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**Marshall University Biomedical Sciences
Graduate Program**

Faculty Research Projects

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For the new student:

This booklet contains brief descriptions of the research projects carried out in the laboratories of the Biomedical Sciences faculty. We hope these pages will begin to acquaint you with all of the wonderful research opportunities available at Marshall University and help you choose your research rotations. Please use the information here to learn about our work, to contact faculty whose work is of interest to you and to become familiar with all of the expertise available to you as you enter a research career. Should you need assistance, please don't hesitate to contact us.

We welcome you to the Biomedical Sciences Graduate Program and wish you every success in your career.

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Research Clusters

The Biomedical Sciences Graduate program offers students interdisciplinary education in five main areas related to human health and disease. These areas of concentration are called Research Clusters. It is one of the strengths of our program that faculty may conduct research in one or more areas and many of the faculty profiles you will find in this booklet list one or more cluster affiliations. Students within the program select one of these five areas as their concentration within the BMS program. The five Research Clusters are listed below with the name and contact information for the coordinator and the web page you may access for additional information.

1. Cancer Biology –

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Website: http://bms.marshall.edu/research_groups/cancerbiology.aspx

2. Cardiovascular Disease, Obesity and Diabetes –

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3. Infectious and Immunological Diseases –

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4. Neuroscience and Developmental Biology –

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5. Toxicology and Environmental Health Science –

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Participating Faculty Members

Name	Department/Section	School	Pg
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Pier-Paolo Claudio	Biochemistry/Surgery	School of Medicine	2
Simon Collier	Biology	College of Science	3
Piyali Dasgupta	Pharmacology	School of Medicine	4
Beverly C. Delidow	Biochemistry	School of Medicine	5
Richard Egleton	Pharmacology	School of Medicine	6
Philippe T. Georgel	Biology	School of Medicine	7
Lawrence M. Grover	Physiology	School of Medicine	8
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Research Clusters: Cardiovascular Disease, Obesity and Diabetes;

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blough@marshall.edu<http://www.marshall.edu/cdn>**Research Overview**

Areas of inquiry include the elucidation of new "biomarkers" for disease, the development of new sensors for point of care testing (nanowires, titanium dioxide nanotubes, microfluidics), the molecular mechanisms associated with contractile signal transduction in skeletal, smooth, and cardiac muscle, and the effects that nanomaterials may have on cellular function and the environment. In addition, we also are in the process of developing new ways to "package" (e.g. nanoencapsulation) and deliver drugs. To investigate these topics, we utilize a variety of different animal and cell culture models and employ molecular, morphological and physiological tools. Our research applies directly to cardiovascular disease, sarcopenia, and diabetes. Graduates and past lab members have gone on to medical school, Ph.D. programs and industrial positions.

1. **Mechanotransduction in striated muscle:** The long-term objective of this project is to investigate the mechanisms skeletal muscle uses to convert mechanical stimuli into biochemical signals. It is possible that these processes are altered with exercise training and during aging. We believe an attenuation of mechanotransduction processes may be a factor in the attainment and progression of aged associated muscle atrophy and dysfunction.
2. **Mechanisms of muscle atrophy and hypertrophy:** The long term objective of this project is to determine the identity and timing of the signal transduction events regulating smooth muscle growth in response to alterations in loading and exercise.
3. **Development of bionanomotors for biomedical applications:** The long-term objective of this project is to develop the means to use bionanomotors to transport single molecules across a substrate under controlled conditions.

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The focus of our translational research laboratory, located in the Translational Genomic Research Institute (TGRI) at Marshall University, is to understand the molecular mechanisms governing malignant transformation in order to tailor novel therapeutic strategies. To effectively design novel biological drugs, a further understanding of the mechanism of cancer pathogenesis is required. Toward this end, we have carried out in the past 20 years studies to understand the crosstalk between those factors that contribute to cancer progression versus those that protect from it.

With this in mind we have been working in our laboratory on the idea of targeting and tailoring more efficacious therapy for cancer. This led us to develop two major research programs that are highlighted below:

1. Gene therapy offers great potential for combating and curing a wide range of pathologic lesions. One of the major limiting factors in gene therapy has been the development of safe and effective delivery systems.

The emphasis of our recent research efforts is on imaging guided drug delivery. The recent emergence of "molecular imaging" has set the stage for an evolutionary jump in diagnostic imaging and therapy. The ability to incorporate drugs or genes into detectable site-targeted systems represents a new paradigm in therapeutics that will usher in an era of image-based drug delivery.

We have developed a novel gene therapy system based on the use of commercially available ultrasound contrast agents and adenoviruses that enhance the specificity of gene transfer in vitro as well in vivo. Ultrasound-mediated microbubble destruction improves the efficacy and reduces the non-specific expression of gene therapy vectors providing a useful tool for manipulating gene expression in the living animal. We are currently working on further developing this useful targeting gene therapy tool to help closing the gap that still exist between laboratory bench and bedside application.

2. A more recent focus of our laboratory is on cancer stem cell biology and its implications for cancer therapy. We are interested in developing a deeper molecular understanding of cancer stem cells that we now know to be the major source of recurrence and treatment failure due to their ability to re-grow the tumor population. We explore pathways and genes important for the proliferation, survival, and self-renewal of cancer stem cells also in normal adult stem cells in order to identify differences that could be exploited therapeutically.

More importantly, we are interested in analyzing and overcoming resistance mechanisms to radiotherapy and chemotherapy in cancer stem cells. A discovery in our laboratory of how to isolate and propagate cancer stem cells allows us now to test and select the most effective chemotherapy options available to eradicate not only the traditional target of tumor bulk but also the highly resistant cancer stem cells. The overarching goal of our studies is the development of novel therapeutic strategies for eliminating cancer stem cells in the clinical setting offering the promise of personalized cancer treatment options for the individual cancer patient's needs.

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Genetic control of cell polarity in development and disease

The Collier lab uses both invertebrate (*Drosophila*) and vertebrate (cell culture) model systems to investigate the importance of planar cell polarity in development and disease. Planar cell polarity (PCP) refers to the orientation of a cell within the plane of an epithelial cell layer, and the precise control of PCP during development is critical for normal tissue function. For example, loss of normal PCP disrupts neural tube closure in vertebrates and is responsible for cases of familial spina bifida in humans.

Projects: Research in the Collier lab focuses upon how epithelial cells become polarized, and what signals determine the direction of cell polarity within a tissue. Projects in the lab involve a wide range of molecular (protein, DNA, RNA), cellular and genetic (*Drosophila*) techniques, and also employ a variety of imaging techniques including confocal and electron microscopy.

1. **Directional control of Planar Cell Polarity by Prickle protein variants:** Mutations in the human *Prickle1* gene cause progressive myoclonus epilepsy-ataxia syndrome, an inherited form of epilepsy. *Drosophila prickle* gene mutations also cause seizures, implying that *prickle* function is conserved between vertebrates and invertebrates. The Prickle gene product is required for normal PCP, and the Collier lab has shown that the direction of PCP in *Drosophila* depends upon which variant (isoform) of the Prickle protein is active within the tissue (1-3). This project aims to characterize the activities of the different Prickle protein isoforms using molecular and genetic approaches.
2. **Function of the PCP Effector proteins Fritz and Fuzzy:** The PCP Effector proteins, Fritz and Fuzzy, were first identified in the Collier lab (4, 5), and are required for normal PCP in both vertebrates and invertebrates. In vertebrates, Fritz and Fuzzy control cilia formation, and mutations in the human *fritz* gene are associated with the ciliopathic diseases, Meckel-Gruber syndrome and Bardet-Biedl syndrome. This project aims to further characterize the activity of the human Fritz and Fuzzy proteins using a vertebrate cell culture system.

