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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 21st 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 2006
Schedule

Wednesday, November 1, 2006

1:00-6:15 p.m.: Check-in — Grand Ballroom Registration Desk, 2nd floor, J. Wayne Reitz Union

2:30-2:45 p.m.: Opening Remarks
Indra K. Vasil, Ph.D., and Kenneth I. Berns, M.D., Ph.D.

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

2:45-3:30 p.m.:
Thomas J. Kelly, M.D., Ph.D.
Director, Sloan-Kettering Institute
DNA replication and cell cycle checkpoints

3:30-4:15 p.m.:
Jeffrey D. Palmer, Ph.D.
Distinguished Professor, Department of Biology, Indiana University
Plant mitochondrial genomes: unexpected bounties of lateral gene transfer

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

Thursday, November 2, 2006

8:00 a.m.-3:00 p.m.: Check-in — Grand Ballroom Registration Desk, 2nd floor, Reitz Union

8:00-8:30 a.m.: Continental Breakfast

Session II Chair: Nick Muzyczka, Ph.D.

8:30-9:15 a.m.:
Steven A. Goldman, M.D., Ph.D.
Professor, Departments of Neurology, Neurosurgery, and Pediatrics,
and Chief of the Division of Cell and Gene Therapy, University of Rochester Medical Center
Isolation, induction and use of adult neural progenitor cells

9:15-9:45 a.m.:
Dennis A. Steindler, Ph.D.
Executive Director, Evelyn F. and William L. McKnight Brain Institute
and Professor, Department of Neuroscience, University of Florida
***Embryonic, adult and cancer stem cells* in vitro *and* in vivo**

9:45 a.m.-10:15 p.m.:
John M. Davis, Ph.D.
Professor, School of Forest Resources and Conservation, University of Florida
Genetic analysis of adaptive traits in forest trees

10:15-10:45 a.m.: Coffee Break

10:45-11:30 a.m.:
Michael S. Waterman, Ph.D.
Professor, Departments of Biological Sciences, Mathematics, & Computer Science, University of Southern California
Whole genome optical mapping

11:30 a.m.-12:00 p.m.:
Julie A. Johnson, Pharm.D.
Professor and Chair, Department of Pharmacy Practice,
and Director, Center for Pharmacogenomics, University of Florida
Pharmacogenomics

12:00-2:00 p.m.: Poster Session and Lunch

Session III Chair: Pamela Soltis, Ph.D.

2:00-2:45 p.m.:
Gerald F. Joyce, M.D., Ph.D.
Professor, Departments of Chemistry & Molecular Biology, The Scripps Research Institute
and Investigator, The Skaggs Institute for Chemical Biology
Genetics* in vitro*: synthesizing the RNA world

2:45-3:15 p.m.:
Douglas E. Soltis, Ph.D.
Professor, Department of Botany, University of Florida
Darwin's abominable mystery: developmental genetic/genomic insights into angiosperm evolution

3:15-3:45 p.m.:
Sean M. Sullivan, Ph.D.
Associate Professor, Department of Pharmaceutics, University of Florida
Development of tumor vasculature targeted nanoplexes for treatment of malignant brain cancer

