer of basic science findings to clinical applications. It is a model for centers which seek to combine the resources of basic science and clinical departments. As such, it forms an integral and welcome part of SUNY Downstate's academic enterprise.

Sincerely,



John C. LaRosa, M.D.
President

DEAN'S STATEMENT

In all areas of biomedical research including cardiovascular and muscle research, it has been said that we are at the end of the beginning. The mapping of the human genome has opened a biomedical revolution comparable to the industrial revolution. In the decades to follow, we can look forward to advances in cardiovascular and muscle research undreamed of only years ago. The Center for Cardiovascular and Muscle Research is poised to play a significant role in this new era.

Sincerely,

Eugene B. Feigelson, M.D.
Dean, College of Medicine



DIRECTORS REPORT

Biomedical research is presently undergoing a revolution in its approach to diseases and their treatment. This is no less true for the cardiovascular sciences in which recent advances in the understanding of the basic molecular processes of cardiac and muscle cells have provided a wealth of information applicable to the study and treatment of cardiovascular and muscular diseases. As the secrets of the human genome become revealed to us, both basic and clinical scientists stand to reap the rewards of this knowledge. Using DNA technology it is possible to find model systems in which an event or disease in question is ascribed to a single or a few genes. This provides a potential to target genes for diagnostic purposes and to perform therapeutic gene therapy in multiple diseases, such as, hypertension, ischemia, hypertrophy, atherosclerosis, and muscular dystrophy.

Since its inception in 1993, the Center for Cardiovascular and Muscle Research (CCMR) has provided a scientific infrastructure that has facilitated the convergence of basic with clinical research. This convergence has brought the latest technologies of the ongoing revolution in molecular genetics to bear on problems in clinical cardiology and muscle diseases. Increasingly, a strong and enduring collaborative effort is being forged between the basic scientists and the clinicians of our medical center. This is the overriding aim of the CCMR.

In its role as a training ground for future physician scientists, the Center acts as a focal point for the transfer of on-hand molecular expertise to medical fellows in their pursuit of clinical questions pertinent to cardiovascular and muscle disorders. During the past five years, scores of clinical fellows and faculty spent six months to a year doing on-hand research work in basic science laboratories on projects of mutual interest.

Research interest groups were created in which the CCMR investigators from different disciplines collaborate and ultimately compete for extra-mural funding to support their research efforts. Two major grant proposals, one for Specialized Center of Research (SCOR) and the other for training for fellows and

students (Training Grant) were recently submitted to NIH for funding consideration.

Two new faculty, Drs. Sowers and Hussain, with international reputation and expertise in diabetic cardiomyopathy and lipoprotein metabolism respectively, were added to the Center and efforts for recruitment of others with expertise in atherosclerosis and gene targeting techniques are in progress.

Another important objective is to provide an education forum by inviting speakers from outside to give state-of-the-art seminars on subjects that relate to the biology of the cardiovascular system in the broadest sense. Scores of distinguished researchers were invited to our campus for seminar presentations. A highly successful intramural symposium entitled "Impact of Molecular Biology on Cardiovascular and Muscle Research" was conducted in June, 1995 at the Campus which attracted a large audience and facilitated useful exchange of information.

In the following pages of this brochure, we have highlighted the research programs of the CCMR members affiliated with various departments of the College of Medicine in both basic science and clinical divisions. I hope this publication will convey to the readers the breadth, depth and excitement of research being pursued at our Center.



VASCULAR BIOLOGY ASSOCIATED WITH METABOLIC DISORDERS

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RESEARCH INTERESTS:

Vascular biology associated with metabolic disorders. Current work examines the interaction of insulin-like growth factor (IGF-1) and angiotensin II (AngII) / Estradiol (E2) in regulation of gene expression/activity of the nitric oxide synthase enzymes and of hyperglycemia, insulin/IGF-1 resistant states and under conditions where AngII is overexpressed in cardiovascular

tissue. Work supported by the NIH, VA Merit and ADA also examines the role of estradiol and gender in mediating the interactive signalling of Ang II and IGF-1 in cardiomyocytes, vascular smooth muscle cells and endothelial cells. Molecular techniques include adenovirus transfection and knockout of various intermediate pathways such as the PI3-kinase pathway.

Funded by multiple grants from the National Institute of Health and the Veterans Administration.

RECENT SELECTED PUBLICATIONS:

1. Ali SS, Igwe RC, Walsh MF, Sowers JR. Troglitazone and vascular reactivity: role of glucose and calcium. *Metabolism* 1999;48(1):125-130.
2. Ren J, Sowers JR, Natavio M, Brown RA. Influence of age on inotropic response to insulin and insulin-like growth factor 1 in spontaneously hypertensive rats: role of nitric oxide. *PSEBM* 1999;221:46-52. Sowers JR, Draznin B. Insulin, cation metabolism and insulin resistance. *J Basic & Clin Physiol & Pharmacol* 1999;9(2-4):223-233.
3. Ren J, Walsh MF, Hamaty M, Sowers JR, Brown RA. Augmentation of the inotropic response to insulin in diabetic rat hearts. *Life Sciences* 65(4):369-380, 1999.
4. Grundy SM, Benjamin IJ, Burke GL, Chair A, Eckel RH, Howard BV, Mitch W, Smith SC, Sowers JR. Diabetes and cardiovascular disease. A statement for healthcare professionals from the American Heart Association *Circulation* 100:1134-1146, 1999.
5. Ren J, Dominguez LJ, Sowers JR, Davidoff AJ. Metformin but not glyburide prevents high glucose-induced abnormalities in relaxation and intracellular Ca²⁺ transients in adult rat ventricular myocytes. *Diabetes* 48:2059-2065, 1999.
6. Jacober SJ, Sowers JR. An update on perioperative management of diabetes *Arch Intern Med* 159:2405-2411, 1999.
7. Ren J, Samson WK, Sowers JR. Insulin-like growth factor 1 as a cardiac hormone: physiological and pathophysiological implications in heart disease. *J Mol Cell Cardiol* 31:2049-2061, 1999.
8. Ren J, Jefferson L, Sowers JR, Brown RA. Influence of age on contractile response to insulin-like growth factor 1 in ventricular myocytes from spontaneously hypertensive rats. *Hypertension* 34:1215-1222, 1999.
9. Hamaty M, Guzman CB, Walsh MF, Bode AM, Levy J, Sowers JR. High glucose-enhanced acetylcholine stimulated cGMP masks impaired vascular reactivity in tail arteries from short-term hyperglycemic rats. *Int Jnl Experimental Diab Res* 1:69-79, 2000.
10. Sowers JR, Lester M. Hypertension, hormones and aging. *J Lab Clin Med* 135:379-386, 2000.
11. Bakris GL, Williams M, Dworkin L, Elliott WJ, Epstein M, Toto R, Tuttle K, Douglas J, Hsueh W, Sowers JR for the National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. Preserving renal function in adults with hypertension and diabetes: A consensus Approach. *Am J Kidney Dis* 36(3):645-661, 2000.
12. Sowers JR. Common pathophysiological issues in diabetes and cardiovascular disease. *Cardiol Rev* 17(4):13-23, 2000.
13. Muniyappa R, Xu R, Ram JL, Sowers JR. Inhibition of Rho protein stimulates iNOS expression in rat vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 278:H1762-H1768, 2000

MOLECULAR MECHANISMS OF INTESTINAL LIPOPROTEIN ASSEMBLY

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RESEARCH INTERESTS:

Intestinal cells assemble chylomicrons to transport dietary fat and fat-soluble vitamins. We have developed a cell culture model to study the assembly and secretion of these lipoproteins. The assembly of lipoproteins is critically dependent on two proteins, apolipoprotein B (apoB) and microsomal triglyceride transfer protein (MTP). It is known that physical interactions between apoB and MTP are important for lipoprotein assembly. We are in the process of identifying the binding sites involved in these protein-protein interactions in order to understand their physiologic role. Our goal is to understand the role of cell-surface receptors, intracellular chaperones, other apolipoproteins, and lipid transfer proteins in the biosynthesis of chylomicrons and the incorporation of fat-soluble vitamins into these lipoproteins. In addition, we would like to understand the mode of action of inhibitors that specifically inhibit chylomicron assembly and secretion, and evaluate their therapeutic potentials in the lowering of plasma cholesterol and triglyceride levels.

Funded by multiple grants from the National Institute of Health and the American Heart Association.

RECENT SELECTED PUBLICATIONS:

1. Luchoomun, J., Zhou, Z., Bakillah, A., Jamil, H., and Hussain, M. M. Assembly and secretion of VLDL in nondifferentiated Caco-2 cells stably transfected with human recombinant apo B48 cDNA. *Arterioscl. Thromb. Vasc. Biol.* 17:2955-2963. 1997.
2. Hussain, M. M., Bakillah, A., and Jamil, H. Apolipoprotein B binding to microsomal triglyceride transfer protein decreases with increases in length and lipidation: implications in lipoprotein biosynthesis. *Biochemistry.* 36:13060-13067. 1997
3. Bakillah, A., Jamil, H., and Hussain, M.M. Lysine and arginine residues in the N-terminal 18% of apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *Biochemistry.* 37:3727-3734. 1998.
4. Hussain, M.M., Bakillah, A., Nayak, N., and Shelness, G.S. Amino acids 430-570 in apolipoprotein B are critical for its binding to microsomal triglyceride transfer protein. *J. Biol. Chem.* 273:25612-25615. 1998.
5. Luchoomun, J., and Hussain, M.M. Assembly and secretion of chylomicrons by differentiated Caco-2 cells: nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. *J. Biol. Chem.* 274:19565-19572. 1999
6. Hussain, M.M. A proposed model for the assembly of chylomicrons. *Atherosclerosis* 148:1-15. 2000.