1. K. Doyle, J. Hogan, M. Lester, S. Collier, *Dev Biol* **317**, 354 (May 1, 2008).
2. J. Hogan, M. Valentine, C. Cox, K. Doyle, S. Collier, *PLoS Genet* **7**, e1001305 (Feb, 2011).
3. M. Valentine, S. Collier, *Fly (Austin)* **5**, (Oct 1, 2011).
4. S. Collier, D. Gubb, *Development* **124**, 4029 (Oct, 1997).
5. S. Collier, H. Lee, R. Burgess, P. Adler, *Genetics* **169**, 2035 (Apr, 2005).

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Cigarette smoking is strongly correlated with the onset of lung cancer. About 90% of small cell carcinomas (SCLC) and 60% of non-small cell carcinomas (NSCLC) are associated with smoking. Nicotine, an active component of cigarettes, has been found to promote the growth of human SCLC cells via a specific receptor namely the $\alpha 7$ - nicotinic acetylcholine receptor (nAChR) on human lung cancer cells.

Studies in my laboratory are focused on signaling pathways recruited by tobacco components like nicotine and NNK, which facilitate the proliferation and progression of human SCLCs. Some of the research projects in our laboratory include:

1. Acetylcholine signaling machinery in NSCLCs.
2. Anti-cancer and anti-angiogenic activity of $\alpha 7$ -nAChR antagonists in human SCLCs
3. The effect of long term nicotine-exposure on the expression of $\alpha 7$ -nAChR in NSCLCs

Such studies are especially relevant to human health because about 30% of lung cancer patients continue to smoke after diagnosis and many others use nicotine-based cessation devices, which could potentially exacerbate the progression of lung cancer in patients.

In addition, we are members of the "Nutrition and Cancer" center at Marshall University (www.marshall.edu/cncc). As part of this research group we explore the anti-cancer effects of capsaicin (the spicy ingredient of chili peppers) in human SCLCs. Our data shows so far that capsaicin can suppress the growth of human SCLC cells. The long-term goal of our research is to have a better understanding of human lung cancer and to development of new therapies in this disease.

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webpage

- 1. Role of β -catenin in melanoma:** Melanoma represents only 5% of skin cancers, but is responsible for at least 70% of deaths related to skin cancer. This is largely because, while early melanoma is both preventable and treatable, later stages are highly invasive and resistant to treatment. β -catenin is a cell adhesion and signaling protein that is commonly misregulated in melanoma, and is well known to play a central role in other cancers. However a comprehensive study of the role of β -catenin in melanoma has not been done. We are examining the location and action of β -catenin in mouse and human melanoma cell lines, as well as its response to the antitumor agent, retinoic acid. Our data indicate that retinoic acid reduces the tumor-promoting functions of β -catenin by inducing a coordinate inhibition of the signaling pathway that activates it, called Wnt. We recently identified a small secreted Wnt inhibitor called SFRP1 as a key retinoid-inducible regulator in all stages of human melanoma cells. This work is particularly exciting because SFRP is effective in blocking tumor cell behavior even in human melanoma lines that do not respond to retinoic acid. We are currently examining the action of SFRP in melanocytes and melanoma cells by a number of means. The techniques employed in the lab include subcellular fractionation and western blotting, fluorescence microscopy, invasion and migration assays, siRNA, transfection and reporter gene assays, RNA analysis by real time PCR and microarray. An additional exciting part of this work is our collaboration with Dr. Reinhard Laubenbacher to generate working computer models of Wnt signaling in melanoma.
- 2. Regulation of pituitary cell function by the multifunctional protein, β -catenin:** β -catenin functions as a cell adhesion molecule and as a transcriptional regulator. Prolactin-secreting pituitary tumor cells require β -catenin to maintain high levels of hormone production. This is of interest because: 1. Hyperprolactinemia results in reproductive dysfunction and is one of the presenting symptoms of prolactin-secreting tumors. These tumors are usually controlled with drugs having significant side effects; finding an alternative treatment would offer patients relief. 2. Pituitary tumor cells grow continuously and use prolactin as an autocrine growth factor. Treatment to control prolactin levels usually controls tumor growth, but a small percentage of tumors escape drug sensitivity. Controlling growth is critical to treatment of pituitary tumors because of their location next to the optic nerve and brain vasculature. We are currently exploring the mechanism of the link between β -catenin and prolactin gene expression, using a variety of cellular and molecular techniques.

* QR (Quick response) codes contain links to websites, video, or text information. They may be scanned using free bar code reader applications on a smart phone or wireless tablet with a built-in camera.

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The blood brain barrier (BBB) is a specialized capillary bed that regulates the transfer of substances to and from the brain. Once thought to be a static barrier, recent studies have shown that the BBB is highly regulated, and is a major player in a number of neurological diseases such as stroke, Parkinson's, Multiple Sclerosis, Alzheimer's and epilepsy. Understanding the physiology and pathophysiology of the BBB will provide important insight into disease progression and provide potential targets for therapeutic intervention. My Laboratory focuses on the regulation of the BBB during health and disease. I currently have two available projects.

1. In the last 20 years there has been a dramatic increase in the use of "Neutraceutical" products for treatment of various disorders and for as dietary supplement. Epigallocatechin gallate (EGCG), a green tea catechin has become a popular dietary supplement, has been shown to have effects on numerous cellular functions including inhibition of various transcription factors that regulate Phase 1, 2 and 3 of metabolism. The enzymes and transporters involved in metabolism play a key role in the transport barrier functions of the BBB. This project investigates how the BBB is regulated by EGCG and how this impact delivery of drugs to the Brain.
2. Diabetes is a major health concern worldwide. A number of peripheral microvascular complications are common in diabetes including diminished perfusion, abnormal endothelial proliferation, and increased permeability. There have been few studies, however, investigating the effects of diabetes on the BBB. This is surprising, because there is considerable evidence linking diabetes as a major risk factor for numerous CNS vascular diseases including stroke, Alzheimer's, and other cognitive disorders. Those studies that have been carried out indicate that even well controlled diabetic patients can have impaired BBB function. My research focuses on how diabetes modulates the molecular and functional properties of the BBB in animal models of diabetes.

Some Recent Publications:

1. Hawkins, B.T. and **R.D. Egleton**. Pathophysiology of the Blood-Brain Barrier: Animal Models and Methods. *Current Topics in Developmental Biology*. 80: 277-309, 2008.
2. Seelbach, M.J., T.A. Brooks, **R.D. Egleton** and T.P. Davis. Peripheral inflammatory hyperalgesia modulates morphine delivery to the brain: A role for P-glycoprotein. *J. Neurochemistry* 102 (5): 1677-1690, 2007.
3. Hawkins, B.T., T.F. Lundeen, K.M. Norwood, H.L. Brooks and **R.D. Egleton**. Increased blood-brain barrier permeability and altered tight junctions in experimental diabetes in the rat: contribution of hyperglycemia and matrix metalloproteinases. *Diabetologia*, 50(1):202-211, 2007.

