Intercampus Doctoral Program in Biochemistry

University of Maryland, Baltimore | University of Maryland, Baltimore County

Program Faculty and Their Research Interests

University of Maryland, Baltimore (UMB), School of Medicine

Department of Biochemistry and Molecular Biology

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Sequence-specific DNA Receptors and Bacterial Gene Expression

Haempohilus influenzae is a bacterium found exclusively in the throats of human beings. Certain strains of this organism can cause ear infections, pneumonia, and meningitis. Many years ago it was discovered that this organism could acquire new genetic information (genes) by directly importing it as naked DNA from its surrounding environment. The ability to obtain DNA this way is called natural genetic transformation, and though many human pathogens can use transformation, a detailed molecular understanding of the process is lacking. Through biochemical, genetic, and recombinant DNA techniques, it is the long range goal of my laboratory to understand at the molecular level those events leading to the development of *Haemophilus influenzae* cells genetically competent for DNA transformation.

The transformation process is complex, involving temporal control of gene expression, protein-DNA recognition, macromolecular transport, and genetic recombination. My students and I identify, clone and study the different genes that affect the transformation process in *H. influenzae.* Current experiments employ protein over-expression and purification, epitope tagging and gene fusion analysis to elucidate the role of each gene in transformation. We are especially interested in identifying key structural and regulatory features of a gene and that may inform us of its cellular location and expression pattern.

Karudapuram, S., and G.J. Barcak. 1997. The Haemophilus influenzae dprABC genes constitute a competence-inducible operon that requires the product of the tfoX (sxy) gene for transcriptional activation. J. Bacteriol. 179:4815-4820.

Black, Lindsay W., Ph.D.

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Viral DNA Packaging and Assembly
Phage Molecular Biology and Protein Folding

Work is focused on the mechanism of bacteriophage morphogenesis, emphasizing DNA packaging and the structure of packaged DNA. We are interested in employing the phage packaging system to construct new cloning vectors. Our aim is to clone long fragments of DNA, of use in genome DNA mapping; in principle, more than a megabase of DNA could be cloned into the T4 head.

We are also able to express, proteolytically process, and package protein fusions into the phage head. These encapsidated fusion proteins can be injected into bacteria, thereby allowing study of their refolding in vivo. We are also studying the mechanism of a sequence-specific gene amplification using PCR as an assay. In addition, through the use of expression vectors, we have completed a characterization of the membrane inserted initiation complex for phage prohead assembly. We are attempting to assemble phage precursor structures and assemble phage through the use of expression vectors.

Hsingchi Lin, Venigalla Rao and Lindsay W Black, Analysis of Capsid Portal Protein and Terminase Functional Domains: Interaction Sites Required for DNA Packaging in Bacteriophage T4, J. Mol. Biol. 289, 249-260 (1999)

Bucci, Enrico, M.D. Ph.D.

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Zero-Link Polymerized Hemoglobins as Oxygen Carriers *In Vivo*

Modified hemoglobins have been proposed as red cells replacement in transfusional practices . At present there are two main kind of chemical manipulation described in the literature. They are intramolecular crosslinking and polymerization. The intramolecular crosslink produces stabilized tetrameric hemoglobin molecules with MW=65,000 Da, which do not dissociate into dimers. Polymerization implies the formation of large aggregates with molecular weights ranging in the million Dalton. Both procedures have been developed in order to prolong the intravascular retention time and avoid extravasation of the infused material.

To obtain our polymers (U.S. Patent # (08/733-413)) we discarded the technologies based on glutaraldehide Northfield, Biopure) and raffinose (Hemosol). Glutaraldehide is very toxic and raffinose cumbersome to use and the yield of polymeric material is very low.

We have used a zero-link technology, which allows the activated carboxyl groups of one molecule to form pseudo peptide bonds (very stable) with the amino residues of another molecule in the vicinity. No chemical residues are left in between the hemoglobin molecules, the byproducts of the reaction are extremely soluble, easy to eliminate and non toxic. The yield approaches 70% of the starting material.

The superpolymer has a molecular size near and above 21,000,000 Da. In light scattering measurements it appears as a very compact, probably roughly spherical material. It is very soluble, with low viscosity and a very low oncotic activity. *This last characteristic is very important because it leaves plenty of room for additives which may be necessary for preservation and lyophylization of the material.* Most important: experiments on the rat and on the cat indicate that there is no extravasation and that the polymers *do not produce increase of the mean arterial pressure of the infused animals.*(*Matheson B., Kwansa H.E., Bucci E. paper in preparation)*

The polymerization procedure can be applied to human, bovine and other mammalian hemoglobins. It will be just a matter of choice, based on yield, hemoglobin availability and public acceptance. The various steps of the preparation involve chemical treatments, purifications and a final pasteurizations. All procedures which can be automatically performed by appropriate equipment.

Obvious uses of these polymers are emergency treatments, during surgery, and hemodilutions, with all of their corollaries Also, treatment of ischemic areas around strokes and heart infarcts, where tissue damage prevents circulation of blood, but not of low viscosity cell-free polymeric hemoglobins. An important application is in bone marrow transplants. In fact it would avoid the blood typing and immuno- compatibility tests necessary to avoid the risk transplanting unwanted bone marrow.

Under study are also the new physiologic and physico-chemical characteristics of the oxygen transport by cell free hemoglobin solutions and ways to modulate the oxygen binding properties of hemoglobin polymers.

*B. Matheson, A. Razynska, H. Kwansa & **E. Bucci.** Appearance of dissociable and crosslinked hemoglobins in renal hilar lymph. J. Lab. Clin. Inv. 135:459-464 (2000)*

***Bucci E,** Watts TL, Kwansa HE, Fasano A, Matheson BA, Rebel A, Koehler RC.Cell-free Hemoglobin, Oxygen Off-Load and Vasoconstriction. Anasthesiol Intensivmed Notfallmed Schmerzther. Nov;36 Suppl 2:S123-4. (2001)*

E. Bucci1, T. L. Watts2, H E. Kwansa1 and A. Fasano2.. Different efficacy in vitro of hemoglobin based oxygen carriers and red cells. Oxygen Transport to Tissue Vol. XXIII: Oxygen Measurements in the 21st Century, in press (2002).*

*Arosio D., Kwansa H. E., Gering ., Piszczek G., and **Bucci E**. Static and dynamic light scattering approach to the hydration of hemoglobin and its supertetramers in the presence of osmolites. Biopolymers 67:1-11,(2002)*

Carrier, France, Ph.D.

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- ***Role of RNA-binding proteins in the genotoxic stress response***
- ***Study of mechanisms that increase chromatin accessibility to anticancer drugs***
- ***Modulation of tumor suppressor functions by protein-protein interactions***

The capacity to constantly monitor the damage inflicted to one's genetic material (genotoxic stress) is one of the most critical mechanisms regulating the homeostasis of lived cells. Free radicals and peroxides, generated during normal physiological processes and inflammation, as well as environmental pollutants, ultra-violet light, and ionizing radiation are the most common sources of DNA damaging agents. If not sensed and properly repaired, this damage can cause malignant transformation (cancer), in part through mutations at specific sites in certain oncogenes. In normal human cells, the genotoxic stress response is rather complex. It includes the induction of several genes that have been associated with a number of important cellular events such as cell cycle control, signal transduction, replication, mutagenesis, transcription, DNA repair and viral activation. In general, stress activated genes and proteins play a protective role to prevent the transmission of damaged DNA to the next generation of cells.

My laboratory is interested in two particular aspects of the cellular response to genotoxic stress in human cells. **1**) We are studying the role of stress-activated RNA-binding proteins (RBP) in the genotoxic stress response and **2**) We are studying the interactions of stress-activated proteins with chromatin DNA as a potential mechanism to increase the efficiency of anticancer drugs. **1**) We have identified three stress-activated RBP proteins, A18 hnRNP, nucleolin and nucleophosmin (NPM). These proteins play important roles in translation, replication and repair. Of particular interest is the interaction of NPM with the tumor suppressor p53. p53 is the most mutated gene is human cancer, it is estimated that more than 50% of human tumors have a mutation in that gene. Our data indicate that NPM inhibits p53 function by preventing its activation at lower doses of UV radiation and thus sets a threshold for p53 activation by genotoxic stress. **2**) We have recently demonstrated that brief opening of the chromatin structure by histone deacetylase inhibitors, prior to treatment with anticancer drugs that target DNA, increases the anti cancer drugs efficiency by more than ten fold in cell lines that are clinically resistant to these drugs. We have also shown that certain small acidic proteins can increase chromatin accessibility to enzymes that are targeted for anti cancer treatments. We are currently exploring the capacity of these proteins to open up the chromatin structure to enhance anticancer drugs' efficiency in tumor cell lines that are resistant to the drugs.

In addition, we are actively pursuing a third project in collaboration with Dr. David Weber where we study the interaction of the tumor suppressor p53 with the S100 calcium binding proteins.

Both, molecular biology and protein biochemistry techniques are used for these studies.

Carrier, F., Georgel, P.T., Pourquier, P., Blake, M., Kontny, H.U., Antinore, M.J., Gariboldi, M., Myers, T. G, Weinstein, J.N., Pommier, Y,and Fornace, A.J., Jr.

Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. Mol. Cell. Biol. 19: 1673-1685, 1999.
http://mcb.asm.org/cgi/reprint/19/3/1673.pdf

Lin, J., Blake, M., Tang, C., Zimmer, D., Rustandi, R.R., Weber, D.J and Carrier, F. Inhibition of p53 transcriptional activity by the S100B calcium binding protein. J. Biol Chem. Sep 14;276(37):35037-41, 2001.
http://www.jbc.org/cgi/reprint/276/37/35037.pdf

Yang, C. and Carrier, F. The UV-inducible RNA binding protein A18 (A18 hnRNP) plays a protective role in the genotoxic stress-response. J.Biol.Chem., Dec 14: 276(50):47277-47284, 2001.
http://www.jbc.org/cgi/reprint/276/50/47277.pdf

Yang, C., Maiguel, D.A., and Carrier, F. Identification of Nucleolin and Nucleophosmin as genotoxic stress-responsive RNA binding proteins. Nucl.Acids Res., 30 (10):2251-2260, 2002.
http://nar.oupjournals.org/cgi/reprint/30/10/2251.pdf

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Biochemical Mechanism of Troponin-linked Calcium Regulation of Muscle Contraction

The long-term goal of my research is to elucidate the molecular mechanism of troponin-linked Ca^{2+} regulation of skeletal and cardiac muscle contraction. The interaction of actin and myosin that occurs during muscle contraction is regulated by changes in intracellular Ca^{2+} concentration. In resting muscle, [Ca^{2+}] is 0.1 M or less, and actin and myosin do not interact. When [Ca^{2+}] increases to 1 M or more in response to nervous stimulation, actin-myosin interaction is switched on, activating the myosin ATPase and providing the energy for muscle contraction. In vertebrate skeletal and cardiac muscle, regulation involves binding of Ca^{2+} to troponin in the thin filaments, which also contain actin and tropomyosin. Troponin is a noncovalent complex of three different protein subunits: TnC binds Ca^{2+}, TnI binds to actin and inhibits actin-myosin interaction, and TnT binds to tropomyosin. Several experimental approaches are being used in my laboratory to gain a detailed understanding of Ca^{2+}-dependent interactions among the thin filament proteins: (1) Site-directed mutagenesis to create mutants of the three troponin subunits. Thiol mutants provide functionally interesting sites for attachment of spectroscopic probes and thiol-directed chemical crosslinkers. Tryptophan mutants provide intrinsic fluorescent probes for distance measurements. (2) Covalent crosslinking to determine sites of contact among the thin filament proteins. This work will involve mass spectrometry, as well as the more traditional protein chemistry methods, for the structural elucidation of crosslinkedspecies. (3) Fluorescence resonance energy transfer (FRET) between natural and genetically engineered intrinsic and extrinsic donor-acceptor pairs to measure distance distributions between specific sites on the thin filament proteins. (4) NMR and Crystallization studies to prepare troponin subunits and complexes suitable for three-dimensional structure determination by NMR and X-ray diffraction.

Kobayashi, T., Zhao, X., Wade, R., and Collins, J.H.: AInvolvement of Conserved, Acidic Residues in the N-Terminal Domain of Troponin C in Calcium-Dependent Regulation@. Biochemistry 38: 5386-5391, 1999.

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Ions in Biological Systems
Enzyme Flexibility and Catalysis

We are studying the role of water in biological systems, with an emphasis on ion hydration. We are examining ion specific effects on the rate of association of the proteins barnase and barstar as studied by stopped-flow mixing using fluorescence detection. The purpose of these studies is to show that ion-protein interactions are controlled by short range hydration forces rather than by long range electric field effects.

We are also studying how protein flexibility affects both enzymatic catalysis and crystallization. Enzymes are marginally stable globular proteins that continuously show "breathing" (partial unfolding) motions. We are using steady state kinetics, transition state analog inhibitors, limited proteolysis, nuclear magnetic resonance, and tryptophan phosphorescence to characterize these breathing motions and to determine their role in damaging enzyme side reactions that contribute to disease and in enzyme crystallization. Some enzymes show conformational drift to more rigid forms under crystallization conditions; we are using the physical techniques mentioned above to characterize the enzyme substates involved and to determine the mechanism by which crystallization conditions drives these changes.

Kiriukhin, Michael Y. and Collins, Kim D. (2002) *Dynamic Hydration Numbers for Biologically Important Ions.* Biophysical Chemistry, in press.

Farrance, Iain, Ph.D.

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Transcriptional Regulation in Cardiac Muscle under Normal and Diseased Conditions

The research in my laboratory focuses on transcriptional regulation in cardiac muscle under normal and diseased conditions. Prior to birth the heart increases in size due to cell division of the cardiac m yocytes. After birth the cardiac myocytes withdraw from the cell cycle and the heart increases in size due to increase in size (and contractile protein content) of each individual myocyte. This process is called cardiac hypertrophy. Heart disease caused by ∀1-adrenergic agonists, chronic high blood pressure, ischemic damage also causes cardiac hypertrophy. This type of hypertrophy (sometimes termed "pathological" hypertrophy) is marked by the upregulation of genes expressed during fetal life and eventually causes a decrease in cardiac function that can lead to heart failure. Studies of gene regulation during this process is

especially relevant since heart failure is a leading cause of death.

Some of the transcription factors that regulate expression of cardiac genes have been identified. These regulatory factors include the TEF-1, MEF-2 families, serum response factor (SRF), GATA-4, NFAT and USF. While the role of individual factors in regulating the expression of gene expression has been grossly determined, how the activity of these factors is regulated to achieve the complex expression patterns seen during cardiac development and in heart disease is still not known.

I am investigating the role of one family of transcription factors, the TEF-1 family, in cardiac-specific gene expression and in cardiac hypertrophy. TEF-1 binding sites are required for the expression of many contractile protein genes in muscle and for the response of these some genes to 1-adrenergic agonists. During both of these processes, the activity of TEF-1 is modulated by: 1) interactions of TEF-1 with its different types of DNA binding sites, and 2) protein:protein interactions (TEF-1 with, itself, with other TEF-1 family members, with cofactors, with other types of transcription factors, and with the basal transcriptional machinery). These interactions are the focus of the research in my laboratory.

My laboratory uses cultured cardiac myocytes for these studies because treatment of these cells with α1-adrenergic agonists mimics the cellualr hypertrophy and gene expression changes seen in the diseased heart. Using molecular biology techniques (DNA transfections, assays for DNA binding proteins, interaction cloning, and structure/function analyses by deletion and mutagenesis) and this (*in vitro*) system we can study the role of TEF-1 and its interactions in the regulation of cardiac contractile protein genes during disease. The information from these studies could lead to improved treatment of heart disease in patients.

Farrance, I.K.G., and Ordahl, C.P., The role of transcripiton enhancer factor-1 (TEF-1) related proteins in the formation of M-CAT binding complexes in muscle and non-muscle tissues, JBC,271:8266-8274, 1996.

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Cellular Mechanisms of Signal Transduction

The focus of the laboratory is on signal transduction pathways and specifically understanding the mechanisms that mediate and control calcium signals in cells. Calcium is a fundamental signal transduction mediator in most cells and we are interested in how, where, and when calcium signals are generated, and how other signaling pathways interact and control the development of calcium signals. We are investigating the intracellular processes that control levels of cytosolic calcium, utilizing a variety of cellular, molecular and physical biochemical techniques on several different cell models including cultured smooth muscle cells. A large segment of the work involves analysis of single cells using specific probes and antibodies to assess spatial and temporal aspects of calcium signal generation. In addition, we are examining the expression of key signaling proteins in relation to calcium signal generation and analyzing the function and distribution of specific receptors, channels, and ion pump proteins after their transfection into cells. Our studies also center on the use of sophisticated high-resolution subcellular imaging analysis to map the organelles (in particular, endoplasmic reticulum subdomains) which function as calcium pools within cells. These investigations are aimed at analyzing the dynamic spatial arrangements of calcium signaling organelles in relation to membrane trafficking events mediated by G proteins and during the massive changes in organelle structure and organization that occur within phases of the cell cycle. In particular, we are pursuing an interesting relationship between the function, distribution, and calcium content of organelles and the progression of cells through the cell cycle or their exit from the cell cycle into the stationary G_0 growth state. By understanding this important connection between the activity of calcium-regulatory organelles and the ability of cells to alter their growth state, we are identifying ways to modify smooth muscle cell proliferation; this is key to preventing the development of atherosclerosis which involves growth and migration of smooth muscle cells inside blood vessels.

Patterson, R.L., van Rossum, D.B., and Gill, D.L. (1999) AStore-operated Ca^{2+} entry: evidence for a secretion-like coupling process@ Cell 98, 487-489

Inesi, Giuseppe M.D.

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Molecular Characterization of the Mechanism of Ca^{2+} Transport by a Group of ATPases Associated with Intracellular Membranes

Our laboratory is interested in the molecular characterization of the mechanism of Ca^{2+} transport by a group of ATPases associated with intracellular membranes. These ATPases play an important role in control of the cytosolic Ca^{2+} concentration and, thereby, in regulation of cellular functions by Ca^{2+}. Our experimental approach includes isolation of intracellular membranes; purification of proteins; kinetic measurements of ion fluxes and coupled enzyme reactions; studies of protein structure and function by chemical derivatization and site directed mutagenesis; gene transfer to express recombinant protein and to influence cellular functions.

Suming Hua, Daniele Fabris, and Giuseppe Inesi (1999) ACharacterization of Calcium, Nucleotide, Phosphate, and Vanadate Bound States by derivation of sarcoplasmic reticulum ATPase with ThioGlo1.@ Biophysical Journal,77, 2217-2225.

Klein, Michael, Ph.D.

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The research interests in my laboratory concern the mechanisms controlling calcium ion fluxes in excitable cells, especially skeletal muscle and cardiac myocytes.

Electrical stimulation of these cells causes calcium ions to be released from the intracellular calcium-sequestering organelle, the sarcoplasmic reticulum. The resulting elevation of cytosolic calcium serves as a "second messenger" in these cells, causing contraction. The techniques for
measuring these changes in intracellular calcium involve the use of electrophysiological methods to stimulate the cell, and confocal optical imaging techniques to measure changes in the fluorescence of a calcium indicator within the cytosol. We have recently discovered in skeletal muscle that calcium ions are released in small, discrete amounts. During small depolarization of the membrane potential, these calcium release events, or calcium "sparks",
appear as brief elevations of calcium of about 100 nanomolar, lasting 20 milliseconds. In addition to sparks caused by changes of membrane potential, we have also observed sparks caused by cytosolic calcium
itself, activated by a mechanism known as calcium-induced calcium release.
In cardiac myocytes we are examining the effects of expression of exogenous proteins on the calcium elevation in adult and embryonic cardiac myocytes. Over-expression of isoforms of SERCA, the
sarcoplasmic reticulum calcium pump, results in dramatic changes in the calcium handling of these cells. We are also investigating the effects of SERCA mutants.
These studies will allow us to examine the regulation of calcium release and uptake, so as to characterize the role of calcium ions in controlling muscle contraction.