Presentation Abstracts

DNA replication and cell cycle checkpoints

Kelly TJ
Sloan-Kettering Institute, New York, NY

Cell cycle checkpoints are complex signal transduction pathways that protect the integrity of the genome by regulating cell cycle progression when the duplication or segregation of the chromosomes is perturbed or the chromosomal DNA is damaged by endogenous or exogenous factors. Checkpoints function to preserve cell viability and to prevent genetic alterations that can lead to cancer. We have studied two cell cycle checkpoints in the model organism *S. pombe*: (1) the replication checkpoint which stabilizes the replisome and delays mitosis when DNA synthesis is inhibited by nucleotide starvation or other factors and (2) the DNA damage checkpoint that blocks cell cycle progression when the genome is damaged by ultraviolet (UV) irradiation. The replication checkpoint is activated at blocked replication forks by the protein kinase Rad3 (ATR in humans) acting through a mediator protein, Mrc1 (claspin in humans), which appears to be a component of the replisome. Rad3 phosphorylates Mrc1 to generate docking sites for a second protein kinase Cds1 (Chk2 in humans). Cds1 is then activated in two stages by Rad3 phosphorylation followed by autophosphorylation. The activated Cds1 phosphorylates downstream targets that block cell cycle progression and prevent fork collapse. This novel two-step kinase cascade provides sensitivity and noise immunity, properties required for the replication checkpoint. Eukaryotic cells irradiated with high doses of UV exhibit responses, referred to as the G1/S, intra-S or G2/M checkpoints, that function to delay particular cell cycle transitions. It is generally thought that the checkpoint-mediated delays provide time for repair of UV photoproducts. Using computer-aided time-lapse microscopy to follow individual *S. pombe* cells, we found unexpectedly that these checkpoint responses are not activated when cells are irradiated with moderate UV doses that approximate sunlight exposure and could reasonably be expected to occur in nature. Instead, cells irradiated in all phases of the cell cycle progress without significant delay into the following S phase and then arrest for hours after the completion of bulk DNA synthesis. This post-replication replication checkpoint response is entirely dependent upon initiation of DNA replication and the checkpoint kinase, Chk1. Failure of the post-replication checkpoint response results in gross chromosomal damage and loss of viability only after UV lesions are propagated through S phase and the subsequent mitosis. Thus, the DNA damage checkpoint appears to be triggered by structures (probably single-stranded gaps) in the chromosomal DNA that are generated when replication forks encounter UV lesions. It follows that the likely physiologic role of the checkpoint is to protect the genome by providing time for post-replication repair.

Biography of Thomas J. Kelly, M.D., Ph.D

Dr. Thomas J. Kelly, M.D., Ph.D., joined Memorial Sloan-Kettering Cancer Center in 2002 as director of Sloan-Kettering Institute after a 30-year career at The Johns Hopkins University School of Medicine, where he served as director of the Department of Molecular Biology and Genetics and director of the Institute for Basic Biomedical Sciences. Dr. Kelly's research focuses on how the genome is duplicated during the eukaryotic cell cycle with particular emphasis on the ways DNA replication is initiated and controlled. Using animal viruses as models, Dr. Kelly's laboratory developed the first cell-free systems for studying the biochemistry of DNA replication in human cells. More recently he has focused on the links between DNA replication and the progression of the cell cycle in human cells and in the fission yeast, *Schizosacchromyces pombe*, which shares many properties with higher eukaryotes. He was a co-winner of the 2004 Alfred P. Sloan Jr. Prize for his seminal contributions to the understanding of the molecular mechanisms of eukaryotic DNA replication. Dr. Kelly is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the American Philosophical Society.

Plant mitochondrial genomes: unexpected bounties of lateral gene transfer

Palmer JD

Department of Biology, Indiana University, Bloomington, IN

Horizontal gene transfer (HGT) is now recognized as a major evolutionary genetic force driving genomic and phenotypic change in prokaryotes and many unicellular eukaryotes. In contrast, there is little published evidence that HGT is common or important in the major groups of multicellular eukaryotes (animals, plants, and fungi). We have discovered that HGT of mitochondrial genes in plants is both widespread and recent and have now expanded this work in several directions. Our recent work has provided insight into mechanisms of HGT (it frequently occurs by direct physical contract between parasitic plants and their host plants and seems to involve direct fusion between donor and recipient mitochondria), has identified a plant whose mitochondrial genome has been radically shaped by HGT (it contains numerous genes acquired by HGT, and from a wide variety of donors, from other flowering plants to mosses and algae), and has shown that many horizontal transfers give rise to chimeric genes in which pieces of foreign genes are pasted into native copies.