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Research Interests:

Research in my laboratory is centered on the epigenetic regulation of gene expression in the context of cell differentiation and development. Our main research focus is on the role of chromatin composition and structure on nuclear functions, with an emphasis on transcription regulation (Hagerman *et al.*, 2009). It has long been established that both chromatin remodeling and the equilibrium between chromatin folding and unfolding act as regulating mechanisms of gene activation or repression (Georgel, 2007). These conformational changes are tightly linked with histone post-translational modifications (PTM). The PTMs can act as beacons to promote the recruitment of specific transcription factors (activators or repressors) which modulate gene expression (see review). We recently started to investigate the effect of diet on these epigenetics changes in the context of breast (collaborative project with Dr. Hardman) and prostate cancer. Our results strongly suggest that dietary changes can lead to reduced breast cancer incidence in our mice model, and incur positive effects on prostate cancer cell lines.

Other research projects investigate the mechanism of action of various chromatin-associated proteins, such as MeCP2 and Sir3, on chromatin compaction (Adkins *et al.* 2009; Georgel *et al.*, 2003 JBC). Finally, we investigate the connection between chromatin remodeling proteins CHD-1 and CHD-2 and cell differentiation, using mice salivary glands as model systems.

Representative publications:

1. Chromatin Stability at Low Concentration Depends on Histone Octamer Saturation Levels. Hagerman, T., Fu, Q., Molinié, B., Lindsay, S., Georgel, P.T. **Biophysical Journal**, Vol. **96**. Pp 1944-1951, (2009)
2. Role of chromatin/epigenetic modifications on DNA accessibility. Georgel, P.T. **Drug News Perspectives** Vol. **20**. Pp 549-556 (2007)).
3. Role of nucleic acid binding in Sir3-dependent interactions with chromatin fibers. Adkins, N.L., McBryant, S., Johnson, C. N., Leidy, J.M., Woodcock, C.L., Robert C.H., Hansen, J.C., and Georgel, P.T. **Biochemistry**, Vol. **48**. Pp 276-288, (2009).
4. Silencing and Re-expression of Retinoic Acid Receptor Beta2 in Human Melanoma. Fan J, Eastham, L., Varney, M., Hall, A., Adkins, N.L., Sollars, V., Georgel, P., Niles, R.M. **Pigment Cell Melanoma Res.** Vol. **23**. Pp 419-429 (2010)
5. Chromatin Compaction by Human MeCP2: Assembly of novel secondary chromatin structures in the absence of DNA methylation. Georgel, P.T., Horowitz, R.A., Woodcock, C.L., Adkins, N.L., Wade, P.A. and Hansen, J.C. **Journal of Biological Chemistry**. Vol. **278** (34). pp 32181-32188 (2003)

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Currently, there are two active projects in my laboratory:

1. **Mechanisms of action of antidepressant medications:** Mood disorders, including depression, are extremely common, affecting 5-10% of the population. A number of antidepressant medications are currently used to treat depression, however many patients do not respond to medication. In addition, although the immediate effects of these medications are known (most alter serotonin and/or norepinephrine neurotransmission), therapeutic effects of these drugs occur with a delay of several weeks. While the reasons for this delayed effect are not known, current research hypotheses focus on changes in synapses function and structure (plasticity). In this project, we are examining synaptic function and plasticity, and the expression of plasticity related molecules in brain areas that are affected by depression and are targets for antidepressant medications. By increasing our understanding of how antidepressant medications affect brain function, we hope to contribute to improved therapies for depression.
2. **Mechanisms of memory formation:** Memory formation occurs through long-lasting changes in the strength of synaptic communication between neurons. In this project we study synaptic strengthening (potentiation) in order to understand how the brain is altered during formation of new memories. We focus on the hippocampus, which is the major brain structure involved in memory formation. Our goal is to understand the cellular and molecular events that occur during memory formation, in particular, the roles of calcium-permeable ion channels and calcium regulated signaling pathways. This project involves a combined experimental and computation approach to gain a detailed understanding of processes occurring at the neuronal, synaptic and molecular levels. The computational portion of the project is being done at Ohio University in the laboratory of Dr. Bill Holmes (Department of Biology). The experimental portion of the project is being done in my laboratory. By determining the brain mechanisms used for normal memory function we will improve our understanding of how memory is adversely affected by neurological disorders and diseases.

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Although long known to be present in blood vessels and cancer cells, the role of histamine as a potential factor involved in regulation of function in these cells has remains unestablished. In view of extensive evidence suggesting multiple important roles for histamine in cellular physiology and pathophysiology, the long-term goal of the research in our laboratory is to determine the function of non-mast cell histamine vascular and cancer cells. Currently, research is aimed at elucidating regulatory mechanisms of histamine synthesis/metabolism and the function of histamine and/or its metabolites in regulating migration and invasiveness of melanoma cells.

This research involves:

1. Immunocytochemistry, Western blot and qualitative and quantitative PCR to investigate regulation of expression of histamine synthetic and metabolic enzymes
2. Enzyme activity measurements to assess post-translational regulation of the histamine synthetic and metabolic enzymes
3. Enzyme activity and spectrophotometric measurements to assess the capacity of histamine and/or its metabolites to interact with and regulate heme-containing enzymes
4. Manipulation of histamine synthesis/metabolism and measurement of appropriate cellular responses to determine potential roles(s) of histamine and/or its metabolites to regulate cell function. Results from this research should establish a basic role for the histamine and/or its metabolites in the regulation of cell function and provide the groundwork for future studies aimed at determining the role of this system in the cancer and cardiovascular diseases.

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A goal of my research is to identify the mechanisms of action of omega3 fatty acids to increase the efficacy and reduce the side effects of cancer chemotherapy and to prevent cancer. It seems too simple, yet there are good biological explanations for how the dietary omega-3 fatty acids can profoundly affect carcinogenesis. These activities include altering: the potential for lipid peroxidation and free radical damage to cancer cells, prostaglandin production in cancer and normal cells, cell membrane fluidity, membrane transport, membrane permeability, the activities of the peroxisome proliferator activated receptor and/or nuclear factor κ B, membrane receptor function, estrogen metabolism and the inflammatory process. My future plans include investigating the individual contribution of these mechanisms to increasing the efficacy of cancer chemotherapy in mouse models and to translate this research to pilot trials in people.

Current Research:

My current research is to assess the ability of increased consumption of omega 3 to prevent cancer. Currently I have a grant from the DOD Breast Cancer Research Program, a grant from the American Institute for Cancer Research, an R01 from the NIH and a pending R21. The DOD grant is to assess the differential effects of canola oil versus corn oil on breast cancer development. We have reported that maternal consumption of canola oil instead of corn oil reduces the risk of breast cancer in mouse offspring. The American Institute for Cancer Research (AICR) project is to assess the effect of increased consumption of walnuts (high in omega 3 fatty acids and β -sitosterol) against growth of cancer. The RO1 project is to assess the effects of purified long chain omega 3 fatty acids on breast cancer development. Preliminary results, using a transgenic mouse model of mammary cancer, indicate that inclusion of long chain omega 3 fatty acids in the maternal diet can significantly reduce the lifetime risk of mammary gland cancer in the offspring. Even without maternal consumption of omega 3, consumption of omega 3 by the offspring after weaning also reduces the risk for mammary gland cancer. The pending R21 developmental project is a pilot clinical trial to determine whether omega 3 fatty acids can reduce elevated NF κ B in patients with chronic lymphocytic leukemia. Much work remains to determine the mechanisms of omega 3 fatty acids for protection from mammary gland cancer, to identify biomarkers that might be used in human cancer prevention trials and whether or not increased consumption of omega 3 fatty acids via dietary change or supplementation is a feasible cancer preventive strategy for humans.

