



November 30 - December 1, 2005

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### **UFGI Strategic Plan**

The discovery of the three-dimensional double helix architecture of DNA in 1953 was not only a defining moment for biology, but arguably one of the most significant scientific discoveries of all time. It fundamentally and permanently changed the course of biology and genetics. The unraveling of DNA's structure, combined with its elegant mechanism for self-replication and the existence of a universal genetic code for all living beings, have together provided the basis for the understanding of fundamental cellular processes, mutation and genetic repair, genetic variation, the origin of life and evolution of species, and the structure/function/regulation of genes. The double helix is also proving to be of immense significance to advances in agriculture, medicine and such other diverse fields as anthropology, criminology, computer science, engineering, immunology, nanotechnology, etc. It was the study of DNA that led to the development of tools that brought about the biotechnology revolution, the cloning of genes, and the sequencing of entire genomes. Yet, most knowledgeable people agree that what has been achieved in DNA science thus far is only the beginning. Bigger and better applications, which will impact directly on the quality of human life and sustainability of life on earth, are yet to come. In order to attain these objectives, the digital nature of DNA and its complementarity are beginning to be exploited for the development of biology as an information-based science. Indeed, a paradigm shift is already taking place in our view of biology, in which the natural, physical, engineering and environmental sciences are becoming unified into a grand alliance for systems biology. Indeed, biology in the 21<sup>st</sup> century will be surely dominated by this expanded vision. The Genetics Institute is committed to fostering excellence in teaching and research, and in promoting cross-campus interdisciplinary interactions and collaborations. In the pursuit of these objectives, it offers a graduate program in genetics, and has identified the following four key areas for teaching, research and development: Bioinformatics, Comparative Genomics, Population and Statistical Genetics, and Epigenetics.

## **FLORIDA GENETICS 2005 Schedule**

### **Wednesday, November 30, 2005**

1:00-6:00 pm: Check-in - Grand Ballroom registration desk, 2nd floor J. Wayne Reitz Union

2:30-2:45 pm:

Opening Remarks - Indra K. Vasil, Ph.D. and Kenneth I. Berns, M.D., Ph.D.

Session I: Kenneth I. Berns, M.D., Ph.D., Session Chair

2:45-3:30 pm:

Gary Felsenfeld, Ph.D., Chief

Physical Chemistry Section and Laboratory of Molecular Biology, NIDDK, National Institutes of Health

***Chromatin boundaries and the regulation of gene expression***

3:30-4:15 pm:

Eric J. Richards, Ph.D., Professor

Department of Biology, Washington University

***Epigenetic variation and inheritance***

4:15-6:00 pm: Poster Session and Reception

### **Thursday, December 1, 2005**

8:00 am-3:00 pm: Check-in - Grand Ballroom registration desk, 2nd floor J. Wayne Reitz Union

8:30-9:00 am: Continental Breakfast

Session II: Thomas P. Yang, Ph.D., Session Chair

9:00-9:45 am:

Karolin Luger, Ph.D., Associate Professor

Department of Biochemistry and Molecular Biology, Colorado State University and Howard Hughes Medical Institute

***Structure and dynamics of nucleosomes and nucleosome assembly factors***

9:45-10:15 am:

Robert A. Waterland, Ph.D., Assistant Professor

Department of Pediatrics, Baylor College of Medicine

***Early nutrition and your epigenomes***

10:15-10:30 am: Coffee Break

10:30-11:00 am:

Keith D. Robertson, Ph.D., Assistant Professor

Department of Biochemistry and Molecular Biology, University of Florida

***Novel targets of DNA methylation in brain tumors***

11:00-11:30 am:

Rongling Wu, Ph.D., Associate Professor  
Department of Statistics, University of Florida

***Statistical models for mapping imprinted quantitative trait loci***

11:30-noon:

Jorg Bungert, Ph.D., Assistant Professor  
Department of Biochemistry and Molecular Biology, University of Florida

***Regulation of the  $\beta$ -globin gene locus during development and differentiation***

Noon-2:00 pm: Poster Session and Lunch

Session III: Robert Ferl, Ph.D., Session Chair

2:00-2:30 pm:

David C. Bloom, Ph.D., Associate Professor  
Department of Molecular Genetics and Microbiology, University of Florida

***Role of epigenetics and chromatin insulators in regulating Herpes Simplex Virus type 1 latent gene expression***

2:30-3:00 pm:

Thomas P. Yang, Ph.D., Professor  
Department of Biochemistry and Molecular Biology, University of Florida

***Regulation of imprinted gene expression in the Angelman/Prader-Willi syndrome region***

3:00-3:30 pm:

Karen Moore, Ph.D., Assistant Professor  
Department of Animal Sciences, University of Florida

***Altering chromatin remodeling in cloned bovine embryos***

3:30-3:45 pm: Coffee Break

3:45-4:15 pm:

Jianrong Lu, Ph.D., Assistant Professor  
Department of Biochemistry and Molecular Biology, University of Florida

***Regulation of E2F by chromatin factors with tumor suppressor activity***

4:15-4:45 pm:

Naohiro Terada, M.D., Ph.D., Associate Professor  
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida

***DNA methylation in pluripotent embryonic stem cells***

## **Presentation Abstracts**

### **Chromatin boundaries and the regulation of gene expression**

Felsenfeld G

Physical Chemistry Section and Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD

The presence within the genome of regions of active chromatin in close proximity to regions of condensed 'heterochromatin' raises the question of how these two kinds of domains can be kept from interacting. Similar questions arise when two independently related gene systems are close enough to one another to result in 'cross-talk' between regulatory elements in the two systems. Insulators are DNA sequence elements that are capable either of blocking such cross-talk ('enhancer-blocking insulators') or of preventing the spread of heterochromatin ('barriers'). The two activities are distinct and separable. Enhancer-blocking insulation in vertebrates involves the DNA binding protein CTCF; recent evidence suggests that it may function through a loop-formation mechanism that interferes with enhancer-promoter interaction. The second kind of insulator activity, (the establishment of a barrier to heterochromatin) appears likely to involve interference with mechanisms in which histone modifications are propagated over chromatin domains, a key element of epigenetic effects. Our recent studies have identified a variety of protein factors that interact either directly or indirectly with boundary elements, and that result in local epigenetic changes, including modifications of histones and methylation of DNA, in a manner consistent with such mechanisms. In addition to the evidence this provides concerning the function of boundaries, it also leads to novel insights about the series of modifications that accompany gene activation.

### **Biography of Gary Felsenfeld, Ph.D.**

Gary Felsenfeld received his Ph.D. in Physical Chemistry from the California Institute of Technology, and then completed a National Science Foundation post-doctoral fellowship at Oxford University. During his long and distinguished career, Dr. Felsenfeld has had appointments with the National Institute of Mental Health, the University of Pittsburgh, Harvard University and Cornell University. In 1961 he became Chief of the Section on Physical Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, and in 1997, was named Chief of the Laboratory of Molecular Biology at NIDDK. He continues in both positions today. Dr. Felsenfeld was elected to membership in the National Academy of Sciences in 1976, and has served as Chair of both the Biochemistry Section and the Biology Section of the NAS. He is a Fellow of the American Association for the Advancement of Science, and of the American Academy of Arts and Sciences.

## **Epigenetic variation and inheritance**

Richards EJ

Department of Biology, Washington University, St Louis, MO

Our group has been studying the regulation and function of cytosine methylation by combining different genetic approaches in *Arabidopsis*. One arm of this analysis has been characterization of mutations isolated by forward genetic screens for altered DNA methylation. We have identified two loci important for maintenance of cytosine methylation: DDM1, which encodes a SWI2/SNF2-class nucleosome remodeling factor, and the Dnmt1-class cytosine-DNA-methyltransferase gene, MET1. Although the morphology of *Arabidopsis* mutants with reduced cytosine methylation is similar to wild-type, propagation of mutant lines by self-pollination is accompanied by the onset of stochastic developmental abnormalities. These abnormalities are caused by the accumulation of inherited lesions, some of which are epigenetic. More recently, we have begun exploiting natural variation in the different *Arabidopsis* strains to examine the prevalence, stability, and inheritance of epigenetic variation in cytosine methylation.

### **Biography of Eric J. Richards, Ph.D.**

After receiving his undergraduate degree in Molecular Biology and Anthropology from Vanderbilt University, Eric Richards joined Fred Ausubel's laboratory in the Department of Molecular Biology at Massachusetts General Hospital (Department of Genetics, Harvard Medical School). His graduate thesis focused on the isolation and characterization of telomeres from the flowering plant, *Arabidopsis*. After completing his degree, Dr. Richards moved to Cold Spring Harbor Laboratory as a Cold Spring Harbor Fellow, and he was later appointed as a Staff Member. In 1992, he joined the faculty of Washington University, where he is currently a Professor of Biology.

## **Structure and dynamics of nucleosomes and nucleosome assembly factors**

Park Y-J, Chodaparambil J, Luger K

Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biology,  
Colorado State University, Fort Collins, CO

It is widely recognized that the packaging of genomic DNA, together with core histones, linker histones, and other functional proteins into chromatin profoundly influences nuclear processes such as transcription, replication, DNA repair, and recombination. How chromatin structure modulates the expression of knowledge encoded in eukaryotic genomes, and how these processes take place within the context of a highly complex and compacted genomic chromatin environment remains a major unresolved question in biology. The basic repeating unit of chromatin, the nucleosome, has evolved to promote chromatin fluidity by a variety of mechanisms, for example transient exposure of nucleosomal DNA, and nucleosome sliding. Both phenomena are aided by histone chaperones. Nucleosome assembly protein 1 (NAP-1) is a key component in the modulation of chromatin structure. It shuttles histones into the nucleus, assembles nucleosomes, and promotes chromatin fluidity by removal and replacement of histone H2A-H2B dimers, thereby affecting the transcription of many genes. The 3.0 Å crystal structure of yeast NAP-1 reveals a homodimer with a novel fold. A long  $\alpha$ -helix is responsible for dimerization via a novel antiparallel non-coiled coil, and an  $\alpha/\beta$  domain is implicated in protein - protein interactions. The four-stranded anti-parallel  $\beta$ -sheet that characterizes the  $\alpha/\beta$  domain is found in all histone chaperones, despite absence of homology in sequence, structural context, or quaternary structure. This is the first structure of a member of the large NAP family of proteins, and suggests a mechanism by which histones are bound, and by which the shuttling of histones to and from the nucleus is regulated. Biochemical and biophysical experiments further suggest additional roles of NAP-1 in 'scavenging' misassembled chromatin.

### **Biography of Karolin Luger, Ph.D.**

Karolin Luger received her Ph.D. in 1989 from the University of Basel, studying protein folding. She completed a four-year post-doctoral fellowship at the Swiss Federal Institute of Technology in Zurich (ETHZ). She served as research assistant professor for four years at ETHZ, investigating nucleosome structure. From 1999-2003, she served as Assistant Professor at Colorado State University, and was promoted to Associate Professor in 2003. She was named a Monfort Professor in 2004, and a Howard Hughes Medical Investigator in 2005. The long-term goal of her laboratory's research is to investigate the structural properties of the nucleosome and of chromatin higher order structures, and to understand in molecular detail the fundamental question of how transcription, replication, recombination, and repair take place within the context of highly compacted chromatin. Dr. Luger's laboratory is particularly interested in mechanistic and structural aspects of these fundamental questions. They are using multi-pronged approaches including (but not limited to) x-ray crystallography, fluorescence resonance energy transfer, analytical ultracentrifugation, as well as conventional biochemistry and molecular biology. Their efforts are complemented in a synergistic manner by several ongoing collaborations with chemists and cell biologists.

## **Early nutrition and your epigenomes**

Waterland RA

Baylor College of Medicine and USDA Children's Nutrition Research Center, Houston, TX

Epigenetics, literally meaning "above genetics", describes the study of various mitotically heritable gene regulatory mechanisms that are layered on top of the DNA sequence information. Just as genetic variation contributes to individual susceptibility to chronic disease, it is increasingly evident that so too does individual epigenetic variation. We know very little, however, about the factors that contribute to interindividual variation in epigenotype. It is critically important to determine if nutrition and other environmental influences during development affect the establishment of human epigenotype.

Epigenetic mechanisms and fundamental concepts of developmental epigenetics will be discussed. Attention will be focused on the epigenetic mechanism of CpG methylation, in that nutrition plays a critical role in providing the methyl donors necessary for this covalent modification of DNA. Recent data demonstrating early nutritional and other environmental influences on the development of locus-specific CpG methylation and associated phenotypes will be reviewed. Future research in environmental epigenomics will aim to identify 1) the environmental factors (nutritional, toxicological, etc.) with the greatest impact on epigenetic mechanisms, 2) the specific regions in the human genome that are epigenetically labile to such influences, 3) the life stages during which human epigenomes are most susceptible to environmental perturbation, and 4) systemic vs. tissue-specific epigenetic modifications. Sophisticated epigenomic approaches will be employed to enable the simultaneous quantitation of epigenotype at many loci throughout the genome.

### **Biography of Robert A. Waterland, Ph.D.**

Dr. Robert Waterland is an Assistant Professor at the USDA Children's Nutrition Research Center at the Baylor College of Medicine in Houston, Texas. Dr. Waterland has dual faculty appointments in the Department of Pediatrics/Nutrition and the Department of Molecular and Human Genetics at Baylor.

Dr. Waterland received his B.S. degree in Physics from the Virginia Polytechnic Institute and State University. After earning his Ph.D. degree in Human Nutrition from Cornell University, he conducted postdoctoral research in developmental genetics at Duke University Medical Center, first as a Dannon Fellow in Interdisciplinary Nutrition Science, then as an NIDDK postdoctoral fellow.

Dr. Waterland's research focuses on understanding how nutrition during critical periods of prenatal and early postnatal development affects gene expression, metabolism, and chronic disease susceptibility in adulthood. Specifically, he is working to elucidate the mechanisms by which early nutrition influences the establishment and maintenance of epigenetic gene regulatory mechanisms. He is a member of the American Society for Nutritional Sciences, the American Society for Clinical Nutrition, the American Society for Biochemistry and Molecular Biology, and the International Society for the Developmental Origins of Health and Disease. Dr. Waterland's research program is supported by funding from NIH-NIDDK, USDA, and The March of Dimes Birth Defects Foundation.

**\* = U.F. Genetics Institute Faculty**

### **Novel targets of DNA methylation in brain tumors**

Robertson KD<sup>1,\*</sup>, Kim T-Y<sup>1,2</sup>, Zhong S<sup>1</sup>

<sup>1</sup>University of Florida Shands Cancer Center and Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Seoul National University College of Medicine, Seoul, Korea

Malignant gliomas are among the most devastating forms of cancer, often producing severe and progressive disability and death in many cases within one year. They are difficult to diagnose and treat. While they rarely metastasize, they do invade normal brain tissue and also exhibit a striking resistance to current therapies. The incidence of glioma peaks in children and in the 50-60 age range therefore this tumor is also a major cause of mortality in children. The standard therapy usually consists of cytoreductive surgery followed by radiotherapy, however complete resection is usually impossible due to the infiltrating nature of the tumor and resection can lead to severe neurological damage. Recent advances have revealed various genetic alterations of oncogenes and tumor suppressor genes in brain tumors, such as over-expression of the EGFR. Further identification of molecular targets, both genetic and epigenetic, would enable the prevention, early diagnosis, and tailored treatment of brain tumors to be approached at the molecular level. Epigenetic alterations, such as DNA methylation-mediated silencing of tumor suppressor genes, are particularly attractive because they are reversible with currently available inhibitors. We have used a combined approach of pharmacologic inhibition of DNA methylation and histone deacetylation and expression microarrays to identify new targets of epigenetic silencing using astrocytoma/high grade glioma cell lines as a model system. Based on our microarray results we identified several novel targets of aberrant promoter DNA methylation. Using methylation-specific PCR and bisulfite genomic sequencing we demonstrate that these genes are methylated at high frequency in primary tumors. Furthermore, functional assays indicate that these genes possess growth-suppressive properties. Our studies represent the basis for future work aimed at determining the utility of DNA methylation of these genes as biomarkers as well as revealing novel growth regulatory pathways that are inactivated in brain cancers.

### **Statistical models for mapping imprinted quantitative trait loci**

Liu T, Wu R\*

Department of Statistics, University of Florida, Gainesville, FL

The role of imprinting in shaping development has been ubiquitously investigated in plants, animals and humans. However, a statistical method that can detect and estimate the effects or variances of imprinted quantitative trait loci (iQTL) distributed over the whole genome has not been extensively developed. In this talk, I will present two general statistical methods for mapping and identifying iQTL responsible for complex traits using a genetic linkage map. The first is constructed by two reciprocal backcrosses, initiated with two inbred lines, allowing the estimation and test of the imprinted effect of an iQTL (fixed-effect model). The second is framed for a hierarchical outbred pedigree in which the genetic variance due to an unknown number of maternally or paternally-derived alleles at an iQTL is estimated (random-effect model). Statistical properties of both the fixed- and random-effect models are investigated through simulation studies and real data analyses. The proposed methods provide a standard procedure for the detection of iQTL involved in the genetic control of complex traits.

## **Regulation of the $\beta$ -globin gene locus during development and differentiation**

Crusselle-Davis VJ, Andersen F, Zhou Z, Bungert J\*

University of Florida Shands Cancer Center, Department of Biochemistry and Molecular Biology, Powell Gene Therapy Center, and Center for Mammalian Genetics, University of Florida, Gainesville, FL

The locus control regions (LCRs) of the human and murine  $\beta$ -globin gene loci are complex regulatory elements composed of several DNaseI hypersensitive (HS) sites. Recent *in vitro* and *in vivo* experiments show that the LCR core HS sites recruit RNA polymerase II and chromatin modifying protein complexes. Several observations suggest that the LCR functions as the primary attachment site for macromolecular complexes involved in the modification of chromatin structure and transcription of the globin genes. We test this hypothesis using different experimental systems. First, we analyze the recruitment of RNA polymerase II and other basal transcription factors to the globin locus during the DNA synthesis phase in synchronized erythroleukemia cells. Second, we analyze the interaction of RNA polymerase II, basal transcription factors, and modified histones with the globin gene locus during *in vitro* differentiation of mouse embryonic stem cells. Third, we utilize a novel *in vitro* assay to analyze the transfer of RNA polymerase II from immobilized LCR constructs to a  $\beta$ -globin gene. The results from these studies are consistent with the hypothesis that RNA polymerase II is first recruited to the LCR and subsequently transferred to the globin genes. The establishment of a specific globin locus configuration, called the "active chromatin hub" (de Laat and Grosveld, 2003, *Chromosome Res* 11(5):447-59), could facilitate the transfer of RNA polymerase II from a highly accessible LCR complex to high affinity globin gene promoters.

## **Role of epigenetics and chromatin insulators in regulating Herpes Simplex Virus type 1 latent gene expression.**

Bloom DC\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Herpes Simplex Virus type 1 (HSV-1) is a double stranded DNA virus that establishes a life-long latent infection within sensory neurons. During latency the 152 kb genome is maintained as a circular episome associated with nucleosomes. HSV-1 latent gene expression is tightly repressed except for the latency-associated transcript (LAT). The latency-associated transcript (LAT) promoter and the LAT enhancer are enriched in acetyl histone H3 (K9, K14) during latency, whereas all lytic genes analyzed (ICP0, UL54, ICP4, and DNA Polymerase) are not. This suggests that the HSV-1 latent genome is organized into transcriptionally permissive and transcriptionally repressed chromatin domains. Such an organization implies that chromatin insulators, similar to those in cellular chromosomes, may separate these distinct transcriptional domains of the HSV-1 latent genome. We sought to identify *cis* elements that could be involved in partitioning the HSV-1 latent genome into distinct chromatin domains. Sequence analysis coupled with chromatin immunoprecipitation (ChIP) and luciferase reporter assays revealed that: 1) the Long and Short Repeats and Unique Short regions of the HSV-1 genome contain clustered CCCTC and CTCCC motifs, 2) CTCF binding motif clusters similar to those in HSV-1 are conserved in other alpha herpesviruses, 3) binding of CTCF to these motifs is enriched on latent HSV-1 genomes *in vivo*, and 4) a 1.5 kb region containing the CTCF motif cluster in the LAT region possesses enhancer-blocking and silencer insulator activities. The finding that CTCF, a cellular protein associated with chromatin insulators, binds to motifs on the latent genome and insulates the LAT enhancer suggests that CTCF may facilitate the formation of distinct chromatin boundaries during Herpesvirus latency.