THE ROLE OF HYPOMAGNESEMIA IN CARDIOVASCULAR DISEASES

BELLA T. ALTURA, PH.D. Professor of Physiology & Pharmacology
BURTON M. ALTURA, PH.D. Professor of Physiology & Pharmacology

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RESEARCH INTERESTS:

Dr. Bella Altura and Dr. Burton Altura study the effects of drugs and divalent cations on the cardiovascular system. They have previously demonstrated that atherosclerosis and audiogenic stress may lead to hypertension and heart disease via action of Mg²⁺. These studies are done in intact animals and human subjects as well as in vitro, in heart and blood vessels, cultured vascular smooth muscle cells, endothelial cells, cardiac myocytes or neurons. Drs. Altura have produced substantial data regarding the importance and effects of Mg²⁺ in cardiovascular biology and disease processes in humans and animals.

The research in Dr. Bella Altura's laboratory is focusing in the roles of cytosolic free magnesium ([Mg²⁺]_i) and serum ionized Mg²⁺ in cardiovascular homeostasis and

disease. Much of our recent research centers on the role of this action in women's health and disease. We have found that estrogens and progesterone play dominant roles in regulating blood and vascular smooth muscle cell levels of free Mg²⁺ ions. Disturbances in the menstrual cycle and menopause lead to perturbation of the normal hormonal regulation of Mg²⁺ in women. Certain disease processes in women, particularly those associated with cardiovascular disease, demonstrate abnormalities in Mg²⁺ metabolism. Much of these advances have been obtained through the use of Mg²⁺ ion-selective electrodes which our laboratory helped to pioneer.

The research in Dr. Burton Altura's laboratory is focusing in on the molecular and cellular mechanisms whereby substances of abuse and alcohol induce cerebral inflammatory states and strokes. We have found recently that alcohol and cocaine induce these disease processes by first causing a rapid and profound lowering of brain and cerebral vascular intracellular, cytosolic free magnesium ions ([Mg²⁺]_i) which in turn trigger entry and intracellular release of Ca²⁺. This appears to be followed by activation of the sphingomyelinase (Smase)-sphingolipid pathway and oxidation of membrane fatty acid double-bonds, leading to formation of PAF-like phospholipids and further entry of Ca²⁺ which in turn activates PKC isoforms and NF-κB. The result is a sustained, localized inflammatory response characterized by leukocyte rolling-adhesion on venules, intense microvascular ischemia or venular rupture leading to focal hemorrhages. Our studies employ ³¹P-NMR, ¹H-NMR, Ca²⁺ and Mg²⁺ ion-selective electrodes, imaging of single cells with fluorescent molecular probes, and state-of-the-art lipid biochemistry methods.

Another focus of the laboratory is the role dietary intake of Mg plays in atherogenesis and blood pressure regulation. We have found that Mg²⁺ regulates the state of oxidation of vascular smooth muscle membranes and generation of a variety of sphingolipids which exert vasomotor actions on numerous blood vessels in the brain and peripheral circulation. Recent findings, in our laboratory, suggest that nitric oxide generation or its inhibition plays an important role in the cardiovascular and molecular-cellular action of Mg²⁺. Mg²⁺ appears to modulate atherogenesis and blood pressure regulation via its actions on PKC isoforms and nuclear transcription via NF-κB activation or inhibition.

Funded by National Institute on Alcohol Abuse and Alcoholism and NOVA Biomedical.

RECENT SELECTED PUBLICATIONS OF DR. BELLA ALTURA:

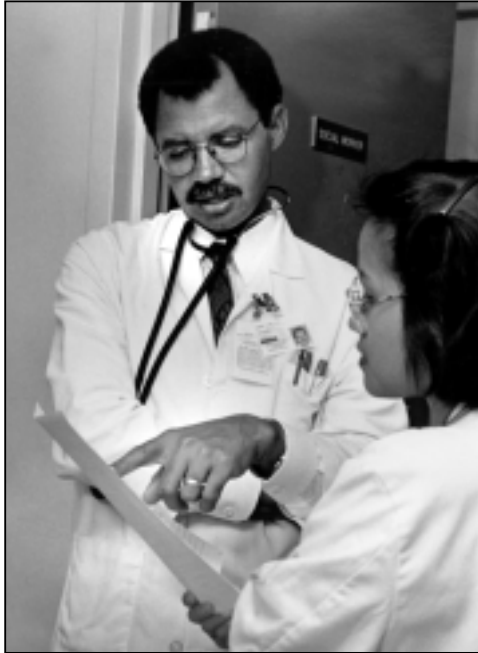
1. Altura BT, Z I Memon, A Zhang, TP-O Cheng, R. Silverman. 1997. Low levels of serum ionized magnesium are found in patients early after stroke which result in rapid elevation in cytosolic-free calcium and spasm in cerebral vascular smooth muscle cells. *Neuroscience Letters* 230: 37.
2. Muneyyirci-Delale O, Nacharaju, VL, Altura BM, Altura BT. 1998. Sex steroid hormones modulate serum ionized magnesium and calcium levels throughout the menstrual cycle in women. *Fertility and Sterility* 69:958-962.
3. Muneyyirci-Delale O, Nacharaju VL, Dalloul M, Altura BM, Altura BT. 1999. Serum ionized magnesium and calcium in women after menopause: inverse relationship of estrogen with ionized magnesium. *Fertility and Sterility* 71:869-872.

RECENT SELECTED PUBLICATIONS OF DR. BURTON ALTURA:

1. GA Morrill, RK Gupta, AB Kostellow, G-Y. Ma, A Zhang, BT Altura, and BM Altura. 1997. MG²⁺ modulates membrane lipids in vascular smooth muscle: a link to atherogenesis. *FEBS letters* 408:191-194.
2. GA Morrill, RK Gupta, AB Kostellow, G-Y. Ma, A Zhang, BT Altura and BM Altura. 1998 Mg²⁺ modulates membrane sphingolipid and lipid second messengers in vascular smooth muscle cells. *FEBS letters* 440:67-171.
3. Z-W. Yang, BT Altura and BM Altura. 1999. Low extracellular magnesium contraction of arterial muscle: role of protein kinase c and protein tyrosine phosphorylation. *Eur J Pharm* 378:273-281.

METABOLIC BASIS OF RACIAL DIFFERENCES IN CHOLESTEROL LEVELS

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RESEARCH INTERESTS:

Dr. Clark's research interest is in clinical cardiology. He has been running a comprehensive study on the metabolic basis of racial differences in HDL - cholesterol levels in African-Americans and non-Hispanic whites. Other projects he leads include ethnic influences on atherosclerosis, the effects of menopause on serum lipoprotein levels in black and white women; and heart disease in patients with diabetic renal-retinal syndrome.

Funded by the National Institute of Health through the University of Texas.

RECENT SELECTED PUBLICATIONS:

1. Clark LT. Primary Prevention of Cardiovascular Disease in High-Risk Patients: Physiologic and Demographic Risk Factor Difference Between African American and White American Populations. *The American Journal of Medicine* 1999, 107(2A):(225-245).
2. Gaba MK, Gaba S, Clark LT. Cardiovascular Disease in Patients With Diabetes: Clinical Considerations. *J. Assoc Academic Minority Physicians*. 1999; 10:15-22.
3. Clark LT, Nseir G, Chesler R. Recreational and Competitive Athletics in Older Athletes with Cardiovascular Disease. In: RA Williams ed: *The Athlete and Cardiovascular Disease*. Lippincott Williams & Wilkins, Philadelphia, 1999, pp. 109-130.
4. Turitto G, Cebeci B, Clark LT, El-Sherif N. Non-invasive testing in athletes: signal averaged ECG and ambulatory recordings. In: R.A. Williams (ed): *The Athlete and Heart Disease*. In: RA Williams ed: *The Athlete and Cardiovascular Disease*. Lippincott Williams & Wilkins, Philadelphia, 1999, pp. 183-196

TRANSFORMING GROWTH FACTOR- β REGULATION IN HYPOXIA

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RESEARCH INTERESTS:

We have shown that expression of transforming growth factor - beta (TGF- β) gene is regulated by hypoxia in human endothelial cells. The effort in the laboratory is concentrated on determining the underlying molecular mechanisms of this transcriptional regulation. The second area of investigation is the mechanism of hypoxia-induced signal transduction. We have identified smad 2, 3 and 4 proteins as mediators of hypoxia-induced gene expression. Interaction of this pathway with other signal transducing pathways is being investigated.

The role of TGF- β in central nervous system is being explored by determining the relationship between TGF- β and hypophyseal pituitary axis (HPA) in non-human primates; and by determining TGF- β gene expression in the brain in rats induced to develop status epilepticus.

Funded by the American Heart Association.

RECENT SELECTED PUBLICATIONS:

1. Batuman OA, Ferrero A, Diaz A, and Jimenez SA: Regulation of transforming growth factor-b1 gene expression by glucocorticoids in human T and glial cells. *J Immunol* 155:4397, 1995.
2. Batuman OA, Go D, Clark LT, Clements P, Feit A, Lederer D, and Smith E: Transforming growth factor-b1 and severity of coronary artery disease in an inner-city population of women. (In Press: *Heart Disease* for publication in Mar-Apr 2001).
3. Cooper B, Panetta T, Ramirez J, and Batuman OA: Localization and temporal distribution of transforming growth factor-b1 during development of initial hyperplasia in a rat vein graft model. (Submitted to the *Journal of Vascular Surgery*).
4. Smith E, Batuman O, Trost R, Coplan J, and Rosenblum L: Correlation between circulating cortisol and transforming growth factor-b1 levels in differentially reared primates. (Submitted to *Brain, Behavior, and Immunity*).

SIGNALING MECHANISMS AND TRANSCRIPTIONAL ADAPTATION IN HEART DEVELOPMENT AND DISEASE

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RESEARCH INTERESTS:

The research in our laboratory is focused on investigation of the molecular mechanisms underlying heart development and disease. We have identified two novel transcription factors that appear to play a critical role in myogenic development. One transcription factor CLP-1 (Cardiac Lineage Associated Protein), is expressed very early i. e., prior to cardiac fate assignment, in development.