Lacampagne, A., Klein, M.G., Ward, C.W. and Schneider, M.F. 2000. Two mechanisms for termination of Ca2+ sparks in skeletal muscle. Proc. Nat'l.Acad. Sci. 97:7823-2828.

Cavagna, M., O'Donnell, J.M., Sumbilla, C., Inesi, G., and Klein, M.G. 2000. Exogenous Ca2+ ATPase isoform effects on Ca2+ transients of embryonic chicken and neonatal rat cardiac myocytes. Journal Physiology 528:53-63.

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Fluorescence Spectrosccopy - Basics and Applications to Biochemistry and Medicine

Our research involves the use and development of fluorescence spectroscopic methods. At present we are placing major emphasis on studies of the interactions of flurophores with metallic particles. The use of these interactions promises to provide new opportunities in biochemical and biomedical research.

A second major emphasis is the establishment of the Center for Fluorescence Spectroscopy. This center provides state-of-the-art time and frequency-domain measurements to users from this campus and from other institutions in this country as well as other countries.

Our basic science projects include the use of fluorescence to study:

1. Conformational distributions of proteins, nucleic acids, and transfer RNA.
2. Dynamics of proteins and membranes.
3. Transient effects in diffusion in solutions, and in proteins and membranes.
4. Synthesis of fluorescent sensor molecules.

Lakowicz, Joseph, R., 2001. Radiative Decay Engineering: Biophysical and Biomedical Applications, Analytical Biochemistry, 298, 1-24.

Lakowicz, J., R., Shen, Y., D'Auria, S., Malicka, J., Fang, J., Gryczynski, Z., Gryczynski, I., 2002. Radiative Decay Engineering. Analytical Biochemistry, 301, 261-277.

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Mechanism of DNA Repair of Oxidized Bases
Linkage of DNA Repair with Carcinogens
Coupling of DNA Mismatch Repair with DNA Replication

Current interests in my laboratory are DNA base excision repair, mismatch repair, protein-DNA interactions, protein-protein interactions, and carcinogenesis. Oxidative damage is a major source of DNA damage in living organisms and the inability to repair DNA damage properly can enhance rates of tumor development. Mutations in human repair genes could lead to genetic instability in cancer. We are studying base excision repair pathways in both prokaryotes and eukaryotes for repairing oxidative DNA damage. The 8-oxo-G (GO) lesion is the major stable product of DNA oxidative damage and has the most deleterious effects because it can mispair with adenine. *E. coli* MutY is an adenine DNA glycosylase that removes the misincorporated adenines opposite the template G or GO lesions. Repair mechanism, structural determination, and protein-DNA interactions are currently studied.

We have identified MutY homologs (MYH) in yeast, human and calf nuclear and mitochrondrial extracts. Fission yeast mutant defective in the *MYH* has a mutator phenotype and is more sensitive to hydrogen peroxide than the wild type strain. The linkage of MYH repair to cancer is currently pursued. Some malignant prostate and breast cancer cell lines have much lower expression of hMYH than non-malignant cells. Our hypothesis is that under-expression of hMYH in human cells can promote tumor progression due to the poor repair of the oxidized bases. We have shown that phosphorylation can affect hMYH glycosylase activity on A/G and A/GO-containing DNA. Our recent finding that hMYH interacts with many repair, replication, and checkpoint proteins will shed light on the repair mechanism. Human MYH interacts with AP endonuclease (APE1), proliferating cell nuclear antigen (PCNA), replication protein A (RPA), mismatch repair proteins hMSH2/hMSH6, and Hus1. Thus, base excision and mismatch repair pathways may couple to the DNA replication. The physical interaction between hMYH and hMSH2/hMSH6 may coordinate both pathways on repairing A/GO mismatches. This may explain the paradox that hMYH can be directed to repair the misincorporated adenines on the daughter strand but not on the parental strand. The checkpoint proteins Hus1

forms a heterotrimer with Rad1 and Rad9 that are structurally related to homotrimer sliding clamp PCNA. We propose a model that DNA repair enzymes may act as molecular sensors to signal damage response pathways. MYH may first recognize the GO lesions and then recruits Rad9/Rad1/Hus1 to DNA damage sites. Through the study of the mechanism of DNA mismatch repair, our understanding of cancer, aging, and genetic diseases can be advanced.

Parker, A., Y. Gu, W. Mahoney, S. H. Lee, K. K. Singh, and A-L. Lu (2001). Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J. Biol. Chem. ***276****, 5547-5555.*

Gu, Y. and A-L. Lu. (2001). Differential DNA recognition and glycosylase activity of the native human MutY homolog (hMYH) and recombinant hMYH expressed in bacteria. Nucl. Acids Res. ***29****:2666-2674.*

Gu, Y., A. Parker, T. M. Wilson, H. Bai, D. Y. Chang, and A. L. Lu. (2002). Human MutY homolog (hMYH), a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins hMSH2/hMSH6. J. Biol. Chem. ***277****: 11135-11142.*

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The molecular biology and genetics of drug resistance in cancer

The interests of the laboratory are primarily focused upon the molecular biology of anti-tumor drug resistance. The ongoing projects include:

A. Folate and Antifolate transport in animal tumor cell lines

- The use of differential mRNA display and cDNA array technology to identify and characterize those genes whose expressions levels are affected by depletion of serum folate levels, and to determine what effects those changes have on antifolate sensitivity and apoptosis as well as global gene expression.
- Understanding the post-transcriptional regulatory mechanisms that control expression of folate receptor alpha in response to serum folate levels.
- Understanding the mechanisms whereby the expression of Metallothionein protects cells from the effects of folate depletion.

B. Prostate cancer

- Determining how alterations in serum folate levels affect the sensitivity of prostate tumor cell lines and mouse xenographs to new generation antifolates designed as de novo purine synthesis inhibitors.
- Determination of the effect of folate status on radiation sensitivity and drug efficacy
- Understanding the extent to which serum folate levels affect the expression of Metallothionein in prostate tumor cell lines and to determine its subsequent affect on intracellular Zn levels and Krebs Cycle mediated ATP production.

Zhu, W-Y, and Melera, P. W. Metallothionein is overexpressed by hamster fibroblasts selected for growth in 15pM folinic acid and provides a growth advantage in low folate (1999) Cancer Research 59:4194-4199

Zhu, W-Y, and Melera, P. W Wild type expression levels of Metallothionein I and II in mouse cells enhances growth in physiological folate through a cell cycle mediated pathway. (2001) Proc. American Assoc. Cancer Res. 42:735

Zhu, W-Y., Alliegro, M. A., and Melera, P. W. The rate of folate receptor alpha (FRá) synthesis in folate depleted CHL cells is regulated by a translational mechanism sensitive to media folate levels, while stable overexpression of its mRNA is mediated by gene amplification and an increase in transcript half life (2001) J. Cellular Biochem. 81:205-219

Zhu, W-Y, and Melera, P. W. Basal levels of metallothionein I and II expression in mouse embryo fibroblasts enhance growth in low folate through a cell cycle mediated pathway. Cell Biology International 25:1261-1269 (2001).

Zhu W-Y, Bunni, M., Priest, D.G., DiCapua, J.L., Dressler, J.M., Chen, Z., and Melera, P.W., Folate depletion and antifolate resistance, In Press.

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Intracellular Signaling Cascades in Heart Muscle Cells and Their Roles in Excitation-cotracxtion Coupling and in Cardiac Pathologies Such as Heart Failure and Cardiac Inflammation

Our laboratory is interested in the membrane receptors for toxins, drugs, and hormones that mediate important functional responses in heart and brain cells.

We are studying drugs and hormones that regulate Ca channels in heart cells that beat spontaneously in culture. For example, we study the peptide hormone, angiotensin II, to learn how its binding to its receptor causes increased Ca channel activity. Our work is multidisciplinary, involving biochemical and electrophysiological methods.

In another project, we are investigating the mechanism of action of the potent neurotoxin, tetanus toxin. We have shown that tetanus toxin binds to specific receptors on cultured neural cells, and that it inhibits release of neurotransmitter. We plan to identify the molecular events that lead to a loss of neurotransmission in neuronal tissue.

DuBell,W.H., Gaa,S.T., Lederer,W.J., and Rogers, T.B. (1998) Independent Inhibition of Calcineurin and K+ Currents by the Immunosupressant FK-506 in Rat Ventricle Am. J. Physiol., 275, H2041-H2052

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Local and Global Cytosolic Calcium Signaling in Skeletal Muscle Fibers and Neurons

Calcium ions serve as intracellular messengers for a wide range of cellular functions, spanning time scales ranging from milliseconds to hours, days or even weeks. We are studying the cellular and molecular mechanisms underlying the generation and transduction of several responses mediated by intracellular Ca2+ in skeletal muscle fibers, sympathetic ganglion neurons, cerebellar Purkinje neurons and in cell culture systems.