Biography of Jeffrey D. Palmer, Ph.D

Jeffrey Palmer received his Ph.D. from Stanford University. He joined the faculty of Indiana University in 1989, is a Distinguished Professor in the department of biology, and holds the Class of 1955 Endowed Professorship. He served as chairman of the department from 1996 to 2003. Under his leadership the department grew substantially in size and stature. His academic interests in biology focus on plant evolution, molecular evolution, and genomics. He was the recipient of a Presidential Young Investigator Award from the National Science Foundation (1985-1990). He received the David Starr Jordan Prize in 1990, the Wilhelmine E. Key Award from the American Genetics Association in 1998, a Merit Award from the Botanical Society of America in 2003, the Stebbins Medal in 2005, and a Guggenheim Fellowship for 2005-2006. He was elected to the American Academy of Arts & Sciences in 1999, to the National Academy of Sciences in 2000, and to the American Association for the Advancement of Science in 2005. With more than 200 influential scientific papers to his credit, Dr. Palmer was named in 2002 as one of the top 15 most highly cited researchers worldwide in the field of plant and animal sciences by the Institute for Scientific Information. He is widely considered to be a world leader in evolutionary genomics.

Isolation, induction and use of adult neural progenitor cells

Goldman SA

Departments of Neurology, Neurosurgery, and Pediatrics, University of Rochester Medical Center, Rochester, NY

The major progenitor pools of the human brain, each of which has now been isolated, include ventricular zone neural stem cells, VZ neuronal progenitors, hippocampal neuronal progenitors, and parenchymal glial progenitor cells (GPCs). The most abundant of these are the GPCs, which can generate astrocytes and oligodendrocytes in a context-dependent fashion. When isolated and transplanted into neonatal shiverer mice, whose brains lack myelin, human GPCs produced oligodendrocytes that mediated substantial and extensive myelination, and substantially extended the survival of the implanted mice. Yet whereas adult glial progenitors generated only oligodendrocytes and astrocytes *in vivo*, upon removal from the tissue environment they produced neurons as well as glia, and expanded as neurospheres. Glial progenitor cells thus seem restricted to glial phenotype by their local environment, but nonetheless retain multilineage competence in isolation. By purifying these cells and assessing their patterns of differential gene expression, relative to their parental tissue environments, we identified a set of signaling pathways that regulate their homeostatic self-renewal *in vivo*, and whose perturbation allows the directed induction of astrocytic and oligodendrocytic fate. Endogenous stem cells of the ventricular subependyma may also be targeted for induction. These cells may be induced to generate new neurons by over-expressing BDNF, and this process is potentiated by the BMP inhibitor noggin, which suppresses glial differentiation. The new neurons mature as medium spiny neurons, and integrate into the existing striatal neuronal network. Remarkably, the new neurons successfully project to the globus pallidus, extending processes over several mm of normal adult striatum. This strategy for inducing new medium spiny neurons in the adult neostriatum has also been assessed in the R6/2 mouse, a transgenic model of Huntington's disease. R6-2 mice treated with adenoviral BDNF and noggin exhibit both improved motor performance and longer survival than untreated controls, suggesting the potential efficacy of this strategy for replacing medium spiny neurons lost to Huntington's. These experiments argue that as our understanding of the biology of adult neural progenitor cells becomes more extensive, our ability to target, induce and implant these cells for therapeutic purposes will become increasingly manifest.

Biography of Steven A. Goldman, M.D., Ph.D.

Dr. Steven A. Goldman is a professor of neurology, neurosurgery, and pediatrics, and chief of the division of cell and gene therapy at the University of Rochester Medical Center. At Rochester, he holds the Dean Zutes Chair in Biology of the Aging Brain, and serves as attending neurologist at Strong Memorial Hospital. He moved to Rochester in 2003 from the Weill Medical College of Cornell University, where he was the Nathan Cummings Professor of Neurology and Neuroscience, and attending neurologist at New York Presbyterian Hospital. A summa cum laude graduate of the University of Pennsylvania, he obtained his Ph.D. from Rockefeller University in 1983, and his M.D. from Cornell in 1984. His thesis work, with Fernando Nottebohm at Rockefeller, included the first report of neuronal production in the adult vertebrate brain. Dr. Goldman then interned in medicine and completed his residency in neurology at New York Hospital-Cornell and at the Memorial Sloan-Kettering Cancer Center. In 1988, after serving as chief resident in neurology, he joined the faculty at Cornell and New York Hospital, where in 1997, at 39, he was promoted to professor of neurology. Dr. Goldman's laboratory is interested in neural regeneration and cell genesis in the adult nervous system, with focus on neural stem and progenitor cells and their use in treating demyelinating and degenerative diseases of the brain and spinal cord. He has published over a hundred papers in his field as first or senior author. He is a recipient of the Jacob Javits Neuroscience Investigator Award of the NIH, and has been elected to the American Neurological Association and American Society for Clinical Investigation. He has served as a consultant to both Aventis Pharmaceuticals and Merck Research Labs, and is a co-founder of Q Therapeutics in Salt Lake City.