The pattern of CLP-1 expression based on whole mount in situ hybridization coincides remarkably well with the established morphogenetic field of early heart formation. Treatment of chicken blastoderm with antisense CLP-1 oligonucleotides resulted in an apparent cardiac bifida condition and abnormal bulbous heart morphology. At the molecular level, the expression of the downstream well documented cardiac specific markers, such as, cNKX2.5 and GATA 4, was ablated, suggesting that CLP-1 may be an important upstream regulator of these proteins in the genetic program underlying cardiogenesis.

In addition to development, our laboratory is interested in signal transduction mechanisms that are active in the pathological states of heart development, such as, hypertrophy and ischemia. We have recently demonstrated that angiotensin II (Ang II) activates the Jak/STAT pathway resulting in enhanced binding of STAT-3, STAT-5A and STAT-6 to the angiotensinogen (ANG) promoter which causes the induction of the autocrine loop of renin-angiotensin system (RAS) during development of myocardial hypertrophy. Nuclear extract isolated from the adult hypertrophic SHR hearts showed a consistent high level of activated STAT proteins whereas those from STAT 6 null (-/-) mutant mice have a significant reduced level of activated STATs. Our follow-up studies with isolated and perfused rat heart subjected to ischemic stress also showed an induction of the Jak/STAT pathway and the consequent activation of the RAS components assayed by STATs/binding to the target site DNA. Pre-conditioning of the heart to ischemic stress produced a substantial reduction in STAT/DNA binding. Taken together, it appears that signaling rendered by the Jak/STAT pathway constitutes an essential step in modulation of the transcriptional machinery essential for development of various pathologies associated with angiotensin II.

Funded by multiple grants from the National Institute of Health.

RECENT SELECTED PUBLICATIONS:

1. Mascareno, E., Dhar, M., and Siddiqui, M.A.Q. STAT Protein Dependent Activation of Angiotensinogen Promoter: A cellular Signal for Hypertrophy in Cardiac Muscle. *Proc. Nat'l. Acad. Sci., U.S.A.* 95, 5590-5594, 1998.
2. Ghatpande, S., Goswami, S., Shafiq, S., Rong, G., and Siddiqui, M.A.Q. Identification of a Novel Cardiac Lineage Associated Protein (CLP-1); A Candidate Regulator of Cardiogenesis. *Develop. Biol.* 208, 210-220, 1999.
3. Ghatpande, S., Goswami, S., Mascareno, E., and Siddiqui, M.A. Q. Signal Transduction Adaptation in Embryonic Heart Development and during Myocardial Hypertrophy. *Mol. Cell Cardiol.* 196, 93-97, 1999.
4. Wagner, M., Mascareno, E., and Siddiqui, M.A.Q. Cardiac Hypertrophy: Signal Transduction, Transcriptional Adaptation and Altered Growth Control. *N.Y. Acad. Sci. U.S.A.* 874, 1-10, 1999.
5. Zaugg, M., Xu, W., Lucchinetti, E., Shafiq, S., Jamali, N., and Siddiqui, M.A.Q. b-Adrenergic Receptor Sub-Types Differentially Affect Apoptosis in Adult Rat Ventricular Myocytes. *Circulation* 102, 344-350, 2000.

LEPTIN: A REGULATOR OF THE CARDIOVASCULAR SYSTEM

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RESEARCH INTERESTS:

The direct relationship between obesity and cardiovascular diseases, such as, hypertension and coronary heart disease (CHD), is not understood at the molecular level. The receptors for leptin have been found in several tissues, including those in the cardiovascular system, suggesting that leptin may play a role in the regulation of cardiovascular functions. We have recently established that activation of the Jak/Stat signaling pathway is an essential component in maintaining the autocrine loop of the local tissue renin-angiotensin system (RAS) during cardiac hypertrophy. We further observed that the STAT proteins that are essential to maintain the autocrine loop of the heart tissue RAS are also the target of leptin. This observation along with the evidence on the presence of leptin receptors in the cardiovascular tissues prompted us to examine whether leptin is a potential regulator of the RAS and/or the cardiovascular function. Activation of the Jak/Stat pathway by leptin can induce the tissue RAS thereby linking obesity with hypertension, and perhaps with the mechanism underlying hypertrophic growth of the heart.

This research is funded by NIH grants awarded to M.A.Q. Siddiqui.

RECENT SELECTED PUBLICATIONS:

1. Eduardo Mascareno, Manya Dhar and M.A.Q. Siddiqui. (1998) Signal transduction and activator of transcription (STAT) Protein-Dependent Activation of Angiotensinogen Promoter: A Cellular Signal for Hypertrophy in Cardiac Muscle. *Proc. Natl. Acad. Sci. USA* 95, 5590-5594.
2. Ghatpande S, Goswami S, Mascareno E, Siddiqui M.A.Q. Signal transduction and transcriptional adaptation in embryonic heart development and during myocardial hypertrophy. *Mol Cell Biochem* (1999) 196:93-97.
3. Wagner M, Mascareno E, Siddiqui M.A.Q. Cardiac hypertrophy: signal transduction, transcriptional adaptation, and altered growth control. *Ann. N Y Acad. Sci.* (1999) 874:1-10
4. Manya Dhar, Eduardo Mascareno, and M. A. Q. Siddiqui. (1997) Two Distinct Factor-Binding Elements in Cardiac Myosin Light Chain 2 Gene Are Essential For Its Repression In Skeletal Muscle: Isolation Of cDNA Clone For Repressor Protein Nished. *J. Biol. Chem.* 27, 18490-18497.
5. E. Mascareno, and M.A.Q. Siddiqui The role of Jak/Stat signaling in heart tissue renin-angiotensin system. (2000) *Mol. Cell. Biochem.* (In Press).

ROLE OF RETINOBLASTOMA TUMOR SUPPRESSOR PROTEIN IN HEART DEVELOPMENT

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RESEARCH INTERESTS:

The specific aim of my studies is to elucidate the role of the retinoblastoma tumor suppressor protein in the epithelial-to-mesenchymal transformation of endocardial cells that occurs during formation of endocardial cushions. The process by which the endocardial cell layer transforms to form endocardial cushions is the first step in septation, the process by which discrete regions of the early heart tube ingress to form septa. The formation of septa marks the functional transition of the heart from a simple tube to a functioning four chambered pump: septation leads to the formation of the atria and ventricles, to the construction of the heart valves controlling blood flow between these heart chambers, and to the generation of the aorticopulmonary vessels that bring blood to and from the heart. By studying the molecular mechanisms that endocardial cells use to control their proliferation, differentiation, and function, we can make progress toward our long-term goal of understanding the molecular basis of endocardial cushion formation and septation, processes crucial to the proper development and function of the heart and cardiovascular system.

Funded by grants from NIH awarded to M.A.Q. Siddiqui.

RECENT SELECTED PUBLICATIONS:

1. Ghatpande S., Wagner M., and Siddiqui, M.A.Q. (1997). Molecular Adaptation of Transcriptional Apparatus in Cardiac Hypertrophy and Embryonic Development. In *Advances in Organ Biology*, Vol. 6, "Myocardial Preservation and Cellular Adaptation," p. 145-153, E.E. Bittar and D.K. Das, eds. (1998).
2. Wagner, M. Detection and measurement of retinoic acid production by isolated tissues using retinoic acid-sensitive reporter cell lines. In *Methods in Molecular Biology, Retinoid Protocols*. The Humana Press Inc. Vol. 89, p. 41-53, 1998.
3. Wagner, M., Mascareno, E. and Siddiqui, M.A.Q. (1999). Cardiac Hypertrophy: Signal Transduction, Transcriptional Adaptation, and Altered Growth Control. In *Heart in Stress*, New York Academy of Sciences, 874, 1-10, 1999.
4. Chandrasekaran, V., Zhai, Wagner, M., Kaplan, P.L., Napoli, J.L., and Higgins, D. Retinoic Acid Regulates the Morphological Development of Sympathetic Neurons. *J. Neurobiology* 42, 383-393, 2000.

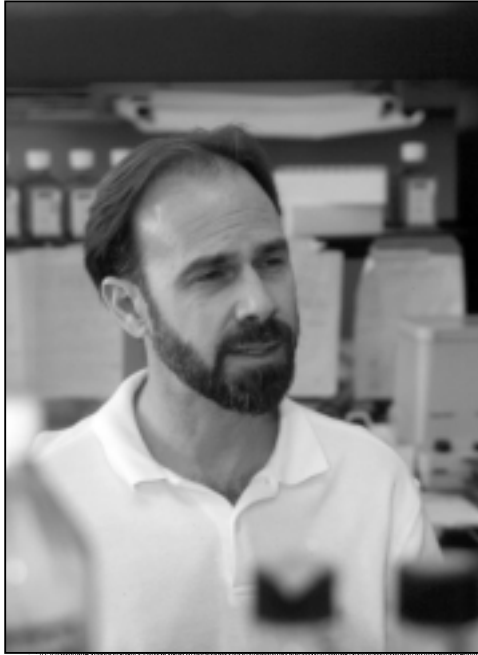
REGULATION OF Na-K, ATPase SUBUNIT BIOSYNTHESIS

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RESEARCH INTERESTS:

The research in our laboratory investigates the molecular mechanisms that regulate Na, K-ATPase subunit biosynthesis in cardiac cells. Na-K, ATPase is an intrinsic plasma membrane enzyme that plays an essential role in animal cell physiology. By its continuous function coupled to the hydrolysis of ATP, Na-K, ATPase is directly responsible for maintaining transmembrane gradients of Na and K, resting membrane potentials, control of cell volume and a significant portion of basal energy utilization.