Generation of the Ca2+ transient in skeletal muscle: Skeletal muscle is activated within a few ms after membrane depolarization by a massive release of Ca2+ ions from the intracellular storage location, the sarcoplasmic reticulum. We are studying three steps in the activation mechanism for Ca2+ release in isolated single functioning skeletal muscle fibers: (1) Activation of membrane voltage sensor molecules (the dihydropyruidine receptors), studied electrophysiologically by the "gating current" generated by the voltage dependent rearrangement of charged regions of this protein; (2) Opening patterns of individual Ca2+ release channels or of a group of a few functionally linked Ca2+ release channels in response to depolarization or ligand activation, detected by laser scanning confocal microscopy as Ca2+ "sparks" (highly localized elevations of cytosolic Ca2+); (3) the mechanism coupling the voltage sensors to the release channels, studied by pharmacological and biochemical manipulation in functioning fibers. (supported by NIH/NINDS grant "Control of calcium release in skeletal muscle fibers")

Molecular basis of skeletal muscle fiber types: In another project we are studying the molecular mechanisms underlying the regulation of fiber type-specific gene expression in mammalian fast-twitch and slow-twitch skeletal muscle fibers, which differ in the speed of contraction and relaxation and which express different isoforms of most muscle-specific proteins. Signaling pathways specifying fast and slow fiber type gene expression, as well as differences in the generation and transduction of the [Ca2+] transients in the two fiber types, is being investigated in fast- and slow-twitch muscle fibers isolated from adult muscle, maintained in cell culture and subjected to various patterns of electrical stimulation. (supported by NIH/NINDS grant "Calcium removal and regulation of muscle and neurons")

Calcium signaling in neurons: Mechanisms of Ca2+ signaling in neurons are being investigated in cultured neurons isolated from frog sympathetic ganglia or in cultured Purkinje neurons isolated from mouse or chick cerebellum. Topics currently under examination in our lab include mechanisms for terminating the cytosolic [Ca2+] signal, the spatial location of the intracellular store from which Ca2+ ions are released and local differences in Ca2+ signals in different regions of the cell body of the same neuron. (supported by NIH/NINDS grant "Calcium removal and regulation of muscle and neurons").

Laboratory techniques used in my laboratory: *Cell Biophysics and Physiology:* action potentials, voltage clamp, gating current, Ca2+ indicators and global intracellular [Ca2+] transients, laser scanning confocal microscopy of local Ca2+ release events (Ca2+ sparks), computer controlled rapid local cell perfusion and high speed (video rate) "xy" or line scan ("xt") confocal microscopy of local Ca2+ transients. *Cell Biology:* isolation and culture of adult rat and mouse fast-twitch and slow-twitch skeletal muscle fibers, frog sympathetic ganglion neurons and mouse and chick cerebellar Purkinje neurons, antibody stain, confocal microscopy and expression of fluorescent analogs of muscle transcription factors. Biochemistry and Molecular Biology: single muscle fiber protein analysis, RTPCR and (in collaboration) heterologous gene and protein expression and reporter gene expression in cultured cell lines and in muscle fibers. *Computer Analysis and Mathematical Modeling:* Multiple parameter curve fitting, modeling the calcium binding and transport systems in neurons and muscle fibers, digital image processing, numerical deblurring of fluorescence microscopic images.

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McDonough, S.I., Cseresnyes, Z. and Schneider, M.F. (2000). Origin sites of calcium release and calcium oscillations in frog sympathetic neurons. J. Neurosci 20:9059-9070

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Dr. Shamoo's current research interests are in the area of Ethics, Science, and Public Policy. More specifically his interests are in: development of good research practices, ethics and public policy, objectivity and conflict of interest, and the ethics of the use of humans in reserach especially persons with mental illness. Dr. Shamoo teaches graduate students on "Responsible Conduct of Research" and holds conferences and workshops on ethics in research. Dr. Shamoo works with policy makers to establish standards of ethical conduct in research.

Shamoo, S. E. , " Adverse Events Reporting- The Tip of an Iceberg", Accountability in Research, 8:197-218.

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Fluorescence-based Fiber Optic Metal Ion Biosensors Employing Proteins as the Recognition Element

The major thrust of my research is aimed at the development of fluorescence-based sensors for research and diagnostic use. These sensors usually function by transducing the presence or level of a particular chemical analyte in solution as a change in the fluorescence of a susceptible dye/recognition molecule, which we can measure through an optical fiber. Like fiber optic borescopes, such sensors can be used with patients *in vivo*, and they also permit continuous, realtime measurement of the analyte, in the manner of a pH meter.

In addition, the fluorescent transduction mechanism permits imaging in cells and tissues. Thus we are the first to image release of zinc from a hippocampal cell culture in response to an electrical stimulus, and to quantitate the level of this release ratiometrically in the microscope. Our sensors have detection limits for Cu and Zn in the picomolar range, and are immune to interference from Ca and Mg, making them suited for *in vivo* measurements. Our goals are several-fold: to develop very sensitive, very selective, flexible sensors; to develop sensors for analytes that are inaccessible or whose levels change too rapidly for classical clinical chemistry technology; and to advance the state of the art of these devices for applications in research and treatment.

R.B. Thompson,,W. O. Whetsell, B. P. Maliwal, C. A. Fierke, and C. J. Frederickson, "Fluorescence microscopy of stimulated Zn(II) release from organotypic cultures of mammalian hippocampus using a carbonic anhydrase-based biosensor system," J. Neurosci. Methods 96 35-45 (2000)

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NMR, Structure/function Studies of S100 and p53 Proteins

One project in our laboratory involves studies of the structure and function of S100B(ßß), a glial-derived protein from the brain. S100B(ßß) is a dimeric Ca^{2+}-binding protein that is overproduced during gliosis in patients with Alzheimer disease, Down syndrome, and Aids related dementia. In addition, S100B(ßß) and/or other members of the S100 protein family (S100 ß, S100L, etc.) are found at high concentrations in several tumor cell lines including lung, bladder, kidney, cervix, breast, head and neck, larynx, lymph, and mouth. Thus, overproduction of S100 proteins may cause problems in the regulation of cell growth in these diseases. Presumably, the function of S100B(ßß) is related to its ability to bind a variety of target proteins in a Ca^{2+}-dependent manner. One such target is the tumor suppressor protein, p53. We have shown that upregulation of S100B abrogates p53 transcription activation in tumor cell lines and that S100B(ßß) binds and inhibits both the protein kinase C-dependent phosphorylation and the oligomerization of p53 *in vitro*. Therefore, the focus of our laboratory is to determine, at atomic resolution, the mechanism by which S100B can affect p53 transcription activation and promote uncontrolled cell growth. In this regard, we have determined the three-dimensional structure of apo-S100B(ßß), the S100B(ßß)-Ca^{2+} complex, and the S100B(ßß)-Ca^{2+}-p53 peptide complex using NMR spectroscopy. The structural studies of S100B(ßß) are imperative for the efficient design of biochemistry and molecular biology experiments that are also completed in our laboratory. Currently, knowledge about the structure and function of S100B(ßß) are being used to design molecules that inhibit S100B(ßß) from binding to p53. Perhaps one of these molecules will be practical as a drug for regulating uncontrolled cell growth *in vivo*. Similarly, structure/function studies are underway for other members of the S100 protein family.

Rustandi, R.R., Baldisseri, D.M., and Weber, D.J. Structure of the negative regulatory domain of p53 bound to S100B Nature Structural Biology, 7:570-574. (2000)

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- **RNA-binding proteins regulating mRNA decay kinetics**
- **Regulation of protein-binding by local higher-order RNA structures**
- **Post-transcriptional regulation of LDL receptor expression**

In my laboratory, we are interested in the cellular factors that regulate mRNA turnover rates, and the signaling systems that may transiently modulate the activity of these factors. Our experimental approaches vary from cell and molecular biology (cultured cell systems, transfection, RNA interference) to biochemical (gel mobility shift, protein-protein and protein-RNA cross-linking), and biophysical systems (fluorescence anisotropy, resonance energy transfer). Some projects that we are pursuing include:

(1) Association of specific proteins with AU-rich mRNA-destabilizing sequences

Many mammalian mRNAs encoding oncoproteins, cytokines, and inflammatory mediators are unstable, due to AU-rich elements (AREs) located in their 3' untranslated regions (3'UTRs). The ability of an ARE to destabilize an mRNA in *cis* is dependent upon interactions between this element and any of a number of cytoplasmic ARE-binding factors. For example, interaction of the protein HuR with an ARE is associated with mRNA stabilization, while binding of other proteins, including AUF1 and tristetraprolin, are associated with acceleration of mRNA decay. While these and several other factors are known to associate with AREs, few details are available regarding either their mechanisms of action or the conditions influencing ARE-binding of one *trans*-acting factor over another. Characterization of the interactions between these proteins and their ARE substrates is an essential step towards a broader understanding of the mechanisms linking ARE-recognition with the initiation or inhibition of mRNA decay processes.

(2) **AREs can adopt higher-order RNA structures that influence their association with ARE-binding factors.**

In multivalent cationic environments, some AREs fold into higher-order RNA structures in a sequence-specific manner. Folding into higher-order ARE structures inhibits association of some, but not all ARE-binding proteins. In order to more fully understand the importance of ARE structural potential on protein association, we are characterizing

structural features of folded AREs, and learning how condensed RNA structures regulate their protein-binding preferences. We hope that answering these questions will improve our understanding of cellular mRNA catabolism, and how cells achieve selective destruction of specific mRNAs from complex mRNA populations. In the longer term, we would like to test methods of selectively modifying the turnover rates of individual transcripts associated with the pathogenesis of specific disease states.

(3) **RNA-protein interactions contribute to the regulation of hepatic LDL receptor expression.**

Beyond ARE-directed mRNA turnover, we are also interested in the involvement of other RNA-targeted mechanisms in the regulated expression of genetic targets that may directly impact human health and disease. One such example is the post-transcriptional regulation of hepatic LDL receptor expression. Internalization of low density lipoprotein (LDL) by hepatic LDL receptors is essential for systemic LDL clearance and cholesterol excretion via bile acid formation. As such, regulating the expression of hepatic LDL receptors represents an attractive target for the development of novel, aggressive, cholesterol-lowering therapeutic strategies.
Previously, we demonstrated that activation of the protein kinase C pathway in a human liver cell culture model induces LDL receptor mRNA levels involving both transcriptional activation and mRNA stabilization. Also, we showed that receptor mRNA binds components of the cytoskeleton, and that this association influences the rate of receptor mRNA decay. Distinct sites within the receptor mRNA 3'UTR contribute to regulation of both its turnover and cytoskeletal localization. Using these sites, we are identifying proteins that specifically interact with functional domains in the LDL receptor mRNA 3'UTR, and describing their physiological functions in the post-transcriptional regulation of hepatic LDL receptor production.