Whole genome optical mapping

Waterman MS

Departments of Biological Sciences, Mathematics, and Computer Science, University of Southern California, Los Angeles, CA

An innovative new technology, optical mapping, is used to infer the genome map of the location of short sequence patterns called restriction sites. The technology, developed by David Schwartz, allows the visualization of the maps of randomly located single molecules around a million base pairs in length. The genome map is constructed from overlapping these shorter maps. The mathematical and computational challenges come from modeling the measurement errors and from the process of map assembly.

Biography of Michael S. Waterman, Ph.D.

Michael Waterman holds an endowed associates chair at the University of Southern California. He came to USC in 1982 after positions at Los Alamos National Laboratory and Idaho State University. He has a bachelor's degree in mathematics from Oregon State University, and a Ph.D. in statistics and probability is from Michigan State University. He was named a Guggenheim Fellow (1995), was elected to the American Academy of Art and Sciences (1995), and was elected to the National Academy of Sciences (2001). He is also a Fellow of the American Association for the Advancement of Science and Fellow of the Institute of Mathematical Statistics. He has held visiting positions at the University of Hawaii (1979-80), the University of California at San Francisco (1982), Mt. Sinai Medical School (1988), Chalmers University (2000), and in 2000-2001 he held the Aisenstadt Chair at University of Montreal. He is professor-at-large at the Keck Graduate Institute of Life Sciences and in fall 2000 he became the first Fellow of Celera Genomics. In 2002 he received a Gairdner Foundation International Award and in 2005 he was elected to the French Académie des Sciences. In fall 2003, he became faculty master of Parkside International Residence College, a residental college that is home to over 600 undergraduates and serves as a center for internationally oriented cultural, academic and social events. Professor Waterman works in the area of computational biology, concentrating on the creation and application of mathematics, statistics and computer science to molecular biology, particularly to DNA, RNA and protein sequence data. He is the co-developer of the Smith-Waterman algorithm for sequence comparison and of the Lander-Waterman formula for physical mapping. He is a founding editor of "Journal of Computational Biology," is on the editorial board of seven journals, and is co-author of the texts "Computational Genome Analysis: An Introduction" and "Introduction to Computational Biology: Maps, Sequences and Genomes."

Genetics *in vitro*: synthesizing the RNA world

Joyce GF

It is believed that an RNA-based genetic system, usually referred to as the "RNA world," preceded the DNA and protein-based genetic system that has existed on Earth for the past 3.5 billion years. Questions concerning how the RNA world arose and the degree of complexity it attained can be addressed through laboratory experiments in prebiotic chemistry and RNA biochemistry. Central to the operation of the RNA world is the ability of RNA to catalyze the replication of RNA, thereby enabling RNA-based evolution. Through methods of *in vitro* evolution, we have developed RNA enzymes that catalyze the template-directed joining of RNA substrates. One such molecule was converted to a format that allows it to produce additional copies of itself by joining two component subunits. It subsequently was converted to a cross-catalytic format whereby two RNA enzymes direct each other's synthesis from a total of four component substrates. These molecules, however, operate with a highly restricted set of RNA substrates and cannot undergo evolution on their own. Using a variety of approaches, we are employing *in vitro* evolution to develop other RNA enzymes that have enhanced potential to replicate and evolve in a self-sustained manner.

Biography of Gerald F. Joyce, M.D., Ph.D.