## Regulation of imprinted gene expression in the Angelman/Prader-Willi syndrome region

Heggstad AD<sup>1</sup>, Dostie J<sup>2</sup>, Dekker J<sup>2</sup>, Dennis TR<sup>3</sup>, Rodríguez-Jato S<sup>1</sup>, Gray BA<sup>3</sup>, Zori RT<sup>3,\*</sup>, Driscoll DJ<sup>3,\*</sup>, Yang TP<sup>1,3,\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA

<sup>3</sup>Department of Pediatrics, University of Florida, Gainesville, FL

The Angelman and Prader-Willi syndromes (AS/PWS) are two distinct neurogenetic disorders associated with a ~2 Mb domain on chromosome 15. This domain is composed of at least eight imprinted genes that are coordinately regulated by a bipartite imprinting center (IC) located at the 5' region of the *SNURF-SNRPN* gene. The PWS-IC appears to play a role in establishing and/or maintaining the paternal epigenotype and may function as a positive regulator of genes expressed exclusively from the paternally-inherited chromosome (e.g., *SNURF-SNRPN*, *MKRN3*, *MAGEL2*, *NDN*), whereas the AS-IC is believed to play a role in establishment of the maternal epigenotype on the maternally-inherited chromosome. We are examining the role of long-range intrachromosomal interactions across the AS/PWS domain in the coordinate regulation of imprinted genes by the PWS-IC. Fluorescence *in situ* hybridization (FISH) studies demonstrate that the PWS-IC is closely juxtaposed with a distal cluster of imprinted genes (*MKRN3*, *MAGEL2*, and *NDN*) preferentially on the paternally-inherited chromosome, indicating a parent-of-origin-specific spatial organization of the AS/PWS domain. preferential interaction of the PWS-IC with the distal gene cluster on the paternal chromosome and indicates Analysis of the domain by Chromosome Conformation Capture (3C) assays confirms the that the major site of interaction with the PWS-IC is within or near the *MKRN3* locus located ~1.3 Mb upstream of the PWS-IC. This interaction occurs in human lymphoblasts where all three distal genes are expressed at low levels, suggesting that the function of the PWS-IC may not be only that of an activator/enhancer of the distal genes. Thus, we propose that this long-range intrachromosomal interaction between the PWS-IC and *MKRN3* locus constitutes a basal interaction involved in establishing and/or maintaining the paternal epigenotype and paternal-specific patterns of gene expression in the AS/PWS domain, irrespective of the expression levels of genes in the distal gene cluster. We also have analyzed DNase I hypersensitive (DH) sites associated with the PWS-IC region in human lymphoblasts and in various mouse cells types that express genes in the distal cluster at different levels. These studies suggest that the PWS-IC may function as a chromatin holocomplex composed of multiple DH sites that coordinately regulate imprinted expression of genes on the paternal chromosome in a manner similar to that of an active chromatin hub. Furthermore, the composition and configuration of the chromatin holocomplex formed by the PWS-IC may be cell-type specific and also play a role in the cell-type-specific regulation of imprinted genes on the paternal chromosome in the AS/PWS domain.

## **Altering chromatin remodeling in cloned bovine embryos**

Moore, K\*

Department of Animal Sciences, University of Florida, Gainesville, FL

Cloning is a powerful tool for improving animal agriculture, by allowing expansion of superior livestock of the desired sex and phenotype. It can also be utilized for producing embryos from animals that are infertile due to age, illness or death. However, there are still great inefficiencies in the production of calves using this technology, due to high rates of early embryonic loss and abortion, making it too costly for use by most commercial cattle producers. In spite of these problems, limited progress has been made towards understanding the factors associated with these abnormalities. Embryonic genome reprogramming is one area that is critical for normal embryonic development, and demethylation is just one aspect of reprogramming that must occur in the very early embryo. Recently, cloned embryos were shown to have reduced chromatin demethylation. Aberrant chromatin remodeling may arise from culture or manipulation of somatic cells, oocytes and/or embryos, resulting in abnormal development, and reduced survival. Our research is concentrating on the status of chromatin remodeling of the embryonic genome in cloned and in vitro produced bovine embryos and whether it can be regulated artificially. The success of this research will bring a greater understanding of the impacts culture and in vitro manipulations have on somatic cells and developing embryos. This will allow for further optimization of these conditions, such that reprogramming will occur normally and allow for development of normal calves to term. Improved efficiencies will also lead to a wider utilization of this technology for improving cattle productivity, both in the beef and dairy industries, which translates into increased profits for the US cattle industries.

## **Regulation of E2F by chromatin factors with tumor suppressor activity**

Lu J

Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Regulation of chromatin states is crucial to normal development and malignant transformation. The E2F transcription factors play a key role in control of cell proliferation and differentiation, and previous studies have suggested that the Rb tumor suppressor may recruit a number of distinct chromatin regulators to modulate E2F activity and cell cycle progression. In the present study, we have conducted a genome-wide RNAi screen in cultured *Drosophila* cells for essential E2F repressors. Among the genes identified are the Domino chromatin remodeling complex, as well as the fly tumor suppressor L3mbt and the related CG16975, both of which share similar structure with the Polycomb group (PcG) protein Scm involved in chromatin condensation and gene silencing. These factors physically associate with E2F proteins, bind to E2F-responsive promoters and repress endogenous E2F target genes. Epistasis analysis indicates that they act mainly in the same pathway, but independently of Rb. In *Drosophila*, *Domino* mutation enhances an over-proliferation phenotype caused by E2F overexpression and suppresses a loss-of-function *cyclin E* mutation, suggesting that Rb-independent chromatin factors may be essential for E2F regulation *in vivo*. Taken together, our studies suggest that chromatin conformation is a key feature of E2F regulation and tumor suppression, and provide further evidence connecting epigenetic dysregulation to neoplastic growth.

## **DNA methylation in pluripotent embryonic stem cells**

Terada N

Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

The capacity for cellular differentiation is governed not only by the repertoire of available transcription factors but the accessibility of cis-regulatory elements. Studying changes in epigenetic modifications during stem cell differentiation will help us to understand how cells maintain or lose differentiation potential. Murine embryonic stem (ES) cells are originally derived from the inner cell mass of a developing blastocyst and have the ability to differentiate into all cell types of an adult animal. Pluripotency of ES cells can be maintained *in vitro* when the cells are cultured in a serum-containing medium supplemented with leukemia inhibitory factor (LIF). When LIF is removed from the medium, the ES cells begin to differentiate *in vitro* into all three embryonic germ layers. This *in vitro* ES cell differentiation system serves as an excellent model to study the regulation of gene expression required for stem cell self-renewal and pluripotency. Recent studies on molecules involved in epigenetic modifications have revealed a unique expression pattern of DNA methyltransferases, histone deacetylases, and methyl-binding proteins in ES cells. ES cells also have a differential genome-wide DNA methylation pattern when compared to their descendant differentiated cells. However, exact genomic loci of such differentially methylated regions remain largely unknown. Using a methylation-sensitive restriction fingerprinting method, we recently identified a novel adenine nucleotide (ADP/ATP) translocase gene *Ant4* that was selectively hypomethylated and expressed in undifferentiated ES cells. Furthermore, we demonstrate that DNA methylation plays a primary role in transcriptional silencing of the *Ant4* gene during ES cell differentiation.

## Poster Session

\* = U.F. Genetics Institute Faculty

### 1. Comparative evolutionary genetics of mutation in rhabditid nematodes

Baer CF<sup>1,\*</sup>, Phillips N<sup>1,2</sup>, Ostrow G<sup>1</sup>, Shaw F<sup>3</sup>, Custer A<sup>1</sup>, Avalos A<sup>1</sup>, Blanton D<sup>1</sup>, Boggs A<sup>1</sup>, Bour W<sup>1</sup>, Damas M<sup>1</sup>, Keller T<sup>1</sup>, Levy L<sup>1</sup>, Mezerhane E<sup>1</sup>, Rosenbloom J<sup>1</sup>

<sup>1</sup>Department of Zoology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Biology, Arcadia College, Philadelphia, PA

<sup>3</sup>Department of Mathematics, Hamline University, St. Paul, MN

The ubiquity of deleterious mutations and their importance to the evolutionary process are well-appreciated. A long-standing question in evolutionary biology is why the mutation rate has not evolved to zero. The explanation is usually assumed to be that there is a "cost of fidelity", i.e., at some point additional investment in fidelity of DNA replication is not cost effective in terms of fitness. There is no theoretical reason to expect the cost of fidelity to be the same among taxa. To begin to understand the factors underlying the mutation rate we allowed mutations to accumulate in the relative absence of natural selection for 250 generations in two strains from each of three species of self-fertile nematodes in the family Rhabditidae. The results are clear: the rate of decay of fitness is about twice as fast in *C. briggsae* as in *C. elegans*, and the pattern for mutations underlying body size is very consistent with the pattern for fitness. Fitness was assayed in two thermal environments, one of which is stressful for one species but not another. There was little if any effect of thermal environment. The estimated genomic mutation rate is consistent with previous estimates for *C. elegans* and much lower than the consensus rate for *Drosophila melanogaster*, the only other species for which there are reliable data. There were no differences between the two strains of *C. briggsae* in either the rate of mutation or the indel bias at dinucleotide microsatellite loci. We discuss possible reasons for the discrepancy between the worm and fly results and suggest future avenues of research.

### 2. Molecular improvement of turf-quality in bahiagrass by altering gibberellin content

Agharkar M, Altpeter F\*, Zhang H

Agronomy Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Bahiagrass (*Paspalum notatum* Flugge) is a low input, drought tolerant and disease resistant warm season turfgrass used for residential lawns and along highways in the southeastern US. However turf quality of currently available bahiagrass cultivars is poor, due to prolific seedhead production, open growth habit and light green color. The objective of this study was to improve the turf quality of bahiagrass by over-expression of a gibberellin catabolizing enzyme, gibberellin 2-oxidase (GA2ox).

A genomic clone of GA2ox1 and cDNA clone of GA2ox8 were isolated from *Arabidopsis*. They were sub-cloned under the control of the constitutive ubiquitin or 35S promoters. Co-transfer of constitutive nptII and GA-2 oxidase expression cassettes, into seed derived callus cultures from turf-type bahiagrass (cv. 'Argentine') was followed by selection with paromomycin sulphate during callus subculture and regeneration (Altpeter and James, 2005, *Inter Turfgrass Soc Res J* 10:485-9). Transgenic plants were confirmed by NPTII ELISA (Agdia), PCR, RT-PCR and altered phenotype.

Transgenic plants over-expressing the gibberellin 2-oxidase exhibit a dwarf phenotype with a darker green leaf color. These plants were successfully established in soil. A hydroponics system is being used for the comparative evaluation of several transgenic lines and the wildtype for root growth and tillering. Data correlating gibberellin 2-oxidase over-expression in transgenic bahiagrass with physiological parameters will be presented.

### **3. Cloning and identification of a micro-RNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus**

Samols MA<sup>1</sup>, Hu J<sup>1</sup>, Skalsky RL<sup>1</sup>, Marshall V<sup>2</sup>, Parks T<sup>2</sup>, Wang CD<sup>2</sup>, Whitby D<sup>2</sup>, Renne R<sup>1</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology and UF Shands Cancer Center, University of Florida, Gainesville, FL

<sup>2</sup>Viral Epidemiology Section, AIDS Vaccine Program, SAIC-Frederick, NCI-Frederick, MD

MicroRNAs are small non-coding regulatory RNA molecules that bind to 3' UTRs of mRNAs to either prevent their translation or induce their degradation. Previously identified in a variety of organisms ranging from plants to mammals, miRNAs are also now known to be encoded by viruses. The human  $\gamma$ -herpesvirus Epstein-Barr Virus has been shown to encode miRNAs which potentially regulate both viral and cellular genes. To determine whether Kaposi's sarcoma-associated herpesvirus (KSHV) encodes miRNAs, we cloned small RNAs from latently and lytically KSHV infected primary effusion lymphoma derived cells and endothelial cells. Sequence analysis revealed 11 miRNAs of 19 to 23 bases in length that perfectly align to KSHV. Surprisingly, all candidate miRNAs mapped to a single genomic locale within the latency-associated region of KSHV (Samols *et al.*, 2005, *J Virol* 79(14):9301-5). The existence of this miRNA cluster has also been reported by two additional groups while this work was under review (Pfeffer *et al.*, 2005, *Nat Methods* 2(4):269-76; Cai *et al.*, 2005, *Proc Natl Acad Sci USA* 102(15):5570-5).

To determine whether these novel viral miRNA genes are evolutionarily conserved we cloned the miRNA containing region from several PEL cell lines, KS tumors and PBMC from subjects with KS or MCD that were previously characterized by K1 and K15 genotyping. Sequence analysis demonstrated that these miRNAs are highly conserved between different virus sub-types and different malignant entities.

By performing RT-PCR-based RNA mapping studies, we detected several transcripts that may express the miRNA cluster as a spliced intron, from a long pre-mRNA that originates upstream of the LANA promoter and extends through the kaposin locus. These data suggests that the KSHV-encoded miRNAs are co-regulated with the expression of ORFs 73, 72, 71, and the kaposin proteins all of which regulate different host cellular signal transduction pathways in latently infected cells.

In summary, these data suggest that viral and/or host cellular gene expression may be regulated by miRNAs during both latent and lytic KSHV replication. The high sequence conservation between different KSHV sub-types further underlines that miRNAs may play an important role in the viral lifecycle.

#### **4. Identification and characterization of a novel gene expressed exclusively in the ZPA in the vertebrate limb**

Rock JR, Harfe BD\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Classical experiments identified the zone of polarizing activity (ZPA) as responsible for patterning the anteroposterior limb axis. Subsequent experiments determined that Sonic hedgehog (Shh) is responsible for the polarizing activity associated with the ZPA. To date, Shh is the only gene known to be expressed exclusively in the ZPA in the limb. To identify other genes expressed in the ZPA, we used a transgenic mouse allele in which we had inserted GFP into the Shh locus. Embryos with this transgene expressed GFP in all cells that normally express Shh. Cells expressing Shh were sorted from E10.5 limbs and labeled complementary RNAs from GFP-positive (the ZPA) and GFP-negative cell populations were hybridized to Affymetrix GeneChips. Analysis of the data revealed that Shh and ~15 other genes were expressed at higher levels in the ZPA than in the rest of the limb. One of these genes, an uncharacterized EST with eight transmembrane domains, was analyzed further. We have provisionally named this gene TM1. RNA *in situ* hybridization revealed that TM1 is expressed in the limb in a pattern temporally and spatially identical to Shh. TM1 is a member of a family of transmembrane proteins that shares no similarity to any of the known transmembrane proteins functioning in the Shh-signaling pathway. In the chick limb, we have found that the TM1 homolog is also expressed exclusively in the ZPA. RNA *in situ* hybridization analysis of TM1 expression in mutant mouse embryos suggests that Fgf-signaling is necessary for normal TM1 expression, but Shh is not.

#### **5. Development of microsatellite DNA markers for genetic studies on the Florida manatee (*Trichechus manatus latirostris*)**

Pause KC<sup>1</sup>, Nourisson C<sup>2</sup>, Clark AM<sup>3</sup>, Bonde RK<sup>4</sup>, McGuire PM<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Centre d'Océanographie de Marseille, France

<sup>3</sup>BEECS Genetic Analysis Laboratory, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

<sup>4</sup>United States Geological Survey, Florida Integrated Science Center, Sirenia Project, Gainesville, FL

Florida manatees are one of the most widely recognized endangered species in the United States. Management and recovery efforts have been successful over the years in increasing manatee population sizes, but the genetic diversity of the species has been questioned. Previous genetic studies have demonstrated low genetic diversity, failing to resolve the population structure. Microsatellite libraries are being developed and screened to produce loci for population studies, pedigree analysis, and individual identification using a genetic fingerprint.

Eight previously published microsatellite primers are being screened with over 200 Florida animals along with three additional unpublished loci. These primers have shown low allelic diversity, justifying the identification of additional polymorphic loci. Additional primers have been developed, and among these new primers, seven appear to be polymorphic for the Florida population. New microsatellite libraries are being produced in an effort to identify more polymorphic loci. These primers are being tested on the Florida population, and will be instrumental in confirming the genetic appropriateness of the established management units.

## **6. Identification of a chromatin insulator with enhancer-blocking activity located within the HSV-1 LAT region**

Amelio AL, Bloom DC\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Herpes Simplex Virus type I, HSV-1, establishes a life-long latent infection within neurons, where the genome is maintained as a nucleosome-associated circular episome, with only the Latency-Associated Transcript (LAT) expressed abundantly. Previous studies have demonstrated that the region encoding the LAT 5' exon possesses enhancer activity, and is implicated in maintaining this region in a transcriptionally permissive chromatin structure during latency. Despite the proximity of the LAT enhancer to the lytic gene ICP0, chromatin-immunoprecipitation (ChIP) analysis of the latent HSV-1 genome reveals that the LAT region is hyperacetylated (enriched in histone H3 acetyl K9, K14) while the ICP0 region is underenriched in this acetylated histone, suggesting the existence of a chromatin insulator with enhancer-blocking function. Insulators are a specific class of DNA sequence elements that can protect genes from regulatory signals emanating from the surrounding environment. Sequence analysis of the HSV-1 genome identified a reiterated CTCF motif cluster located downstream of the LAT enhancer and upstream of the ICP0 coding sequence. The cellular protein CTCF is a regulatory protein and a component of all currently identified vertebrate insulators. ChIP analysis of dorsal root ganglia from mice latently infected with HSV-1 strain 17+ has revealed that the identified motif cluster binds CTCF during HSV-1 latency. A luciferase reporter assay demonstrates that this element possesses enhancer-blocking activity, suggesting that this element forms a distinct regulatory domain in the HSV-1 LAT locus during latency. In combination with the LAT enhancer, regulation of this insulator may offer a novel mechanism for controlling HSV-1 latency and reactivation. Finally, we have identified the existence of seven other potential insulator elements in the HSV-1 genome that flank the IE genes suggesting the possibility that the IE genes of HSV-1 are regulated in separate chromatin domains.

## **7. Modeling cystic fibrosis related diabetes in CFTR-deficient mice: effects of CFTR genotype on glycemic control after sub-lethal beta cell injury or pulmonary sensitization and challenge**

Stalvey M<sup>1</sup>, Muller C<sup>1</sup>, Wasserfall C<sup>2</sup>, Brusko T<sup>2</sup>, Campbell-Thompson M<sup>2</sup>, Schatz D<sup>1</sup>, Atkinson M<sup>2</sup>, Flotte T<sup>1,3,\*</sup>

<sup>1</sup>Department of Pediatrics, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

<sup>3</sup>Powell Gene Therapy Center, University of Florida, Gainesville, FL

Cystic fibrosis related diabetes (CFRD) is increasing. No model existed to test hypotheses. Our hypothesis was that CFTR-deficient (CFTR<sup>-/-</sup>) mice have alterations of cytokine responses and islet function that would manifest as hyperglycemia after environmental stresses. Specifically, mice would have greater hyperglycemia following streptozotocin (STZ) induced islet injury compared to controls; and lung challenged would have greater hyperglycemia than non-lung challenged CFTR<sup>-/-</sup> mice. We hypothesized that hyperglycemia would be associated with a pro-inflammatory response. Eight C57BL/6J, six FVB/NJ and nine CFTR<sup>-/-</sup> mice received STZ with seven C57BL/6J, six FVB/NJ and eight CFTR<sup>-/-</sup> mice receiving LR as placebo. Four CFTR<sup>-/-</sup> mice were sensitized with *Aspergillus fumigatus* antigen (Af) and five CFTR<sup>-/-</sup> mice were given PBS, one month prior to lung challenge. Intraperitoneal glucose tolerance tests (GTT) were performed four weeks after STZ or LR and 48 hours after lung challenges. Isolated splenocytes were

stimulated with Con-A. Supernatants were collected at 48 hours for cytokine measurement and cells were labeled with  $^3\text{H}$ -thymidine at 72 hours. Glucose was higher in STZ-treated CFTR<sup>-/-</sup> mice following fast ( $p < 0.05$ ). No differences were seen in GTT of mice receiving LR. Lung-challenged CFTR<sup>-/-</sup> mice demonstrated similar blood glucose levels as controls. Lymphocyte proliferation was increased in STZ-treated CFTR<sup>-/-</sup> mice ( $p < 0.05$ ). STZ-treated CFTR<sup>-/-</sup> produced higher levels of  $T_H1$  cytokines IL-2 and INF-gamma,  $T_H2$  cytokines IL-4 and IL-10, and the non-specific cytokines TNF-alpha, GM-CSF, and IL-6 ( $p < 0.05$ ). STZ-treated CFTR<sup>-/-</sup> mice produced higher levels of IL-10, and GM-CSF ( $p < 0.05$ ) compared to lung challenged. Higher fasting and GTT levels seen in STZ-treated CFTR<sup>-/-</sup> mice make this an excellent model of CFRD. The response to islet injury is suggestive of an inherent difference in function and viability of CFTR-deficient beta cells. The pro-inflammatory state may contribute to the rapid decline in the clinical course in CFRD.