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*Wilson, G.M., Sutphen, K., Moutafis, M., Sinha, S., and Brewer, G. (2001) Structural remodeling of an A+U-rich RNA element by cation or AUF1 binding. J. Biol. Chem., **276,** 38400-38409.*

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*Wilson, G.M., Sun, Y., Lu, H., and Brewer, G. (1999) Assembly of AUF1 oligomers on U-rich RNA targets by sequential dimer association. J. Biol. Chem. **274,** 33374-33381.*

*Wilson, G.M., Vasa, M.V., and Deeley, R.G. (1998) Stabilization and cytoskeletal-association of LDL receptor mRNA are mediated by distinct domains in its 3' untranslated region. J. Lipid Res. **39,** 1025-1032.*

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Conformation and Dynamics of Complex Polysaccharides and Glycoproteins by NMR Spectroscopy and Computer Molecular Modeling

The biological question at which my research is directed is that of the functional significance of the carbohydrate of glycoproteins. We have approached the problem through the exploration of the chemical structure, the three dimensional conformation, and the noncovalent forces which stabilize the folded forms of the oligosaccharides. High resolution ^{1}H and ^{13}C NMR spectroscopy, especially 2-dimensional heteronuclear methods, is applied to glycopeptides and oligosaccharides isolated by high performance liquid chromatography. High resolution NMR spectroscopy has been combined with computer model building and circular dichroism spectroscopy in studies of the three dimensional conformation and dynamics of complex carbohydrates.

Manuel Martin-Pastor and C. Allen Bush AA new strategy for the Conformational Analysis of Carbohydrates based on NOE and NMR Coupling Constants. Application to the flexible polysaccharide of Streptococcus mitis.@Biochemistry 38: 8045-8055 (1999).

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M. Martin-Pastor and C. A. Bush ``Conformational studies of Lewis X and Lewis A trisaccharides using NMR residual dipolar couplings" (2002) Biopolymers , 89-98.

H. F. Azurmendi and C. A. Bush ``Tracking Alignment from the Moment of Inertia Tensor (TRAMITE) of Biomolecules in Neutral Dilute Liquid Crystal Solutions" (2002) J. Amer. Chem. Soc., {\bf 124}, 2426-2427.

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Protein chemistry, mechanistic properties and inhibition of glutathione-dependent enzymes that are potential anti-tumor targets.

Over the past several years this laboratory has been investigating the catalytic mechanism and stereochemical features of several enzymes that use glutathione as a cofactor.

Of particular interest is the glutathione-dependent glyoxalase pathway. This elementary metabolic pathway found in all cell functions to chemically remove cytotoxic methylglyoxal arising as an unavoidable by-product of the normal metabolism of cells. The pathway is of particular interest because one of the two enzymes in the pathway is deficient in cancer cells. From a basic science perspective, experiments are directed at examining both the kinetic and stereochemical properties of the glyoxalase enzymes that allow the pathway to operate at such a high level of efficiency in normal cells. From a practical perspective, special glutathione derivatives are being synthesized as inhibitors of the glyoxalase enzymes in order to lay the foundation for the development of a novel chemotherapeutic strategy that is based on differences in the activities of the glyoxalase enzymes between normal cells and cancer cells.

A second research interest is the synthesis and testing of special Amechanism-based@ inhibitors of HMG-CoA
reductase, in order to clarify selected stereochemical features of the active site of this enzyme. This work may provide a basis for the development of a new class of cholesterol-lowering drugs.

Creighton, D.J., Hamilton, D.S., Kavarana, M.J., Sharkley, E.M., and Eiseman, J.L. "Glyoxalase Enzyme System as a Potential Target for Anti-tumor Drug Development," Drugs of the Future, 25, 385-392 (2000); Creighton, D.J., and Hamtilton, D.S., "Brief History of Glyoxalase I and What We Have Learned About Metal Ion-Dependent, Enzyme Catalyzed Isomerizations," Arch Biochem.Biophys., 387, 1-10.

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My research interests lie primarily in the development of optical sensors and optical sensing techniques and their application to both biomedical and environmental research. In the field of biomedical research, our group will be focused on optical sensing at both the microscopic and macroscopic levels. Microscopic analyses will be aimed at developing new spectroscopic tools capable of monitoring cellular reactions without disturbing routine cellular function. This work includes the development of novel "nanoscale" optical sensors capable of being inserted into single living cells as well as innovative high resolution non-invasive imaging techniques. Such analyses can provide us with fundamental insights into various biological reaction pathways, and could lead to the development of future treatments for medical disorders.

Macroscopic analyses are aimed at the development of non-invasive or minimally invasive spectroscopic techniques for the rapid and accurate diagnosis of diseases, such as cancer, *in vivo*. One such technique that we are currently interested in developing is multiphoton photoacoustic spectroscopy. This technique will allow for differentiation of subsurface tissues based upon their absorption spectra. Near infrared light will be used to penetrate deep into human tissue, while detection of an acoustic signal will allow for the sensitive analysis of deep absorption events. In addition to the development of novel spectroscopic techniques and instrumentation for

such analyses, a great deal of this research involves elucidating key spectroscopic differences between normal and diseased tissues and relating these differences to chemical and/or morphological differences..

The third major area of interest in our research group is the remote detection of environmental contaminants (e.g., VOCs). In particular, our group is interested in the development of fiber optic sensors capable of providing an array of complimentary multidimensional data. Unlike conventional remote sensors, the fusion of the various types of spectroscopic data returned (i.e., fluorescence, Raman, atomic emission, etc.) will provide the information necessary to perform accurate analyses of complex, unknown environments.

*T. Vo-Dinh, J.P. Alarie, B.M. Cullum and G.D. Griffin, "Antibody-Based Nanosensor for Measurement in a Single Cell," Nature Biotechnology, **18**, p. 764-767 (2000).*

*T. Vo-Dinh and B. M. Cullum, "Biosensors and Biochips for Bioanalysis," Fresen. J. Anal. Chem., **366**, p. 540-551 (2000).*

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Bio-Medical Applications of Mass Spectrometry
Protein-Nucleic Acid Interactions
Nucleic Acid Adducts, Metal Binding

Our laboratory is dedicated to the development of mass spectrometric methods for the characterization of biopolymers and macromolecular complexes and to their applications to the biomedical field. In particular, mass spectrometry is applied to the investigation of interactions between nucleic acids and nucleic acid-binding proteins, such as transcription factors, repair enzymes, and other nucleic acid-processing enzymes. Fundamental questions, such as mechanism and stoichiometry of binding, sequence specificity, and binding constant, are addressed together with the investigation of effects produced on binding and repair kinetics by the presence of covalent adducts and other nucleic acid modifications.

Mass spectrometry enables to follow a reaction in real time, allowing for the observation of short-lived intermediates. Kinectic and mechanistic information can be readily obtained for enzymes that bind to DNA, recognize the lesion, and repair or replace damaged nucleotides that would otherwise produce replication mismatches and specific toxins: ricin A chain (a model for N-glycosylase enzymes) and -sarcin (a model for phosphodiesterase/excision enzymes). Mass spectrometry allows for direct and expedite analysis of mixture analytes at levels comparable to those present in a living cell and without using radio-labels or immuno-assays.

Tandem techniques (MS/MS) are employed in our lab to characterize the structure of covalent adducts of DNA with recognized carcinogens and drugs used in cancer therapy. Gas phase sequencing via collision-induced dissociation and/or enzymatic ladder protocols are used to locate the site of adduction. Combined approaches are used to obtain the footprint of the repair enzymes on the target nucleic acid.

This type of structural and mechanistic information will hopefully contribute to the understanding of how an event at the molecular level, the chemical modification of a susceptible region of nucleic acid, can trigger the cascade of biological events culminating in cancer.

Fabris, D.; Hathout, Y.; Fenselau, C. AInvestigation of Zinc Chelation in Zinc-Finger Arrays by Electrospray Mass Spectrometry@, Inorg.Chem. 38, 1322-1325 (1999).

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mechanisms of organic reactions

In general, we are interested in the mechanisms of organic reactions, particularly those with some biological relevance. While our investigations involve biologically important species and the results impact on the larger puzzle of biology relevant to human health, these investigations often unveil aspects of that are clearly fundamental to organic reactivity. Much of our recent work has centered on the chemistry of reactive intermediates encountered in the activation of carcinogenic nitrosamines. Nitrosamines are widely encountered in the human environment, for example - in foods (beer, unfortunately), in cosmetics and health care products, in tobacco products, and in industrial settings such as rubber-, leather- and metal-working compounds and fluids. There is also growing recognition that they may be formed endogenously (in the human body) by the combination of amines with nitrites, the latter either consumed in the diet or formed as a result of oxidation of the recently recognized ubiquitous effector nitric oxide.

-Acetoxynitrosamines have been widely used in studies of nitrosamines as convenient precursors for the -hydroxynitrosamines that are thought to be less stable. -hydroxynitrosamines, the products of enzymatic (P450) hydroxylation of nitrosamines, have until recently been little studied. They are mostly unstable and difficult to prepare and handle. But, we have employed rapid mix technologies to overcome this problem.

Chemistry of alkane diazoates, derived from _-hydroxynitrosamines, and their diazonium ion and carbocation products are also of interest. Ultimately we are interested in what controls atom-site selectivity in the alkylation of DNA, the reaction which initiates the biological cascade that is carcinogenesis.

Other projects involve iminium ion chemistry, synthesis of modified nucleosides that may represent promutagenic lesions, and the solution chemistry of anti-cancer agents.