Gerald F. Joyce is a professor in the departments of chemistry and molecular biology and an investigator with the Skaggs Institute for Chemical Biology at The Scripps Research Institute in La Jolla, Calif. He received his B.A. from the University of Chicago in 1978 and both an M.D. and Ph.D. from the University of California, San Diego, in 1984. He carried out postgraduate medical training at Mercy Hospital in San Diego and postdoctoral research training at The Salk Institute before joining the faculty of The Scripps Research Institute in 1989. Dr. Joyce's research involves the test-tube evolution of nucleic acids and the application of these methods to the development of novel RNA and DNA enzymes. He also has a longstanding interest in the origins of life and the role of RNA in the early history of life on Earth. He has published over 100 scientific papers and is the inventor or co-inventor of 11 issued patents. He was elected to the U.S. National Academy of Sciences in 2001, and in 2005 received the H.C. Urey Award, presented every six years by the International Society for the Study of the Origin of Life. Dr. Joyce has lectured extensively around the world, including at the Pontifical Academy and the Royal Swedish Academy of Sciences.

Embryonic, adult and cancer stem cells *in vitro* and *in vivo*

Steindler DA

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL

The adult rodent and human brain harbors a population of stem/progenitor cells (NSC's) that can both self-renew and generate progeny along the three lineage pathways of the central nervous system (CNS), but their *in vivo* identification has proven elusive. Our recent studies have further characterized a multipotent astrocytic stem cell (MASC) that resides within "brain marrow" (the adult subventricular zone) using an *in vitro* system that recapitulates neuropoiesis *in vitro* as it occurs *in vivo*. Live cell imaging, immunophenotypic analysis, and electrophysiological studies have revealed a cell that exhibits astrotypic characteristics, but can give rise to large numbers of neuroblasts. These cells occupy a distinct neurogenic niche in the adult CNS, but injuries and disease may induce what appear to be fully differentiated astrocytes to assume a neurogenic role whereby they upregulate their expressions of developmentally-regulated proteins and attempt to recapitulate neurogenic programs. Neurogenic astroctye-like cells change their biochemistry throughout the neuraxis at the end of the second postnatal week in mice and assume a more differentiated phenotype *in vivo* where their activities are related to standard operating procedures associated with the normal CNS. *In vitro* conditions can be created, relying on information gained from studies of embryonic and cancer stem cells, that restore neurogenic programs of apparently differentiated brain cells, and the goal of new studies is to resolve the precise molecular cascades responsible for lifelong plasticity of CNS cells. This way, reactive neurogenesis could be exploited for neural repair in many different neurological disorders. In addition to the hope offered by stem-like cells in the mature human brain, these cells may also be involved in tumorigenesis that warrants further investigation.

Genetic analysis of adaptive traits in forest trees

Davis JM*

School of Forest Resources and Conservation, University of Florida, Gainesville, FL

The health of our terrestrial ecosystems exerts many direct and indirect influences on the human population on earth, with impacts ranging from the global carbon balance to renewable energy. Since forests are such a prominent class of terrestrial ecosystem, the application of genomics approaches in forest tree species is expected to have significant, positive impacts on the understanding of ecosystem function. Genomics approaches are now feasible in *Populus* and *Pinus*, two distinct genera of forest trees with complementary phenotypic traits, ecological niches and genomic toolkits available for their analysis. The extent of natural genetic diversity in both of these systems is remarkably high, which creates the opportunity to make causal connections between natural allelic variation (i.e. genotype) and adaptive traits (i.e., phenotype) in properly designed experiments. The outcomes of this research are expected to enhance the efficiency of genetic improvement programs, and to improve understanding of forest ecosystem form and function.

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

Pharmacogenomics

Johnson JA*

Director, UF Center for Pharmacogenomics, University of Florida, Gainesville, FL

Pharmacogenomics is a field aimed at discovery of the genetic basis for variable drug response, with the potential to improve the safe and effective utilization of existing drug therapies, and identify new drug targets. This session will provide an overview of literature from the field, experimental approaches that are used, and future clinical potential of pharmacogenomics. There will be a particular focus on work at the University of Florida, which is focused on the pharmacogenomics of drugs used in the treatment of hypertension, heart failure and clotting disorders. There will also be discussion of the UF Center for Pharmacogenomics, and its mission and activities.