## **8. An evaluation of hypervariable plastid amplicons as phylogenetic markers**

Dhingra A<sup>1</sup>, Ramdial J<sup>1</sup>, Peir G<sup>2</sup>, Soltis P<sup>3,\*</sup>, Soltis D<sup>4,\*</sup>, Folta KM<sup>1,\*</sup>

<sup>1</sup>Department of Horticultural Sciences, University of Florida, Gainesville, FL

<sup>2</sup>Department of Biology, Duke University, Durham, NC

<sup>3</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

<sup>4</sup>Department of Botany, University of Florida, Gainesville, FL

In a recent report we described a PCR-based method for rapidly generating chloroplast sequence from a wide range of plant species. Several of the amplicons generated exhibited a high-frequency of fragment-length polymorphism, even between species within the same family. These finite hypervariable regions were evaluated as molecular markers to confirm and/or challenge derived phylogenetic relationships between species. Two types of analyses were performed to validate the applicability of this approach. First, the amplicons were generated and sequenced from a range of 27 dicots to examine the limits of the method across a large set of species. A second approach analyzed the sequences generated from closely-related species, namely a series of Caryophyllales and a number of *Fragaria* species. The results indicate that these primer pairs represent a rapid means to generate highly variable sequences from closely related species, and that at least a subset of them may serve as useful markers in phylogenetic and/or molecular evolution analyses.

## **9. ASAP: Amplification, Sequencing & Annotation of Plastomes**

Dhingra A, Folta KM\*

Department of Horticultural Sciences, University of Florida, Gainesville, FL

Background: Availability of DNA sequence information is vital for pursuing structural, functional and comparative genomics studies in plastids. Traditionally, the first step in mining the valuable information within a chloroplast genome requires sequencing a plasmid library or BAC clones. These activities involve complicated preparatory procedures like chloroplast DNA isolation or identification of the appropriate BAC clones to be sequenced. Rolling circle amplification (RCA) is being used currently to amplify the chloroplast genome from purified chloroplast DNA and the resulting products are sheared and cloned prior to sequencing. Herein we present a universal high-throughput, rapid PCR-based technique to amplify, sequence and assemble plastid genome sequence from diverse species in a short time and at reasonable cost from total plant DNA, using the large inverted repeat region from strawberry and peach as proof of concept. The method exploits the highly conserved coding regions or intergenic regions of plastid genes. Using an informatics approach, chloroplast DNA sequence information from five available eudicot

plastomes was aligned to identify the most conserved regions. Cognate primer pairs were then designed to generate ~1 - 1.2 kb overlapping amplicons from the inverted repeat region in 14 diverse genera.

Results: One hundred percent coverage of the inverted repeat region was obtained from *Arabidopsis*, tobacco, orange, strawberry, peach, lettuce, tomato and *Amaranthus*. Over 80% coverage was obtained from distant species, including *Ginkgo*, loblolly pine and *Equisetum*. Sequence from the inverted repeat region of strawberry and peach plastome was obtained, annotated and analyzed. Additionally, a polymorphic region identified from gel electrophoresis was sequenced from tomato and *Amaranthus*. Sequence analysis revealed large deletions in these species relative to tobacco plastome thus exhibiting the utility of this method for structural and comparative genomics studies.

Conclusions: This simple, inexpensive method now allows immediate access to plastid sequence, increasing experimental throughput and serving generally as a universal platform for plastid genome characterization. The method applies well to whole genome studies and speeds assessment of variability across species, making it a useful tool in plastid structural genomics.

## **10. Deletion of the endogenous *HPRT* promoter on the active X chromosome leads to complex alterations of histone modification patterns**

Rodriguez-Jato S<sup>1</sup>, Shan JX<sup>1</sup>, Kang SH<sup>1</sup>, Brant JO<sup>1</sup>, Heggstad AD<sup>1</sup>, Yang TP<sup>1,2,\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Center for Mammalian Genetics, University of Florida, Gainesville, FL

In this study, we examined the role of the promoter in maintaining transcriptionally active epigenetic states. We targeted a 323 bp deletion of the endogenous *HPRT* promoter on the active X chromosome in human HT1080 cells which removed nearly all known transcription factor binding sites in the promoter and abolished transcription. Previous analysis of this deletion showed loss of the DNase I hypersensitive site in the *HPRT* promoter region, but little or no change in general DNase I sensitivity or DNA methylation patterns across the locus. We now have examined the effect of this deletion on histone modification patterns using ChIP assays on the control parental and mutated cells. Analysis of nine sites across the wild type locus detected high levels of dimethylated H3 lysine 4 (H3-K4diMe) over the promoter and 5' transcribed region which decreased to background levels ~12 kb downstream of the promoter. Additionally, high levels of acetylated H4 (H4 Ac), H3-K9Ac and H3-K4triMe in control wild type cells were confined to the 5' transcribed region. Deletion of the minimal promoter resulted in a significant decrease in the levels of H3-K9Ac and H3-K4triMe. However, the mutation also led to an increase in H4Ac levels (predominantly in the 5' flanking region), and had no effect on H3-K4diMe levels. Our data suggest that H4 hyperacetylation and H3-K4diMe are not dependent on regulatory elements in the minimal promoter (and/or active transcription), and are not sufficient to maintain DNase I hypersensitivity of chromatin in the region. In contrast, maintaining H3-K9Ac and H3-K4triMe appear to require one or more regulatory elements associated with the minimal promoter region and/or active transcription. Thus, different mechanisms may be involved in maintaining specific types of histone modification patterns on active genes.

## **11. Over-expression of the DREB1a abiotic stress regulon in transgenic bahiagrass (*Paspalum notatum* Flugge)**

James VA, Altpeter F\*

Agronomy Department, University of Florida, Gainesville, FL

Bahiagrass is an important turf and forage grass in the southeastern US and in subtropical regions around the world. The productivity and persistence of bahiagrass, however, is limited by environmental stresses. Over-expression of the DREB1a (CBF3) transcriptional activator of the C/DRE stress response pathway has been successfully used in several plant species for enhancing tolerance to freezing, salt and drought stress. In order to explore the applicability of this system in bahiagrass, a DREB1a transcription factor ortholog was isolated from xeric, wild barley and placed under control of the drought-, cold- and salt-inducible barley HVA1 promoter. The combination of the DREB1a transcription factor gene with a stress-inducible promoter might avoid negative effects during non-stress conditions. The HVA1-DREB1a expression unit was introduced into the apomictic bahiagrass cultivar 'Argentine' via biolistic gene transfer and transgenic plants over-expressing DREB1a have been identified by real time RT-PCR. In contrast to wildtype plants, DREB1a-expressing transgenic plants survived severe salt stress (200mM NaCl) under hydroponic growth conditions. The response of these plants to dehydration stress under controlled environment conditions and to freezing stress under field conditions are currently being evaluated.

## **12. The HSV-1 LAT region undergoes a rapid, transient decrease in histone acetylation and transcription following explant of murine sensory ganglia**

Giordani NV, Amelio AL, Kubat NJ, O'Neil JE, Bloom DC\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Only the latency-associated transcript (LAT) region of the Herpes Simplex Virus Type 1 (HSV-1) genome is abundantly transcribed during latency. While the LAT locus plays a significant role in reactivation, its precise function in the process is unknown. It was previously shown that both the LAT promoter and the LAT 5' exon/enhancer are hyperacetylated, or enriched, in histone H3 diacetyl K9/K14 relative to HSV-1 lytic genes when examined by chromatin immunoprecipitation (ChIP) analysis in latently-infected murine dorsal root ganglia (DRG). Enrichment in this type of modified histone is generally associated with transcriptionally permissive chromatin, while underenrichment is observed in less transcriptionally permissive regions. In the present study, we examined changes in transcriptional permissiveness of the LAT locus following explant-induced stress. DRG from latently infected (HSV-1 strain KOS or 17syn+) and LAT transgenic mice were assessed for histone H3 (K9, K14) acetylation at early times post-explant. Both the LAT promoter and the LAT enhancer displayed dramatic decreases (> 10-fold) in acetylation over the times examined (0-4 hours post-explant), suggesting that decreases in transcriptional permissiveness of this region occur during reactivation. Reverse transcription followed by real-time PCR was used to examine transcription from the LAT region at early times post-explant of DRG from both latently-infected and LAT transgenic mice. A rapid (up to 1000-fold) decrease in the amount of both the LAT 5' exon and the 2.0-kb intron RNAs was observed relative to cellular housekeeping RNAs. These findings suggest that explant of HSV-1 latently-infected or LAT transgenic mouse DRG induces rapid decreases in both histone acetylation and transcription at the LAT locus, and that chromatin- and transcriptional-level changes at the LAT locus may be early events in HSV-1 reactivation.

### 13. Gene expression domains and the evolution of woody stems

Quesada T<sup>1,2</sup>, Dervinis C<sup>2</sup>, Li Z<sup>3</sup>, Casella G<sup>3,\*</sup>, DiFazio S<sup>4</sup>, Tuskan J<sup>4</sup>, Peter G<sup>1,2,\*</sup>, Davis J<sup>1,2,\*</sup>, Kirst M<sup>1,2,\*</sup>

<sup>1</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

<sup>2</sup>School of Forest Resources and Conservation, University of Florida, Gainesville, FL

<sup>3</sup>Department of Statistics, University of Florida, Gainesville, FL

<sup>4</sup>Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

During wood formation, the meristematic cambium differentiates into a complex of specialized cell types through differential regulation of genes. To evaluate potential relationships between tissue-specific expression and genomic location, we generated gene expression profiles in stems (nodes and internodes) from the *Populus trichocarpa* reference genotype Nisqually-1 and contrasted them to three other vegetative tissues (roots, mature and young leaves) using whole-genome DNA chips. Among the 53,394 genes evaluated, 18,244 (34%) were significantly expressed above background levels (FDR 5%) across all vegetative tissues, whereas only 3,483 genes (6.5%) were stem specific. Our results suggest that there are apparent chromatin domains that are transcriptionally active in all tissues, as well as some specific to stems. Stem specific-domains may provide evidence for epigenetic regulation of wood development. Although a large common set of genes was expressed across all vegetative tissues, significant differences in expression were detected when contrasting expression in stem vs. root (5,352 genes), stem vs. young leaf (5,619) and stem vs. mature leaf (9,331). To evaluate the role of subfunctionalization in the evolution of woodiness we compared gene expression between pairs of anciently duplicated genes. Paralogs that maintained similar functions are expected to be equally expressed in each tissue, while subfunctionalization may lead to differential expression. Strikingly, less than 10% of paralogs were differentially expressed in leaves and roots, while 25% were differentially regulated in stems, suggesting a significant role of gene duplication and subfunctionalization in the evolution of woodiness. Statistical analyses are being developed to test the significance of gene expression among tissues and the significance of correlated expression among adjacent genes within linkage groups. These analyses will be used in studying the potential role of epigenetic regulation of tree responses to various treatments such as wounding, hormone applications, and nitrogen fertilization.

### 14. Cloning and characterization of an *R*-gene from peanut homologous to the tomato *Mi* nematode resistance gene

Varma TSN<sup>1</sup>, Gallo M<sup>1,2,\*</sup>

<sup>1</sup>Agronomy Department, University of Florida, Gainesville, FL

<sup>2</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Knowledge of peanut (*Arachis hypogaea* L.) resistance genes (*R*) is very limited and their use for peanut genetic improvement lags behind most crop plants. In this study, our objective is to clone and characterize *R*-genes in peanut that specifically confer resistance to the root knot nematode, *Meloidogyne arenaria*. Degenerate PCR primers were used to amplify the nucleotide binding site (NBS) encoding regions from *Arachis hypogaea* var. NemaTAM, which is an interspecific cultivar with high levels of resistance to *M. arenaria*. Analysis of 192 clones revealed that 80 sequences belong to the section *Arachis*. Twenty-one other clones have sequences with significant homology to other genes found in *Medicago*, *Vigna*, *Pisum*, *Oryza*, *Solanum*, *Lycopersicum*, *Citrus*, *Cicer*, *Arabidopsis* and *Vitis*. Of the remaining clones, eight have sequences related to genes found in animals. Interestingly, one peanut clone shared conserved motifs found in the well-characterized tomato nematode resistance gene, *Mi*. The peanut

resistance genes characterized will be tested for use in marker-assisted selection (MAS) to improve disease and nematode resistance.

### **15. Gene-pair haplotypes: novel, complex markers for linkage mapping in octoploid strawberry**

Tombolato DCM<sup>1</sup>, Stewart PJ<sup>1</sup>, Davis TM<sup>2</sup>, Folta KM<sup>1,\*</sup>

<sup>1</sup>Horticultural Sciences Department, University of Florida, Gainesville, FL

<sup>2</sup>Department of Plant Biology, University of New Hampshire, Durham, NH

Molecular markers have long been employed in marker-assisted selection and construction of genetic linkage maps. Such markers are not yet available in cultivated strawberry (*Fragaria x ananassa*) because of its formidable octoploid (2n=8x=56) genome. The octoploid genome generally exhibits disomic segregation-segregation within, but not between, the diploid genomes that comprise the polyploid. A system for mapping in the octoploid has been devised around the "gene-pair haplotype" (GPH), markers based on variability detected within and between the four representative diploid subgenomes. A GPH consists of a suite of intergenic polymorphisms (SSRs, SNPs, indels, and restriction sites) that comprise a fingerprint for each unique allele in a given intergenic region. When considered together, the specific combination of variable markers allows discrimination of scorable alleles that exist within, and between, subgenomes. The implementation of this novel genetic marker will allow genetic mapping in the complex octoploid background.

### **16. Comparative fluorescence *in situ* hybridization (zoo-FISH) studies of the Florida manatee, *Trichechus manatus latirostris***

Kellogg ME<sup>1</sup>, Dennis TR<sup>2</sup>, Gray BA<sup>2</sup>, Duke DG<sup>3</sup>, Pause KC<sup>4</sup>, Bonde RK<sup>5</sup>, Zori RT<sup>2,\*</sup>, McGuire PM<sup>4</sup>

<sup>1</sup>Department of Physiological Sciences, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pediatrics, Division of Genetics, University of Florida, Gainesville, FL

<sup>3</sup>Interdisciplinary Center for Biotechnology Research, Hybridoma Core Laboratory, University of Florida, Gainesville, FL

<sup>4</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>5</sup>U.S. Geological Survey, Florida Integrated Science Center, Sirenia Project, Gainesville, FL

Comparative fluorescence *in situ* hybridization (zoo-FISH) studies are being performed to characterize the cytogenetic molecular nature of the Florida manatee, *Trichechus manatus latirostris*. This technique is being used to compare the evolutionary conservation between human and manatee chromosome segments. Microdissection, the physical isolation of partial or whole chromosomes using a microscopic needle, was used to collect manatee chromosome 23 from multiple manatee cartilage cells. The collected DNA was then PCR amplified, fluorescently labeled, and hybridized to homologous sequences within human chromosomes. Hybridization stringency conditions were optimized by varying temperature, salt and formamide concentrations. Preliminary results to date show hybridization of manatee chromosome 23 only to itself, among manatee chromosomes, as well as to human chromosome nine. These studies will facilitate high-resolution genetic mapping of the manatee genome as well as intraspecific and interspecific comparisons to other, non-human, species.

## **17. Statistical model to detect quantitative trait nucleotides that encode cancer growth**

Li H, Wu R\*

Department of Statistics, University of Florida, Gainesville, FL

Since the recognition of cancer as a genetic disease, growing evidence shows that most cancer is the result of an intricate interaction of low-penetrance genetic variants with environmental exposures. These low-penetrance cancer genes, each usually with a minor effect and cooperating with others in a complicated web, have been difficult to detect. Here, we present a powerful statistical model which incorporates functional mapping into the quantitative trait nucleotide (QTN) mapping framework. Given the block-like pattern of linkage disequilibrium distribution in the genome, a small number of haplotype-tagging SNPs (htSNPs) can be utilized to identify a large portion of haplotype diversity. By incorporating the dynamic mechanisms of tumor development, we can significantly decrease the number of unknown parameters related to cancer genes and, thus, increase the statistical power of modeling. Our model has a power to discern the risk haplotype of interacting QTNs that encode cancer growth. Monte Carlo simulations were performed to investigate the statistical behavior of our model.

## **18. "Fading Borders": a hypothesis of floral gene expression in *Amborella trichopoda* (Amborellaceae) and other basal angiosperms**

Buzgo M<sup>1</sup>, Kim S<sup>1</sup>, Chanderbali AS<sup>1,2</sup>, Soltis PS<sup>2,\*</sup>, Soltis DS<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

Floral morphology and development of *Amborella trichopoda*, the sister to all other extant angiosperms, show that organ identities are not strictly defined by whorls, in contrast to the eudicot *Arabidopsis* and most other model plants. Similar observations for other basal angiosperms suggest that the gradual transition of organ identities may be the ancestral condition of flowering plants. These observations lead to the "Fading Borders" concept: a gradual transition of organ identities may result from gradually overlapping patterns of expression of floral organ identity genes. Patterns of expression of transcription factor genes in several basal angiosperms (e.g., *Amborella*, *Nuphar*, *Persea*) are consistent with the predictions of the Fading Borders concept. For example, the transcription of B and C class genes was detected throughout the flower, in a broader pattern that included other organs than only those predicted by the ABC model (orthologs of *PISTILLATA* and *APETALA3* in tepals and carpels, *AGAMOUS* in some tepals). Other floral organ identity genes similarly exhibit broader expression patterns in basal angiosperms than in eudicots. We propose that evolutionary changes that restricted expression of these transcription factor genes to specific regions of the floral meristem resulted in flowers with clearly defined whorls of organs, as explained by the ABC model for eudicots. The Fading Borders concept, formulated from integrated studies of floral development and gene expression, may help to clarify the early evolution and subsequent canalization of floral form.

## 19. Floral development in *Persea americana* (avocado): the male mystery in Lauraceae

Buzgo M<sup>1</sup>, Chanderbali AS<sup>1,2</sup>, Zheng Z<sup>1</sup>, Kim S<sup>1</sup>, Oppenheimer GD<sup>1,\*</sup>, Soltis DE<sup>1,\*</sup>, Soltis PS<sup>2,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

Flowers of Lauraceae are small and consist of organs in distinct, trimerous whorls, with clearly differentiated organ identities. This syndrome is also found in other groups of magnoliids (e.g., Aristolochiaceae, Annonaceae) and in monocots. In Lauraceae, a pair of nectar-secreting appendages occurs at the base of the third whorl of stamens. Are they best considered accessory staminodes? Or are they additional pollen sacs, implying that the stamens are branched? The initiation sequence of the androecium and nectary-like or stamen-like structures outside of the androecium could help to answer these questions. The delimitation of the androecium towards the gynoecium and the perianth is discussed with respect to other basal angiosperms. The reliability of *in situ* hybridization of an *AGAMOUS* homolog (MADS-box gene required for the organ identities "stamen" and "carpel") is critically discussed. The expression of a single *AGAMOUS* homolog in the tepals may be insufficient to infer their homology with stamens, but may instead hint at the evolutionary processes of flower miniaturization and multiplication.

## 20. Molecular phylogeny of *Conradina* and other scrub mints (Lamiaceae) from the southeastern USA: evidence for hybridization in pleistocene refugia?

Edwards CE<sup>1,2</sup>, Soltis DE<sup>1,\*</sup>, Soltis PS<sup>2,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

*Conradina* (Lamiaceae) consists of six allopatric species endemic to the southeastern United States, four of which are federally endangered or threatened. The limits and status of several taxa have been contested based on morphological grounds, and clarification of these limits is necessary for the design and implementation of effective and fiscally responsible protection and management plans. The objectives of this study were to investigate the monophyly of *Conradina* and its relationship to other related mints which are endemic to the southeastern United States, to understand the patterns of diversification in *Conradina*, and to clarify species relationships. A molecular phylogeny was inferred by sequencing regions from the nuclear and plastid genomes from multiple accessions of each species of *Conradina* and multiple individuals from species of *Clinopodium*, *Dicerandra*, *Piloblephis*, *Stachydeoma*, *Monarda*, *Pycnanthemum*, and *Mentha*. The nuclear and plastid phylogenies conflict, which may be due to shared ancestral polymorphism and lineage sorting, or more likely, introgression that occurred very recently or during the Pleistocene.

## 21. Quantitative genetic analysis of spontaneous activity in *Drosophila melanogaster*: genetic, sex, and environmental effects

Jones KM<sup>1</sup>, Higgins L<sup>2</sup>, Wayne ML<sup>1,\*</sup>

<sup>1</sup>Department of Zoology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Biology, Indiana University, Bloomington, IN

We know genetics contribute to behavior. However, we do not have a clear understanding of how genes and environment interact to produce complex behaviors. We also do not know the

relative contributions of genetics and the environment to the variation in behavior. We investigated spontaneous activity in small groups of the fruit fly *Drosophila melanogaster*. We used nine near-isogenic lines recently isolated from a natural population. We conducted scan samples of behaviors, including feeding, resting, walking, grooming, courting, mating, and flying. From these behaviors, we calculated a composite score of activity for quantitative genetic analysis. Genetic differences accounted for 14.41% of the variation observed. A significant genotype by sex interaction was observed contributing to 8.60% of the variation signifying genetic variation for sex dimorphism in behavior. There was no effect of rearing environment on activity. Additionally, different genotypes and sexes within genotype also exhibited different rank orders of the behaviors that contributed to activity.