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My long-term research interest is in understanding the etiology and mechanisms underlying type 2 diabetes and obesity, concomitantly related diseases. Type 2 diabetes is the most common form of human diabetes, accounting for over 90% of cases and affecting 250 million people worldwide. Obesity at such epidemic proportions creates serious public health problems. Both diseases, furthermore, are associated with chronic complications and cardiovascular disease, increasing morbidity as well as mortality.

There is substantial evidence demonstrating that genetic factors are strongly involved in the development of type 2 diabetes and obesity, and I have focused my attention on the link between gene dysfunction and these diseases.

Currently, I seek to understand the molecular basis of an obesity susceptibility gene, named *tabw2*, derived from the TALLYHO mouse model for polygenic type 2 diabetes and obesity. *Tabw2* gene appears to interact with high fat/ high sucrose diets to make mice overtly obese. In that respect it is an excellent model for human obesity, which most often results from interactions between genetic susceptibility and an obesigenic environment – *i.e.*, diets enriched in calories from fat and sugar. Therefore, understanding the molecular basis for diet-induced obesity in *tabw2* mutant mice may uncover new cellular regulatory pathways that can then be exploited in the control of human obesity.

Also, genetic and physiological characterizations of diabetes in TALLYHO mice are ongoing. It is well-recognized that the development of type 2 diabetes is acquired by a combination of insulin resistance in the target tissues and failure of insulin secretion from pancreatic beta-cells. Obesity is known to be a prominent cause of insulin resistance, but only 25-30% of obese individuals progress to type 2 diabetes. Recent emerging evidence indicates that insulin resistance in peripheral tissues alone is not sufficient to cause diabetes and demonstrates that defects in glucose sensing and/or the growth and survival of beta-cells per se go wrong in type 2 diabetes.

My continued research will include gene discovery, genetic resource development, and related biochemical and physiological studies associated with type 2 diabetes and obesity.

Emine C. Koc, Ph.D.

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Protein Synthesis in Mammalian Mitochondria

The role of mitochondria in aging, heart disease, diabetes, neurodegenerative disorders, obesity, and cancer is becoming more apparent due to their central role in energy metabolism. In mammals, mitochondria are responsible for providing over 90% of the energy in the form of ATP, which is generated by the process of oxidative phosphorylation (OXPHOS). Mitochondria have their own 16.5 kb circular genome and translation machinery/ribosomes essential for the synthesis of 13 essential proteins of the OXPHOS complexes. Mammalian mitochondrial ribosome (55S) is composed of ~80 mitochondrial ribosomal proteins (MRPs), about half of which have homologs in bacterial ribosomes. There is growing evidence suggesting the involvement of MRPs in various metabolic diseases, apoptosis and cancer. Clearly, changes in the expression of MRPs influence mitochondrial metabolism and alter the balance between apoptosis and tumor formation due to the changes in energy production.

Our multidisciplinary research takes advantage of biochemical, molecular and cell biological, and mass spectrometry-based proteomics technologies. Using this “systems biology” approach, we have paved the way to study mitochondrial protein synthesis by identifying components of the translation machinery/ribosomes in mammalian mitochondria. More recently, we revealed that covalent post-translational modifications of MRPs, mainly by reversible acetylation and phosphorylation, regulate protein synthesis and therefore OXPHOS dictated by mitochondrial acetyl-coA, NAD⁺ and ATP levels. Our current research interests are aimed at determining how components of mitochondrial translation/ribosomes affect OXPHOS and apoptosis in normal and disease conditions. As we learn more about the regulatory roles of MRPs and their post-translational modifications, new strategies will be devised to manipulate mitochondrial function/dysfunction in metabolic diseases, cancer, and aging.

Sample Publications:

1. Surovtseva, Y.V., T.E. Shutt, J. Cotney, H. Cimen, S.Y. Chen, **E.C. Koc**, and G.S. Shadel (2011). Mitochondrial Ribosomal Protein L12 selectively associates with human mitochondrial RNA polymerase to activate transcription. *Proc. Natl. Acad. Sci. USA* (epub ahead of print).
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Oxidative stress in obesity-associated hypertension:

The incidence of hypertension is nearly two times greater in obese individuals than in persons of normal weight. There is also a significant correlation between weight gain and the evolution of borderline high blood pressure into established hypertension. Arterial hypertension is usually associated with a number of cardiovascular risk factors grouped in the “insulin-resistance syndrome” or “syndrome X”. Patients with Syndrome X present high plasma insulin and triglyceride levels, low high-density lipoprotein (HDL) cholesterol, glucose intolerance, central obesity, and hemostatic and fibrolytic disturbances. The exact mechanisms for obesity’s effect on blood pressure are not known. Experimental and clinical evidence has linked an enhanced production of reactive oxygen species (ROS) to certain diseases of the cardiovascular system including hypertension. Experimental studies demonstrated that ROS, mainly through the production of superoxide anion, can cause important alterations in the cellular signal transduction systems leading to conditions favoring vasoconstriction.

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Pathophysiological mechanisms underlying the development of obesity-associated hypertension:

With the alarming rise in the incidence of obesity it is becoming increasingly important to develop suitable animal models to investigate mechanisms underlying the development of obesity-associated diseases such as hypertension (high blood pressure) and to serve as subjects for screening drugs which might hold the key for the treatment of these diseases. Using genetically obese Zucker rats and lean controls, we have demonstrated that obese rats are more sensitive to experimental conditions that induce hypertension than age- and gender-matched lean rats. Moreover, the magnitude of the hypertensive response is much greater in obese rats than in lean rats. We also have shown that blood pressure is elevated in obese rats relative to lean rats when both groups are fed a diet that is high in fat and salt content.

The primary focus of our current research is on the specific mechanisms through which diets high in fat and salt content can induce the development of hypertension in obese rats. One hypothesis that we are testing is that hypertension results because high fat diets alter the production of Nitric Oxide (NO) and/or the responsiveness of blood vessels to the actions of NO. NO is an important vasodilator that may prevent a rise in blood pressure in response to a number of conditions that would otherwise cause blood pressure to increase to hypertensive levels. We use whole animal studies to monitor physiological parameters that may be affected by the ingestion of a high fat, including blood pressure, energy intake and expenditure, circulating levels of specific hormones, and the excretion of substances that could affect blood pressure, such as NO, catecholamines and sodium. We use isolated blood vessels to compare the contractile responses of blood vessels from lean and obese rats to hormones and other substances known to affect blood pressure and blood vessel function. In addition, we examine the expression of specific genes for proteins that affect blood vessel function such as receptors for hormones that induce blood vessel contraction and the enzyme (NO synthase) that catalyzes NO production. The effect of diet on the level of systemic or tissue specific oxidative stress is currently being assessed by measuring plasma levels and urinary excretion of 8-epi-prostaglandin F₂α and changes in the nitrotyrosine content of specific tissue proteins respectively.