Students and post-docs in my group are engaged in an eclectic mix of activities including organic synthesis, analytical and physical chemistry. They thereby develop expertise with a wide range of techniques, instrumentation and strategies in modern chemical research.

Cai, H.; Fishbein, J.C. _-Acyloxydialkylnitrosamines: Effects of Structure on the Formation of N-Nitrosiminium Ions and a Predicted Change in Mechanism J. Am. Chem. Soc. 1999, 121, 1826.*

Chahoua, L.; Cai, H.; Fishbein, J.C. Cyclic _-Acetoxynitrosamines: Mechanisms of Decomposition and Stability of _-Hydroxynitrosamine and Nitrosiminium Ion Reactive Intermediates J. Am. Chem. Soc. 1999,121, 5161.*

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The central focus of my laboratory is the study of gene regulation at the atomic level using X-ray crystallography and other biophysical techniques. I am currently investigating two different regulatory systems, one involved in Circadian Rhythms, and the other involved in the immune system.

Behavior and physiological processes in mammals occur in rhythmic (circadian) cycles that correlate with the light-dark cycle of a day. This is represented at the transcriptional level by changes in expression of specific genes in a rhythmic cycle. I am investigating the structural mechanism that underlies the on/off switch of certain genes by two proteins involved in the Circadian cycle. The nature of this switch provides a direct link between metabolism and alterations in Circadian rhythms.

Major Histocompatibility Complex II (MHCII) molecules play an essential role in the development, regulation and activity of mammalian immune system. Lack of expression, or overexpression, of MHCII has serious, usually fatal, consequences. I am studying the structure and mechanism of action of the multiprotein complex, the MHCII enhanceosome, which, together with the transcriptional coactivator CIITA, is responsible for regulating the expression of the genes that code for MHCII.

Garvie, C.W., Pufall, M., Graves, B.J., and Wolberger, C. (2002). Structural analysis of the autoinhibition of Ets-1 and its role in protein partnerships. Accepted at J. Biol. Chem. (available online, ahead of print)

Garvie, C.W., Hagman, J. and Wolberger, C. (2001). Structural studies of the Ets-1/Pax5 complex formation on DNA. Molecular Cell, ***8*** *(6), 1267-76.*

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Research in my group is focused along four general lines of study; the prediction of protein tertiary structure and function, the development and refinement of biological force fields, an investigation into the spectroscopic properties of biological molecules, and finally, development of the next generation of theoretical methods to aid in the determination of experimental protein structures.

Mine is a theoretical group, where we are interested in the development of evolutionary algorithms for protein structure prediction. Evolutionary, or in this case, Genetic Algorithms, computationally mimic natural selection. The simulation evolves with multiple objective function optimization in order to reach the desired solution, a native-like protein structure.

Once a tertiary structure of the biological molecule is determined, then the real fun can begin. Now it is possible to calculate the vibrational spectrum in such a way as to take into account the anharmonicity of the molecule. This does, however, require accurate force fields. Therefore research in my group is also aimed at developing high level molecular force fields for biological macromolecules. I am also interested in the way in which vibrational modes couple together both intra-molecularly or to external modes of either other molecules or to the solvent. Questions which we are addressing include: what effect does mode coupling have on the spectroscopy, and more importantly on the possibility of vibrational energy transfer, either internally or externally to other molecules.

Finally, research in my group also examines protein conformational changes, when for example,the protein binds a ligand or other macromolecules (ie DNA). In collaboration with the Center for Neutron Research at NIST, we are currently developing a next generation molecular modeling package for use by the Small Angle Neutron Scattering (SANS) community.

Kruger, S.T.; Gregurick, S.K.; Shi, Y.; Wang, S.; Waldkowski, B.; Schwartz, F., "Specific DNA binding to CRP induces conformational changes in the CRP in solution," Nature, 2000, (in press).

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Biomedicinal Chemistry with applications in antiviral and anticancer therapy, as well as Biomedical Technology with applications in artificial blood

My research interest is synthetic organic chemistry. Our specific current research interests include:

(A) "Fat" and "Slim" Nucleosides and Nucleotides as Potential Anticancer and Antiviral Agents: These novel ring-expanded ("fat") and ring-contracted ("slim") nucleoside/nucleotide analogues, designed and synthesized in this lab, are potential chain-terminators of nucleic acid synthesis when incorporated into a tumor or viral DNA/RNA during transcription (or reverse transcription in case of retroviruses including HIV that causes AIDS). Mode of chain termination, and hence the viral or tumor replication, is believed to be the base-mispairing accompanied by considerable deviation of the base-sugar bond from the natural array. In addition, because of their unique structural, spatial, and conformational characteristics and constraints, "fat" and "slim" nucleosides/-tides are excellent probes for nucleic acid metabolism, structure, and function.

(B) Synthesis of novel bifunctional reagents to cross-link proteins and nucleic acids: blood substitutes and cancer chemotherapy. We are currently involved in the synthesis of novel bi-functional organic reagents to cross-link hemoglobin subunits as well as double stranded nucleic acids. The modified cell-free hemoglobins are to be used as blood substitutes for emergency transfusions. The cross-linking is anticipated to prevent the otherwise rapid renal elimination of cell-free hemoglobins, while also decreasing the latter's oxygen affinity to afford adequate oxygen transport from lungs to tissues. Cross-linking of nucleic acid double helices has implications in checking viral and tumor growths, besides assisting in

investigations of nucleic acid metabolism, structure, and function.

R. K. Sood, V. S. Bhadti, A. I. Fattom, R. B. Naso, H. Chen, B. E. Korba, E. Kern, and R. S. Hosmane, "Novel Ring-Expanded Nucleoside Analogs Exhibit Potent and Selective Inhibition of Hepatitis B Virus Replication in Cultured Human Hepatoblastoma Cells," Antiviral Res., 2002, 53, 159-164.

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Structure-function studies on single-stranded nucleic acid binding proteins

RNA chaperones

I am interested in structure-function studies on a class of nucleic acid-interactive proteins: single-strand specific binding proteins (also known as helix-destabilizing proteins). As a class, the binding proteins have a wide range of biological roles, yet they share a number of commonalities. These include homologous functional domains, i.e., regions responsible for nucleic acid interaction, binding cooperativity, and interaction with other proteins.

The binding proteins under current investigation include bacteriophage T4 gene 32 protein, the classical single-stranded DNA binding protein. Although gene 32 protein was first isolated thirty years ago, it continues to provide us with fascinating insights. The protein has a critical function in the replication (copying) of DNA, as well as in the repair of damaged DNA molecules and in DNA recombination. Having identified an amino acid sequence (the "*LAST*" motif) involved in both protein-nucleic acid and protein-protein interaction, we are particularly concerned with understanding the relationship of these two binding activities, and in further delineating the structural basis of the protein's binding cooperativity and nucleic acid binding surface, as well as the control of the protein's ability to lower the denaturation temperature, T_m, of double helical DNA.

We also have a long-term interest in RNA binding proteins, in particular those with RNA "chaperone" activity. These include the nucleocapsid (NC) proteins of retroviruses, as well as pre-messenger RNA binding proteins (*e.g.*, hnRNP A1). In our studies, we employ a very broad spectrum of experimental approaches, extending from traditional phage experiments and curtting-edge recombinant DNA techniques to a wide range of biochemical and biophysical methods.

L.A. Waidner, E.K. Flynn, M. Wu, X. Li, and R.L. Karpel, "Domain Effects on the DNA-Interactive Properties of Bacteriophage T4 Gene 32 Protein, J. Biol. Chem. 276, 2509-2516 (2001).

M. Wu, E.K. Flynn and R.L. Karpel," Details of the Nucleic Acid Binding Site of T4 Gene 32 Protein Revealed by Proteolysis and DNA T_m Depression Methods", J.Mol.Biol. 286, 1107-1121 (1999).

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Research in our laboratory uses complementary methods of transient laser spectroscopy and traditional bioanalytical methods to investigate photocleavage of nucleic acids and proteins. Specifically, functionalized imide and diimide systems, with highly tunable redox properties, are employed to induce cleavage of the biological polymers by targeting the nucleotide bases in DNA and oxidizable aromatic side chains in proteins. Our efforts include developing parallel systems that will induce site-specific (for sequencing applications) and non-specific (for footprinting applications) lesions in oligonucleotides following the absorption of a photon by the imide or diimide chromophores.

In parallel studies, we are preparing amino acid conjugates of the photosensitizers. Amino acids with charged or hydrophobic side chains will be used to facilitiate association with different protein residues. Redox reactions with tyrosine and tryptophan are photoinduced. Using analytical methods (electrophoresis, HPLC, and mass spectrometry), reaction products are identified. Once separated and identified, the cleavage products are correlated with the initial site of damage. This work will provide valuable insight into the design of artificial "photoproteases," which is an area that remains virtually unexplored. In conjunction with developing chemical systems to carry out the reactions described above, we are integrating these systems into time-resolved assays, where macromolecular dynamics, following the initiation of a mixing or folding event, can be directly probed.

Rogers, J. E.; Weiss, S. J.; Kelly, L. A. "Photoprocesses of Naphthalene Imide and Diimide Derivatives in Aqueous Solution and DNA," Journal of the American Chemical Society 2000, 122, 427 – 436.

Pollack, Ralph M., Ph.D.

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Enzyme mechanisms, and model systems for enzymes

Our laboratory is interested in the mechanism of enzymatic proton transfer to/from carbon. These proton transfers are difficult nonenzymatically, but enzymes are able to accelerate them enormously. In order to probe the basis of this rate acceleration, we investigate both model systems and enzymes themselves. The major system that we employ is the enzyme 3-oxo- ^{5}-steroid isomerase. Site-directed mutagenesis allows us to specifically modify this enzyme to produce mutants, which we investigate kinetically and structurally to determine the roles of specific amino acid residues in catalysis. We also use model systems to try to mimic the catalytic power of the enzyme.