## 22. Differences in verapamil response between CYP3A5 genotypes

Langae TY<sup>1,\*</sup>, Yarandi HN<sup>2</sup>, Katz D<sup>3</sup>, Dehoff RM<sup>4</sup>, Kehoe JM<sup>1</sup>, Klocman AS<sup>1</sup>, Pepine CJ<sup>4</sup>, Johnson JA<sup>1,\*</sup>

<sup>1</sup>Center for Pharmacogenomics, University of Florida, Gainesville, FL

<sup>2</sup>Department of Statistics, University of Florida, Gainesville, FL

<sup>3</sup>Abbott Laboratories, Abbott Park, IL

<sup>4</sup>Department of Cardiology, University of Florida, Gainesville, FL

Background/Aims: In the CYP3A5 gene, the A>G (\*3) and G>A (\*6) SNPs result in severely decreased expression of CYP3A5 enzyme relative to a normal functional allele. We tested whether these polymorphisms are associated with differences in response to verapamil, a calcium channel blocker that is a CYP3A substrate and inhibitor.

Methods: All patients from the hypertension clinical trial INVEST for whom the only change to therapy at the initial visit was the addition of verapamil and who consented to genetic analysis were genotyped for CYP3A5 \*3 and \*6 SNPs. CYP3A5 haplotypes were determined by computational methods (PHASE II) and any CYP3A5 allele containing either CYP3A5 \*3 (G) or \*6 (A) variant allele was designated as nonfunctional. The data were analyzed using SAS PROC GLM with baseline values, dose, ethnicity, age, height, weight and sex included in the model.

Results: Allele frequencies for CYP3A5 \*3 (G) or \*6 (A) were 0.30, 0.69, 0.90 and 0.15, 0.04, 0.0 for Blacks (n=36), Hispanics (252) and Whites (173), respectively. When controlled for baseline blood pressure (BP) values, there were significant differences in treated (Tx) diastolic BP (DBP), and mean BP (MBP) based on number of functional CYP3A5 alleles (Table), but not systolic BP (SBP). Findings remained significant when considering multiple covariates in the model, and in addition to CYP3A5 genotype, baseline BP, weight and ethnicity were also significant covariates for DBP and MBP response.

Number of CYP3A5 functional Alleles	Tx SBP	Tx DBP	Tx MBP
0 (n = 290)	136 ± 15	79 ± 9	98 ± 9
1 (n = 138)	136 ± 19	79 ± 9	98 ± 10
2 (n = 38)	141 ± 22	83 ± 9	103 ± 12
Overall p value	0.13	0.006	0.008
Least square means ± SD			

Conclusion: These data suggest CYP3A5 genotypes may influence hemodynamic responses to verapamil.

### **23. Characterization of and AAV2/9 mediated gene therapy for the cardiac phenotype in a mouse model of Pompe Disease**

Pacak C<sup>1,2,3</sup>, Mah C<sup>2,3,\*</sup>, Sakai Y<sup>2</sup>, Cresawn K<sup>1,2,3</sup>, Lewis M<sup>2,3</sup>, Germain S<sup>2,3</sup>, Byrne B<sup>1,2,3,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>Powell Gene Therapy Center, University of Florida, Gainesville, FL

<sup>3</sup>Department of Pediatric Cardiology, University of Florida, Gainesville, FL

The long term goal of this project is to develop a clinically relevant gene therapy approach for the treatment of Pompe Disease. Pompe Disease is a form of muscular dystrophy and metabolic myopathy caused by mutations in the acid alpha glucosidase (GAA) gene. An insufficient amount of GAA leads to the accumulation of glycogen in lysosomes and consequent cellular dysfunction.

Here we present a characterization study of the cardiac phenotype in our GAA knockout mouse model (GAA<sup>-/-</sup>) at various ages through analysis of ECG traces, MRI data and use of the periodic acid shift (PAS) stain to visually assess glycogen content in tissue sections. Through ECG analysis we are able to observe a shortened PR interval by three months of age mimicking the conduction phenotype observed in the human Pompe population. By two weeks of age abnormal amounts of glycogen can be observed in the lysosomes of cardiac cells as demonstrated by the PAS stain. MRI analysis shows a significant increase in myocardial mass by 12 months of age.

Currently, we are interested in using this model of cardiac dysfunction in order to develop cardiac specific gene therapy which can potentially one day be applied to many genetically inherited cardiomyopathies. We have previously shown AAV can be administered systemically using a relatively noninvasive delivery route, transcend the vasculature and transduce tissues throughout the body.

Next, we sought to identify the rAAV serotype with the highest natural affinity for cardiac tissue. By performing a serotype comparison analysis, we found that LacZ transgene delivery using rAAV2/9 results in approximately 200 fold higher levels of expression in cardiac tissue than an identical dose with AAV1. Currently, experiments are underway in which GAA<sup>-/-</sup> mice have been injected with various doses of rAAV2/9-CMV-hGAA in order to non-invasively assess our gene delivery method.

### **24. The Progenetix data collection and bioinformatics toolbox: data mining from oncogenomic screening experiments**

Baudis M\*

Division of Pediatric Hematology/Oncology and UF Shands Cancer Center, University of Florida, Gainesville, FL

Over the last decades, thousands of (molecular-) cytogenetic studies have been aimed at the description of structural and numerical abnormalities of tumor karyotypes. In agreement with the multistep model of oncogenesis, in most malignancies repetitive chromosomal aberration patterns supposedly reflect the cooperation of different genes. A systematic analysis of these patterns for oncogenomic pathway description requires the large scale compilation of (molecular-) cytogenetic tumor data, and the development of tools for transforming those data into a format suitable for data mining purposes.

The analysis of smaller sets of CGH results by means of bioinformatics methods, reductionist approaches have been used for the generation of branched aberration pathways and clinical

prediction models. For large datasets and the generation of more general oncogenomic model systems, the preconceived notion of a limited set of hot spot regions or the unspecific reduction of data complexity is bound to show limited success.

Although array/matrix CGH technologies have an improved spatial resolution compared to chromosomal methods, so far they have added little to the understanding of complex oncogenomic relations. Additionally, the detection of single clone abnormalities has added a new layer of complexity.

The Progenetix database has been developed into the largest source of molecular-cytogenetic tumor data. Currently the website allows access to the cytogenetic data from more than 13,000 tumor specimen and provides unique online tools aimed at the visualization and bioinformatic mining of cytogenetic data. Here, I will review the availability of data derived from oncogenomic screening techniques and especially describe the development and content of the Progenetix collection. Additionally, I will present our current approach to develop advanced computational models for the structural analysis of large oncogenomic datasets, and examples for clinical correlation of cytogenetic imbalances.

## **25. Development of a custom microarray of floral genes for *Persea americana* (avocado)**

Chanderbali A<sup>1</sup>, Soltis D<sup>2,\*</sup>, Soltis P<sup>1,\*</sup>

<sup>1</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

<sup>2</sup>Department of Botany, University of Florida, Gainesville, FL

As part of the Floral Genome Project's goals to investigate the origin, conservation, and diversification of the genetic architecture of the flower, a microarray approach to study floral gene expression is being developed for *Persea americana* (avocado). This species is one of a set of plant 'exemplars' selected, based on recent phylogenetic studies, to represent the basal angiosperm groups, and bridge the gap between the plant models *Arabidopsis* and rice. The avocado microarray, developed by Agilent Technologies, contains *in situ* synthesized 60-mer oligo nucleotide probes, representing approximately 6200 unique avocado floral transcripts collected and sequenced by the Floral Genome Project (<http://fgp.bio.psu.edu/fgp/index.html>). We have analyzed gene expression in floral buds versus leaves using two arrays in a dye-swap experiment. Based on the criterion of two-fold difference, it was found that ca. 300 of the 6200 floral transcripts were up-regulated in flowers, and ca. 100 were up-regulated in leaves. Among the florally up-regulated genes are *Persea* homologues of APETALA3, PISTILLATA and SEPALLATA3, bona-fide floral genes in *Arabidopsis*. These results suggest that this *Persea* array is providing reliable insights into the gene expression profiles of floral and vegetative tissues and should therefore be considered an excellent tool for planned comparative analyses of gene expression among the floral organs.

## **26. Control of breathing in a mouse model of Glycogen Storage Disease Type II**

DeRuisseau LR<sup>1</sup>, Mah C<sup>2,\*</sup>, Cresawn KO<sup>3</sup>, Fuller DD<sup>4</sup>, Byrne BJ<sup>5,6,\*</sup>

<sup>1</sup>Department of Physiological Sciences, University of Florida, Gainesville, FL

<sup>2</sup>Department of Molecular Therapy, University of Florida, Gainesville, FL

<sup>3</sup>Department of Pediatric Cardiology, University of Florida, Gainesville, FL

<sup>4</sup>Department of Physical Therapy, University of Florida, Gainesville, FL

<sup>5</sup>Department of Pediatric Cardiology, University of Florida, Gainesville, FL

<sup>6</sup>Powell Gene Therapy Center, University of Florida, Gainesville, FL

Glycogen Storage Disease Type II (GSD II) is a lysosomal storage disorder resulting from deficiency of the enzyme acid alpha glucosidase (GAA). The lack of GAA is associated with glycogen accumulation throughout the body. Skeletal muscle is particularly affected and diaphragm dysfunction is often life-threatening and thus necessitates mechanical ventilation. Accordingly, the first purpose of this study was to determine if breathing is affected in a mouse model of GSD II (the *Gaa*<sup>-/-</sup> knockout mouse). Using barometric plethysmography, minute ventilation (MV; mL/min), breathing frequency (F; breaths/min) and tidal volume (TV; mL/min) were measured in awake control and *Gaa*<sup>-/-</sup> mice. During quiet breathing (inspired O<sub>2</sub> = 21%; balanced N<sub>2</sub>), MV (control vs. *Gaa*<sup>-/-</sup>: 60 ± 6 vs. 43 ± 4), F (234 ± 11 vs. 195 ± 8), and TV (0.26 ± 0.01 vs. 0.22 ± 0.02) were attenuated in *Gaa*<sup>-/-</sup> mice. Ventilation deficits in GSD II patients have been attributed to respiratory muscle weakness, however, several case reports suggest that the central nervous system is also affected. Therefore, we are currently testing the hypothesis that the neural control of the respiratory muscles is impaired in *Gaa*<sup>-/-</sup> mice. Initial experiments quantified glycogen (µg/g wet wt) in the spinal cord of control (14.3 ± 1.8) and *Gaa*<sup>-/-</sup> (30.0 ± 5.0) mice. Ongoing experiments are quantifying phrenic motor output in *Gaa*<sup>-/-</sup> and control mice. Preliminary data indicate that *Gaa*<sup>-/-</sup> mice have substantially impaired phrenic discharge compared to control mice. Our data suggest that ventilatory deficits in *Gaa*<sup>-/-</sup> mice may reflect both muscular and neural mechanisms. Accordingly, in addition to respiratory muscle weakness, GSD II patients may have an impaired ability to recruit the respiratory muscles during breathing. (Supported by NIH HL59412, DK58327; NIH T32 HD043730 / UF Alumni Assoc. Fellowship.)

## **27. Expression of floral MADS-box genes in basal angiosperms: implications for the evolution of floral regulators**

Kim S<sup>1</sup>, Koh J<sup>1</sup>, Yoo M-J<sup>1</sup>, Kong H<sup>2,3</sup>, Hu Y<sup>2</sup>, Ma H<sup>2</sup>, Soltis PS<sup>4,\*</sup>, Soltis DE<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Department of Biology, the Institute of Molecular Evolutionary Genetics, and the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA

<sup>3</sup>Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences, Beijing, China

<sup>4</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

The ABC model of floral organ identity is based on studies of *Arabidopsis* and *Antirrhinum*, both of which are highly derived eudicots. Most of the genes required for the ABC functions in *Arabidopsis* and *Antirrhinum* are members of the MADS-box gene family, and their orthologs are present in all major angiosperm lineages. Although the eudicots comprise 75% of all angiosperms, most of the diversity in arrangement and number of floral parts is actually found among basal angiosperm lineages, for which little is known about the genes that control floral development. To investigate the conservation and divergence of expression patterns of floral MADS-box genes in basal angiosperms relative to eudicot model systems, we isolated several floral MADS-box genes and examined their expression patterns in representative species, including *Amborella* (Amborellaceae), *Nuphar* (Nymphaeaceae), and *Illicium* (Austrobaileyaceae), the successive sister groups to all other extant angiosperms, plus *Magnolia* and *Asimina*, members of the large magnoliid clade. Our results from multiple methods (relative-quantitative-RT PCR, real-time PCR, and RNA *in situ* hybridization) revealed that expression patterns of floral MADS-box genes in basal angiosperms are broader than those of their counterparts in eudicots and monocots. In particular, 1) *AP1* homologs are generally expressed in all floral organs and leaves, 2) *AP3/PI* homologs are generally expressed in all floral organs, and 3) *AG* homologs are expressed in stamens and carpels of most basal angiosperms, in agreement with the expectations of the ABC model; however, an *AG* homolog is also expressed in tepals of *Illicium*.

The broader range of strong expression of *AP3/PI* homologs is inferred to be the ancestral pattern for all angiosperms and is also consistent with the gradual morphological intergradations often observed between adjacent floral organs in basal angiosperms.

## **28. Sequence and expression studies of A-, B-, and E-class MADS-box genes in *Eupomatia* (Eupomatiaceae): support for the bracteate origin of the calyptra**

Kim S<sup>1</sup>, Koh J<sup>1</sup>, Ma H<sup>2</sup>, Hu Y<sup>2</sup>, Endress PK<sup>3</sup>, Hauser BA<sup>1,\*</sup>, Soltis PS<sup>4,\*</sup>, Soltis DE<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Department of Biology, the Institute of Molecular Evolutionary Genetics, and the Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA

<sup>3</sup>Institute of Systematic Botany, University of Zurich, Switzerland

<sup>4</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

Basal angiosperms exhibit considerable diversity in the type, arrangement, and number of floral parts. Elucidating the genetics of floral development in these lineages will provide key insights into the early evolution of the flower. *Eupomatia* (Magnoliales, Eupomatiaceae) has flowers that bear a calyptra, an unusual organ that encloses the floral bud. The structural homology and evolutionary derivation of the calyptra are unknown, although some have proposed that it is a bract while others favor a derivation from the perianth. To address the evolutionary origin of the calyptra, we isolated, sequenced, and characterized the expression of A-, B-, and E-class MADS-box genes from *Eupomatia bennettii* and a close relative, *Magnolia grandiflora* (Magnoliaceae). The expression patterns of organ identity genes in floral organs of *Eupomatia* and *Magnolia* were very similar. However, the expression patterns of these MADS-box genes indicated that the ABC model is not strictly applicable to either *Eupomatia* or *Magnolia*. For example, A-class homologues were expressed in carpels and leaves of both *Eupomatia* and *Magnolia*. In the calyptra, expression levels of B- and E-class genes were low and almost identical to those observed in leaf tissue. In contrast, high levels of expression for B- and E-class genes were observed in the stamens, staminodes, and carpels. These gene expression data agree with recent developmental data and the interpretation of the calyptra as a bract. We also report the presence of various forms of alternatively spliced mRNAs in the cDNA pool from floral organs, and the implications of these mRNAs are discussed.

## **29. DNA sequences from Miocene fossils: an *ndhF* sequence of *Magnolia latahensis* (Magnoliaceae) and an *rbcl* sequence of *Persea pseudocarolinensis* (Lauraceae)**

Kim S<sup>1</sup>, Soltis DE<sup>1,\*</sup>, Soltis PS<sup>2,\*</sup>, Suh Y<sup>3</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Florida Museum of Natural History, University of Florida, Gainesville, FL

<sup>3</sup>Natural Products Research Institute, Seoul National University, Seoul, Korea

We report a partial *ndhF* sequence (1528 bp) of *Magnolia latahensis* and a partial *rbcl* sequence (699 bp) of *Persea pseudocarolinensis* from the Clarkia fossil beds of Idaho, USA (Miocene; 17-20 MYBP). The *ndhF* sequence from *M. latahensis* was identical to those of extant *M. grandiflora*, *M. schiediana*, *M. guatemalensis*, and *M. tamaulipana*. Parsimony analysis of the *ndhF* sequence of *M. latahensis* and previously reported *ndhF* sequences for Magnoliaceae placed *M. latahensis* within *Magnolia* as a member of the Theorhodon clade. This result is reasonable considering that: 1) the morphology of *M. latahensis* is very similar to that of extant *M. grandiflora* and 2) a recent molecular phylogenetic study of Magnoliaceae showed that the maximum sequence divergence of *ndhF* among extant species is very low (1.05% in subfamily Magnolioideae) compared with other angiosperm families. We re-analyzed the previously reported *rbcl* sequence of *M. latahensis* with

sequences for all major lineages of extant Magnoliales and Laurales. This sequence is sister to *Liriodendron*, rather than grouped with a close relative of *M. grandiflora* as predicted by morphology and the results of the *ndhF* analysis. In the *rbcl* sequence of *P. pseudocarolinensis*, three to six nucleotides differed from extant *Persea* species and two nucleotides differed from extant *Sassafras albidum*. Phylogenetic analyses of *rbcl* sequences for all major lineages of Magnoliales and Laurales placed the fossil *P. pseudocarolinensis* within Lauraceae and as sister to *S. albidum*. These results reinforce the suggestion that *Clarkia* and other similar sites hold untapped potential for molecular analysis of fossils.

### **30. Phylogeny of dinoflagellates inferred from mitochondrial cytochrome oxidase I and application of oligonucleotide probes for the identification of *Karenia brevis***

Yu F<sup>1</sup>, Steidinger KA<sup>2</sup>, Pang J<sup>3</sup>, McGuire PM<sup>4</sup>

<sup>1</sup>Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

<sup>2</sup>Florida Marine Research Institute - Fish & Wildlife Conservation Commission, St. Petersburg, FL

<sup>3</sup>Kunming Institute of Zoology, Kunming, Yunnan, China

<sup>4</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

A portion of the mitochondrial gene coding a subunit of cytochrome oxidase I has been sequenced for phylogenetic reconstruction using maximum parsimony (MP) and maximum likelihood (ML) methods and Bayesian analysis. These molecular data suggest that dinoflagellates represent a monophyletic group and are specifically allied with apicomplexans and ciliates. Dinoflagellates fall into five major genetic groups: peridinales, gonyaulacales, gymnodiniales, prorocentrales, and suessiales. Both MP and ML analyses provide strong bootstrap support for the monophyly of the fucoxanthin dinoflagellates with *Karenia brevis*, *Karlodinium micrum*, and *Gyrodinium galatheanum*. The incongruent phylogenetic reconstructions based on mitochondrial, nuclear, and chloroplast genes are associated with their very different evolutionary rates. Also, in an effort to distinguish *K. brevis* from other dinoflagellates, we compiled the sequence data for the large and small subunit ribosomal RNA, internal transcribed spacer (ITS), and mitochondrial *cox1* and cytochrome-b genes and found that only the ITS sequences could be used to design new species-specific oligonucleotide probes for *K. brevis*. Such probes should be useful for detecting community structure, identifying new isolates, and monitoring harmful algal blooms.

### **31. *KFR1*, A novel Kelch-domain, F-box protein associated with light-regulated transcript stability in plants**

Madzima T<sup>1</sup>, Kaufman LS<sup>2</sup>, Folta KM<sup>1,\*</sup>

<sup>1</sup>Plant Molecular and Cellular Biology Program and Horticultural Sciences Department, University of Florida, Gainesville, FL

<sup>2</sup>Laboratory for Molecular Biology, University of Illinois at Chicago, Chicago, IL

The Lhcb transcript levels in *Arabidopsis* and pea seedlings are regulated by blue light by two genetically-antagonistic mechanisms. Transcript levels increase in the dark-grown seedling in response to a short, single low fluence pulse of blue light ( $10^4 \mu\text{mol m}^{-2}$ ), yet are destabilized by a single pulse of blue-high-fluence light ( $10^5 \mu\text{mol m}^{-2}$ ). *Kfr1* (Kelch domain, F-box RNA-associated 1) encodes a novel F-box/Kelch domain protein which, through yeast three-hybrid screens, has been shown to interact with the pea 5' UTR. Genetic tests using *kfr1* mutants demonstrate that association between KFR1 and the 5' UTR is necessary for the blue-light induced Lhcb transcript destabilization. This lack of transcript degradation can be mimicked by treatment with MG-132 (a proteasome inhibitor) indicating that KFR1 is likely acting as predicted

by its topology; ubiquitinating a factor associated with transcript stability. The *kfr1* mutants also exhibit defects in blue-light-mediated hypocotyl elongation, suggesting that this protein may play a role in other processes associated with photomorphogenic development. This study demonstrates that light regulates transcript stability via ubiquitination of proteins that confer stability to specific, light-labile transcripts. These genetic elements may also have wide effects on plant development during acclimation to the light environment.

### **32. G protein $\beta 3$ subunit (GN $\beta 3$ ) polymorphism and hypertension**

Ortega MA, Langae TY\*, Gong Y, Johnson JA\*

Center for Pharmacogenomics and Department of Pharmacy Practice, University of Florida, Gainesville, FL

**Purpose:** Hypertension (HTN) is a complex disorder and a major risk factor for coronary heart disease, stroke, and chronic renal failure. The GN $\beta 3$  gene encodes for the  $\beta 3$  subunit of heterotrimeric G protein that is involved in cell signaling. There have been conflicting data in the literature concerning the role of G protein  $\beta 3$  subunit (GN $\beta 3$ ) polymorphism (C825T) with increased risk for hypertension. In this study we investigated the association between GN $\beta 3$  C825T polymorphism and hypertension in African-American and Caucasian hypertensive (HT) and normotensive (NT) subjects.