Because the kidney plays an important role in blood pressure regulation, additional studies are being conducted to evaluate kidney structure and function. Kidney function is assessed by measuring the activity of specific enzymes in kidney tissue and the concentration of specific metabolites in the urine. Upon completion of a given study, the expression of specific genes that affect kidney function are also evaluated. Kidney structure is assessed microscopically using kidney sections stained with dyes and labeled antibodies.

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Our laboratory is investigating the mechanism by which dietary constituents such as vitamin A, resveratrol (red wine), quercetin (apples), etc arrest growth of melanoma cells and prevent melanocytes from being converted to melanoma cells. Melanoma is rapidly increasing in incidence, especially in the under 40 year old population. In its early stage, it is curable by surgery, but once it spreads, it is notoriously resistant to treatment. If we can prevent its occurrence and/or progression in the population at high risk for melanoma through dietary or skin application of bioactive phytochemicals, this could lower the burden of melanoma morbidity and mortality.

A new area of investigation is production of hypoxia-inducible factor (HIF-1alpha) by melanoma cells under conditions of normal oxygen tension. HIF-1alpha is normally produced under hypoxic conditions and allows tumor cells to recruit a blood supply (angiogenesis) and shift their metabolism to produce energy under hypoxic conditions (glycolysis). The role of HIF-1alpha expression by melanoma cells in normal oxygen conditions may contribute to their malignant properties. We are also investigating the ability of selected phytochemicals and vitamin C (ascorbic acid) to decrease the expression of HIF-1alpha in human melanoma cells.

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The Norton Group is funded to perform fundamental studies in two related areas: design, fabrication and characterization of nanoscale structures for sensor applications, and the development of novel DNA Identification Technologies. Real time readout of molecular recognition and binding events is a common theme shared by these projects. Native and modified/engineered molecules are employed to develop biomimetic systems to address current, pressing problems in rapid detection. These projects are best suited for research students with an interest and ability to work in an exceptionally multidisciplinary environment.

1. **Nanoscale Structure Design, Fabrication and Characterization:** The long-term goal of these studies is to produce nanostructures capable of transducing molecular recognition events into optical or electronic signals in real time. The near term objective is to generate a research platform capable of placing sensing and reporting moieties with a resolution on the order of a chemical bond, 1 - 3 angstroms, composing true molecular reporter systems.

Techniques we employ in these studies include laser scanning confocal microscopy, cell culture, molecular cloning, photo- and molecular lithography, nanoparticle labeling, TEM, SEM and AFM microscopy, gel electrophoresis, and molecular modeling. These studies are performed in collaboration with researchers from across the nation and utilizing resources in the Molecular and Biological Imaging Center (MBIC) (www.marshall.edu/mbic/).

2. **Rapid DNA Signature Recognition:** Fourth generation sequencing technologies have the potential to read entire genomes in less than a minute. This is a project to test the potential speed and error rates of modified/engineered polymerases. The eventual goal is to develop an integrated system to detect specific DNA codes in clinical or environmental systems. An example application would be rapid, inexpensive detection of a specific human SNP (single nucleotide polymorphism), one of which is responsible for the majority of Human Hereditary Hemochromatosis in the US. Since approximately one in 8 to 12 Americans is a carrier of this mutation, it can lead to potentially fatal iron overload disease in approximately 0.5% of the population. Because it is treatable (if detected early), there should be an extreme push to develop methods of fast, non-invasive genetic testing for this common, but relatively under-diagnosed disorder. Many similar applications are emerging.

Web: www.vandaliaresearch.com/about.asp
www.parabon.com/nanolabs/
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Current Research:

1. Succinimide-induced nephrotoxicity: The succinimide ring is incorporated into hundreds of chemicals used as drugs, agricultural fungicides, and industrial agents. Toxicity to the kidney (nephrotoxicity) has been associated with exposure to succinimide antiepileptic agents and some agricultural fungicides. Recent work has determined that succinimide metabolites produced in the liver and transported via blood to the kidney are responsible for inducing the kidney damage. Studies have also shown that females are more sensitive than males to succinimide-induced nephrotoxicity, and the stereochemistry of the metabolites contributes to nephrotoxic potential (S-enantiomers more toxic than R-enantiomers). This project seeks to determine the exact nature of the toxic metabolites, sub-cellular renal targets of the metabolites, how metabolites gain entry into the kidney and the toxicogenomics of succinimide-induced nephrotoxicity.
2. Chloroanilines are commonly used chemical intermediates in the manufacture of dyes, drugs, agricultural herbicides and fungicides, and thousands of other products. Exposure to a chloroaniline can result in a number of toxicities including toxicity to the blood, spleen, liver and kidney. This project seeks to determine the chemical species (parent compound or metabolite) responsible for liver and kidney damage and the mechanism by which hepatotoxicity and nephrotoxicity occurs. Studies are also directed to understanding which enzyme systems (e.g. cytochrome P450s; flavin monooxygenases, prostaglandin H synthase, peroxidases, etc.) are responsible for bioactivation of chloroanilines and/or their metabolites (aminochlorophenols, chloronitrobenzenes) to hepatotoxic (liver) and/or nephrotoxic (kidney) chemical species.
3. Methadone is a drug used to reduce the dependence of heroin addicts on heroin. However, some methadone users die unexpectedly when using normal doses of methadone. Preliminary studies have suggested that there may be a defect in the inactivation of methadone in the liver in these individuals who die unexpectedly. The purpose of this study is to determine if genetic polymorphisms are responsible for these deaths. Studies are focused on single nucleotide polymorphisms (SNPs) in cytochrome P450 genes in the DNA of deceased methadone users, and where the gene products (CYP enzymes) are responsible for metabolizing methadone to inactive metabolites.

Website link: http://bms.marshall.edu/research_groups/toxicology/rankin.aspx

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The goal of my research is to understand the regulation of male germ cell differentiation and the uncontrolled growth of male germ cells in testicular tumors using cell and molecular biology techniques. Microarray data from my lab showed that male germ cells in the early stages of their differentiation to spermatozoa express high levels of the oncogenes Ski and Sno. Ski and Sno are members of a family of transcription factors that can either activate or repress expression of target genes. Recently, we have shown that Ski, and possibly Sno, expression may be altered in male germ cell tumors. Testicular cancer is a disease that affects young men in their reproductive years. Therefore, this work has important implications for both the control of male fertility and the treatment of testicular cancer. Future studies will characterize the role of SKI and SNO in male germ cells. Recently, funding for this project was obtained as part of the COBRE grant to Marshall University.

1. **Establish Ski and Sno expression patterns in testicular tumors and cell lines:**

Experiments designed for this project will examine Ski and Sno gene expression in germ cell tumors in a large sample population and determine whether expression levels correlate with tumor classification. Ski/Sno gene expression patterns will be determined using Real Time PCR on RNA extracted from tumor cells and normal seminiferous tubules obtained by laser capture microdissection microscopy, a state of the art technique for isolating cells from tissue sections. Immunostaining and western blotting will determine protein levels and localization. Cell lines from testicular tumors will be examined to determine their suitability as an experimental system for investigating the role of Ski/Sno in germ cell proliferation and differentiation.