R. M. Pollack, L. D. Thornburg, Z. R. Wu, M. F. Summers, Mechanistic Insights from the Three-Dimensional Structure of 3-Oxo- 5-steroid Isomerase, Archives of Biochemistry and Biophysics, 370, 9 (1999).

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My general interests are in bioinorganic and biophysical chemistry. The main technique that will be employed in my laboratory will be electron paramagnetic resonance (EPR) spectroscopy, a technique useful for macromolecular structure determination and study of transition metal ions with unpaired electrons. Transition metal ions are important in biological systems because they participate in electron transfer reactions (e.g. iron-sulfur clusters), structural stabilization and recognition (e.g. zinc fingers) and chemical transformations (e.g. cytochrome P450). The projects in my lab will incorporate inorganic, physical and biological chemistry techniques.

1) Amyloid association studied by EPR spectroscopy
Amyloid proteins condense to form fibrils and plaques that are believed to be linked to neurodegeneration in Alzheimer's disease. Transition metal ion reactivity is believed to be a source of amyloid plaque neurotoxicity. Our goal is to determine the rates of association and dissociation of nitroxide spin-labeled amyloid proteins using EPR spectroscopy to understand the importance of amino acid side-chain mobility in the aggregation events. We are also interested in elucidating structural changes that occur when transition metal ions bind to amyloid proteins.

2) Multi-stranded DNA with paramagnetic metal ions
The aim of this project is to assemble multiple strands of DNA into discrete architectures upon binding metalloporphyrins or divalent transition-metal ions. Folding of nucleic acid sequences at the ends of chromosomes initiated by free-base porphyrins has been found to inhibit telomerase, an enzyme that is overexpressed in cancer cells. Instead of using free-base porphyrins, we will use metalloporphyrins and metallophthalocyanines to induce the guanine-quartet structural element implicated in telomerase inhibition. A further consequence of adding metalloporphyrins to DNA is that the resulting DNA structures could serve as scaffolds for novel biological magnetic materials. The triple helix is a multi-stranded DNA motif that has been targeted as a means to inhibit protein expression. Although Mg^{2+} is required in triple-helices, its coordination environment is unknown. Substitution of spectroscopically active metal ions like Cu^{2+}, Co^{2+} and VO^{2+} for Mg^{2+} can be used to reveal why Mg^{2+} is required for triplex assembly. Ultimately, we will use the information about Mg^{2+} binding to design paramagnetic metal-ion binding sites into triple helices to create new magnetic materials.

Szalai, V. A.; Thorp, H. H. "Electron Transfer in Tetrads: Adjacent Guanines are not Hole Traps in G Quartets" Journal of the American Chemical Society 2000 ***122****, 4524-4525.*

Smith, Paul J., Ph.D.

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Molecular recognition using model systems
DNA binding compounds

My interests are in the areas of bioorganic and physical organic chemistry. Currently, two research projects are underway in my laboratory. The first involves the design, synthesis, and evaluation of small host molecules that form strong non-covalent complexes with phosphotyrosine. Such compounds have the potential to serve as valuable tools in the study of cellular signaling pathways. Tyrosine phosphorylation has been shown to initiate the formation of dimeric and multimeric protein signaling complexes responsible for cell growth and differentiation; these protein-protein binding events require the formation of direct contacts between the phosphorylated tyrosine residue and an SH2 domain on the secondary (downstream) protein. Small molecules that complex phosphotyrosine can therefore act as antagonists for SH2-containing proteins, preventing the formation of signaling complexes, and thus interrupting signalling. The objective of the second project is the development of molecules that bind to double-stranded DNA sequence-specifically. These compounds can serve as probes of DNA structure and may ultimately provide a means to gene-specific delivery of anti-tumor agents. Using computer-based molecular modeling, we have identified several small chemical units that have the potential to recognize base-pair specific structural features of the DNA minor groove. Assembly of these monomers into linear oligomers will ideally allow specific binding to any desired DNA target sequence; specificity will be evaluated using a variety of techniques including chemical footprinting.

Steven L. Hauser, Edward S. Cotner, Paul J. Smith "Synthesis of a Capped Dicationic Derivative of -Cyclodextrin" Tetrahedron Lett. 1999, 40, 2865-2866.

Summers, Michael F., Ph.D.

Investigator/Howard Hughes Medical Institute; Co-Director
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Dr. Michael F. Summers received his B.S. degree in Chemistry from the University of West Florida in 1980, his Ph.D. degree in 1984 from Emory University, and was a postdoctoral fellow at the NIH from 1984-1987. He joined the faculty at the University of Maryland Baltimore County as an Assistant Professor in 1987, and was promoted to Associate Professor with tenure in 1991 and to Full Professor in 1996. In 1994 he was appointed to the Howard Hughes Medical Institute, and he is currently a Full Investigator. He served as a member of the NIH Reviewers Reserve from 1990-1995, and was a member of NIH Study Section BBCA from 1995-1999. He is also an Editor for the *Journal of Molecular Biology* and a member of the editorial advisory boards of *Protein Science* and *Journal of Biomolecular NMR*. Dr. Summers was a recipient of the ASM Hinton Award for Mentoring (2002), the White House Presidential Award for Mentoring (2000), and the Protein Society (DuPont-Merck) Young Investigator Award (1996). Dr. Summers' research focuses on NMR studies of proteins and macromolecular interactions, with a major emphasis on the structural proteins that comprise the human immunodeficiency virus (HIV-1). His group determined the 3D structure of the HIV-1 nucleocapsid protein, the HIV-1 and HTLV-II matrix proteins, and the core domain of the HIV-1 capsid protein.

They also recently solved the structure of the HIV-1 nucleocapsid protein bound to RNA stem-loop recognition elements of the HIV-1 Ψ-site. Other studies have focused on metalloproteins and proteins with unusual thermal stability derived from hyperthermophilic bacteria.

D'Souza, V., Melamed, J., Habib, D., Pullen, K., Wallace, K., Summers, M. F., "Identification of a High-Affinity Nucleocapsid Protein Binding Site within the Moloney Murine Leukemia Virus Ψ-RNA Packaging Signal. Implications for Genome Recognition,"J. Mol. Biol. ***314****, 217-232 (2001).*

Whalen, Dale L., Ph.D.

Professor

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Research in our laboratory has been centerd on the study of the solution reactions of epoxides in general, with the goal of better understanding the detailed mechanisms of their hydrolysis reactions and reactions with acidic and nucleophilic reagents. More specifically, we are interested in studying the reactions of diol epoxide metabolites of polycyclic aromatic hydrocarbons, widely spread enbironmental contaminants, are often metabolized to diol epoxides. Certain of these hydrocarbons are highly carcinogenic, and this biological activity is attributed to diol epoxide metabolites which react with DNA and other cellular macromolecules. Our studies of diol epoxide metabolites and model epoxide systems in solution are designed to provide us with a better understanding of their reactions in biological systems.

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Bashirelahi, Nasir, Ph.D.

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Knowledge of the mechanism of action of steroid hormone receptors has important implications in the diagnosis of a variety of diseases. However, the regulatory aspects by which hormone binding modulates transcriptional activation remains unknown. In recent years, many estrogenic chemicals, of both biological and non-biological origin, have been discovered in the environment. We are investigating whether commonly-encountered xenoestrogens affect the interaction of estrogen with its receptors of the interactions of the receptor with the genomic response element, potentially distruptin normal cellular regulation processes and playing a roe in the development of both benign prostatic hyperplasia (BHP) and/or prostatic carcinoma.

N. Bashirelahi, B. Koffman, and R.J. Sydiskis. 1997. Steroid Receptors. Endocrine Toxicology, 2nd ed. Edited by J.A. Thomas and H.D. Colby. 8 1997 Taylor and Francis.

Enwonwu, Cyril O., D.D.S, Ph.D.

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Interactions between malnutrition and infections at cellular and sub-cellular levels

Cellular and molecular effects of nutrients: Cellular and metabolic processes are regulated by food nutrients either directly or through the mediation of hormones which interact with specific organelles and cell-surface receptors. Our research activities focus on prevalent public health nutritional problems, and more importantly, on the role of diet/nutrition in health promotion and disease prevention. Using sophisticated, sensitive tools for exploring whole body, cellular and molecular effects of specific nutrients, our current research interests include (a) impact of nutritional status, particularly ascorbate deficiency, on muscariniccholinergic and ß-adrenergic receptors as well as signal transductive events in the salivary glands, and its relevance to xerostomia; (b) protein-energy malnutrition - its effects on specific and nonspecific host defense systems; (c) cytokine biology in malnutrition; (d) role of antioxidant food nutrients in disease prevention; (e) nitrogen and energy metabolism in sickle cell disease; and (f) diet/nutrition in the management of disease states including infectious, degenerative, and metabolic diseases.

Enwonwu, C.O., Afolabi, B.M, Salako, L.A., Idigbe, E.O., AL-Hassan, H., Rabiu, R.A.. Hyperphenylalanaemia in children with falciparum malaria. Quarterly Journal of Medicine, 1999; 92:495-503.

Affiliate Faculty

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Oxidative Stress in carcinogenesis and in the metabolism of anticancer drugs

The primary interests of my laboratory are free radicals in medicine and biology, as well as the interaction of anticancer agents with DNA and glutathione. Our interest also includes metal carcinogenesis. During the past year research included:

1. The effect of oxidative stress on cells. This includes carcinogenesis, genotoxicity, cytotoxicity and how these three relate to one another.

2. Detection of hydroxyl radicals at very low concentrations (mM).

3. Measuring 8-OH-deoxyguanine levels in human cancer and cells exposed to oxidative stress.

4. Apoptosis induced by toxic metals and anticancer drugs.

5. Hydroxyl radicals generated by toxic metals to study possible mechanisms of genotoxicity.

Amstad, P., Liu, H., Ichiminya, M., Brezesky, I., Trump, B., Buhimschi, I. and Gutierrez, P. " BCL-2 is involved in preventing oxidant-induced cell death and in decreasing oxygen radical production", Redox Report, 2002, 6: 351-362.