**Methods:** A total of 680 subjects between 35 and 65 years old were analyzed for this study. Normotensive subjects were defined as having a sitting BP < 140/90 mm Hg with no previous diagnosis and a family history of hypertension. Subjects were considered hypertensive if they had a family history of HTN and were diagnosed hypertensive and had a sitting BP  $\geq$  140/90 mm Hg, or taking antihypertensive medications. Genotyping for GN $\beta 3$  polymorphism was determined by PCR followed by pyrosequencing. Logistic regression was used to model the probability of being hypertensive by race.

**Results:** Variant allele (T) frequency was 0.34, and 0.73 for Caucasians and African-Americans respectively. In African-Americans, there was a significant association between GN $\beta 3$  genotypes and HTN. After adjusting for age, gender, and BMI the odds ratio and 95% confidence interval of being HT for TT vs CC, and TC vs CC genotypes were 2.775 (1.119-6.8883) and 2.738 (1.074-6.980) respectively. We did not observe any association between GN $\beta 3$  genotypes and HTN in Caucasians.

**Conclusion:** These results suggest that African-Americans with TT (the variant alleles) may have higher risk for developing hypertension.

### **33. Antigenic variation in *Babesia bovis*: characterization of an active site of transcription and identification of a novel sub-family of *ves* genes**

Al-Khedery B<sup>1</sup>, Allred DR<sup>1,2,\*</sup>

<sup>1</sup>Department of Pathobiology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

Expression of rapidly variant isoforms of the heterodimeric Variant Erythrocyte Surface Antigen 1 (VESA1) protein at the surface of *Babesia bovis*-infected erythrocytes is thought to facilitate its long-term persistence in immune cattle. The *ves1 $\alpha$*  gene encoding the VESA1a subunit was previously identified, but the locus from which it is expressed remained unknown, as did the gene

encoding the VESA1b subunit. We recently characterized the genomic locus containing the site of active *ves1 $\alpha$*  transcription (SOT) in the C9.1 clonal parasite line (Al-Khedery and Allred, in press). The C9.1 *ves1 $\alpha$*  gene is in very close juxtaposition with a divergently-oriented (head-to-head), closely related but structurally different gene, which we have termed C9.1 *ves1 $\beta$* . We hypothesize this novel gene, which is also actively transcribed in the C9.1 line, to encode the VESA1b polypeptide. Since surface-expression of the 1b subunit is consistently varied in concert with that of the 1a subunit, the overlap of the 5' UTR sequences of the juxtaposed genes in the active SOT may provide a mechanism for their co-regulation. In contrast with the consistent three exon structure of *ves1 $\alpha$*  genes, members of the *ves1 $\beta$*  subfamily have multiple exons and poorly conserved intron locations. Interestingly, similar to the SOT genes, the majority of these genes are arranged in a tightly-juxtaposed head-to-head organization, with a higher frequency of  $\alpha/\beta$  pairings. These results provide a background to begin deciphering the control of VESA1 subunit expression, modification, and possible epigenetic regulation during antigenic variation. (Supported by NIH #R01 A1055864 and USDA #97-35204-4768 and #2001-35204-10144 grants.)

#### **34. The maize *Viviparous10* locus encodes the *Cnx1* gene required for molybdenum cofactor biosynthesis**

Tseung CW<sup>1</sup>, Porch TG<sup>2</sup>, Schmelz EA<sup>3</sup>, Settles AM<sup>1,\*</sup>

<sup>1</sup>Horticultural Sciences Department, University of Florida, Gainesville, FL

<sup>2</sup>USDA Tropical Agriculture Research Station, Mayaguez, PR

<sup>3</sup>Center of Medical, Agricultural, and Veterinary Entomology, USDA-ARS, Gainesville, FL

Abscisic acid (ABA), auxin, and nitrate are important signaling molecules that affect plant growth responses to the environment. The synthesis or metabolism of these compounds depends upon the molybdenum cofactor (Moco). We show that maize (*Zea mays*) *viviparous10* (*vp10*) mutants have strong precocious germination and seedling lethal phenotypes that cannot be rescued with tissue culture. We devised a novel PCR-based method to clone a transposon tagged allele of *vp10* and show that *Vp10* encodes the ortholog of *Cnx1*, which catalyzes the final common step of Moco synthesis. The seedling phenotype of *vp10* mutants is consistent with disruptions in ABA and auxin biosynthesis as well as a disruption in nitrate metabolism. ABA and auxin levels are reduced in *vp10* mutants, and *vp10* seedlings lack Moco-dependent enzyme activities that are repairable with exogenous molybdenum. *vp10* and an *Arabidopsis cnx1* mutant, *chlorate6* (*chl6*), have similar defects in aldehyde oxidase (AO) enzyme activity, which is required for ABA synthesis. Surprisingly, *chl6* mutants do not show defects in abiotic stress responses. These observations confirm an orthologous function for *Cnx1* and *Vp10* as well as define a characteristic viviparous phenotype to identify other maize *cnx* mutants. Finally, the *vp10* mutant phenotype suggests that *cnx* mutants can have auxin as well as ABA biosynthesis defects, while the *chl6* mutant phenotype suggests that low levels of AO activity are sufficient for normal abiotic stress responses.

#### **35. Molecular and genetic analysis of the *rough endosperm3* mutant of maize**

Fajardo D, Latshaw S, McCarty D\*, Settles AM\*

Horticultural Sciences Department, University of Florida Gainesville, FL

The *rough endosperm* (*rgh*) class of seed mutants disrupts normal endosperm and embryo development and is characterized by seeds with a pitted or etched surface. We have identified the *rgh3* mutant in the UniformMu population by means of phenotype screening as well as SSR and B-A translocation mapping to chromosome arm 5L. The UniformMu population is a

Robertson's *Mutator*, transposon-active population that it is introgressed into W22 color-converted inbred (McCarty *et al.*, 2005). Mature kernel sections of the *rgl3* mutants show characteristic endosperm defects such as overproliferation of aleurone cells, and a defective embryo. However, a fraction of the *rgl3* mutant kernels are able to produce viable embryos that germinate into small seedlings, turn pale green, and eventually die, indicating that the *rgl3* mutant is likely to affect plastid function at some level. Analysis of B-A mosaic kernels of *rgl3* suggests that the *Rgl3* gene is non-tissue autonomous and is required in the endosperm and the embryo to rescue the embryo developmental defects. Based on these data, we hypothesize that the *rgl3* locus will reveal a signaling function in both endosperm and embryo development, and potentially, this signaling may involve the plastid.

### **36. Marker assisted evaluation of transgene containment in apomictic bahiagrass**

Sandhu S<sup>1</sup>, Altpeter F<sup>1\*</sup>, Blount A<sup>2</sup>

<sup>1</sup>Agronomy Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

<sup>2</sup>Agronomy Department, IFAS, Quincy, FL

An understanding of the reproductive biology of the target species is essential for risk assessment of genetically modified plants and supports the development of effective strategies for transgene containment.

Bahiagrass is the predominant forage grass in the southeastern United States. Sexual, diploid (2n=20) and tetraploid (2n=40) apomictic cytotypes of bahiagrass (*Paspalum notatum* Flugge) are grown.

'Argentine' is a commercially important tetraploid apomict cultivar of bahiagrass. Apomixis is the plant's ability to reproduce asexually through seeds, whereby the embryos develop without the contribution of the male gamete. Thus, apomixis is useful to fix superior genotypes and depending on the degree of sexuality could also offer a means for gene containment. We currently investigate the degree of sexuality in apomict 'Argentine' bahiagrass under field and greenhouse conditions. Glufosinate resistant 'Argentine' bahiagrass was generated by biolistic gene transfer (James and Altpeter, 2005, *Intern Turfgrass Res J* 10:485-9) and herbicide resistance was used as a marker to study the gene flow from transgenic tetraploid to wildtype tetraploid or diploid bahiagrass genotypes. Data on herbicide resistance of seed progeny will be presented.

### **37. Isolation and characterization of photoperiodic flowering regulators from *Fragaria***

Winslow AR, Bies DH, Folta KM\*

Horticultural Sciences Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Cultivated strawberry (*Fragaria x ananassa*) has a crop value of \$1.4 billion, about \$200 million in Florida alone. Despite its economic importance, little is known about the genetic mechanisms that control the transition between vegetative and reproductive growth. The signal transduction pathways that regulate the floral transition are well understood in model systems, and show a strong degree of conservation (and even interchangeability) of genetic components between plant species. We have initiated a study to characterize the genetic mechanisms of photoperiodic floral induction in cultivated strawberry. Homologs of the important meristem identity genes

*Constans*, *Suppressor of Constans*, *Flowering Locus T* and others have been isolated from strawberry EST libraries and characterized. This study describes their relationship to other floral regulatory homologs and the attempts to demonstrate their function in *Arabidopsis* and strawberry.

### **38. A transgenic approach to understanding imprinting regulation in the PWS locus**

Futtner CR<sup>1</sup>, Chamberlain SJ<sup>2</sup>, Johnstone KA<sup>1</sup>, Brannan CI<sup>1,\*</sup>, Resnick JL<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Genetics, University of North Carolina, Chapel Hill, NC

Prader Willi (PWS) and Angelman (AS) syndromes are both neuro-developmental disorders arising from the improper expression of oppositely imprinted genes located on human chromosome 15 q11-13. Imprint regulation of this region is under the control of a bi-partite imprinting center consisting of an Angelman imprinting center (AS-IC) located approximately 35 kb upstream of the paternally expressed *Snrpn* exon 1, and a Prader Willi imprinting center (PWS-IC) located just 5' to and including *Snrpn* exon 1. The PWS-IC has been shown to be a positive element promoting expression of a set of genes on the paternal allele, while the AS-IC provides suppression of the PWS-IC on the maternal allele thereby suppressing expression of the same set of genes. Both are required for proper establishment and/or maintenance of the imprint. In the mouse, both gene order and imprinted expression have been conserved with the syntenic region being located on murine chromosome 7C. While the location of the PWS-IC has been conserved in the mouse, the position of the murine AS-IC remains unknown. We have taken a transgenic approach to locating the AS-IC and further dissecting out components of the PWS-IC. Using a recombineering method utilizing the lambda phage Red genes we have created a series of deletions within a *Snrpn* containing BAC that we previously have shown recapitulates the imprinted expression of the endogenous locus in single copy. Analysis of mice carrying these transgenes will help to elucidate the minimal sequences necessary to confer correct imprinting in the PWS locus.

### **39. Comparative regulatory behavior of recombinant C<sub>3</sub> and C<sub>4</sub> PEPC isoforms in *Hydrilla***

Rao SK, Reiskind JB, Bowes G\*

Department of Botany, University of Florida, Gainesville, FL

The two PEPC isoforms, *hvpepc3* and *hvpepc4* found in leaves of the facultative C<sub>4</sub> aquatic plant, *Hydrilla*, are 95% homologous at both nucleotide and deduced amino acid level. Expression pattern, kinetic differences, malate sensitivity and phosphorylation status indicate that HVPEPC4 is the photosynthetically active form. Interestingly, the full-length *Hydrilla* PEPC sequences show the C<sub>3</sub>-invariant Ala at position "774"; none have the C<sub>4</sub>-determinant Ser characteristic of all published sequences encoding C<sub>4</sub> PEPC. In order to investigate the regulatory and kinetic differences of HVPEPC3 and HVPEPC4, the coding sequences of *hvpepc3* and *hvpepc4* were inserted into the pTV119N vector (Takara). The resulting expression plasmid designated as pTHV3 and pTHV4 were transformed into the PEPC deficient *E. coli* host-F15. A preliminary assessment of the protein expression suggests that active PEPC is produced by the host grown at room temperature (24 °C) and in the absence of the inducer IPTG. However, increase in temperature to 37 °C and addition of 1 mM IPTG boosted the level of expression. Optimization of protein expression, purification and kinetic studies are currently in progress. (Supported by USDA NRICGP 2002-35318-12540.)

#### 40. DNA methylation regulates germ cell-specific gene expression

Maatouk DM<sup>1</sup>, Kellam LD<sup>1</sup>, Mann MRW<sup>2</sup>, Lei H<sup>3</sup>, Li E<sup>3</sup>, Bartolomei MS<sup>2</sup>, Resnick JL<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>HHMI and Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA

<sup>3</sup>Epigenetics Program, Models of Disease Center, Novartis Institute for Biomedical Research, Cambridge, MA

Germ cell development in mammals is accompanied by the erasure and re-establishment of epigenetic marks. Murine germ cells initially differentiate in the extraembryonic mesoderm and subsequently migrate to the fetal gonad. Migratory germ cells have high levels of DNA methylation, but then undergo an extensive wave of genomic demethylation shortly after arrival in the fetal gonad on E10. By E13, sexual differentiation is underway as male germ cells mitotically arrest and female germ cells enter meiotic prophase. Here we provide evidence that this wave of genomic demethylation is rate limiting for continuing germ cell differentiation. We previously reported that premigratory germ cells precociously express the postmigratory marker GCNA1 when cultured in the presence of DNA demethylating agents. This result suggests that the natural demethylation of the germ cell genome at E11-12 is rate limiting for continuing differentiation. Consistent with this notion, we now report that several germ cell-specific genes exhibit high levels of DNA methylation prior to expression at 10.5 dpc, but are hypomethylated when expressed three days later. To test the hypothesis that DNA methylation regulates germ cell-specific gene expression *in vivo*, we examined embryos deficient in DNA methyltransferase 1 (Dnmt1). GCNA1 expression is detectable by E8 in Dnmt1 knockout embryos, two to three days earlier than in wild type embryos, demonstrating that DNA demethylation regulates the timing of germ cell differentiation *in vivo*. Surprisingly, GCNA1 is also ectopically expressed in somatic tissues in Dnmt1 knockout embryos. RT-PCR studies suggest that other germ cell-specific genes are also ectopically expressed in Dnmt1 deficient embryos, indicating that DNA methylation is necessary to silence some germ cell-specific genes in somatic cells. These results indicate that the dynamic changes in DNA methylation during germ cell development are essential not only for epigenetic reprogramming, but also for regulation of germ cell development.

#### 41. SNP discovery of BK channel beta 1 subunit

Gong Y, Beitelshes AL, Langae TY\*, Johnson JA\*

Center for Pharmacogenomics and Department of Pharmacy Practice, University of Florida, Gainesville, FL

**Aims:** The large-conductance, Ca<sup>(2+)</sup>-dependent K (+) (BK) channel plays a key role in the control of vascular tone. The genetic variation of the gene encoding the beta 1 subunit of BK channel (*KCNMB1*) has been reported to be associated with hypertension. However, variants in *KCNMB1* have not been systematically characterized to date. We undertook a single nucleotide polymorphism (SNP) discovery effort to characterize polymorphisms in *KCNMB1* using genomic DNA from 60 ethnically diversified individuals.

**Methods:** We amplified exons, intron/exon junctions, upstream region and 3' untranslated regions of *KCNMB1* using polymerase chain reaction (PCR). Polymorphism discovery was performed by denaturing high performance liquid chromatography (DHPLC) using partially denaturing conditions to screen for variations. We then used direct DNA sequencing to further characterize the nature and exact location of the polymorphisms and estimate the allele frequencies of the SNPs. Haplotype and linkage disequilibrium analysis was conducted using the

HAP, Arlequin and Haploview software. Conservation and *in silico* functional analysis was performed in PhyloVista and PolyMapr.

Results: We identified a total of 25 SNPs in *KCNMB1*. Seven of the polymorphisms (28%) are novel SNPs not previously reported. There were three coding-region SNPs including one synonymous and 2 non-synonymous SNPs. Twelve of the SNPs are located in intronic or untranslated regions. Allele frequencies range from less than 1.7% to 50% and 19 SNPs had a minor allele frequency greater than 5%. A lack of strong linkage disequilibrium among the 25 SNPs was observed in all three race/ethnicity group.

Conclusions: The *KCNMB1* gene contains frequently occurring SNPs that may play a role in interpatient variability in drug response in drugs with vascular activity or in disease risk. Clinical association studies are necessary to determine the whether such associations exist.

#### **42. Metabolic engineering of the chloroplast genome to enhance abiotic stress tolerance**

Fouad WM, Altpeter F\*

Agronomy Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Elevated beta-alanine levels were recently reported to be associated with enhanced heat tolerance. *Escherichia coli* l-aspartate-alpha-decarboxylase (ADC), coded by the *panD* gene, catalyzes the decarboxylation of l-aspartame to generate beta-alanine and carbon dioxide. ADC is a pyruvoyl-dependent enzyme that undergoes post translational modifications (self-processing) required for its activities. The *panD* gene is also unique to prokaryotes. Insertion of the *panD* gene into the chloroplast genome might have several benefits over nuclear transformation. As a prokaryotic gene, *panD* transcription, translation and post translational modifications are expected to be more efficient within the chloroplast. Therefore, the objective of the current study is to integrate the *E. coli panD* into the tobacco chloroplast *via* homologous recombination. The *panD* gene was isolated from *E. coli* by PCR using gene specific primers and subcloned under the control of plastid Prn promoter and *rbcL* 3' UTR to generate a chloroplast expression unit. This cassette was physically linked to the *aadA* selectable marker expression cassette and both cassettes were flanked by the tobacco *rbcL* / *accD* sequences to direct site specific homologous recombination into the tobacco chloroplast genome using biolistic gene transfer. Fifteen independent transplastomic plants were regenerated following selection for spectinomycin. PCR analysis using primers from the region flanking the targeted transgene integration site confirmed site specific integration of the *panD* expression unit into the chloroplast genome. These shoots were subject to a second round of regeneration to obtain homotransplastomic plants. Currently we are performing Southern blot analysis and evaluation of the heat tolerance of the transplastomic lines.

#### **43. Response to artificial selection on egg size in the serpulid polychaete *Hydroides elegans***

Miles CM, Wayne ML\*

Department of Zoology, University of Florida, Gainesville, FL

Egg size plays a critical role in many of the quantitative modeling approaches that have been used to try and identify selection pressures and processes that influence developmental mode

evolution in marine invertebrates. It has been hypothesized that nonfeeding larvae evolved under selection for increased egg size; this may have occurred many times. Egg size is part of a larger complex of life history traits that has co-evolved under the influence of natural selection and is directly related to parental fitness. The objective of this study is to use artificial selection to increase egg size and explore the implications across the life cycle by comparing larval, juvenile and adult traits in replicate control vs. selected lines of the polychaete *Hydroides elegans*. Since we know that there is significant additive genetic variation for egg size in this population, we hypothesize that a tradeoff with another fitness-related trait is acting to maintain genetic variation in egg size.

#### **44. Proteolytic mapping of the adeno-associated virus capsid**

Van Vliet K<sup>1</sup>, Blouin V<sup>1,2</sup>, Agbandje-McKenna M<sup>3</sup>, Snyder RO<sup>1,4,5,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>Laboratoire de Therapie Genique, INSERM U649, Nantes Cedex 1, France

<sup>3</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>4</sup>Powell Gene Therapy Center, University of Florida, Gainesville, FL

<sup>5</sup>Department of Pediatrics, University of Florida, Gainesville, FL

The 3D structures of the viral capsid for three AAV serotypes have previously been determined by X-ray crystallography and cryo-electron microscopy. These studies of AAV and those of autonomous parvoviruses have yielded important structural information of the virions in a low-energy conformation. However, for several non-enveloped and enveloped viruses, biochemical analyses of virions in solution under physiological conditions have shown that capsids are dynamic. Major capsid rearrangements can occur during receptor binding, cell entry, disassembly, assembly, genome packaging, maturation, and egress. We demonstrate that proteolytic digestion of mature AAV2 virions with trypsin results in the generation of an apparently intact capsid that is altered in receptor binding and antibody recognition, has reduced infectivity, and possesses a unique set of capsid protein fragments. The products of digestion were mapped using unique antibodies, protein sequencing, mass spectroscopy, and 3D structure modeling. Proteolytic analysis utilizing trypsin or chymotrypsin was capable of distinguishing AAV1 from AAV2 as seen by differential susceptibility and unique fragment patterns. In addition, empty AAV2 capsids could be distinguished from full (DNA containing) capsids by an increased susceptibility of VP2 to trypsin, and more rapid digestion by chymotrypsin. These data demonstrate the power of this combined approach for understanding capsid dynamics during the AAV life cycle, and may have implications in the use and engineering of AAV vectors for gene transfer.

#### **45. Invertases as genetic determinants of sink strength**

Bocock PN<sup>1</sup>, Huang LF<sup>1</sup>, Koch KE<sup>1,2,\*</sup>, Davis JM<sup>1,3,\*</sup>

<sup>1</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

<sup>2</sup>Department of Horticultural Sciences, University of Florida, Gainesville, FL

<sup>3</sup>School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Plants utilize carbon by partitioning the reduced carbon obtained through photosynthesis into different locations within the cell and subsequently allocating it to sink tissues throughout the plant. We are utilizing *Populus* as a model system in which to study invertase and its role in sink strength determination with the aim of applying this knowledge to tree breeding and genetic modifications.