2. **Establish the signaling pathway by which SKI and SNO promote male germ cell proliferation:**

Nothing is known about the pathway in which SKI and SNO acts in germ cells or testicular tumors. SKI and SNO can either activate or repress gene transcription as part of multi-protein regulatory complexes. Among the proteins that interact with SKI is the retinoic acid receptor. Retinoic acid (vitamin A) is an important signaling pathway in male germ cells as vitamin A deficiency causes sterility due to a block in germ cell proliferation. This Aim will focus on the protein interactions of SKI with the retinoid signaling pathway in male germ cells using a vitamin A deficient mouse model. Protein interactions will be determined by immunoprecipitation and western blotting.

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Endocrine disrupting chemicals (EDC) are compounds, natural or synthetic, which through mimicking or inhibiting natural hormone action, disrupt reproduction, development and homeostasis. Dioxins are particularly potent environmental pollutants that function as EDCs and are present in humans at levels that range from parts per trillion to parts per billion. While it is clear that dioxins are found in humans, and they harmful to wildlife species, their impact on human health is still a controversial subject. This uncertainty is due, at least in part, to too few human studies, and a need for a deeper mechanistic understanding of how exposure to pollutants – like dioxins – may impact human health. Consequently, as detailed below one of our interests are to study the mechanisms of EDC action within the ovary.

We, and others, have reported that fetal exposure to dioxins has a striking negative effect on adult female reproduction; however the mechanism of disruption is not clear. Consequently, one of our objectives is to adopt and optimize techniques that will purify ovarian follicles from mouse ovaries and recapitulate folliculogenesis in vitro to delineate the site of endocrine disruptor action within the ovary and to determine if this disruptive event is due to an epigenetic DNA modification, and alterations in hormone signaling and synthesis within ovarian follicles. Addressing these questions will provide greater insights into how fetal exposure to pollutants results in adult endocrine disruption.

A second direction in our laboratory is to explore the role of the Aryl Hydrocarbon Receptor (AHR) in mediating interactions between tumor cells and their microenvironment. The survival of tumor cells requires paracrine support from their surrounding microenvironment which consists of macrophages, fibroblasts, endothelial cells, adipocytes and extracellular matrix (ECM). The interactions between mammary adipocytes and cancerous epithelial cells are a particularly important stromal-cancer cell interaction in mammary cancer given the abundance of the pro-tumorigenic factors that are released from adipocytes. We have recently discovered that adipocyte-supported growth of breast cancer cells requires AHR signaling within breast cancer cells. Currently, our laboratory, in collaboration with Dr. Santanam, are testing whether adipocyte-secreted factors through activation of several transcription factors, and transcriptional programs, are reducing cellular levels of reactive oxidative species (ROS) within tumor cells, which enables them to evade death and proliferate. Addressing these new hypothesizes will provide new insights into the paracrine interactions between tumor cells and their microenvironment, which will lead to new therapeutic strategies for the treatment of cancer.

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Cardiovascular Disease, Obesity and Diabetes

1. **Novel biomarkers in the blood, fat tissue and arteries:** One of the major projects in our laboratory is to identify unique biomarkers that play a role in obesity, diabetes or coronary artery disease. In particular we are interested in identifying and studying the role of microRNAs (small nucleotides that regulate gene and protein expression) in these diseases. We use either animal models of obesity, diabetes or cardiovascular disease or obtain samples from patients with these diseases (Collaboration with physicians from Dept. Cardiology, Endocrinology & Thoracic Surgery) to look for these biomarkers.
2. **Diet, Exercise and Cardiovascular disease:** A long time interest of our laboratory is to study the effect of diet and exercise on cardiovascular disease, in particular “atherosclerosis” (blockage of the arteries). We have mouse models that express high levels of antioxidant enzymes (enzymes that decrease oxidative stress) with an obese background. In this project we propose to use these mice to study the effect of dietary fat or exercise on cardiovascular risk.
3. **Sex differences in epigenetic markers in aging fat tissue:** Our laboratory is interested in studying sex differences in risk to cardiovascular disease. In this project we utilize tissues from aging rats (both male and female rats) to study the differences in epigenetic markers that regulate fat tissue function.

Women’s Health

4. **Oxidative stress, Pain and Endometriosis:** Endometriosis is a disease that affects 10-15% younger women. This disease is mostly accompanied by infertility and chronic pain. Endometriosis is also a risk for ovarian cancer. We have long standing interest in studying the etiology of this disease by using both animal models of endometriosis and samples from patients with endometriosis (collaboration with Department of Obstetrics & Gynecology). We are currently studying unique pain specific microRNAs that play a role in this disease.

OTHER COLLABORATIVE PROJECTS:

1. Epigenetic biomarkers in Type 1 and 2 Diabetes and endothelial function: with Dr. Richard Egleton
2. Aryl hydrocarbon receptor and its role in adipose and vasculature interactions: with Dr. Travis Salisbury
3. Nicotinic receptor signaling in Atherogenesis: with Dr. Piyali Dasgupta

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Ambient temperature and physical activity have a surprising impact on bone length, but it is unclear how such common variables regulate growth of the postnatal skeleton. We study environmental inputs on bone elongation in growth plates, the regions of cartilage where bone lengthening occurs. Our long-term goal is to identify the physiological mechanisms underlying temperature- and exercise- enhanced bone elongation in the growth plate, with the intent of finding new ways to potentially treat growth impediments in children. We employ whole animal and bone culture models to test specific hypotheses about environmental effects on the growth plate matrix, its vasculature, and nutrient supply. We use a variety of tools such as live animal imaging; histology and immunostaining; fluorochrome bone labeling; micro-CT analysis; and fluorescent microsphere blood flow assays. Our research is important for an evolutionary understanding of limb length variation among mammals in different environments and may aid in developing more effective treatments for childhood growth disorders.

Laboratory Projects**Imaging skeletal growth plates using *in vivo* multiphoton microscopy**

Multiphoton microscopy is an emerging technology for live animal imaging that offers exciting possibilities for the study of growth plate dynamics *in vivo*. We have worked for the past year with collaborators at Cornell University to establish a platform for imaging intact skeletal growth plates. We are now using this method to assess how systemic regulators arrive at and move within the cartilage matrix of the growth plate under various experimental conditions. This system provides a new mechanism for understanding the physiological regulation of bone growth through the ability to dynamically measure changes in solute delivery to the growth plate of a living animal.

Determining how temperature alters cartilage growth *in vitro*

The objective of this study is to determine how temperature modulates bone elongation using an established bone culture model. We grow intact metatarsal bones from neonatal mice *in vitro* to isolate local effects of temperature on the growth plate, since this system is largely free of systemic inputs. Analysis by multiphoton microscopy enables visualization of intact growth plate cartilage, which can be manipulated and assessed in real time. Cell morphology and protein expression are evaluated using standard histology and immunohistochemistry on fixed preps and whole mount samples.