Prolonged exposure of a variety of cells to conditions which are inhibitory to Na-K, ATPase function, such as digitalis derivatives or low extracellular K, yields an initial increase in the intracellular concentration of Na and a subsequent upregulation of Na-K, ATPase activity and subunit content. Similar increases in Na-K, ATPase expression have been noted in vivo with administration of digoxin to experimental animals and humans. Our current results indicate that low K mediates an increase in b1 subunit gene transcription that is dependent on extracellular Ca. Transient transfection studies with 5' deletion constructs revealed that a 21 base pair region of the proximal b1 gene promoter between nucleotides -62 to -42 is necessary for transcriptional upregulation in response to low K.

Another area of interest in is the characterization of the signal transduction pathways that are responsible for control of Na-K, ATPase expression in hypertrophied cardiac myocytes in the absence and presence of ischemia. Myocardial hypertrophy is an enlargement of cardiac myocytes without cell proliferation that is associated with a wide array of cardiovascular diseases such as hypertension, valvular dysfunction and end-stage heart failure. Hypertrophied myocardium is more prone to lethal arrhythmias in response to ischemia; however, the molecular events underlying this phenomenon have not been defined. We postulate that reduction of Na-K, ATPase contributes to an increase in sudden cardiac death due to hyperarrhythmias. We are currently conducting experiments in animals and cell culture to test this hypothesis

Funded by the American Heart Association.

RECENT SELECTED PUBLICATIONS:

1. Gidh-Jain, M., Huang, B., Jain, P., Gick, G. and El-Sherif, N. (1998) Alterations in cardiac gene expression during ventricular remodeling following experimental myocardial infarction. *J. Mol. Cell. Cardiol.* 30:627-637.
2. Chin, S., Apriletti, J. and Gick, G. (1998) Characterization of a negative thyroid hormone response element in the rat Na-K, ATPase $\alpha 3$ gene promoter. *Endocrinology.* 139:3423-3431.
3. Chin, S., He, H. and Gick, G. (1998) Selective induction of Na-K, ATPase $\alpha 3$ subunit mRNA abundance in cardiac myocytes by retinoic acid. *J. Mol. Cell Cardiol.* 30:2403-2410.

MOLECULAR AND CELLULAR ASPECTS OF COMPLETE CONGENITAL HEART BLOCK AUTONOMIC REGULATION OF CARDIAC ION CHANNELS

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RESEARCH INTERESTS:

Congenital Heart Block (CHB) is a fetal heart conduction defect affecting infants of mothers with autoimmune disease. Our efforts are directed towards understanding the pathogenesis of CHB using a combination of molecular and electrophysiological approaches. In addition, we are also interested in ion channel pathophysiology where we aim to understand the signaling

cascade's modulation of the heart by autonomics in health and disease.

Project#1: Autoimmune mediated-CHB is considered to result from the transplacental passage of autoantibodies into the fetal circulation resulting in damage to the otherwise normally developing heart. Accordingly, CHB is of special interest since it and the associated carditis are a model for passively acquired antibody-associated cardiac injury. CHB carries a substantial morbidity and mortality with over 60% of the affected children requiring pacemaker therapy. To date, complete atrio-ventricular (AV) block is irreversible although varying degrees of block have been noted and second degree block has on rare occasion reverted to normal sinus rhythm. However, the non-cardiac manifestations of neonatal lupus are transient, resolving at about six months of life coincident with the disappearance of maternal autoantibodies from the neonatal circulation. Over the past few years, we provided evidence that IgG-enriched fractions and anti-52 kD SSA/Ro antibodies affinity purified from sera of mothers whose children have CHB induce complete atrio-ventricular (AV) block in the human fetal and rat heart perfused by the Langendorff technique and in multicellular AV nodal preparation, inhibit L-type Ca currents at the whole cell and single channel level. Confocal immunofluorescent studies showed sarcolemmal staining of the isolated myocyte to IgG containing anti-Ro/La antibodies but not to normal IgG. Immunization of female BALB/c mice with recombinant SSA/Ro protein generated high titer antibodies which crossed the placenta during pregnancy and were associated with varying degrees of AV conduction abnormalities including complete AV block, in the pups. These findings strongly suggest that maternal autoantibodies from mothers of children with CHB are causally related to the development of CHB and provide new insights into the pathogenesis and etiology of CHB.

Project#2: Voltage-gated Ca channels play a vital role in normal and diseased myocardium. The sympathetic nervous system exerts an important modulatory effect on cardiac Ca channels through both α_1 and β -adrenoceptors. In this research program, our efforts are directed towards understanding the signaling cascades and pathways involved upon autonomic stimulation of the normal and diseased hearts. Our previous reports established the existence of a functional negative feedback regulatory mechanism between α_1 - and β -adrenergic receptors vis a vis Ca channels and this may, at least in part, involve protein kinase C (PKC). As part of our continuing effort to understand how PKC modulates cardiac

function and to determine which PKC isozyme mediated this regulation, we have dialyzed myocytes with peptide inhibitors of PKC isozymes and recorded the effects of PMA on Ca channels. These peptides specifically inhibit the translocation and function of C2-containing isozymes, but not the C2-less isozymes in intact cells. Our data showed that, in the presence of peptide inhibitors of cPKC, the PMA inhibitory effects on Ca channels were antagonized, suggesting a possible role of C2-containing isozymes in this inhibition.

Funded by multiple grants from National Institute of Health and Veterans Administration.

RECENT SELECTED PUBLICATIONS:

1. Qu Y, Ghatpande A, El-Sherif A, Boutjdir M: Gene Expression of Na/Ca Exchanger during Development in Human Fetal Heart. *Cardiovasc. Res.*, 2000.
2. Huang B, Qin D, Deng L, Boutjdir M, El-Sherif N: Reexpression of T-type Ca channel gene and current in post-infarction remodeled rat left ventricle. *Cardiovasc. Res.*, In Press, 2000.
3. Qu YX, El-Sherif N, Boutjdir M: Gene Expression of Na/Ca Exchanger During Development of Human Fetal Heart. *Cardiovasc. Res.*, 45:866-873, 2000.
4. Huang B, Wong s, Qin D, Boutjdir M, El-Sherif N: Diminished basal phosphorylation level of phospholamban in the post-infarction remodeled rat ventricle. Role of beta-adrenergic pathway, Gi protein, phosphodiesterase and phosphatases. *Cir. Res.*, 29:85(9):848-55, 1999.
5. Chen L, El-Sherif N, Boutjdir M: Unitary Current Analysis of L-type Ca²⁺ Channels in Human Fetal Ventricular Myocytes. *J. Cardiovasc. Electrophysiol.*, 10:692-700, 1999.
6. Mazel JA, El-Sherif N, Jill Buyon, Boutjdir M: Conduction Abnormalities in a Murine Model for the Clinical Syndrome of Congenital Heart Block. *Circulation*. 13:99:1914-18, 1999.

ELECTROPHYSIOLOGY AND MOLECULAR BASIS OF ARRHYTHOGENICITY

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RESEARCH INTERESTS:

Dr. El-Sherif's laboratories implement an integrated approach to cardiovascular research that encompasses molecular biology techniques, patch clamp recordings, cellular, and whole organ physiology/pathophysiology research. A major scope of the integrated research approach is to study the consequences of myocardial ischemia on the heart with a special emphasis on electrophysiological alterations.

The *in vivo* laboratory conducts tridimensional isochronal mapping of cardiac activation/repolarization in various experimental models of cardiac arrhythmias including the post-infarction model, hypertrophy models, the tachycardia-induced cardiomyopathy model, and models of the long QT syndrome. This research also overlaps with the optical mapping laboratory, where potentiometric dyes are utilized to record multisite action potentials in a Langendorff heart preparation in various animal models.

The patch-clamp laboratories are involved in various research protocols that investigate alterations in sarcolemmal cardiac ion channels in a variety of pathological models with a special emphasis on alterations of potassium currents kinetics.

Continuing research in the molecular biology laboratories have resulted in several original reports on some of the important molecular changes associated with the process of post-infarction remodeling. The laboratories are currently involved in identifying the signal transduction pathways involved in post-infarction remodeling, ischemic preconditioning, hypertrophy, and the molecular changes associated with the transition from compensated hypertrophy to heart failure.

The long-term goal of the research program is to provide better understanding of basic mechanisms involved in post-infarction remodeling as it relates to sudden cardiac death and heart failure and the use of this information to target novel therapeutic interventions in the clinical setting.

Funded by multiple grants from Veterans Administration.

RECENT SELECTED PUBLICATIONS:

1. El-Sherif N, Turitto G: The long QT syndrome and Torsade de Pointes. *Pacing Clin Electrophysiol* 22:91-110, 1999.
2. Mazel JA, El-Sherif N, Buyon JP, Boutjdir M: Conduction abnormalities in a Murine model of the clinical syndrome of congenital heart block. *Circulation* 99:1914-1918, 1999.
3. El-Sherif N, Caref EB, Chinushi M, Restivo M: Mechanism of arrhythmogenicity of the short-long cardiac sequence that precedes ventricular tachyarrhythmias in the long QT syndrome. *J Am Coll Cardiol* 33:1415-1423, 1999.
4. Huang B, Wang S, Qin D, Boutjdir M, El-Sherif N. Diminished basal phosphorylation level of phospholamban in the post-infarction remodeled rat ventricle. Role of β -adrenergic pathway, GI protein, phosphodiesterase, and phosphatases. *Circ Res.* 85:848-55. 1999

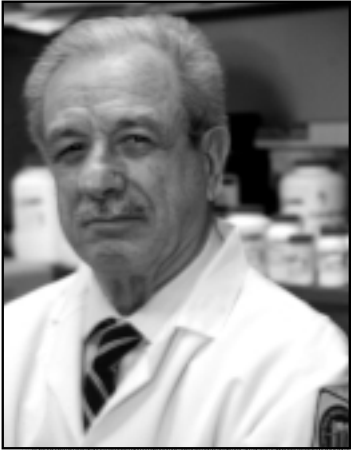
ELECTROPHYSIOLOGY IN CARDIAC AUTOMATICITY

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RESEARCH INTERESTS:

Dr. Vassalle is a cardiac electrophysiologist, interested in the automaticity of different cardiac pacemakers and their control. The research activity of his laboratory is directed at understanding the factors that determine, modify and regulate the pacemaker activity of different cardiac pacemakers under normal conditions as well as under abnormal conditions such as those encountered clinically.