Darwin's abominable mystery: developmental genetic/genomic insights into angiosperm evolution

Soltis DE*

The flower is the central identifying feature of the angiosperms. Yet, little is known about the origin of the flower and the subsequent early and rapid evolutionary diversification of flowering plants (Darwin's "Abominable Mystery"). Synergistic research involving paleobotany, phylogenetics, classical developmental studies, genomics, and developmental genetics has afforded valuable new data regarding floral evolution in general, as well as the early flower in particular. Recent progress in understanding angiosperm phylogeny provides a solid framework for evaluating evolutionary innovation, and identifies the taxa that provide the best insights into key innovations. The interplay of genomics, developmental genetics, and focused studies of morphology, has provided rapid advances in the understanding of flower evolution. Phylogenetic studies of floral gene families suggest that most, if not all, of the fundamental genetic components of the classic Arabidopsis-based ABC model" of floral organ identity were present in the ancestral angiosperm genome. Developmental genetic studies of diverse flowering plants reveal that there is no single model of floral organ identity that applies to all angiosperms; rather, there are likely multiple models that apply depending on the phylogenetic position and floral structure of the group in question. The classic ABC (or ABCE) model may work well for most highly derived angiosperms. However, modifications are needed for basal angiosperms. We offer the "fading borders" as a testable hypothesis for the basalmost angiosperms and, by inference, perhaps some of the earliest (now extinct) angiosperms, as well.

Development of tumor vasculature targeted nanoplexes for treatment of malignant brain cancer

Sullivan, SM*

Department of Pharmaceutics, University of Florida, Gainesville, FL

Plasmid DNA isolated from *E. Coli* is formulated with cationic amphiphiles to form toroids <200nm in diameter, termed nanoplexes. Peptide ligands that bind to receptors expressed on tumor cells and tumor endothelium are derivatized to the nanoplex surface yielding targeted gene delivery to these cells. Rat arterial organ cultures (arteriographs) are used to optimize the targeting parameters of the nanoplexes and also study the role of receptor density in achieving selective gene transfer. Expression of cytotoxic genes engineered to kill the tumor cells and tumor endothelial cells is restricted to only these cells using a proliferating endothelial promoter. The cytotoxic genes are the product of molecular biological engineering with the end result being expression of gene products that are secreted from the transfected cells killing not only the transfected cells but all the nearby surrounding cells. Hence, transfection of a small number of cells results in a large impact on tumor growth.

Posters

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

1. Altering plant architecture of bahiagrass by constitutive expression of a gibberellin-catabolizing enzyme

Agharkar M, Lomba P, Altpeter F*, Zhang H, Kenworthy K*, Sinclair T

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

Bahiagrass is a low input, drought tolerant and disease resistant warm season turfgrass used for residential lawns and along highways in the southeastern US. Turf quality of bahiagrass is compromised by 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). GA2ox1 and GA2ox8 ORF's were isolated from *Arabidopsis* and 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, apomictic bahiagrass (cv. 'Argentine') was carried out by biolistic gene transfer. Transgenic nature of the regenerated plants was confirmed by NPTII ELISA (Agdia), PCR, Southern blot analysis and RT-PCR. Initial phenotypic characterization was carried out with hydroponic or soil grown plants under controlled environment conditions. Five independent transgenic lines were established in a replicated small field plot trail at the PSREU near Citra, FL. Data collected from controlled and field environment on plant height, number of vegetative and generative tillers, seedhead length, flowering time establishment rate and biomass of clippings will be presented.