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About five hundred billion blood cells need to be replaced in the human body each day. This feat is accomplished by the exponential amplification of cells from a pluripotent precursor cell known as the hematopoietic stem cell (HSC). The HSC does not generate the required 5×10^{11} cells directly on a daily basis, but instead generates highly prolific progenitor cells that produce the nine major hematopoietic cell types. Defining the regulation of these progenitor cells is important to our understanding of stem cells, leukemias, and autoimmune diseases. My laboratory investigates these progenitor cells using the mouse as a model system in an effort to better understand the maturation of progenitor cells in bone marrow. This will lead to new chemotherapeutic strategies for leukemia. The following projects are available in my laboratory related to this area of research:

- 1. Examination of the link between lipid metabolism, bone reformation, and leukemia:** We have recently completed a lengthy genetic screen to identify factors that are important to controlling myeloid progenitor cell frequencies in bone marrow with the hypothesis that these genes would be pertinent to leukemia chemotherapy. Not only was our hypothesis validated by the genes identified in the study, but we identified two pathways that were also highly enriched in this screen – lipid metabolism and bone reformation. We plan on examining these pathways to determine if genetic differences in these pathways can impact leukemia predisposition and be used in risk analysis for patients.
- 2. Determination of the frequency of progenitor cells in bone marrow:** Our previous studies support the following in an in vivo context: (A) mice fed diets containing moderate levels of n-3 FAs produce a more mature myeloid progenitor cell compartment relative to those fed diets containing omega-6 (n-6) FAs and (B) there is a reduction in the number of progenitor cells present in mice fed a n-3 FA diet compared to a n-6 FA diet. Many nutritional factors that inhibit cancers have been associated with epigenetic changes, such as posttranslational histone modifications and DNA methylation. Thus, the effect of n-3 FAs on differentiation suggests an epigenetic mechanism. Since acute myelogenous leukemia (AML) is characterized by progressive inhibition of differentiation, we hypothesize that n-3 FA can increase differentiation of bone marrow progenitor cells through epigenetic mechanisms and that this effect is applicable to slowing the progression of AML.

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Projects currently ongoing in my lab:

1. **The effect of herbal agents on susceptibility to toxins:** Billions of dollars are spent in the United States each year by individuals on nutritional and herbal supplements. We are investigating the mechanism for reduction of hepatic acetaminophen toxicity by S-adenosyl-L-methionine (SAME). This project is important since acetaminophen is the number one cause of drug induced liver failure in the U.S. This project is evaluating the cellular effects of SAME that are responsible for attenuating acetaminophen toxicity. We are also comparing SAME to the currently used clinical treatment for acetaminophen to evaluate SAME's effectiveness.
2. **Evaluating methods to reduce the side effects of cancer chemotherapeutic drugs:** This project is evaluating the protective effect of another natural product on cisplatin nephrotoxicity. Cisplatin is a widely used cancer chemotherapeutic agent. Unfortunately, the two principal side effects of cisplatin treatment are renal and peripheral nerve dysfunction. We have found an agent that can reduce the renal toxicity in isolated renal tissue. We are evaluating whether resveratrol impacts cell signaling or alters oxidative stress as part of its protective effect against cisplatin.
3. **Examination of the mechanism for kidney damage by a metabolite of acetaminophen (Tylenol):** Acetaminophen is converted by enzymes in the liver to several different substances. One of these agents is toxic to the kidney. We are investigating the role of oxidative stress in induction of 4-aminophenol renal toxicity. Further studies are also evaluating possible agents to reduce renal toxicity. A second component of this project is to test the hypothesis that part of the mechanism for 4-aminophenol toxicity is mediated by protein modification. The proteins modified by 4-aminophenol, the sub cellular location of these proteins and the impact on cell function are being assessed to evaluate the mechanism of toxicity.
4. **Susceptibility to oxidative stress in diabetes:** Over 1 in 50 Americans are diagnosed with diabetes mellitus. Diabetes is associated with a higher risk of kidney disease and renal failure. The predisposition to renal dysfunction may involve a defect in the ability to protect the kidney from damage by free radicals. A student would examine: a) the predisposition to renal damage by renal toxicants that are known to induce free radical damage; b) investigate the cellular and subcellular abnormalities induced by diabetes; c) initiate studies of possible interventions to slow the progression of renal dysfunction.

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Iron is a nutrient required for several functions crucial to life. As such, iron levels may be manipulated to inhibit cell growth, restrict cell passage through the cell cycle, or even stimulate apoptosis. In addition to its requirement for cell growth, iron is a key mediator of oxidant stress. Iron donates electrons for the generation of the superoxide radical, and serves as both an electron donor and acceptor in the iron catalyzed Haber-Weiss reaction (Fenton chemistry) which generates hydroxyl radicals, and can also lead to the formation of ferryl radicals.¹ Oxidant stress derived from iron can drive processes important to carcinogenesis, such as damage to DNA, mutagenesis, and stimulation of proliferation and inflammation.

Ferritin is the key cellular iron storage protein. Iron captured within ferritin does not generate the reactive radicals that it might otherwise form. We have developed a conditional transgenic mouse model where the generation of transgenic ferritin is tissue specific and temporally regulated. Our data demonstrate that transgenic induction of ferritin yields a phenotype of iron scarcity. Using this model, wherein we can manipulate iron at the cellular level, we hypothesize that we can impact the production of oxidant stress with tissue and temporal specificity.

The long term goal of our laboratory is to apply iron biology models to understand the role of oxidant stress in the etiology of chronic diseases, such as aging, atherosclerosis, cancer, kidney failure, and neurodegeneration. **Current projects include:**

1. elucidating the role of oxidant stress and inflammation in lung carcinogenesis
2. evaluation of the impact of iron sequestration in renal models of ischemia and acute failure
3. determining the role of the liver labile iron pool in systemic iron regulation,
4. unraveling the mechanisms that regulate cellular and systemic ferritin trafficking respectively *in vitro* and *in vivo*.

Tissue culture models as well as mouse models will be used, thus you will be trained to work *in vitro* and *in vivo*. Techniques we employ include evaluation of gene expression by real-time PCR, northern and western blotting, genotyping using PCR and real-time PCR, biochemical assays to measure phase II enzyme activities, oxidant stress measurements (glutathione, protein carbonyl, 4-HNE and TBARs as endpoints), and iron evaluation (hematocrit, hemoglobin, total iron, non-heme iron). This laboratory uses team approaches to accomplish its goals, and thus I value individuals who can work together. Remember – the well executed experiment is all that stands between you and your bliss!

¹ Henle, E.S., and Linn, S. Formation, prevention, and repair of DNA damage by iron/hydrogen peroxide. J Biol. Chem. 272:19095-98, 1997.

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Dr. Yu's laboratory focuses on biofilm genetics, innate immunity and antibiotic resistance in bacteria. We use the techniques of genetics and genomics to analyze how bacteria grow biofilms, and what bacteria use to invade a host causing pneumonia and how to combat the antibiotic resistance in bacteria.

1. **Genetics of Biofilms:** Bacteria in nature often grow as aggregating colonies attached to a surface known as a biofilm. Biofilm formation is a leading cause of chronic disease. To grow a biofilm, bacteria need to produce polysaccharides resulting in the formation of slimes. We are studying how bacteria know when to start making slimes. The model microorganism is a ubiquitous biofilm-forming bacterium called *Pseudomonas aeruginosa*. Chronic infections with biofilm *P. aeruginosa* are the major cause of morbidity and mortality in patients with cystic fibrosis (CF). By examining the molecular switch that controls the transition between biofilm and non-biofilm formation, we want to understand how bacteria make biofilms, thus to control biofilms.
2. **Resistance to Pneumonia:** At any given moment, we breathe in bacteria into our lungs. However, few of us will develop pneumonia. This is because we have a robust defense system to fight off the invading bacteria. The main defenses in the lungs include resident macrophages (big eaters), white blood cells and small proteins with potent antimicrobial activities. We have developed various models in the laboratory to study how these cells eliminate the bacteria, how the host realizes the incoming bacteria by producing a battery of small molecules in order to recruit the white blood cells to the lungs, how to evaluate and boost the activities of novel antimicrobials in pneumonia models. By understanding the innate lung defense mechanisms, we hope to develop model systems that could be used to evaluate novel therapeutics to fight against the bronchopneumonia in patients with CF
3. **Combating antibiotic resistance:** Methicillin-resistant *Staphylococcus aureus* (MRSA) and tobramycin-resistant *P. aeruginosa* (TRPA) are two infectious agents that cause a significant morbidity and mortality in immuno-compromised individuals. There is an urgent need to develop new antibiotics to combat these pathogens. To develop such a therapeutic, we are testing a series of rationally designed peptides with potent activity against bacterial pathogens. We also screen for more of these antimicrobial peptides (AMPs) for candidates with a broad spectrum of anti-MRSA and -TRPA activities. We hope to identify the novel AMPs with increased efficacy and reduced toxicity. We also want to use genetic engineering to modify and improve the natural enemy of bacteria, bacteriophage as a means to circumvent antibiotic resistance. The goal of this research is to develop translational options to fight against the emerging antibiotic resistance.