Using the newly sequenced poplar genome, we have identified eight acid invertase family members through amino acid sequence similarity searches. Three of these family members encode invertases targeted to the vacuole, while the other five invertases are targeted to the apoplast. Poplar invertases share the intron/exon structure generally conserved in plants of seven exons separated by six introns with one exception, *PtIVR1*. This gene encodes an intronless vacuolar invertase which appears to be the first intronless invertase found in any organism.

Though the invertase family has been well characterized in *Arabidopsis*, it is not possible to determine their respective poplar orthologs based on sequence identity. To address this problem, we are using a microcolinearity approach by identifying invertase gene neighbors on the poplar chromosome with identical gene neighbors on the *Arabidopsis* chromosome. We plan to apply a statistical procedure for determining the significance of colinearity, which should in turn help us gain insights into the function and evolution of invertase genes. These predictions will then be verified through the use of Real Time Quantitative PCR. These data combined with other, transgenic aspects of this project will provide new information on the role of invertase in sink strength determination.

#### **46. Adeno-associated virus mediated knockdown of phospholamban using RNA interference**

Andino LM<sup>1</sup>, Byrne BJ<sup>2,3,\*</sup>, Kasahara H<sup>4,\*</sup>, Lewin AS<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pediatrics, University of Florida, Gainesville, FL

<sup>3</sup>Powell Gene Therapy Center, University of Florida, Gainesville, FL

<sup>4</sup>Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL

Regulation of Ca<sup>2+</sup> flux in the heart via blockade of the  $\beta$ -adrenergic receptor affects the phosphorylation state of phospholamban (PLN), a regulator of the sarcoplasmic reticulum calcium ATPase (SERCA2a). Reducing the activity of PLN enhances cardiac function in some animal models of congenital and ischemic cardiac disease.

Objective: The aim of this study is to use viral vectors to deliver short hairpin RNA (shRNA) targeting the PLN mRNA through the RNA interference pathway. We employed Adeno-associated virus serotype 1 (AAV1) and also a self-complementary version of AAV1 (scAAV1).

Methods: Two PLN-specific siRNAs, si248 and si750, and a control siRNA were designed. DNA versions of small hairpins containing the three siRNAs were each cloned in the two types of AAV vectors. All vectors contained the GFP gene in addition to one of the three siRNAs driven by the H1 RNA polymerase III promoter. Co-transfection experiments were performed using a 1:4 molar ratio of PLN:siRNA plasmids. RT-PCR and SYBR green staining were utilized for quantitation of PLN mRNA levels standardized to GAPDH levels. Immunoblots were utilized to assay effects on PLN protein levels. The siRNA plasmids were then packaged into AAV-1 capsids. These viruses were used for infection of primary ventricular neonatal cardiomyocytes.

Results: A ~40-47% reduction was seen in PLN mRNA levels 48 hours post-transfection using the PLN-targeting shRNAs expressed from the scAAV plasmids. The reduction in protein levels was much higher, >80% for both. In the primary cells, levels of RNA and protein were reduced by 46% and 51% respectively after infection with scAAV virus.

Conclusions: RNA interference mediated by both AAV and scAAV proved to be successful in producing targeted reduction of PLN RNA and protein in primary cardiomyocytes. Experiments

are currently underway to assess the effects of RNA interference of PLN *in vivo* following cardiac infection in neonatal mice.

#### **47. Genetic architecture of two fitness-related traits in *Drosophila melanogaster*: ovariole number and body size**

Telonis-Scott M<sup>1</sup>, McIntyre LM<sup>2</sup>, Wayne ML<sup>1,\*</sup>

<sup>1</sup>Department of Zoology, University of Florida, Gainesville, FL

<sup>2</sup>Computational Genomics, Department of Agronomy, Purdue University, West Lafayette, IN

In *D. melanogaster*, ovariole number and thorax length are morphological characters thought to be associated with fitness. Maximum daily egg production in females is positively correlated with ovariole number, while thorax length is correlated with male reproductive success and female fecundity. Though both traits are related to fitness, ovariole number is likely to be under stabilizing selection, while thorax length appears to be under directional selection. Current research has focused on examining the sources of variation for ovariole number in relation to fitness, with a view towards elucidating how segregating variation is maintained in natural populations. Here, we utilize a diallel design to explore the genetic architecture of ovariole number and thorax length in nine isogenic lines derived from a natural population. The full diallel design allows the estimation of general combining ability (GCA), specific combining ability (SCA), and also describes variation due to reciprocal effects (RGCA and RSCA). Ovariole number and thorax length differed with respect to their genetic architecture, reflective of the independent selective forces acting on the traits. For ovariole number, GCA accounted for the majority (67.3%) of variation segregating between the lines, with no evidence of reciprocal effects or inbreeding depression; SCA accounted for a small percentage (3.9%) of the variance, suggesting dominance variation; no reciprocal effects were observed. In contrast, for thorax length, the majority of the non-error variance was accounted for by SCA (17.9%), with only one third as much variance (6.2%) due to GCA. Interestingly, RSCA (nuclear-extranuclear interactions) accounted for slightly more variation (7.5%) than GCA in these data. Thus, genetic variation for thorax length is largely in accord with predictions for a fitness trait under directional selection: little additive genetic variation and substantial dominance variation (including a suggestion of inbreeding depression); while the mechanisms underlying the maintenance of variation for ovariole number are more complex.

#### **48. MicroRNA regulation of murine limb development**

Maatouk DM<sup>1</sup>, McManus MT<sup>2</sup>, Harfe BD<sup>1,\*</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup>Diabetes Center, University of California at San Francisco, San Francisco, CA

MicroRNAs (miRNAs) encode short untranslated mRNAs that have been shown to be essential for the normal development of several organisms. miRNA transcripts are initially processed to produce a precursor hairpin transcript of ~70-80 nt in length (pre-miRNA). The hairpin pre-miRNA is then further processed by the Dicer enzyme, producing a mature ~21 nt miRNA. Mature miRNAs regulate gene expression by binding to target sequences resulting in mRNA degradation or translational inhibition. There are over 250 miRNAs encoded in the mammalian genome, however only a few have been assigned functions. We are interested in uncovering the roles of miRNAs during murine limb development. Mice null for Dicer die by 7.5 dpc, well before limbs form. To investigate the roles of miRNAs at later developmental stages we have generated a conditional null allele of Dicer. Previously, we found that removal of Dicer specifically from the limb mesoderm results in loss of miRNA processing, increased cell death and abnormal gene

expression. To further investigate the role of miRNAs in limb development we are using microarray technology to uncover target genes that are misregulated upon loss of mature miRNAs. Loss of miRNAs may directly or indirectly influence gene expression. To determine which genes obtained in our screen are direct targets of miRNAs, genes will be screened for known miRNA binding sites. By identifying genes which are direct targets of miRNAs our studies will help elucidate the roles of miRNAs during vertebrate limb development.

#### **49. Intrachromosomal interactions in the imprinted Angelman/Prader-Willi Syndrome domain**

Heggstad AD<sup>1</sup>, Dostie J<sup>2</sup>, Dennis TR<sup>3</sup>, Dekker J<sup>2</sup>, Rodríguez-Jato S<sup>1</sup>, Gray BA<sup>3</sup>, Zori RT<sup>3,\*</sup>, Driscoll DJ<sup>3,\*</sup>, Yang TP<sup>1,3,4,\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA

<sup>3</sup>Department of Pediatrics, University of Florida, Gainesville, FL

<sup>4</sup>Center for Mammalian Genetics, University of Florida, Gainesville, FL

The Angelman and Prader-Willi Syndrome (AS/PWS) domain is composed of at least eight imprinted genes that are coordinately regulated by a bipartite imprinting center (IC) located at the 5' region of *SNURF-SNRPN*. Our laboratory has previously postulated that the PWS-IC coordinately stimulates transcription of multiple genes on the paternally-inherited chromosome through formation of an active chromatin hub. To test this hypothesis, we have examined the long-range intrachromosomal interactions within the AS/PWS domain. Because the paternally-expressed genes *MKRN3*, *MAGEL2*, and *NDN* are localized in a cluster greater than 1 Mb away from the PWS-IC we were able to use fluorescence *in situ* hybridization to visualize the long-range spatial organization of the region. We observed a difference in the long-range intrachromosomal association of the distal imprinted cluster and the PWS-IC. There is a preferential association of the PWS-IC and the chromosomal region containing *MKRN3*, *MAGEL2*, and *NDN* on the paternally-inherited allele. To examine specific long-range interactions at higher resolution, we performed Chromosome Conformation Capture (3C) on the AS/PWS domain. 3C analysis of cultured lymphoblasts demonstrated a strong long-range association between the PWS-IC and the *MKRN3* locus only of the distal genes preferentially on the paternally-inherited allele. We postulate that the interaction between the PWS-IC and the *MKRN3* locus represents a constitutive loop that is formed in cells that express the distal genes at low levels, such as cultured lymphoblasts, and this loop may be involved in maintaining the paternal epigenotype in somatic cells. In cells which express the distal genes at high levels we predict that there will be an association between the PWS-IC and all three distal genes on the paternally-inherited chromosome, resulting in the formation of an active chromatin hub which underlies the coordinate regulation of these imprinted genes.

## 50. An efficient high-throughput *in situ* hybridization method and its application in The Floral Genome Project

Zheng ZG<sup>1</sup>, Liu L<sup>2</sup>, Guan HX<sup>1</sup>, Grey PH<sup>1</sup>, Farmerie W<sup>2,\*</sup>, Oppenheimer DG<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Florida, Gainesville, FL

<sup>2</sup>Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

A major component of evolutionary developmental genetics is the analysis of gene expression patterns in multiple species. Microarrays are typically used to analyze gene expression patterns across multiple species. One disadvantage of microarrays is that expression is determined only for tissues from which appropriate quantities of RNA can be isolated. Expression in different cell types within organs cannot be resolved. *In situ* hybridization offers cellular resolution, but is laborious requiring at least several weeks to generate results for a single gene. To utilize *in situ* hybridization for multiple genes in several different species, we optimized the traditional *in situ* hybridization protocol, and streamlined the design and construction of single-stranded RNA probes. Using our high-throughput protocol, a single person can generate *in situ* results for 25 to 50 genes in one week. Our largest timesaving is the result of two main changes to the traditional protocol. First, by using an automatic tissue processor and commercially available embedding molds and rings, hundreds of tissue samples can be processed in parallel. Second, we designed a computer program to choose primers that will amplify unique sequences for each gene to be analyzed. Also, the addition of a T7 promoter to one of the primers allows the PCR template to be used as a template for *in vitro* transcription. Using our optimized protocol, we tested this approach on several hundred genes from more than 20 families in *Arabidopsis*, *Eschscholzia*, *Persea*, *Saruma*, *Cucumis* and *Nuphar*. The results of our gene expression analysis will be presented.

## 51. Inducible control of gene expression to study the function of DNA gyrase in the malaria parasite

Bonilla TD<sup>1</sup>, Bonilla JA<sup>1</sup>, Yowell CA<sup>1</sup>, Rowe TC<sup>2</sup>, Dame JB<sup>1,\*</sup>

<sup>1</sup>Department of Pathobiology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL

Malaria is one of the greatest threats to global public health. The number of effective antimalarial drugs used to treat this disease is dwindling, and few replacement compounds are under development. A goal of our research is to identify essential enzymes of the parasite that differ substantially from those of the host that may serve as novel drug targets. The genome of *Plasmodium falciparum*, the principal causative agent of human malaria, encodes genes that are homologous to those of bacterial gyrA and gyrB subunits of DNA gyrase. DNA gyrase is without a counterpart in the human host and is the target of fluoroquinolone antibiotics. The putative malarial DNA gyrase is predicted to be localized in the apicoplast, an organelle that was derived from an ancestral algal endosymbiont. To characterize the function of the DNA gyrase-like enzyme in *P. falciparum*, we have examined the molecular and cytological response of *P. falciparum* treated with the fluoroquinolone, ciprofloxacin. Proliferation of *P. falciparum in vitro* is inhibited by ciprofloxacin, and cessation of cell proliferation correlates with the loss of plastid DNA copies. Efforts are currently underway to develop inducible gene-knockdown technologies to correlate loss of DNA gyrase function with loss of parasite viability. Tet-regulated gene expression and high-efficiency transposon-based transformation in *P. falciparum* are now feasible, and we have developed a strategy combining these new techniques to: 1) regulate the expression of antisense RNA to interfere with protein translation, 2) regulate the expression of defective enzyme subunits that are designed to introduce dominant-negative mutations, and 3) regulate

the *in situ* expression of endogenous DNA gyrase to conditionally turn off the transcription of one of the subunit genes. The putative *Plasmodium* DNA gyrase, if found essential to parasite viability, provides a unique and feasible target for antimalarial drug design.

## **52. Targeted disruption of the 16 kb locus encoding the four digestive vacuole plasmepsins of the human malaria parasite, *Plasmodium falciparum***

Bonilla JA, Bonilla TD, Yowell CA, Dame JB\*

Department of Pathobiology, University of Florida, Gainesville, FL

Four of the ten aspartic proteases (plasmepsins) encoded by the *P. falciparum* genome are localized in the digestive vacuole. These enzymes are involved in the digestion of hemoglobin as the parasite matures within the erythrocyte. Since the digestion of hemoglobin is a unique, presumably essential function of the parasite and the parasite is killed by aspartic protease inhibitors, the digestive vacuole plasmepsins have been proposed to be targets for the development of novel antimalarial drugs. Single-plasmepsin knockout mutants of each of the four plasmepsins demonstrated that none of the plasmepsins individually are essential for parasite growth in the asexual blood stage and suggested that there is functional redundancy among the digestive vacuole plasmepsins. The amount of hemoglobin digested by the knockout mutants was not significantly different from the parental parasite line as measured by hemozoin accumulation. Further, the digestion of hemoglobin was not accomplished by up-regulating transcription or increasing accumulation of the three remaining digestive vacuole plasmepsins. Subsequent studies involving the creation of knockouts lacking two, three or all four digestive vacuole plasmepsins have expanded our understanding of their role in hemoglobin digestion. The creation of a knockout mutant parasite line lacking all four plasmepsins that are normally localized in the digestive vacuole suggests that *P. falciparum* has a redundant mechanism for digesting hemoglobin involving multiple classes of proteases. Our data suggest that the digestive vacuole plasmepsins are not the primary targets of aspartic protease inhibitors. Potential functions of the digestive vacuole plasmepsins in other parts of the parasite lifecycle are being investigated using the collection of knockout parasite lines now available. Inducible gene-knockdown approaches are being developed in our laboratory to identify other plasmepsins that may be the target of killing by aspartic protease inhibitors.

## **53. Significant association between endothelin receptor A haplotype and death or heart transplantation in heart failure patients**

Davis HM, Shin J, Langae TY\*, Gong Y, Johnson JA\*

Center for Pharmacogenomics and Department of Pharmacy Practice, University of Florida, Gainesville, FL

Background: Impaired cardiac function mediates the activation of compensatory neurohormonal mechanisms that are involved in the progression of heart failure (HF). Plasma levels of endothelin, the most potent human vasoconstrictor, are increased in patients with HF. Endothelin receptor A (EDNRA) is the predominant isoform through which endothelin exerts its effects. Our aim is to determine whether the H323H C/T and 1363 C/T polymorphisms of EDNRA are associated with adverse outcomes defined as heart transplantation or death in patients with HF.

Methods: A study population of 227 patients enrolled from a HF clinic who were followed for first adverse outcome every six months for up to four years were genotyped for the H323H C/T and 1363 C/T polymorphisms of the EDNRA gene. HAP software was used to determine haplotypes.

Cox proportional hazard regression was modeled with genotypes, haplotypes and outcomes, adjusting for non-genetic predictors.

Results: Seventy-eight patients received a heart transplant or died within the median 2.5-year follow-up period. Beta-blocker use was 81% and ACE inhibitor/angiotensin receptor blocker use was 95% within the population at baseline. EDNRA CT haplotype (Hazard ratio (HR) 3.70, 95% CI 1.28-10.70), NYHA functional class (HR 2.39, 95% CI 1.69-3.37) and male gender (HR 2.25, 95% CI 1.28-3.96) are associated with increased risk for adverse outcomes. Conversely, higher creatinine clearance (HR 0.98, 95% CI 0.98-0.99), higher serum sodium concentration (HR 0.92, 95% CI 0.87-0.98) and use of an ACE inhibitor/angiotensin receptor blocker (HR 0.28, 95% CI 0.11-0.70) were associated with decreased adverse outcomes. Neither of the genotypes alone was significantly associated with adverse outcomes.

Conclusion: Combined with established HF risk factors, the EDNRA CT haplotype may increase risk for heart transplantation or death in HF patients receiving modern pharmacotherapy.

#### **54. An incidental EST library for a polyploid oligochaete**

Price DA<sup>1</sup>, Tulsian JO<sup>1</sup>, Saunders ME<sup>1</sup>, Fend SV<sup>2</sup>

<sup>1</sup>Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, FL

<sup>2</sup>U.S. Geological Survey, Menlo Park, CA

Since the lophotrochozoa (mollusks/annelids) are relatively underrepresented in GenBank, we are keen to document an "incidental" worm EST library unintentionally created when the whole body of a frog (*Xenopus tropicalis*), which had been fed live oligochaete worms (*Lumbriculus variegatus*), was used as the source of mRNA for construction of cDNA libraries for EST sequencing. This incidental worm library consists of about 5700 ESTs that could not be identified as originating from *Xenopus tropicalis* by comparison to the sequenced *X. tropicalis* genome. Some of these 5700 ESTs are clearly lophotrochozoan sequences based on their similarity to known molluscan or annelid sequences in GenBank, but others show as much similarity to vertebrate as any known invertebrate sequences. Of the nine problematic sequences we have experimentally examined so far, all are of worm origin.

*Lumbriculus variegatus* is a complex species cluster. Cytogenetic studies on Danish populations showed that there are sexually reproducing even ploidy strains as well as asexual odd ploidy ones. The particular strain represented in the incidental library, "California Blackworms" comes from a commercial supplier of live fish food in California; this strain is probably an asexual pentaploid. There is extensive sequence variation within the DNA prepared from the whole body of an individual worm from this population, and since the variation is not uniform, we think that California Blackworms may be hybrids (allopolyploids). We are comparing gene sequences – both nuclear and mitochondrial – of worm populations from various locations. We have found three different (5% or more) 16S fragment sequences from US worms. Two of these match 16S sequences reported from Eurasian populations. The third has been found in only one population so far – a wild California population (Summit Lake in the Sierra Nevada) and could be ancestral to the other two. We are in the process of comparing nuclear gene sequences from these different populations.

## **55. A novel role for green light in plant shade-avoidance syndrome**

Maruhnich SA, Folta KM\*

Plant Molecular and Cellular Biology Program and Horticultural Sciences Department, University of Florida, Gainesville, FL

Light is a critical signal for seedling development. Plants receive different light qualities and quantities depending on their environment which lead to changes in morphology, biochemistry, and gene expression. Red and blue wavelengths, enriched in open sunlight or an over-story of a canopy, are important for photosynthesis, and seedlings interpret these energies as signals to rapidly develop. Far-red wavelengths, which are present in high quantities in the under-story of a canopy or in densely packed fields, promote shade-avoidance responses. Green light has been considered generally ineffective at inducing developmental change. However, here we demonstrate that green light has profound effects on remodeling seedling and plant stature; including effects on early photomorphogenic development and shade-avoidance, and that the green shade-avoidance response is likely mediated by a system which is genetically separate from known light sensing systems.

## **56. Molecular characterization of a carbonic anhydrase isoform in *Hydrilla*, an aquatic facultative C<sub>4</sub> plant**

Rao VS, Rao SK, Reiskind JB, Bowes G\*

Department of Botany, University of Florida, Gainesville, FL

Carbonic anhydrase (CA, EC 4.2.1.1) is an enzyme that catalyzes the interconversion of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> in living systems. In plants CA plays a role in many important physiological functions including photosynthesis and respiration. In C<sub>4</sub> photosynthesis CA provides HCO<sub>3</sub><sup>-</sup> to phosphoenolpyruvate carboxylase in the cytosol of mesophyll cells. CA isoforms are distributed among various cellular compartments, and may have differing roles in photosynthesis from one another. *Hydrilla verticillata* (L.f.) Royle is a facultative C<sub>4</sub> NADP-ME submersed monocot with an inducible C<sub>4</sub>-based carbon concentrating mechanism (CCM). Under stress conditions of high light, temperature and O<sub>2</sub>, and low CO<sub>2</sub>, a C<sub>4</sub> system is induced. Studies in this laboratory are underway to identify and localize the CA isoforms in *Hydrilla* in order to pinpoint its specific role in the CCM. Employing RT-PCR techniques with degenerate primer pairs designed from published CA sequences, a partial cDNA (hvca1) of 0.7 kb encoding a putative *Hydrilla* CA was obtained. Northern analysis performed using hvca1 as a probe indicated the presence of 1.1 kb transcript. A time course study conducted over 48 hours showed that it was overexpressed during C<sub>4</sub> cycle. Experiments are underway to obtain the full-length version of the above sequence and to characterize it further. (Supported by USDA NRICGP 2002-35318-12540.)