2. Peripheral myelin protein 22 modulates the cytoskeletal organization of epithelia and Schwann cells

Amici S, Bachow S, Notterpek L*

Department of Neuroscience, University of Florida, Gainesville, FL

Peripheral myelin protein 22 (PMP22) is a tetraspan glycoprotein involved in the formation and maintenance of myelin in the peripheral nervous system. If PMP22 is misexpressed, inherited neuropathies arise; however, the function of PMP22 at the plasma membrane remains unclear. We have recently shown that PMP22 and $\alpha 6\beta 4$ integrin are in a complex in Schwann cells of the peripheral nervous system and in a human colon adenocarcinoma cell line (clone A cells). Integrins are transmembrane receptors that link the extracellular matrix with the cytoskeleton. They control signaling events critical for cell survival, morphology and movement, which are often modulated by the Rho family of GTPases. To explore if PMP22 is involved in modifying cell morphology, adhesion and migration, we have employed *in vitro* models to evaluate the intrinsic properties of cells with altered levels of PMP22. Schwann cells derived from PMP22-deficient mice exhibit reduced adhesion and slowed migration. Overexpressed human wild type PMP22 traffics to the cell surface of clone A epithelia and is associated with reduced proliferation. By immunostaining, phosphorylated myosin light chain, a downstream target of Rho kinase, is prominent at focal contacts when PMP22 is overexpressed, as compared to vector control cells. The increase in phosphorylated myosin light chain prompted us to examine cellular adhesive properties. Indeed, the overexpression of PMP22 strengthens the adhesion of epithelia to laminin. These results indicate, through direct or indirect mechanisms, PMP22 modulates cytoskeletal organization, and provide novel insights into the biological function of this disease-linked gene.

3. Transposon insertion needed for normal seed development?

Braunschweig M, Settles AM*

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

Carotenoids are a widely-distributed group of pigments having a long carbon chain and a red, yellow or orange color. In plants, carotenoids play roles in energy transfer in photosynthesis, and are precursors to flavor/aroma molecules and hormones such as abcisic acid (ABA). The maize viviparous rough (vp-rgh) mutant is characterized by a shrunken seed with low starch accumulation, germinates early while on the mother plant, and the white seedling dies because it lacks pigmentation for photosynthesis. We cloned the vp-rgh¬ locus, and the sequence was found to be from the phytoene desaturase (pds) locus. Pds is an enzyme in the catoenoid biosynthetic pathway. Vp-rgh¬ individuals were found to be polymorphic at the pds locus with PCR, having either large(700bp) and/or small(500bp) bands. Normal individuals posses at least one large band, but individuals with only the small band have the lethal mutant phenotype. Sequence alignment showed the large and small alleles to be identical, except the normal allele has one more exon. The normal allele for the pds locus can be amplified with a TIR primer, while the mutant allele can not, suggesting that the normal allele has a transposon insertion. A Southern blot may tell us if the transposon insertion is affecting copy number of the gene. To follow, we must learn how this transposon insertion in a carotenoid biosynthetic gene is affecting seed development.

4. Genomic analysis of the human Hsp70 protein family

Brocchieri L[1,*], Conway de Macario E[2], Macario AJL[2]

[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
[2]University of Maryland Biotechnology Institute, Baltimore, MD

Hsp70 proteins play crucial roles in cell physiology and pathology. In a definitive survey made feasible by the availability of the complete human genome sequence, we identified 47 loci encoding hsp70 candidates. Sixteen of them had an N-terminal nucleotide-binding domain (NBD) typical of Hsp70 conserved in various degrees, but could be separated into sub-groups by distinctive C-terminal domains. Eight of the genes corresponded to typical hsp70s, encoding proteins of approximately 70kDa and possessing the characteristic C-terminal substrate-binding domain (SBD). The products of these eight genes were found to reside in the cytosol/nucleus (HSPA2, HSPA8, HSPA6, HSPA1A, HSPA1B, and HSPA1L), in the endoplasmic reticulum (HSPA5), and in the mitochondrion (HSPA9B). In two other genes the SBD domain was truncated (HSPA14) or missing (STCH). The C-terminal domains were unrelated to the typical Hsp70 SBD in the remaining six genes, of which four (HSPA4, HSPA4L, HSPH1, HYOU1) encoded heavier proteins (100-170 kDa) and two (HSPA12A and HSPA12B) showed regions of differentiation that extended also to large parts of the NBD. Among the remaining 31 sequences, one was characterized by one internal frame-shift and, although reportedly transcribed under certain conditions, its functionality remains to be established. Thirty hsp70-related sequences presented multiple frame-shifts and in-frame stop codons characteristic of pseudogenes. We also analyzed exon-intron structure, protein isoforms, alternative-transcription variants, patterns of expression, and evolutionary relationships of all 47 hsp70 candidates. Exon-intron structure analysis and evolutionary-tree reconstructions revealed a central role for HSPA8 in the evolution of the human hsp70 family. The results indicated that five of the six genes encoding 70-kDa cytosolic proteins and the vast majority of the 30 hsp70 pseudogenes originated from HSPA8 by retrotransposition. This finding unveils the importance of LINE retro-transposon elements in the evolution of the human Hsp70 family.