Over the years, Dr. Vassalle's lab has employed different techniques, including the recording of transmembrane potentials by means of a microelectrode technique and of contractile force, voltage clamp methods, radiotracer techniques, recording from the intact animal, recording of intracellular sodium activity and use of isolated myocytes for recording action potential and currents in the same cells by means of different voltage clamp methods. The problems studied include the mechanisms underlying the pacemaker activity in Purkinje fibers and in the sinoatrial node, the role of the vagus and the frequency-dependent inhibition in ventricular standstill during vagal stimulation, overdrive suppression and overdrive excitation in different pacemakers of the heart, postvagal tachycardia, the action of the neuromediators norepinephrine and acetylcholine at a cellular level, regulation of the Na-K pump activity by different factors and its electrophysiological role, mechanisms of digitalis arrhythmias both in vitro and in vivo, positive and negative inotropic mechanisms of digitalis, effects of different ions on membrane potentials and currents, the oscillatory potential V_{os} and the oscillatory current I_{os} as well as the tail voltage V_{ex} and tail inward current I_{ex} and their underlying mechanisms, the effect of calcium overload on the electrical and mechanical activity of different cardiac tissues, potassium fluxes in the sinus node and atrial tissues and their regulation by neuromediators, the role of different oscillatory pre- and after-potentials in the initiation and maintenance of spontaneous activity in different pacemaker tissues, the role of the Na-Ca exchange current in shaping the action potential under conditions of calcium overload, the changes induced by different drugs as tools to understand cell function as well as their mechanism of action, etc. The understanding of these mechanisms is the precondition for an effective treatment of the often serious clinical disorders of the cardiac electrical activity. To date, the results obtained have been presented in 148 abstracts, published in 164 original papers and discussed in 53 reviews and in 3 edited books.

Funded by the National Institute of Health.

RECENT SELECTED PUBLICATIONS:

1. Aceto, E. and M. Vassalle. Arrhythmogenic actions of norepinephrine and acetylcholine in sheep cardiac Purkinje fibers. *G. Ital. Cardiol.* 28, Suppl 1: 102-109, 1998.
2. Liu Y.M., H. Yu, C-Z. Li, I.S. Cohen and M. Vassalle. Ca^{2+} effects on I_f and I_K in rabbit sinoatrial node myocytes: Implications for SA node automaticity. *J Cardiovasc. Pharmacol.*, 32: 783-790, 1998.
3. Shen, J.-B. and M. Vassalle. On the mechanism of cesium-induced voltage and current tails in single ventricular myocytes. *J. Biomed. Sci.* 6: 161-175, 1999.
4. Liu Q.-Y. and M. Vassalle. Role of Na-Ca exchange in the action potential changes caused by drive in cardiac myocytes exposed to different Ca^{2+} loads. *Can. J. Physiol. Pharmacol.* 77: 383-397, 1999.
5. Zhang, H. and M. Vassalle. Role of dual pacemaker mechanisms in sino-atrial node discharge. *J. Biomed. Sci.* 7:100-113, 2000.

PROTEASES IN MUSCLE WASTING DISEASES

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RESEARCH INTERESTS:

Dr. Stracher studies the role of proteases in muscle wasting diseases and the use of low molecular weight protease inhibitors as potential therapeutic agents in treating these disorders. He has been studying molecular mechanisms responsible for neuromuscular degeneration and the role of thiol proteases such as calpain and its inhibitors in this process. Dr. Stracher has recently tested this hypothesis in the mdx mouse, a model for the human form of Duchenne muscular dystrophy and has found that intramuscular administration of the calpain inhibitor leupeptin inhibited muscle degeneration as assessed by histologic analysis and muscle strength testing (see reference 3). These studies followed the more extensive studies in primates after median nerve transaction in the mid-forearm. Both intramuscular and oral administration of the calpain inhibitor leupeptin led to almost total neuromuscular recovery after 3 months treatment as judged by histochemical analysis. These and other results have given rise to the formulation of a more common cytotoxic pathway known as the Ca⁺⁺/calpain hypothesis which may be responsible for a number of related neuromuscular and neurodegenerative disorders (see reference 5).

Funded by the National Institutes of Health - NINDS.

RECENT SELECTED PUBLICATIONS:

1. Stracher, A., Calpain Inhibitors as Neuroprotective Agents in Neurodegenerative Disorders. *Int'l Tinnitus Journal*, Vol. 3, No. 2, 1-5 (1998).
2. Wang, J., Ding, D., Shulman, A., Stracher, A. And Salvi, R.J., Leupeptin Protects sensory hair cells from acoustic trauma, *NeuroReport* 10, 811-816 (1999).
3. Badalamente, M.A. and Stracher, A., Delay of degeneration and necrosis in mdx mice by calpain inhibition, *Muscle and Nerve* 23, 106 (2000).
4. Cheng, A.J., Huang, T., Stracher, A., Kim, A., Liu, W., Malgrange, B., LeFebvre, P., Shulman, A., and Van DeWater, T., Calpain Inhibitors protect auditory sensory cells from Hypoxia and Neurotrophin-withdrawal induced Apoptosis, *Brain Research* 850, 234-243 (1999).
5. Stracher, A., Calpain inhibitors as therapeutic agents in nerve and muscle degeneration, in, *Ototoxicity*, ed. Henderson, Salvi, *Ann. N.Y. Acad. Sci.*, 884, 52-56, 1999.

DEVELOPMENT AND DIFFERENTIATION OF SKELETAL MUSCLE

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RESEARCH INTERESTS:

Dr. Rushbrook's interests include :

a) Development and differentiation of skeletal muscle. Studies focus on the diversity of expression of myosin heavy chain isoforms in adult and developing fast muscles. Five heavy chain isoforms are differentially expressed in various adult fast muscles and a progression of six developmentally in the pectoralis

major (PM) muscle. A total of seven heavy chain genes are involved. A dramatic transition occurs between heavy chains IV and V in the PM muscle just after hatching. Messenger RNA for isoform IV is no longer present at hatching. Heavy chain protein IV continues as the major myosin until 3 days post-hatch. Between 3- and 5- days post-hatch it is completely replaced by heavy chain V protein for which the message has been upregulating from around the time of hatching. The sudden loss of heavy chain IV protein contrasts markedly with the stability of adult heavy chains, which have a half life of about 30 days. Methylation of the heavy chain is associated with the protein transition. The proteolytic mechanism behind the transition and a role for methylation in stabilization of the incoming protein are under investigation.

b) Mechanisms of regulation of allosteric enzymes, in particular mitochondrial NAD⁺-dependent isocitrate dehydrogenase, a key enzyme in aerobic metabolism. The enzyme displays homotropic allosteric behaviors for substrates isocitrate and NAD⁺, and reacts in a heterotropic fashion positively to ADP and negatively to ATP, NADH and NADPH. The enzyme contains two regulatory subunits, the products of distinct genes, and two catalytic subunits, the products of a single gene but differing in some as yet unknown way at the protein level. The regulatory subunits are present as 3'-alternatively spliced forms with tissue-specific expression. The functional differences associated with these forms are of interest. Ultimate understanding of the enzyme will require expression of the cloned subunits for crystallization and x-ray analysis, in the presence and absence of substrates and regulatory effectors.

Funded by the National Science Foundation.

RECENT SELECTED PUBLICATIONS:

1. Human Cystatin C Forms an Inactive Dimer During Intracellular Trafficking in Transfected CHO Cells. Merz, G.S., Benedikz, E., Schwenk, V., Johansen, E., Vogel, L.K., Rushbrook, J.I. and Wisniewski, H.M. (1997) *J. Cell. Physiology* 173, 423-432.
2. Protein and mRNA Analysis of Myosin Heavy Chains in the Developing Pectoralis Major Muscle. Rushbrook, J.I., Huang, J., Weiss, C., Yao, T.-T., Siconolfi-Baez, L., and Becker, E. (1998) *J. Muscle Res. & Cell Motil.* 19, 157-168.
3. Cellular Processing of the Amyloidogenic Cystatin C Variant of Hereditary Cerebral Hemorrhage with Amyloidosis, Icelandic Type. (1999) Benedikz, E., Merz, G.S., Schwenk, V., Johansen, T.E., Wisniewski, H.M., and Rushbrook, J.I. *Amyloid* 6, 172-182.
4. Bovine NAD⁺-Dependent Isocitrate Dehydrogenase: Alternative Splicing And Tissue Dependent Expression of Subunit 1. C. Weiss, Y. Zeng, J. Huang, M.B. Sobocka and J.I. Rushbrook. (2000) *Biochemistry* 39, 1807-1816.
5. Cloning of the Human Platelet F11 Receptor: A Cell Adhesion Molecule Member of the Immunoglobulin Superfamily Involved in Platelet Aggregation. M.B. Sobocka, T. Sobocki, P. Banerjee, C. Weiss, J.I. Rushbrook, A. Norin, J. Hartwig, M.O. Salifu, M.S. Markell, A. Babinska, Y.H. Ehrlich, E. Kordecki. (2000) *Blood* 95, 2600-2609.