## **57. Gene expression in primordial germ cell development**

Kellam LD, Maatouk D, Resnick JL\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Primordial germ cells (PGCs) play a significant role in the development of an organism as they ensure that an animal can procreate and pass genetic information onto the next generation. In mammals, PGCs have a complex developmental pathway, originating outside the embryo and actively migrating to the genital ridges where they will become mature gametes. It is believed that both male and female PGCs follow a default pathway of development until XY embryos

receive signals from the testis cords inhibiting meiosis. Our lab is interested in this sex common period of PGC development. While little is known about this sex common state of development, studies using the germ cell marker GCNA1 in culture have shown that PGCs do not require the environment of either the embryo or the nascent gonads to continue their development. This finding and others suggests that PGCs develop according to a cell intrinsic program. To further investigate this autonomous development, our lab sought to determine the factors governing regulation of genes that behave similarly to GCNA1. In trying to determine the minimal promoter region from these genes through transfection experiments in cell culture, we found a region that appeared to actually inhibit the expression of the synaptonemal complex protein gene Scp3 in somatic cells. In binding assays, this sequence shows band shifts in nuclear extracts from somatic tissues but not in testis extracts. Sequence analysis shows this region to contain binding sites for the transcription factor E2F-6. The binding site for this family of transcription factors has been shown to be regulated by CpG methylation. It is believed that expression of post-migratory genes in germ cells is coordinate with the methylation status of the CpG island surrounding the first exon, however identifying an upstream inhibitory element controlled by methylation presents a possible back-up mechanism for regulating the Scp3 locus in somatic cells.

### **58. Mechanisms of gene silencing in the Prader-Willi and Angelman syndrome region in mice**

Johnstone KA, DuBose AJ, Futtner CR, Peery EG, Resnick JL\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Chromosome 15q11-13 contains a cluster of imprinted genes involved in Prader-Willi syndrome (PWS) and Angelman syndrome (AS). Imprinting in the region is under the control of a bipartite imprinting centre (IC), composed of the Prader-Willi syndrome IC (PWS-IC), which activates paternal gene expression in the region, and the AS-IC, which is thought to silence the PWS-IC in the maternal germ line.

In common with several other imprinting control regions, the PWS-IC contains a germline differentially methylated region (DMR) acquired during female gametogenesis. The paternally expressed genes in the region are also associated with DMRs, with the silenced maternal allele carrying the methylation imprint. While DMRs are often associated with imprinted genes, there are also several paternally repressed genes for which no DMR has been identified. Instead these genes are frequently associated with a paternal antisense transcript and some antisense transcripts have been shown to silence genes in *cis*.

We are investigating both methylation and antisense transcription as mechanisms of silencing in the PWS/AS region by using the mouse as a model system.

### **59. A low protein diet during pregnancy alters DNA methylation**

Kiefer CM<sup>1</sup>, Haafiz A<sup>2</sup>, Bouldin CM<sup>1</sup>, Brant JO<sup>1</sup>, Novak DA<sup>2</sup>, Yang TP<sup>1,2,3,\*</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pediatrics, University of Florida, Gainesville, FL

<sup>3</sup>Center for Mammalian Genetics, University of Florida, Gainesville, FL

Nutrient deprivation *in utero* has been demonstrated in both human and animal models to have adverse long-term effects on the metabolic and physiological states of offspring, including increased incidence of glucose intolerance and hypertension as adults. However, the mechanisms

by which increased disease susceptibility may arise as a consequence of dietary restriction during gestation are unclear. One possibility is that epigenetic changes in the placenta and/or fetus in response to maternal malnutrition could lead to stable aberrant regulation of genes important for normal placental and fetal development. This is consistent with the growing number of reports linking altered dietary intake of protein or folic acid to changes in DNA methylation, a principal mechanism of epigenetic regulation in mammals. To investigate this possibility, we have examined the effects of a low protein diet during pregnancy on DNA methylation patterns of imprinted genes in the rat placenta. Timed-pregnant Sprague-Dawley rats were fed either a normal protein diet (NPD) or an isocaloric low protein diet (LPD) from day five of pregnancy until they were sacrificed at 19 days of gestation. DNA methylation levels and patterns within differentially methylated regions (DMR) of three well-characterized imprinted genes, *Igf2*, *H19*, and *Snrpn*, were analyzed in placental tissue by sodium bisulfite genomic sequencing. Analysis of these DMR's showed a significant loss of DNA methylation on the normally hypermethylated allele in response to the LPD. However, methyl-acceptance assays showed there were no significant changes in genome-wide methylation levels in response to LPD. Analysis of changes in overall mRNA levels of each imprinted gene in response to LPD yielded significant changes in expression only of the *Snrpn* gene, but allele-specific expression remains to be investigated. These studies indicate that maternal dietary restriction could impact placental growth and development by heritably altering DNA methylation patterns and regulation of imprinted genes.

#### **60. The isolation of an ethylene-regulated gene involved in the synthesis of the floral volatile isoeugenol in *Petunia x hybrida***

Dexter RJ<sup>1</sup>, Loucas HM<sup>1</sup>, Tieman DM<sup>2</sup>, Klee HJ<sup>2</sup>, Clark DG<sup>1</sup>

<sup>1</sup>Department of Environmental Horticulture and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

<sup>2</sup>Department of Horticulture and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Floral aroma is comprised of volatile organic compounds (VOCs) whose synthesis is dependent on highly regulated genes expressed in the flower of *Petunia x hybrida*. Using cDNA microarray analysis, a group of genes with high expression in the flower were identified and screened for involvement in the synthesis of VOCs. RNAi induced gene silencing led to the identification of one gene (*PhIST*) shown to be involved in the synthesis of the floral volatile isoeugenol. This gene coded for a putative 454 amino acid protein with high homology to a group of unknown transferases in *Arabidopsis*. Expression analysis showed that *PhIST* is both spatially and temporally regulated with highest expression in the leaves and petal limb. Treatment with exogenous ethylene resulted in decreased *PhIST* expression throughout the flower compared to levels in *etr1-1* ethylene insensitive flowers. After fertilization, endogenous ethylene synthesis in the flower resulted in a similar decrease in expression. Physiological significance of this pattern of expression will be discussed.

## **61. Transcription of the *ves1 $\alpha$* gene associated with rapid, clonal antigenic variation in *Babesia bovis* appears to be monoallelic**

Drummond PB<sup>1</sup>, Allred DR<sup>1,2,\*</sup>

<sup>1</sup>Department of Pathobiology, University of Florida, Gainesville, FL

<sup>2</sup>Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL

*Babesia bovis* establishes persistent infections of long duration in cattle. Current evidence strongly implicates the heterodimeric IRBC surface antigen, variant erythrocyte surface antigen 1 (VESA1), as a parasite-derived ligand mediating both rapid antigenic variation and cytoadhesion of infected red blood cells (IRBC) to the microvascular endothelium. The VESA1a subunit is encoded by *ves1 $\alpha$*  members of the *ves* multigene family, but previous work was ambiguous regarding the variety of *ves1 $\alpha$*  genes transcribed by individual parasites. To address this question, we applied RT-PCR using "universal" primers annealing to conserved sequences flanking two highly variant regions of the *ves1 $\alpha$*  gene: the "cysteine and lysine-rich domain" (CKRD; flanking intron 2) and the "variant domain conserved sequences" 1 and 2 subdomains (VDCS1 and 2). The universality of the CKRD-flanking primers has been determined on gDNA sequences. In pilot experiments, RT-PCR was performed on bulk RNA collected from asynchronous C9.1 clonal line cultures; amplification products were cloned and sequenced. In contrast with earlier experiments suggesting rapid, significant alterations *in vitro* (Allred *et al.*, 2000, *Mol Cell* 5:153), *ves1 $\alpha$*  transcripts were overall uniform in sequence with only minor variation. Observed variations include occasional single-nucleotide point mutations and a minor proportion (10- 20%) that had undergone very limited segmental sequence replacements. These results strongly suggest that *ves1 $\alpha$*  transcription, like that of variant antigens of many species, is monoallelic and that *in vitro* variation occurs slowly. To answer this question unambiguously and to determine the reasons for disagreement with earlier results, the approaches of single-cell RT-PCR and nuclear run-on probing of *ves1 $\alpha$*  gene arrays will be applied. (Supported by NIH grant #R01 AI55864.)

## **62. Evolutionary changes in the copy number of a family of disease resistance genes in the legume *Phaseolus vulgaris***

Vallejos CE, Jones V

Department of Horticultural Sciences and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Resistance to a group of potyviruses in *Phaseolus vulgaris*, the common bean, is controlled by a dominant allele at the I locus. Genetic and molecular characterization of the dominant allele has revealed that it extends over a distance in excess of 425 Kb, and comprises a large number (~24) of TIR-NBS-LRR sequences. In addition, three independent single copy TIR loci have been detected in cultivated accessions. A survey of wild accessions extending from northern Argentina to northwestern Mexico shows a geographical gradient in copy number of TIR-related sequences. Accessions at the northern and southern extremes of the distribution display between two and three copies of TIR sequences, while those from northern Ecuador to central Mexico display a large and diverse number of copies. These patterns of hybridization suggest that this family of TIR sequences has undergone a series of tandem and ectopic duplications during evolution and domestication.

### **63. Over-expression of a soybean vegetative storage protein gene in bahiagrass (*Paspalum notatum* var. Flugge)**

Luciani G<sup>1</sup>, Altpeter F<sup>1,2,\*</sup>, Wofford DS<sup>1,\*</sup>

<sup>1</sup>Agronomy Department, University of Florida, Gainesville, FL

<sup>2</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Bahiagrass (*Paspalum notatum* var. Flugge) is the predominant forage grass in Florida grown on more than five million acres. Bahiagrass is very persistent and tolerates over-grazing, however its forage quality is not very high. This project aims at the molecular improvement of the leaf protein composition by over-expression of the vspB gene from soybean. The vspB gene encodes a soybean vegetative storage protein containing 7% lysine. An expression vector where the vspB gene is regulated by the constitutive and heat-responsive ubi1 promoter and intron from corn, and also includes a KDEL ER-retention signal for enhancing protein accumulation and a c-myc tag to simplify expression analysis was constructed and stably introduced into bahiagrass by biolistic gene transfer (Altpeter and James, 2005, *Intern Turfgrass Res J* 10:485-9). Transgene expression data from regenerated transgenic bahiagrass plants will be presented.

### **64. Molecular analysis of a polydactylous chicken**

Bouldin CM<sup>1</sup>, Cohn MJ<sup>2,\*</sup>, Harfe BD<sup>1,\*</sup>

<sup>1</sup> Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

<sup>2</sup> Department of Zoology, University of Florida, Gainesville, FL

Spontaneous mutations in vertebrate organisms have been successfully used to elucidate information about limb patterning. We analyzed a spontaneous mutant of the species *G. gallus* commonly referred to as "Dorkings" in an attempt to identify the molecular cause behind the formation of a polarized extra hindlimb digit. Two signaling centers have been implicated in producing polarized ectopic digits: the apical ectodermal ridge (AER) and the zone of polarizing activity (ZPA). The AER forms through the condensation of fibroblast growth factors 4 and 8 (Fgf4 and Fgf8) expressing ectodermal cells located on the distal edge of the limb bud. In Dorking embryos, we have found that both Fgfs are ectopically expressed anteriorly. The ZPA is defined as distal, posterior mesenchymal tissue that expresses the secreted protein sonic hedgehog (Shh). This region of the limb has been implicated in specifying proper patterning of the forming digits. In Dorking embryos, we did not observe any ectopic Shh expression in the limb. In addition, the Shh target gene Patched 1 (Ptc1) was not ectopically expressed in Dorking embryos. These data suggest that expanded Fgf expression may cause polydactyly in Dorking embryos without activation of the Shh-signalling pathway. Currently, we are directly testing the role Fgfs play in ectopic digit formation by recombining Dorking ectoderm with wild type mesoderm in limb grafting experiments.

### **65. Determination of Atp10a imprinting status**

DuBose AJ, Johnstone KA, Resnick JL\*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

ATP10A is a preferentially maternally expressed gene in humans that is under the control of the Angelman Syndrome imprinting center. The imprinting status of the orthologous gene Atp10a (Atp10c, pfatp) is unknown. A deletion has been created that includes Atp10a and mice inheriting this deletion maternally become overweight while mice inheriting the deletion

paternally do not which suggests imprinted expression. There have been conflicting reports at the molecular level regarding the imprinting status of Atp10a. One lab found that Atp10a is biallelically expressed in all tissues examined. They also found that there is no differential CpG methylation in the promoter region and no antisense transcripts of Atp10a. Another lab published that Atp10a is predominantly expressed from the maternal allele in the hippocampus and olfactory bulb. We are currently assessing expression from each parental allele using crosses between C57BL/6J and congenic C57BL/6J Cast c7 mice and an RFLP.

**66. Arsenic hyperaccumulation in *Pteris vittata*: a high throughput method to identify fern arsenic resistance determinants based on cDNA expression cloning in *Escherichia coli***

Rathinasabapathi B<sup>1,\*</sup>, Wu S<sup>1</sup>, Garcia J<sup>1</sup>, Rivoal J<sup>2</sup>, Ma LQ<sup>3</sup>

<sup>1</sup>Horticultural Sciences Department, University of Florida, Gainesville, FL

<sup>2</sup>Institut de Recherche en Biologie Vegetale, Department de Sciences Biologiques, Universite de Montreal, Canada

<sup>3</sup>Department of Soil and Water Science, University of Florida, Gainesville, FL

The Chinese brake fern *Pteris vittata* has an extraordinary ability to hyperaccumulate arsenic. To identify the genes involved in arsenic resistance in *P. vittata*, an expression cloning strategy was employed. An expression cDNA library was made from RNA isolated from fronds of plants exposed for one week to 0.6 mM sodium arsenate in the irrigation solution. Phagemids from the cDNA library were introduced into *E. coli* and the cells were selected on medium containing lethal doses (2.5 or 5 mM) of sodium arsenate. The cDNA clones were identified based on their ability to impart arsenate resistance to *E. coli* BM25.8 or XL-1 Blue. Analysis of the deduced amino acid sequences of cDNA clones identified novel arsenic resistance determinants including cytosolic triosephosphate isomerase, ribosomal proteins and proteins highly homologous to plant proteins currently annotated with no known function.

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**Rao SK.** Poster #39. Comparative regulatory behavior of recombinant C<sub>3</sub> and C<sub>4</sub> PEPC isoforms in *Hydrilla*

**Rao VS.** Poster #56. Molecular characterization of a carbonic anhydrase isoform in *Hydrilla*, an aquatic facultative C<sub>4</sub> plant

**Rathinasabapathi B.** Poster #66. Arsenic hyperaccumulation in *Pteris vittata*: a high throughput method to identify fern arsenic resistance determinants based on cDNA expression cloning in *Escherichia coli*

**Rock JR.** Poster #4. Identification and characterization of a novel gene expressed exclusively in the ZPA in the vertebrate limb

**Rodriguez-Jato S.** Poster #10. Deletion of the endogenous *HPRT* promoter on the active X chromosome leads to complex alterations of histone modification patterns

**Samols MA.** Poster #3. Cloning and identification of a micro-RNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus

**Sandhu S.** Poster #36. Marker assisted evaluation of transgene containment in apomictic bahiagrass

**Stalvey M.** Poster #7. Modeling cystic fibrosis related diabetes in CFTR-deficient mice: effects of CFTR genotype on glycemic control after sub-lethal beta cell injury or pulmonary sensitization and challenge

**Telonis-Scott M.** Poster #47. Genetic architecture of two fitness-related traits in *Drosophila melanogaster*: ovariole number and body size

**Tombolato DCM.** Poster #15. Gene-pair haplotypes: novel, complex markers for linkage mapping in octoploid strawberry

**Tseung CW.** Poster #34. The maize *Viviparous10* locus encodes the *Cnx1* gene required for molybdenum cofactor biosynthesis

**Vallejos CE.** Poster #62. Evolutionary changes in the copy number of a family of disease resistance genes in the legume *Phaseolus vulgaris*

**Van Vliet K.** Poster #44. Proteolytic mapping of the adeno-associated virus capsid

**Varma TSN.** Poster #14. Cloning and characterization of an *R*-gene from peanut homologous to the tomato *Mi* nematode resistance gene

**Winslow AR.** Poster #37. Isolation and characterization of photoperiodic flowering regulators from *Fragaria*

**Yu F.** Poster #30. Phylogeny of dinoflagellates inferred from mitochondrial cytochrome oxidase I and application of oligonucleotide probes for the identification of *Karenia brevis*

**Zheng ZG.** Poster #50. An efficient high-throughput *in situ* hybridization method and its application in The Floral Genome Project

## FG2005 Registrants

Agharkar, Mrinalini. Agronomy/PMCB. mrinal@ufl.edu

Ahmed, Chulbul M. Microbiology and Cell Science. ahmed1@ufl.edu

Ai, Lingbao. Biochemistry and Molecular Biology. ailingbao@hotmail.com

Al-Khedery, Basima. Pathobiology. Al-KhederyB@mail.vetmed.ufl.edu

Allen, Bill. Bioethics, Law and Medical Professionalism. allen@chfm.ufl.edu

Allred, David. Pathobiology. allredd@ufl.edu

Almira, Ernesto. Interdisciplinary Center for Biotechnology Research. eca@ufl.edu

Amelio, Antonio. Molecular Genetics and Microbiology. amelio@ufl.edu

Andersen, Felicie. Biochemistry and Molecular Biology. felimus@ufl.edu

Andino, Lourdes. Molecular Genetics and Microbiology/IDP. landino@ufl.edu

Baer, Charles. Zoology. cbaer@zoo.ufl.edu

Bailey, Lynn. Food Science and Human Nutrition. lbbailey@ifas.ufl.edu

Baker, Henry. Molecular Genetics and Microbiology. hvbaker@ufl.edu

Barko, Tim. Zoology. hippasus@ufl.edu

Batich, Chris. Materials Science and Engineering. cbati@mse.ufl.edu

Baudis, Michael. Pediatric Hematology/Oncology. mbaudis@ufl.edu

Benner, Steven. Chemistry. benner@chem.ufl.edu

Berns, Kenneth I. U.F. Genetics Institute. berns@mbi.ufl.edu

Bloom, David. Molecular Genetics and Microbiology. dbloom@ufl.edu

Bocock, Philip. Plant Molecular and Cellular Biology. pnbocock@ufl.edu

Bonde, Robert. U.S.G.S. – Sirenia Project/Veterinary Medicine. robert\_bonde@usgs.gov

Bonilla, J. Alfredo. Pathobiology. bonillaf@mail.vetmed.ufl.edu

Bonilla, Tonya D. Pathobiology. bonillat@mail.vetmed.ufl.edu

Borum, Peggy. Food Science and Human Nutrition. prb@ufl.edu

Bouldin, Cortney, Molecular Genetics and Microbiology. cortb@ufl.edu

Bowes, George. Botany. gbowes@botany.ufl.edu

Brant, Jason. Biochemistry and Molecular Biology. jobrant@ufl.edu

Brown, Kevin. Biochemistry and Molecular Biology. kdbrown1@ufl.edu

Bunger, Joshua. Pathology. bungler@ufl.edu

Bungert, Jorg. Biochemistry and Molecular Biology. jbungert@ufl.edu

Bush, Marsha. Biochemistry and Molecular Biology/IDP. mlb123@ufl.edu

Buzgo, Matyas. Botany. mbuzgo@ufl.edu

Byatt, John. Office of Technology Licensing. jbyatt@rgp.ufl.edu

Casella, George. Statistics. casella@ufl.edu

Champ, Karen. Horticultural Sciences. kichamp@mail.ifas.ufl.edu

Chanderbali, Andre. Florida Museum of Natural History. achander@botany.ufl.edu

Chase, Christine. Horticultural Sciences/PMCB. ctdc@ifas.ufl.edu

Chen, Su-Shing. Computer and Information Science and Engineering. suchen@cise.ufl.edu