Gu, Y., Desai, T., Gutierrez, P. O. and Lu-Chang, A-L. " Alterations of DNA base excision enzymes hMYH and hOGG1 in hydrogen peroxide resistant and transformed human breast cells" Medical Science Monitor" 2001, 7:861-868.

Kaper, James B., Ph.D.

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Molecular Pathogenesis of Bacterial Infections

Bacteria that cause disease usually produce specific enzymes, toxins, or surface structures that allow them to colonize host tissues, avoid host defenses, and damage host cells. Research in my laboratory concerns the molecular biology of such virulence factors, specifically studies on genetic control and effect on eukaryotic cells. Genes encoding such factors are cloned and sequenced and specific mutations are made in these genes to inactivate the virulence factor. Bacterial strains containing the mutated genes are then studied in a variety of *in vitro* and animal models to determine the contribution of each factor to the disease process and immune response. One example of such studies is cholera toxin, which causes increases in adenylate cyclase activity in eukaryotic cells and water and ion secretion in intestinal cells, resulting in diarrhea. The genes for cholera toxin were cloned, sequenced, and mutated in my laboratory. *Vibro cholerae* strains containing mutated toxin genes are now being evaluated in clinical trials as live, attenuated vaccine strains for the prevention of cholera. Another example is the neuraminidase of *V. cholerae* which alters the sialic acid composition of eukaryotic cells surfaces, thereby increasing the density of toxin receptors on the cell. The gene encoding this factor was sequenced and mutated and the effect of the mutated gene on toxin receptors and fluid secretion studied in *in vitro* and animal models. A final example is a new virulence factor in enteropathogenic *Escherichia coli*. We have recently cloned genes responsible for the attachment of this organism to intestinal epithelial cells and for disruption of the eukaryotic cell cytoskeleton, resulting in changes in actin polymerization within the cell. Our recent data indicates that binding of the *E. coli* to the eukaryotic cell activates a tyrosine kinase with the epithelial cell and we are currently characterizing bacterial genes responsible for this activity.

These studies which focus on the molecular biology of the interaction of bacterial factors and eukaryotic cell function will lead to greater understanding of the pathogenesis of disease due to these organ-isms and improved methods for prevention and diagnosis.

Mellies, J., S. J. Elliott, V. Sperandio, M. S. Donnenberg, and J. B. Kaper. The Per regulon of enteropathogenic Escherichia coli: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE) - encoded regulator (Ler). Molecular Microbiology. 1999, 33:296-306.

Mobley, Harry, Ph.D.

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Molecular mechanisms of bacterial pathogenesis of urinary tract infection and peptic ulcer disease bacterial genetics

E. coli,Proteus mirabilis, Helicobacter pylori

Our laboratory=s interests are the molecular mechanisms of bacterial pathogenesis. Molecular genetic techniques are used to characterize virulence genes of uropathogenic bacteria including those encoding bacterial ureases, a group of enzymes that hydrolyze urea to ammonia and carbon dioxide. Urea, hydrolysis leads to kidney stone formation. We are interested in the urea-specific transcriptional regulation by UreR as well as regulators of fimbrial gene expression.

Techniques in the lab include enzyme purification and kinetics, gene bank preparation, molecular cloning, gene expression, DNA sequence analysis, site specific mutagenesis, and other recombinant DNA techniques.

Other interests include molecular mechanisms of bacterial adherence by pathogenic *E. coli* and gene expression in *Helicobacter pylori*, the etiologic agent of peptic ulceration.

McGee, David J., Carrie A. May, Rachel M. Garner, Janette M. Himpsl and Harry L. T. Mobley. 1999. Isolation of Helicobacter pylori genes that modulate urease activity. J. Bacteriol. 181: 2477-2484.

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Molecular genetics of Alzheimer's Disease

Mutations in the highly homologuous presenilin 1 and 2 genes (PS1 and PS2) cause the majority of early-onset familial Alzheimer's Disease (FAD). Our studies focus on the function of presenilin proteins and on how AD-linked mutations cause disease. We have shown that overexpression of PS2 induces apoptosis and that the FAD PS2 (N141I) mutation increases apoptosis. Moreover, overexpression of presenilins in dividing cells causes a cell cycle arrest, and this arrest is potentiated by the FAD PS1 and PS2 mutations. The mechanisms leading to PS induction of apoptosis and cell cycle arrest are being investigated. Knowledge of the function of presenilins is being uncovered by identifying and studying presenilin-interacting proteins. Using the yeast two-hybrid system, we discovered two novel PS interactors. One of these is calmyrin, a myristoylated calcium-binding protein. We have shown that calmyrin interacts with PS2 in yeast 2-hybrid assays, in vivo colocalization of the two full-length proteins, and by increased binding of the two proteins by affinity-chromatography and coimmunoprecipitation. Furthermore, the two proteins when coexpressed in HeLa cells induce additive cell death. These and other studies have indicated that the two proteins are linked in a common signaling pathway. Current research is underway to determine the role of presenilin-calmyrin interaction in normal and disease states. The second PS interactor represents an exciting new class of proteins that we discovered and named ubiquilin. Ubiqulin binds to both PS1 and PS2 proteins in yeast two hybrid assays, GST pull-down experiments, colocalization of the proteins expressed in vivo, and in immunoprecipitation assays. Ubiquilin is noteworthy since it contains multiple ubiquitin-related domains typically thought to be involved in targeting proteins for degradation by the ubiquitin-proteasome system. Instead, we found ubiquilin promotes increased PS protein accumulations. Protein pulse-labeling experiments indicate that ubiqulin does not affect presenilin protein turnover, but instead may function as a molecular chaperone in aiding presenilinfolding and /or biogenesis. The studies of these interactors are likely to lead to a better understanding of presenilin fuction and how FAD mutations casue disease.

Janicki, SM. Stabler, SM, and Monteiro, MJ. 2000. Familial Alzheimer's Disease Presenilin-1 mutants proteniate cell cycle arrest. Neurobiology of Aging 21:829-836.

Mah, A, Perry, G, Smith, M. and Monteiro, MJ. 2000. Identification of ubiquilin, a novel presenilin interactor that increases presenilin protein accumulation. J. Cell Biology, 151: 847-862.

Passaniti, Tony, Ph.D.

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Research efforts in my laboratory have targeted endothelial cells (EC) and tumor vasculature for therapeutic application. Since angiogenesis is an important component of tumor growth, progression, and metastasis, my laboratory has investigated the mechanisms of EC survival and differentiation. In particular, we have been interested in transcription factor regulation of angiogenesis. We have developed a quantitative assay to measure angiogenesis in vivo and to test inhibitors of angiogenesis. Using this assay, we have shown that infection with an adenoviral vector expressing the wild-type p53 gene resulted in inhibition of angiogenesis. Our studies of EC response to chemotherapeutic drugs have shown that activation of the nuclear factor kappa B is responsible for both preventing apoptosis and maintaining cellular differentiation.

We are currently studying the role of the transcription factor Cbfa (core binding factor alpha) in EC interactions with the extracellular matrix, with other EC, and in angiogenesis. Our current hypothesis is that this transcription factor is expressed in neovasculature and may regulate the expresssion of downstream genes involved in EC migration and angiogenesis. We are testing this hypothesis by using Cbfa dominant negative and overexpression vectors to generate stable EC transfectants. We are continuing these basic studies of how transcription factors regulate EC biology to identify novel downstream genes that may be targets of anti-angiogenesis and, therefore, anti-tumor agents.

Wang, W. and Passaniti, A. (1999) Extracellular matrix inhibits apoptosis and enhances endothelial cell differentiation by a NFkB-dependent mechanism. J. Cell. Biochem. 73:321-331.

Yang, J., Nagavarapu, U., Relloma, K., Sjaastad, M., Moss, W.C., Passaniti, A., and Herron, G.S. (2001) Telomerized human microvasculature is functional in vivo. Nature Biotech., 19:219-224.

Varma, Shambhu D., Ph.D.

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Implications of oxygen radicals and abnormalities of carbohydrate metabolism in the biochemistry of eye lens and cornea

The primary emphasis of my research is to understand the biochemistry of the eye in relation to the vision impairing diseases caused by aging, diabetes and nutritional deficiencies. Studies on the effects of these afflictions on lens metabolism and cataract formation are in progress. The knowledge generated has been useful in devising biochemical and physiological strategies in actually preventing the formation of cataracts in diabetic and galactosemic animals and in animals subjected to oxidative stress. More recently we have demonstrated that the development of cataracts, in vitro as well as in vivo, can be attenuated by certain keto acids such as pyruvic acid. The attenuative effect of these agents has been found to be linked to (1) their property of scavenging various reactive species of oxygen commonly referred to as oxy-radicals, (2) to the inhibition of certain enzymes involved in the pathogenesis of diabetic and galactosemic cataracts and (3) to their ability of preventing oxidative and non-oxidative glycation of structural and enzymatic proteins. Studies on the prevention of lipid oxidation are also in progress. We have also demonstrated that

nutritional antioxidants can play a significant role in the prevention of the above eye diseases. The above studies are also now being extended to determine the significance of oxidative stress in retinal diseases associated with aging and diabetes. The studies involve basic enzymology, measurement of superoxide and its derivatives, determining the radical scavenging properties of various endogenous and exogenous compounds, their ability to modulate enzyme activities and to prevent oxidative stress in vivo and under tissue culture situation. Physiological assessment of the ocular functions is also done under control and experimental situations.

S.D. Varma, P.S. Devamanoharan, A.R. Rutzen, A.H. Ali and M. Henein. Attenuation of Galactose-Induced Cataract by Pyruvate. Free Radical Research. Vol.30, pp.253-263. 1999.

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