GENE DELIVERY AND EXPANSION IN MOUSE MODEL OF MUSCULAR DYSTROPHIES

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RESEARCH INTERESTS:

Dr. Bhagwati's laboratory is involved with developing novel methods for gene delivery and expression in mouse models of muscular dystrophies and motor neuron diseases. The aims of Dr. Bhagwati's research include:

a. Analyzing the complex pathogenesis of the inherited neurological disease, myotonic dystrophy, which predominantly affects skeletal muscle and heart. Efforts are underway to target the myotonic dystrophy triplet repeat expansion mutation, (CTG)_n, by homologous recombination in embryonic stem cells, to the myotonic dystrophy locus in mouse. Transgenic mice harboring this mutation will be analyzed for the patho-physiological features of this disease. These experiments are designed to provide information on the basic mechanisms of skeletal muscle and cardiac pathology in this disease.

b. Improvements in the delivery and expression of plasmid DNA in skeletal and cardiac muscle are crucial for the ability to successfully introducing normal genes or ablating the function of abnormal genes in many human diseases. Attempts are being made to develop more efficient ways for delivery of genes into muscle using liposomes and ligand mediated gene transfer. The efforts in the laboratory focus specifically on transfer of the dystrophin gene in a mouse model of Duchenne muscular dystrophy, and the myostatin gene in muscle atrophy in primary muscle or neurogenic disorders. These experiments are designed to provide insights and viable solutions to the many obstacles and problems associated with such techniques in gene therapy in humans.

Funded by National Institute of Health.

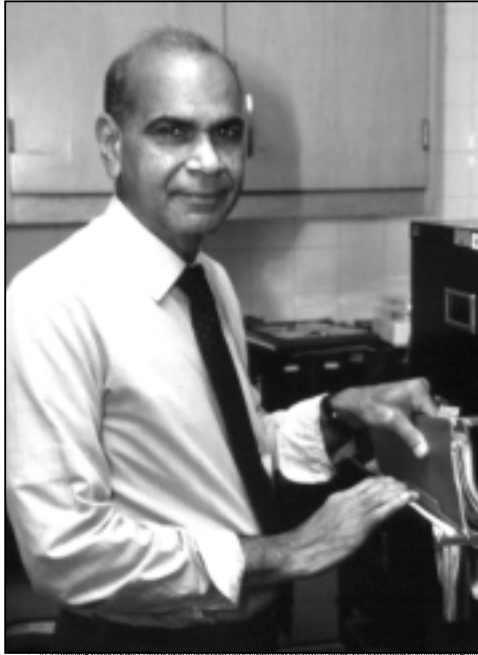
RECENT SELECTED PUBLICATIONS:

1. S. Bhagwati, A. Ghatpande, B. Leung. Identification of two nuclear proteins which bind to RNA CUG repeats : significance for myotonic dystrophy. *Biochemical and Biophysical Res. Comm.* 228, 55-62, 1996.
2. S. Bhagwati, A. Ghatpande, B. Leung. Normal levels of DM RNA and myotonin protein kinase in skeletal muscle from adult myotonic dystrophy (DM) patients. *Biochim. Biophys. Acta* 11317,3: 155-157, 1996.

TEMPORAL AND SPACIAL DISTRIBUTION OF TRANSCRIPTION FACTORS INVOLVED IN MUSCLE DIFFERENTIATION

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RESEARCH INTERESTS:

My research interests are in the general area of muscle development and disease. We have investigated the spatial and temporal distribution of transcription factors involved in muscle regeneration and the effect of the trinucleotide repeat (CTG)_n expansion, the mutation responsible for myotonic dystrophy, on differentiation of skeletal muscle myoblasts. More recently we have concentrated on the apoptotic cell death process in skeletal and cardiac muscles. We have shown that the enhanced β -adrenergic signaling involved in cardiac apoptosis is largely dissociated from the β -adrenergic receptor subtype and selectively mediated by the β_1 -adrenergic receptors. The precise apoptotic pathway involved is being investigated to explore potential targets for therapy in the failing heart.

RECENT SELECTED PUBLICATIONS:

1. Bhagwati S, MS Ghatpande, SA Shafiq, B. Leung. 1996. In-situ hybridization analysis for expression of myogenic regulatory factors in regenerating muscle of mdx mouse. *J Neuropath Exp Neurol* 55: 509-514.
2. Bhagwati S, B Leung, SA Shafiq, A Ghatpande. 1997. Myotonic dystrophy: decreased levels of myotonin protein kinase (MT-PK) leads to apoptosis in muscle cells. *Exp Neurol* 146: 277-281.
3. Ghatpande S, S Goswami, S Mathew, G Rong, L Cai, SA Shafiq, MAQ Siddiqui, 1999. Identification of a novel cardiac lineage - associated protein (cCLP-1): A candidate regulator of cardiogenesis. *Develop Biol* 208:210-221.
4. Bhagwati S. SA Shafiq, W Xu, 1999. (CTG)_n repeats markedly inhibit differentiation of C2C12 myoblast cell line: implications for congenital myotonic dystrophy. *Biochem Biophys Acta* 1453: 221-229.

FOLATE DEFICIENCY AND REGULATION OF FOLATE BINDING PROTEIN RECEPTOR

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RESEARCH INTERESTS:

1. This laboratory has purified and cloned a rat glycosylphosphatidylinositol (GPI) folate binding protein (FBP) (GPI-FBP) receptor. Furthermore, we have studied the effect of folate deficiency on the expression of this protein in the kidney, choroid plexus, and liver. When cultured cells that constitutively express this GPI-FBP receptor are grown in low folate or folate deficient

medium, expression of this folate receptor increases. This occurs in order to ensure that intracellular folate levels are maintained its low extracellular concentrations. In contrast, in the rat kidney, the level of this protein is markedly reduced as plasma folate diminishes in response to low dietary folate. This occurs because the apo-GPI-FBP on the brush borders of the proximal renal tubules is degraded by proteases (i.e., meprin) on these epithelial cells. This amplifies the deleterious consequences of reduced dietary folate since the ability to reabsorb urinary folate is lost. This underscores that maintenance of adequate dietary folate is especially critical.

Placenta (both human and rat) contains the highest relative tissue expression of this GPI-FBP. Therefore, the placenta is being used to determine the effect of different levels of dietary folate deficiency on the expression of this protein, and consequently, on fetal development. Levels of fetal FBP will be measured by a radioimmunoassay developed in this laboratory that employs an antibody specific for this protein.

2. We are planning to study whether a high affinity antiserum to this rat GPI-FBP administered in the periconceptional period affects fetal development. The magnitude of the effect of blocking transcytosis of folate from maternal plasma with this antiserum will be correlated with different levels of plasma folate, achieved by maternal maintenance with varying levels of dietary folate. This information could help provide a rational basis for understanding why dietary folate supplementation reduces NTD(s); i.e., raising plasma folate can overcome delayed or inadequate expression of the folate receptor at this critical periconceptional time when neural tube embryogenesis occurs. The rat is especially appropriate for assessment of the effects of dietary folate deficiency on fetal development since all stages of fetal development in this animal have been extensively studied.

3. A third area of interest is in transcobalamin II (TCII), a plasma protein that binds B12 and facilitates its cellular uptake by binding to a TCII-B12 receptor. Structural and functional polymorphism of TCH that could affect the binding of

B12 and/or the binding of the TC-B12 complex to the receptor are being studied. This is important because of polymorphisms of TCII complex to its receptor. These findings would be particularly relevant to the understanding and treatment of a large cohort of women who have normal plasma levels of vitamin B12 but who nevertheless have elevated plasma homocysteine and/or methylmalonic acid, metabolic disorders that could contribute to coronary artery disease prevalence in this population.

Funded by multiple grants from the National Institute of Health.

RECENT SELECTED PUBLICATIONS:

1. da Costa, M. and Rothenberg, S. P.: Purification and characterization of folate binding proteins from rat placenta. *Biochim. Biophys. Acta* 1292:23-30, 1996.
2. Quadros, E.V., Regec, A.L., Khan, K.M.F. and Rothenberg, S.P.: Transcobalamin II synthesized in the intestinal villi facilitates transfer of cobalamin to the portal blood. *Am. J. Physiol.* 277:G161-G166, 1999.
3. Rothenberg, S.P.: Increasing the dietary intake of folate: Pros and Cons. *Sem. Hematol.* 36:65-74, 1999.
4. Rothenberg, S.P., Quadros, E.V. and Regec, A. Transcobalamin II in, *Chemistry and Biochemistry of B12* Ed: R. Banerjee, John Wiley & Sons, Inc., New York. Pp 441-473, 1999.
5. da Costa, M., Rothenberg, S.P., Sadasvan, E., Qian, O., Regec, A.: Folate deficiency reduced the GPI-anchored folate binding protein in rat renal tubules. *Am. J. Physiol.* 278:C812-C821, 2000.
6. Sadasivan, E. and Rothenberg, S.P.: Cloning and characterization of the complementary DNA and gene encoding a folate binding protein in the rat. *Gene*. In Press.
7. Sobti, P., Rothenberg, S.P., Quadros, E.V. Radioenzymatic Assay for Reductive Catalysis of N5N10methylentetrahydrofolate by Methylentetrahydrofolate Reductase - *J. Biochem. Biophys. Methods*. In Press.

THE ROLE OF PLATELETS IN CARDIOVASCULAR FUNCTION AND DISORDERS

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RESEARCH INTERESTS:

The significant role of platelet activation in cardiovascular disorders is evidenced by the occurrence of numerous clinical disorders caused by platelet dysfunction including thrombosis, heart attack, stroke and excessive bleeding. The integrity of the cardiovascular system is dependent on the activity of platelets which play a critical role in wound healing and thus have a major contribution to hemostasis. Platelet activation *in vivo* has been associated with the presence of platelet auto and alloantibodies directed to platelet surface proteins in the circulation of patients with drug-dependent thrombocytopenia purpura, post-transfusion purpura, neonatal isoimmune thrombocytopenia, chronic immune thrombocytopenia purpura, septicemia and HIV-associated thrombocytopenia. Current investigations in our laboratories focus on two discoveries that have opened new directions in this research. 1) An ecto-protein kinase/phosphoprotein phosphatase system that is involved in the maintenance of platelet homeostasis and blood coagulation. 2) Platelet activation by stimulatory antibodies. We have cloned a full length cDNA for a new platelet glycoprotein protein, termed the F11 receptor (F11R), which resides on the membrane surface, and is a receptor for a potent platelet stimulatory antibody. We have determined that the F11R protein is a member of the immunoglobulin superfamily whose structure is that of a cell adhesion molecule (CAM). Binding of platelets to an antibody M.Ab.F11 matrix resulted in adhesion and cell spreading by filopodia and lamellipodial networks. Our ongoing research is designed to determine the signalling pathways involved in the physiological and pathophysiological functions of the F11R in cell-cell adhesion and in platelet activation, and the role of the extracellular phosphorylation and dephosphorylation system in maintaining platelets in a resting state.