http://www.bms.marshall.edu/research_groups/pathogenesis_and_aging/yu.aspx

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Current Research

The majority of CD4 T cells are conventional CD4 T (Tcon) cells. They can differentiate into 3 subsets of T helper (Th) cells, the Th1, Th2 and Th17 cells. The Th cells are responsible for immunities against infections, therefore are essential for the survival of the host in a nonsterile environment. However, when Th cells are directed against self-antigens and allergens, they cause autoimmune and allergic diseases, such as multiple sclerosis, type 1 diabetes, rheumatoid arthritis, inflammatory bowel diseases and asthma. To keep the “bad” CD4 Tcon cells in check, the immune system has evolved to have a small “police army” of regulatory CD4 T cells or Treg cells to suppress the harmful Tcon cells. The goal of our research is to understand the regulation of the functions of both CD4 Tcon and Treg cells.

1. **Mechanisms for Treg cell-mediated immune suppression:** Despite their importance, it remains a mystery how Treg cells suppress Tcon cells. We believe the key to solving this mystery is a transcription factor named Foxp3. Foxp3 is both necessary and sufficient for Treg cell functions. Therefore, we assume that is the products encoded by the target genes of Foxp3 that mediate the suppression of CD4 Tcon cells. We have been using a variety of technologies, including microarray, ChIP-on-chip and genetic screening to identify the Foxp3 target genes. Once identified, we will determine which of them are important for Treg cell functions.
2. **Cell lineage plasticity and inter-convertibility of Treg and Th cells:** We have recently found that pathogenic Th cells that cause autoimmune and allergic diseases can gain Treg-like immune suppressive function when they were forced to express Foxp3. Conversely, Treg cells can differentiate into Th1 cells, but not Th2 and Th17 cells. These findings showed certain degrees of plasticity between the CD4 Tcon and Treg cell lineages. We are now investigating the mechanisms responsible for the lack of Th2 and Th17 differentiation from Treg cells. In addition, we are exploring the possibility to convert pathogenic Th cells to Treg-like cells for antigen-specific therapy of autoimmune and allergic diseases.
3. **Differentiation of CD4 Tcon into T helper (Th) cell subsets:** The differentiation of all 3 Th cell subsets is driven by a single transcription factor expressed specifically in one subset but not the others. In the past, we identified GATA-3 as the master regulator of Th2 cell differentiation and Hlx as a key regulator of Th1 differentiation. Our current efforts are directed to understanding how the Th subset-specific transcription factors recruit other regulatory proteins to form functional complexes, and how such complex interact with DNA in chromatin to regulate gene expression in the Th cells.

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One of our research interests is to study molecular mechanism of mammalian fertilization. In particular, we focus on epigenetic regulation of gametogenesis and early embryogenesis. Successful completion of this research could contribute to new approaches for human fertility control. We also investigate the Wnt/catenin signaling pathway in human cancers. This research potentially leads to new means for detection, prevention and treatment of cancer. We use a broad spectrum of approaches in our research, such as gene knockout, DNA microarray, RNA interference, cell culture, microscopic imaging, among other traditional techniques in molecular and cellular biology.

- 1. Molecular mechanism of gametogenesis:** Mammalian gametogenesis is a highly ordered, precisely orchestrated developmental process in which germ cells undergo self-renewal, apoptosis, proliferation and differentiation. The final products of this process are functional gamete cells, sperm and eggs. **Project 1A:** A role of NPC-1 in gametogenesis (in collaboration with Dr. Laura Richardson) NPC-1 acts in intracellular cholesterol traffic. Loss of NPC-1 function in human causes Niemann-Pick C disease, a disorder characterized by abnormal lipid storage in various tissues, reduced sexual hormone level, and neurodegeneration. In the mouse model, we found that gametogenesis is defective in mice lacking functional NPC-1 protein. Further investigation for this phenotype is on the way. **Project 1B:** A novel Patched homolog gene Patched-X in spermatogenesis: We found that an unidentified Patched (Ptc) homolog gene, tentatively named as Patched-X, is specifically expressed in male germ cells. Ptc is a receptor for hedgehog (Hh), whose signaling pathway plays a critical role in embryonic pattern formation and adult tissue homeostasis. We are going to test the hypothesis that Patched-X is required for the development of male germ cells by gene knockout technology.
- 2. Molecular mechanism of sperm-egg membrane binding and fusion:** At a critical moment of mammalian fertilization, the plasma membranes of sperm and an egg bind and fuse together to form a one-cell zygote to initiate the development of a new organism. The molecular events involving in the regulation of this fascinating process are elusive.
Project 2A: Regulation of gamete membrane interaction by egg PKC: PKC is a family of threonine/serine protein kinases participating in a wide spectrum of cellular activities. We will test the hypothesis that egg PKC plays a regulatory role in sperm- egg membrane interaction through maintaining the integrity of molecular and morphological topology of the egg plasma membrane.
- 3. G-protein coupled receptor 56 in cancer** (in collaboration with Dr. Maiyon Park) Dysregulation of G-protein coupled receptors (GPCRs) in human cancers has been widely recognized. GPR56 is a novel, orphan GPCR. Our result shows that the transcription of GPR56 gene is significantly decreased in T lymphocytes from patients with chronic myelogenous leukemia or non-Hodgkin's lymphoma. We are currently characterizing GPR56 and its related signaling pathway.

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Projects in control of posture and locomotion:

Our lab focuses upon understanding how the nervous system generates behaviors. We center our work on the control of standing and walking. Rather than attempting to unravel the molecular structure of the nervous system, we are interested in how nerve cells are connected to control movements. We also study the structure of limbs to understand how they are adapted to their functions in standing and walking. We investigate these problems in insects as model systems and use techniques of neurophysiology, high-speed video and anatomy. The major implication of our work is in neurobiology but we also interact with labs that are building walking robots and prosthetic devices. Our funding is through a grant from the Division of Integrative Organismal Biology of the National Science Foundation (NSF).

The current projects in the lab include:

1. Understanding the structure and function of structures containing the elastic protein resilin; this compound is nearly a pure 'rubber' and can form ligaments that act like springs.
2. Understanding the sensory and motor mechanisms underlying support of body weight; we are using small magnets and an electric coil to change the body weight while we record from leg sense organs and muscles. It is very entertaining and we have funds to support student work.