Clancy, Maureen. Horticultural Sciences/PMCB. clancy@ifas.ufl.edu

Clark, Dave. Plant Molecular and Cellular Biology Program. geranium@ufl.edu

Collamat, Golda. Microbiology and Cell Science. mzktiger@ufl.edu

Connors, Shahnjayla. U.F. Shands Cancer Center. sconners@ufl.edu

Cousins, Robert. Nutritional Sciences. cousins@ufl.edu

Crino, Ondi. Zoology. ocrino@ufl.edu

Dala, Jasbir. Neuroscience. jasbirsdalal@yahoo.com

Dame, John B. Pathobiology. damej@mail.vetmed.ufl.edu

Davis, Heather. Pharmacy Practice/Pharmacogenomics. rocskdav@ufl.edu

Davis, John. Forest Resources and Conservation/PMCB. jmdavis@ufl.edu

Davis, Valerie. Biochemistry and Molecular Biology. vjc@ufl.edu

Daya, Shyam. Molecular Genetics and Microbiology. s1daya@ufl.edu

Demir, Ahu. Chemistry. ahudemir@ufl.edu

DeRuisseau, Lara. Physiological Sciences. LDeRuisseau@phhp.ufl.edu

Dervinis, Chris. Forest Resources and Conservation. cderv@ufl.edu

Dexter, Richard. Plant Molecular and Cellular Biology. rdexter@ufl.edu

Dhingra, Amit. Horticultural Sciences. adhingra@ifas.ufl.edu

Driscoll, Dan. Pediatrics. driscdj@peds.ufl.edu

Drummond, Paul. Pathobiology. drummondp@mail.vetmed.ufl.edu

DuBose, Amanda. Molecular Genetics and Microbiology. ajdubose@ufl.edu

Edwards, Christine. Florida Museum of Natural History/Botany. edwardsc@ufl.edu

Edwards, Thea. Zoology. tedwards@zoo.ufl.edu

Estavillo, Gonzalo. Botany. monsa@ufl.edu

Fajardo, Diego. Horticultural Sciences/PMCB. diegof@ufl.edu

Farmerie, Bill. Interdisciplinary Center for Biotechnology Research. wgf@biotech.ufl.edu

Febres, Vicente. Horticultural Sciences. vjfebres@ifas.ufl.edu

Felsenfeld, Gary. National Institutes of Health. GaryF@intra.niddk.nih.gov

Ferl, Robert. Horticultural Sciences. robferl@ufl.edu

Fields, Rob. Biochemistry and Molecular Biology. crfields@ufl.edu

Flanegan, James. Biochemistry and Molecular Biology. flanegan@ufl.edu

Flotte, Terence R. Pediatrics. flotttr@peds.ufl.edu

Folta, Kevin. Horticultural Sciences. kfolta@ifas.ufl.edu

Fouad, Walid. Agronomy. wfouad@ufl.edu

Futtner, Christopher. Molecular Genetics and Microbiology. cfuttner@ufl.edu

Gallo, Maria. Agronomy/PMCB. mgmea@ifas.ufl.edu

Gandhi, Kunjal. Biology. komal@ufl.edu

Georgelis, Nick. Plant Molecular and Cellular Biology. gnick@ufl.edu

Ghaffari, Guity. Pediatrics. ghaffari@pathology.ufl.edu

Ghivizzani, Steven. Orthopaedics and Rehabilitation/Molecular Genetics and Microbiology. ghivisc@ortho.ufl.edu

Giordani, Nicole. Molecular Genetics and Microbiology. giordani@ufl.edu

Gokirmak, Tufan. Plant Molecular and Cellular Biology. gokirmak@ufl.edu

Gong, Yan. Pharmacy Practice/Pharmacogenomics. gong@cop.ufl.edu

Goodenow, Maureen. Pathology, Immunology, and Laboratory Medicine. goodenow@ufl.edu

Gopalakrishnan, Suhasni. Biochemistry and Molecular Biology. suhasni@ufl.edu

Gouze, Elvire. Orthopaedics and Rehabilitation. gouzee@ortho.ufl.edu

Gouze, Jean-Noel. Orthopaedics and Rehabilitation. gouzej@ortho.ufl.edu

Green, Linda. Interdisciplinary Center for Biotechnology Research. lgreen@biotech.ufl.edu

Greenfield, Warren, III. Orthopaedics and Rehabilitation. greenh@ortho.ufl.edu

Guan, Hexin. Botany. hxguan@hotmail.com

Hadjipanayis, Angela. Molecular Genetics and Microbiology. anghadji@ufl.edu

Hannah, Curt. Plant Molecular and Cellular Biology. Hannah@mail.ifas.ufl.edu

Harfe, Brian. Molecular Genetics and Microbiology. bharfe@mgm.ufl.edu

Harmon, Alice. Botany. harmon@ufl.edu

Hauswirth, Bill. Ophthalmology. hauswrth@eye1.eye.ufl.edu

Heggestad, Arnold. Biochemistry and Molecular Biology. arne@ufl.edu

Herbstman, Deborah. Molecular Genetics and Microbiology. dmh@ufl.edu

Herzog, Roland. Pediatrics. rherzog@ufl.edu

Hirsch, Michael. Industrial Engineering. mjh8787@ufl.edu

Hoang-minh, Lan. Biomedical Engineering. hoang@ufl.edu

Hong, Kwon-Ho. Physiology and Functional Genomics. hong@ufl.edu

Hoy, Marjorie. Entomology and Nematology. mahoy@ifas.ufl.edu

Ishov, Alexander. Anatomy and Cell Biology. ishov@ufl.edu

Jaishankar, Sobha. Research and Graduate Programs. sjaishan@rgp.ufl.edu

James, Victoria. Agronomy. vajames@mail.ifas.ufl.edu

Jia, Jinghua. Molecular Genetics and Microbiology. jhjia@ufl.edu

Johnson, Julie A. Pharmacogenomics/Pharmacy Practice. johnson@cop.ufl.edu

Johnstone, Karen. Molecular Genetics and Microbiology. karenj@ufl.edu

Jones, Kelly. Zoology. kmjones@zoo.ufl.edu

Jones, Valerie. Horticultural Sciences/PMCB. valjones@ifas.ufl.edu

Justilien, Verline. Molecular Genetics and Microbiology. verline@ufl.edu

Kahveci, Tamer. Computer and Information Science and Engineering. tamer@cise.ufl.edu

Karthik-Joseph, John. Horticultural Sciences. jkjoseph@ufl.edu

Kellam, Lori. Molecular Genetics and Microbiology. lkellam@ufl.edu  
Kellogg, Margaret. Physiological Sciences. maggsk@ufl.edu

Khalaf, Abeer. Plant Molecular and Cellular Biology. abeera@ufl.edu

Khalil, Ahmad. Pediatrics. akhalil@ufl.edu

Kilberg, Michael. Biochemistry and Molecular Biology. mkilberg@ufl.edu

Kim, Sangtae. Botany. sangtae@botany.ufl.edu

Kim, Taeyou. Biochemistry and Molecular Biology. kimty@ufl.edu

Kirst, Matias. Forest Resources and Conservation. mkirst@ufl.edu

Klee, Harry. Plant Molecular and Cellular Biology. hjklee@ifas.ufl.edu

Kohn, Andrea. Whitney Laboratory for Marine Bioscience. abk@whitney.ufl.edu

Kuipers, Paul. Pediatric Genetics. kuipepj@peds.ufl.edu

Kuldau, John. Psychiatry. john.kuldau@med.va.gov

Kurenova, Elena. Surgery. kurenev@surgery.ufl.edu

Kwiatkowski, Dacia. Molecular Genetics and Microbiology. dlk82@ufl.edu

Laipis, Philip. Biochemistry and Molecular Biology. plaipis@ufl.edu

Langae, Taimour. Pharmacy Practice/Pharmacogenomics. langae@cop.ufl.edu

Lawrence, Charles. Pre-collegiate Education and Teaching (CPET). lawrence@cpet.ufl.edu

Lee, Sheng-Chien. Agricultural and Biological Engineering. rlee168@ufl.edu

Leonard, Christiana. Neuroscience. leonard@mbi.ufl.edu

Li, Hongying. Statistics hli@stat.ufl.edu

Li, Ju. Animal Sciences. li@animal.ufl.edu

Li, Ning. Interdisciplinary Program in Biomedical Sciences. winterlemon2005@yahoo.com

Li, Zhen. Statistics. zhenli79@ufl.edu

Lin, Nianwei. Interdisciplinary Program in Biomedical Sciences. linnw@ufl.edu

Liu, Jian. Mechanical and Aerospace Engineering. ljian99@ufl.edu

Liu, Li. Interdisciplinary Center for Biotechnology Research. liliu@biotech.ufl.edu

Loda, Rebecca. Interdisciplinary Program in Biomedical Sciences. rloda@ufl.edu

Lu, Jianrong. Biochemistry and Molecular Biology. jrlu@ufl.edu

Luciani, Gabriela. Agronomy. gabilu@ufl.edu

Luger, Karolin. Biochemistry and Molecular Biology, Colorado State University and Howard Hughes Medical Institute. Karolin.Luger@colostate.edu

Lyons, Thomas. Chemistry. lyons@chem.ufl.edu

Maatouk, Danielle. Molecular and Genetics and Microbiology. dmaatouk@ufl.edu

Madzima, Thelma. Plant Molecular and Cellular Biology. tmadzima@ufl.edu

Mah, Cathryn. Pediatrics. cmah@ufl.edu

Manak, Michael. Plant Molecular and Cellular Biology. manak@ufl.edu

Maneval, David. Human Nutrition. drmaneval@mail.ifas.ufl.edu

Mariani, Chris. Neuroscience. marianic@mail.vetmed.ufl.edu

Martin, Marcus. Microbiology and Cell Science. marcus1@ufl.edu

Martino, Ashley. Pediatrics. amartino@ufl.edu

Maruhnich, Stefanie. Plant Molecular and Cellular Biology maruhni@ufl.edu

Matchett, Miranda. Agricultural and Biological Engineering. redbed@ufl.edu

Mauthner, Stephanie. Aging and Geriatric Research. smauthner@aging.ufl.edu

Mayfield, John D. Plant Molecular and Cellular Biology. mayf@ufl.edu

McCarty, Donald. Plant Molecular and Cellular Biology. drm@ufl.edu

McGuire, Peter. Biochemistry and Molecular Biology. pmcguire@biochem.med.ufl.edu

McQuown, Jennelle. Animal Sciences. mcquown@animal.ufl.edu

Mei, Jianguo. Chemistry. jgmei@ufl.edu

Meneses, Claudio. Industrial and Systems Engineering. claudio@ufl.edu

Miles, Cecelia. Zoology. [cmiles@zoo.ufl.edu](mailto:cmiles@zoo.ufl.edu)

Mitchell, Melanie. Interdisciplinary Program in Biomedical Sciences. [mkm25@ufl.edu](mailto:mkm25@ufl.edu)

Miyamoto, Michael. Zoology. [miyamoto@zoo.ufl.edu](mailto:miyamoto@zoo.ufl.edu)

Moore, Gloria. Plant Molecular and Cellular Biology. [gamoore@ad.ufl.edu](mailto:gamoore@ad.ufl.edu)

Moore, Karen. Animal Sciences. [kmoore@animal.ufl.edu](mailto:kmoore@animal.ufl.edu)

Moore, Mariellen. Pharmacy. [marielm@ufl.edu](mailto:marielm@ufl.edu)

Morris, Scott. Plant Molecular and Cellular Biology. [azshark1@ufl.edu](mailto:azshark1@ufl.edu)

Morse, Alison. Forest Resources and Conservation. [ammorse@ufl.edu](mailto:ammorse@ufl.edu)

Moyer, Richard. Dean's Office. [rmoyer@ufl.edu](mailto:rmoyer@ufl.edu)

Muller, Christian. Pediatrics. [cmuller@ufl.edu](mailto:cmuller@ufl.edu)

Mulligan, Connie. Anthropology. [mulligan@anthro.ufl.edu](mailto:mulligan@anthro.ufl.edu)

Muzyczka, Nicholas. Molecular Genetics and Microbiology. [muzyczka@ufl.edu](mailto:muzyczka@ufl.edu)

Nam, Hyun-Joo. Biochemistry and Molecular Biology. [hnam@mbi.ufl.edu](mailto:hnam@mbi.ufl.edu)

Navare, Hrishikesh. Pharmaceutics. [hanavare@ufl.edu](mailto:hanavare@ufl.edu)

Ndjamen, Blaise. Microbiology and Cell Science. [ndjamen@ufl.edu](mailto:ndjamen@ufl.edu)

Neibaur, Isaac. Agronomy. [foundegg@ufl.edu](mailto:foundegg@ufl.edu)

Niu, Yuxin. Pharmacy. [niuyx@ufl.edu](mailto:niuyx@ufl.edu)

Njie, Emalick. Neuroscience. [emalick@ufl.edu](mailto:emalick@ufl.edu)

O'Brien, Brent. Plant Molecular and Cellular Biology. [bob2373@ufl.edu](mailto:bob2373@ufl.edu)

O'Brien, Tom. Biochemistry and Molecular Biology. [tobrien@ufl.edu](mailto:tobrien@ufl.edu)

O'Grady, Kevin. Microbiology and Cell Science. [ogrady@ufl.edu](mailto:ogrady@ufl.edu)

O'Malley, David. Horticultural Sciences. [domalley@ufl.edu](mailto:domalley@ufl.edu)

Oppenheimer, David. Botany. [doppen@botany.ufl.edu](mailto:doppen@botany.ufl.edu)

O'Rourke, Jason. Molecular Genetics and Microbiology. [orourke@ufl.edu](mailto:orourke@ufl.edu)

Ortega, Melissa. Pharmacogenomics. [maortega@ufl.edu](mailto:maortega@ufl.edu)

Ostrow, Gigi. Zoology. [ostrowdg@ufl.edu](mailto:ostrowdg@ufl.edu)

Ouellette, Sara. Animal Sciences. souelle@bellsouth.net

Pacak, Christina. Molecular Genetics and Microbiology. cpacak@gtc.ufl.edu

Pan, Wei. Plant Molecular and Cellular Biology. wpan@ufl.edu

Park, Dongkyoo. Interdisciplinary Program in Biomedical Sciences. dkpark@ufl.edu

Paul, Anna-Lisa. Horticultural Sciences/PMCB. alp@ufl.edu

Pause, Kimberly. Biochemistry and Molecular Biology. kpause@ufl.edu

Peter, Gary. Forest Resources and Conservation. gfpeter@ufl.edu

Pinedo, Pablo. Large Animal Clinical Sciences. PinedoP@mail.vetmed.ufl.edu

Price, David. Whitney Laboratory for Marine Bioscience. daprice@ufl.edu

Price, Elvin. Clinical Pharmaceutical Sciences. eprice@ufl.edu

Pring, Daryl. Plant Pathology/USDA-ARS. drpg@ifas.ufl.edu

Qiu, Kai. Psychiatry. qiuk@ufl.edu

Qiu, Xiaolei. Biochemistry and Molecular Biology. qiuxl@ufl.edu

Quesada, Tania. Plant Molecular and Cellular Biology. tquesada@ufl.edu

Ramdial, Jeremy. Microbiology and Cell Science. turtl84@ufl.edu

Ramirez, Gustavo. Plant Molecular and Cellular Biology. gramirez@ufl.edu

Rao, Srinath. Botany. skrao@ufl.edu

Rao, Vidya. Botany. vsrao@ufl.edu

Rathinasabapathi, Bala. Horticultural Sciences. brath@mail.ifas.ufl.edu

Reed, Sarah. Animal Sciences. reed@animal.ufl.edu

Resnick, Jim. Molecular Genetics and Microbiology. resnick@mgm.ufl.edu

Richards, Eric. Biology, Washington University. richards@biology.wustl.edu

Robertson, Keith. Biochemistry and Molecular Biology. keithr@ufl.edu

Rock, Jason. Molecular Genetics and Microbiology. jrock1@ufl.edu

Samols, Mark. Molecular Genetics and Microbiology. samols@ufl.edu

Sandhu, Sukhpreet. Agronomy. ssandhu@ufl.edu

Sankpal, Umesh. Biochemistry and Molecular Biology. sankpalu@ufl.edu

Santiago, Aleixo. Anatomy and Cell Biology. wzaap123@ufl.edu

Santos, C.P. U.F. Shands Cancer Center. cpsantos@ufl.edu

Saran, Jeetpaul. Orthopaedics and Rehabilitation. jeetpaulsaran@gmail.com

Schmalbach, Priel. Neurobiology. priely@ufl.edu

Schultz, Gregory. Obstetrics and Gynecology. schultzg@obgyn.ufl.edu

Seki, Tsugio. Physiology and Functional Genomics. tseki@ufl.edu

Seron, Terri. Whitney Laboratory for Marine Bioscience. tseron@whitney.ufl.edu

Settles, Mark. Horticultural Sciences. settles@ufl.edu

Shan, Jixiu. Biochemistry and Molecular Biology. shanjx@ufl.edu

Shao, Jiahong. Medicine. shaoj@medicine.ufl.edu

Shirazian, Azadeh. Molecular Genetics and Microbiology. azadeh@ufl.edu

Smith, Emily. Molecular Genetics and Microbiology/IDP. eysmith@ufl.edu

Snyder, Richard. Molecular Genetics and Microbiology/Regenerative Health Biotechnology. rsnyder@gtc.ufl.edu

Soltis, Douglas. Botany. dsoltis@botany.ufl.edu

Soltis, Pam. Florida Museum of Natural History. psoltis@flmnh.ufl.edu

Song, Sihong. Pharmaceutics. shsong@ufl.edu

Srivastava, Arun. Pediatrics. ASRIVASTAVA@GTC.UFL.EDU

Stalvey, Michael. Pediatrics stalvms@peds.ufl.edu

Sullivan, Meredith. Botany. ms79@ufl.edu

Suzuki, Masaharu. Plant Molecular and Cellular Biology. msuzuki@mail.ifas.ufl.edu

Swanson, Maury. Molecular Genetics and Microbiology. mswanson@ufl.edu

Tan, Li. Chemistry. tanli@ufl.edu

Telonis-Scott, Marina. Zoology. mtelonis@zoo.ufl.edu

Tennant, Michele. Health Science Center Libraries/U.F. Genetics Institute. Michele@library.health.ufl.edu

Terada, Naohiro. Pathology, Immunology, and Laboratory Medicine. terada@pathology.ufl.edu

Thiaville, Michelle. Biochemistry and Molecular Biology. mimagee@ufl.edu  
Tirumalaraju, Sivananda Varma. Agronomy. tsnvarma@yahoo.com  
Tombolato, Denise. Horticultural Sciences. de@grove.ufl.edu  
Triplett, Eric. Microbiology and Cell Science. ewt@ufl.edu  
Tseung, Chi-Wah. Horticultural Sciences. ctseung@ufl.edu  
Ucar, Deniz. Molecular Genetics and Microbiology. ucarden@ufl.edu  
Vallejos, C. Eduardo. Horticultural Sciences/PMCB. vallejos@ufl.edu  
Van Vliet, Kim. Molecular Genetics and Microbiology. vanvliet@mbi.ufl.edu  
Vasil, Indra. U.F. Genetics Institute. ikv@ifas.ufl.edu  
Vevea, Jenny. Agricultural and Biological Engineering. jennyvevea@hotmail.com  
Visscher, Anne. Horticultural Sciences. annevisscher@mac.com  
Wang, Bei. Molecular Genetics and Microbiology. Bei@ufl.edu  
Wang, Lin. Chemistry. lwang@chem.ufl.edu  
Wang, Xu. Animal Sciences. wangx@animal.ufl.edu  
Waterland, Robert A. Pediatrics, Baylor College of Medicine. waterland@bcm.tmc.edu  
Watson, Rachael. Orthopaedics/IDP. rswatson@ufl.edu  
Wayne, Marta. Zoology. mlwayne@zoo.ufl.edu  
White, Jason. Microbiology and Cell Science. jmwhite@cfl.rr.com  
Winner, Dane. Animal Sciences. winner@animal.ufl.edu  
Winslow, Ashley. Horticultural Sciences. ashwinslow@yahoo.com  
Wu, Rongling. Statistics. rwu@stat.ufl.edu  
Wu, Yanrong. Chemistry. ellenwu@ufl.edu  
Xavier, Nirmala. Entomology and Nematology. xaviern@ufl.edu  
Xiong, Xi. Agronomy. xixiong@ufl.edu  
Yang, Jia-Ding. Agronomy. yangjiading@yahoo.com  
Yang, Qing. Molecular Genetics and Microbiology. qyang@ufl.edu  
Yang, Thomas. Biochemistry and Molecular Biology. tpyang@ufl.edu

Yu, Fahong. Interdisciplinary Center for Biotechnology Research. fyu@ufl.edu  
Zacharias, David. Neuroscience. daz@whitney.ufl.edu  
Zhang, Chun. Molecular Genetics and Microbiology. chunz@ufl.edu  
Zhang, Hangning. Agronomy. hnzhang@ifas.ufl.edu  
Zhang, Jiejun. Pharmacology. jiejin@ufl.edu  
Zhang, Xiaoguo. Botany. xgzhang@ufl.edu  
Zhang, Yanping. Molecular Genetics and Microbiology. yanp@ufl.edu  
Zhao, Jianfei. Plant Molecular and Cellular Biology. jfzhao@ufl.edu  
Zheng, Zhengui. Botany. patrickz@ufl.edu  
Zhong, Sheng. Biochemistry and Molecular Biology. szhong@ufl.edu  
Zhou, Lei. U.F. Shands Cancer Center/Molecular Genetics and Microbiology. leizhou@ufl.edu  
Zhou, Zhuo. Biochemistry and Molecular Biology. zzhou@ufl.edu  
Zimowska, Grazyna. Entomology and Nematology. gjzimowska@mail.ifas.ufl.edu  
Zori, Robert. Pediatric Genetics. zorirt@peds.ufl.edu