Funded by the American Heart Association and Center for Biotechnology, SUNY at Stonybrook, New York.

RECENT SELECTED PUBLICATIONS:

1. Babinska, A., Sobocki, T., Hogan, M., Sobocka, M., Ehrlich, Y.H. and Kornecki, E. Identification of ecto-PKC on the surface of human platelets: role in the maintenance of latent fibrinogen receptors. *Am J of Physiol. Heart Circ. physiol.* 278:H2008-H2019. 2000.
2. Sobocka, M., Sobocki, T., Banerjee, P., Weiss, C., Rushbrook, J., Norin, A., Hartwig, J., Salifu, M.O., Markell, M., Babinska, A., Ehrlich, Y.H. and Kornecki, E. Cloning of the human platelet F11 receptor: a cell adhesion molecule member of the immunoglobulin superfamily involved in platelet aggregation. *Blood*, Vol. 95: 2600-2609. 2000.

SULFHYDRYL/DISULFIDE REACTIONS IN PLATELET RESPONSES

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RESEARCH INTERESTS:

The long-range goal of our research is to present a model for stimulus-response coupling in blood platelets, and, in particular, to define the role of sulfhydryl/disulfide reactions. The rationale is based on early studies showing that surface sulfhydryl groups are required for platelet responses, and on the recent demonstration, in our laboratory, that protein disulfide isomerase (PDI) mediates platelet aggregation and secretion. These results point to the involvement of thiol and disulfid metabolism in platelet function and lead to the hypothesis that thiol-disulfid rearrangements are necessary for platelet responses.

We are presently determining which sulfhydryl-containing proteins are required for activation. Our general strategy here is to compare the concentration dependence for sulfhydryl reagents on inhibition of function and on the labeling pattern. For example, if a particular concentration of sulfhydryl reagent does not inhibit responses but does give maximal labeling of certain proteins, these proteins can be excluded as being critical for that response. Conversely, likely candidates for a causal role in activation would be those proteins where the concentration dependence (of the reagent) for labeling is parallel to that inhibition of function.

We also want to test the hypothesis that activation of the platelet fibrinogen receptor GP IIb/IIIa by PDI is a causal event in stimulus-response coupling. Our recent work showed that PDI is required for aggregation and secretion and that antibodies to PDI partially blocked the binding of PAC-1 (a fibrinogen mimetic). Using inhibitors of PDI we intend further to characterize the role of PDI in GP IIb/IIIa activation.

Funded by the American Heart Association.

RECENT SELECTED PUBLICATIONS:

1. Milev, Y; Essex DW: Protein disulfide isomerase catalyses the formation of disulfide linked complexes of thrombospondin-1 with thrombin-antithrombin-III. *Arch Biochem Biophys* 361: 1200126, 1999.
2. Essex DW; Li, M: Protein disulphide isomerase mediates platelet aggregation and secretion. *Brit J Haematol* 104:448-454, 1999.
3. Essex DW; Miller, A.; Swiatkowska, M., Feinman, RD: Protein disulfide isomerase catalyses the formation of disulfide-linked complexes of vitronectin with thrombin antithrombin. *Biochemistry* 38:10398-10405, 1999.
4. Essex, DW; Li, M: A polyclonal antibody to protein disulfide isomerase induced platelet aggregation. *Thromb Res* (In Press).

LHRH NEURAL MIGRATION IS ASSOCIATED WITH VASCULARIZATION OF THE NASAL MESENCHYME

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RESEARCH INTERESTS:

Luteinizing hormone-releasing hormone (LHRH) is essential for normal development and function of the reproductive systems of vertebrates, including humans. The cells which produce LHRH are unique among brain cells in that they originate in the epithelium of the olfactory placode and migrate across the nasal septum, through the cribriform plate and into the forebrain early in development. Observations in mice and in human embryos in this laboratory, and in a wide variety of vertebrate species in other laboratories, over the last ten years, support the hypothesis that this phenomenon is universal among vertebrates.

In this laboratory, we are currently interested in the role of the developing vasculature on the origin and migration of LHRH neurons. In most early embryos, the appearance of neural cell adhesion molecule (N-CAM) - immunoreactive cell bodies and axons of the migration route coincides with the onset of vasculogenesis in the nasal mesenchyme and precedes the initial detection of LHRH-immunoreactivity in the olfactory placode. This research examines the possibility that the processes of vasculogenesis may be important for normal development of the "cellulovascular strand" along which LHRH neurons migrate into the brain. Further study may reveal endothelial cell-associated proteinases or other compounds on or in the cell membranes which could locally disrupt the basement membrane of the forebrain and facilitate the passage of LHRH cells into the brain.

Funded by the National Institute of Health.

RECENT SELECTED PUBLICATIONS:

1. Dellovade, T. L., Pfaff, D. W., and Schwanzel-Fukuda, M. (1998) The gonadotropin hormone-releasing hormone system does not develop in the small eye (Sey) mouse phenotype. *Dev. Brain Res.* 107:233-240.
2. Dellovade, T. L., Pfaff, D. W., and Schwanzel-Fukuda, M. (1998) Olfactory bulb development is altered in small-eye (Sey) mice. *J. Comp. Neurol.* 402:4022-418.
3. Hardelin, J.P., Julliard, A.K., Moniot, B., Soussi-Yanicostas, N., Verney, C., Schwanzel-Fukuda, M., Ayer-Le Lievre, C., and Petit, C. (1999) Anosmin-1 is a regionally restricted component of basement membranes and interstitial matrices during organogenesis: implications for the developmental anomalies of X chromosome-linked Kallmann syndrome. *Devel. Dynamics* 215:26-44.
4. Schwanzel-Fukuda, M. (1999) Origin and migration of luteinizing hormone-releasing hormone neurons in mammals. *Microscopy Research and Technique* 44:2-10.

REGULATION OF SYNAPSE FORMATION IN THE NEURO-MUSCULAR FUNCTION

KATHRYN MILES, PH.D. Research Assistant Professor

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RESEARCH INTERESTS:

Dr. Miles' research focuses on the regulation of protein phosphorylation of the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction. The overall interest of her laboratory is the regulation of synapse formation in the mammalian neuromuscular junction.

Another area of interest for Dr. Miles is the characterization of an isoform of protein kinase C (nPKC θ) that is specifically expressed in skeletal muscle. Dr. Miles has demonstrated that this enzyme is localized neuromuscular junctions and that its expression in skeletal muscle is regulated by nerve, she is focusing her efforts on determining the functions of this enzyme in the neuromuscular synapses.

RECENT SELECTED PUBLICATIONS:

1. Miles K. and M. Wagner. Overexpression of nPKC θ is permissive for myogenic differentiation. *Journal of Cellular Biochemistry* 79:71-79, 2000.
2. Hilgenberg L, S Yearwood, S Milstein, and K. Miles. Neural influence on protein kinase C isoform expression in skeletal muscle. *Journal of Neuroscience* 16:4994-5003, 1996.
3. Hilgenberg L. and K. Miles. Developmental regulation of a protein kinase isoform localied in the neuromuscular junction. *Journal of Cell Science* 108:51-61, 1995
4. Miles, K. S.M. Audigier, P. Greengard and R.L. Huganir. Autoregulation of phosphorylation of the nicotinic acetylcholine receptor. *Journal of Neuroscience* 14:3271-3279, 1994

CORE RESEARCH FACILITIES

DIVISION OF LABORATORY ANIMAL RESOURCES



The primary function of the Division of Laboratory Animal Resources (DLAR) is to provide care and services for animals used in biomedical research. These services include the procurement of animals and their maintenance under conditions appropriate for the conduct of experimentation and teaching. To conduct biomedical research at our animal facilities, a person/company must have an approved research protocol. Please call for information and current prices.

PROCUREMENT OF ANIMALS :

Prices for different species can be obtained from DLAR or directly from vendor.

HOUSING OF ANIMALS:

Daily board charges vary according to species. At present the following species are housed: cats, chickens, dogs, frogs, goats, guinea pigs, hamsters, mice, non-human primates, pigs, rabbits, rats, sheep, snakes, S.A. opossum. Other species of animals may be housed if appropriate space is available.

LIBRARY RESOURCE MATERIAL DEALING WITH LABORATORY ANIMALS:

Use is available upon request.

OTHER SERVICES :

Animal blood and tissues available upon request, use of surgical suite, preparation of animals for surgery, assistance with surgical procedures, diagnostic services, x-ray service.

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CORE RESEARCH FACILITIES

DNA SEQUENCING FACILITY



The Department of Anatomy and Cell Biology maintains a DNA sequencing laboratory, based on an Applied Biosystems model 373A automated DNA sequencer. This machine detects fluorescently labeled dideoxy terminators used in a Sanger-type dideoxy sequencing reaction utilizing single stranded (e.g. M13 and asymmetric PCR products) or double stranded (plasmids, PCR products) DNA templates. DNA sequencing is provided on a fee for service basis. DNA samples are usually sequenced within several days. Universal forward and reverse sequencing primers are provided; all other primers must be provided by the user. DNA sequence data is available in both printed and computer form, and can be picked up in person or sent by both electronic and/or regular mail. Help with database searching and analysis is also available.

| DNA TO BE SEQUENCED | # BP (99%) | COST/SAMPLE |
|----------------------------|-------------------|--------------------|
| Single-stranded | 350-450 | \$ 20.00 |
| Plasmid | 350-450 | \$ 20.00 |
| PCR DNA | 300-350 | \$ 20.00 |

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CORE RESEARCH FACILITIES

ELECTRON MICROSCOPY FACILITY



The Department of Anatomy and Cell Biology maintains an electron microscopy facility consisting of four ultramicrotomes, including an LKB 2188 with a cryoultramicrotome system specifically designed for ultrathin frozen sectioning, a critical point dryer, a sputter coater, an Edwards coating system and the JEOL JEM-100c electron microscope with a scanning attachment. Technical assistance is provided by Mr. Wei Quan and photographic services are provided by Mr. Vincent Garafolo.

COST PER SPECIMEN

| | |
|--|--------|
| A. Complete transmission electron microscopic work-up (from fixation to, but not including photography) | \$ 100 |
| 1. Fixation and embedding | \$ 50 |
| 2. Sectioning | |
| a. thick | \$ 20 |
| b. thin | \$ 30 |
| B. Frozen Sections | |
| 1. Fixation and mounting | \$ 50 |
| 2. Sectioning | \$ 50 |
| C. Scanning electron microscopy | |
| 1. Fixation and mounting | \$ 40 |
| 2. Critical point drying | \$ 10 |
| 3. Sputter coating | \$ 10 |

USE OF ELECTRON MICROSCOPE

| | |
|----------------------------------|-------|
| A. With no help (per hour) | \$ 10 |
| B. With help (per hour) | \$ 20 |

DEVELOPING AND PRINTING

| | |
|--|-------|
| A. Negative (1) | \$ 2 |
| B. Roll of 10 negatives (SEM) and developing | \$ 10 |
| C. Print (1) 8x10 | \$ 4 |
| D. Print (1) 5x7 | \$ 3 |

Mr. Wei Quan
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CORE RESEARCH FACILITIES

MICROINJECTION AND MICROMANIPULATION FACILITY



This facility contains a Leitz Laborert inverted microscope with a) a long depth of field, b) phase contrast and Normansky optics, c) a JAVELIN video camera and monitor and d) Leitz micromanipulators in conjunction with a medical systems PL1-100 picoinjector. The system sits on a vibration-free working table. A Flamming Brown Micropipette puller from Sutter Instruments and a Technical Products Microinstruments MICROFORGE are available to produce fine quality micropipettes. The facility is able to introduce cloned DNA, genes, mRNA and purified proteins into cultured cells and to produce transgenic mice.

COST PER EXPERIMENT

- A. Microinjection of macromolecules (cells and macromolecules to be supplied by the investigator) into the:
 - 1. cytoplasm \$ 50/100 cells
 - 2. nucleus \$100/100 cells
- B. Microinjection of macromolecules (oocytes and macromolecules to be provided by the investigator) into *Xenopus* oocytes:
 - 1. cytoplasm \$ 50/100 oocytes
 - 2. nucleus \$100/100 oocytes
- C. Microinjection into the pronuclei of fertilized mouse ovum to produce transgenic mice contact for fee.
- D. Investigator using microinjection facility:
 - 1. With no assistance \$ 10/hr plus \$25 for initial instructions
 - 2. With assistance \$ 20/hr

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CORE RESEARCH FACILITIES

PHOSPHORIMAGER USER CENTER



The PhosphorImager User Center provides "self-service" access to a Molecular Dynamics Storm 860 for the detection and quantitation of radioactive beta (^{14}C , ^{35}S , ^{32}P), gamma radiation (^{125}I , ^{131}I), x-rays as well as fluorescent emissions. A radioactive sample (usually a membrane or a gel) is exposed to a re-usable PhosphorStorage screen which is then "read" by the Storm 860. Radioactive signals can be visualized on a computer screen, manipulated and analyzed using ImageQuant, software supplied by Molecular Dynamics. This system is 10 times more sensitive than standard X-ray film in the detection of ^{32}P and has a linear response over a range of 5 orders of magnitude. The Storm 860 can also detect fluorescent signals by placing the sample directly on the instrument. ImageQuant software will also process these images. The instrument is part of a local area network consisting of 2 additional high speed computer workstations and a 1200 dpi printer that is now connected to the Internet providing remote access to data and simplifying downloading to local computers. Hardware is available for the storage of data on Jaz or Zip disks. Software on the workstation includes Image Quant and MS Excel for manipulation and data storage. Files created by Image can be imported into most graphics programs for either PC or Macintosh operating systems.

Use of the Storm 860 requires rental of a PhosphorStorage screen and a user fee (\$300 for one year).

Dr. Miriam Feuerman
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CORE RESEARCH FACILITIES

PROTEIN SEQUENCING CENTER



The protein sequencing center provides N-terminal sequencing and microchemistry services to support the research of investigators at the SUNY Health Science Center at Brooklyn as well as those of outside institutions. Edman sequencing is performed at the highest sensitivity currently available, on state of the art Applied Biosystems Procise capillary LC instrument. Protein and peptide samples are accepted in solution, or immobilized to a recommended membrane. Microanalytical sequencing is the center's major activity and sample turnover time is short. Data analysis is usually completed within 5 days of receipt of sample. For N-terminally blocked proteins, enzymatic digestion (from samples in solution, in-gel or on membrane) can be performed, followed by HPLC separation of the digest mixture. The center provides free consultations on any protein research problem. These are arranged by appointment.

Consultations prior to preparing samples for analysis by the center are free but required before samples will be accepted for analysis. Aim to schedule this consultation a few weeks before the final steps of a purification.

Dr. Julie I. Rushbrook
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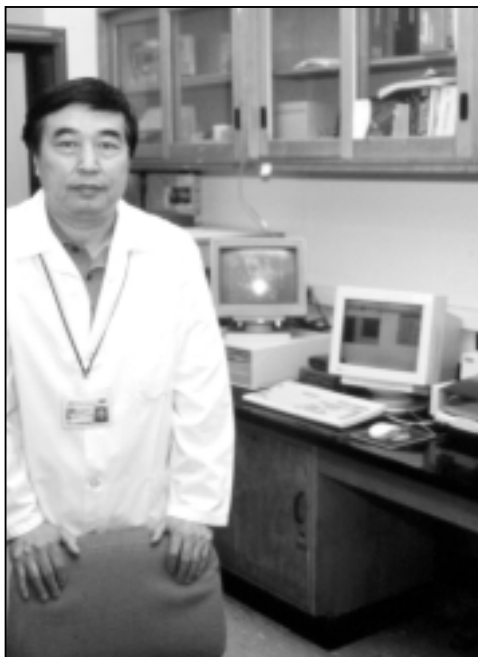
Linda Siconolfi-Baez
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PRICE SCHEDULE

- | | |
|---|-------|
| A. Protein/peptide N-terminal amino acid sequencing: | |
| 1. Set-up (sample loading + first 5 cycles-nonrefundable) | \$165 |
| 2. Per cycle cost after cycle 5 | \$ 24 |
| 3. Database searches, sequence analysis carried out by the Core Facility, cost/analysis | \$ 10 |
| B. Sample preparation: | |
| 1. Prosorb tube | \$ 13 |
| 2. Reduction and alkylation for Cys analysis | \$ 30 |
| C. In-gel tryptic digestion of proteins: | |
| 1. Charge per sample with controls | \$ 75 |
| (price will be adjusted for any enzymes of different cost) | |
| 2. HPLC separation of the fragments | \$175 |
| D. Electroblothing from SDS-gels by Core Facility prior to sequencing: | |
| 1. Charge/gel | \$ 45 |
| 2. Problott paper supplied to other laboratories (1 sheet) | \$ 25 |
| E. Amino acid analysis: | |
| 1. Hydrolysis + analysis/sample | \$100 |
| 2. Multiple samples with common control solutions (/sample) | \$ 80 |
| 3. Specialized analyses (Ident. of P-Ser, P-Thr, P-Tyr) | \$100 |
| F. Protein/peptide purification by HPLC: A microbore HPLC system is available for the purification of peptides and small proteins where the sample is available in small amounts. The system will be operated by the investigator under supervision. Users will purchase their own column and reagents. | |
| Use/day | \$ 30 |

CORE RESEARCH FACILITIES

VIDEO IMAGING FACILITY



A state-of-the-art Fluorescence Imaging Workstation from Photon Technology International, Inc. (PTI) attached to a Nikon Diaphot inverted microscope is suitable for diverse epifluorescence microscopy applications. The system can be used either with a Newicon video camera coupled to an image intensifier (Videoscope) for regular low light applications, or a photomultiplier (PMT) for photon counting photometry. As a light source, this system is equipped with a stabilized, high speed dual wavelength scanning illuminator and a monochromator suitable for fluorescence ratio imaging. A user friendly software running under Microsoft Windows provides real-time acquisition and concurrent analysis of the images with spectrographs. It allows on-line filtering of the images to minimize optical recording artifacts. In addition to epifluorescence and phase contrast optics, this microscope system also has a temperature regulated stage making it suitable for optical recordings in cell cultures as well as in slice preparations.

PRICE SCHEDULE

1. New Users - An initial four (maximum) training session is required \$ 50/hr
2. Others
 - a. without assistance \$ 5/hr
 - b. with assistance. \$ 30/hr

Dr. Angel Cinelli

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CORE RESEARCH FACILITIES

FOTO/ANALYST II SYSTEM



OBJECTIVE :

To provide facilities for computer-based gel documentation and image analysis.

SERVICES :

The Foto/analyst II workstation acquires images of ethidium bromide-stained agarose gels using an integrating camera configured with a zoom lens, and provides live video and continuous-tone hardcopy output. Thermal prints are of continuous-tone 256 greyscale quality. Video images are digitized and can be saved directly to a computer. The system interfaces directly to a Macintosh Quadra 650 computer, and specialty software allows images to be analyzed for other purposes, such as PCR quantitation.

Dr. C. Hellen or Rodney Romain

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