5. The murine autosomal Ant4 gene is essential for survival of male meiotic germ cells

Brower J[1], Rodic N[1], Seki T[2], Fliess N[2] , McCarrey J[3] , Oh P[2], Terada N[1,*]

[1]Department of Pathology, University of Florida, Gainesville, FL
[2]Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL
[3]Department of Cellular and Structural Biology, University of Texas Health Sciences Center at San Antonio, TX

The Adenine nucleotide translocases (Ant) are nuclear encoded DNA gene products that facilitate the transport of ADP and ATP by an antiport mechanism across the inner mitochondrial membrane. We have recently identified a novel member of the Ant family, Ant4 in mouse, which is highly conserved among mammals. In contrast to the closest paralog Ant2, which is encoded by the X chromosome and ubiquitously expressed in somatic cells, Ant4 is encoded by an autosome, chromosome 3. Ant4 expression begins at the transition that occurs between type B spermatogonia as they progress into primary spermatocytes. The primary spermatocytes represent the cell type within the testis that enters meiosis. Concomitant with the entry of the spermatocyte into meiosis is the inactivation of the X and Y chromosomes which begins during prophase I. Indeed, real-time PCR analysis of the different spermatogenic cell types of spermatogenesis, demonstrate a decrease in Ant2 and an increase in Ant4 transcript upon entry into meiosis. Thus Ant4 appears to be evolutionary conserved in mammals in order to compensate the loss of Ant2 expression due to the meiotic sex chromosome inactivation (MSCI) that occurs during male germ cell development. Furthermore, Ant4 ablation by homologous recombination based gene silencing has led to an approximate 3 fold reduction in testis mass. Ant4 deficient male mice exhibit severe morphological aberrations in the process of spermatogenesis, with only spermatogonia present in the adult testis. The Ant4 deficient adult testis exhibit high levels of apoptosis in comparison to wild type and Ant4 heterozygous testis, as shown by TUNEL analysis. These data clearly indicate that the autosomal gene Ant4 is essential for survival of male meiotic germ cells.

6. Network analysis reveals HIV-1 activation of macrophages is independent of toll-like receptors

Brown J[1], Kohler J[1,2], Coberley CR[1], Sleasman J[4], Goodenow M[1-3,*]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL
[2]All Children's Hospital, Gainesville, FL
[3]Pediatrics, Division of Immunology and Infectious Diseases, Gainesville, FL
[4]University of South Florida, St. Petersburg, FL

Macrophages offer important long-lived reservoirs for HIV-1. Establishment of viral reservoirs within macrophages is not a benign process. We hypothesized that genetic networks involved in regulating the immune response and signal transduction cascades would be impacted by HIV-1 infection, and these complex interrelated processes would be better understood from a global genomic perspective. Monocyte-derived macrophages from four individual healthy donors were treated with a macrophage-tropic HIV-1JR-FL virus or mock treated. At days 2, 4, and 7 post infection cells were lysed and RNA was collected and prepared for hybridization on Affymetrix HG-U95A microarray GeneChips. Resulting data was imported into a custom engineered relational database designed for functional genomic analysis of HIV-1 infection. Through database queries, a subset of genes that varied by more than 5-fold relative to mock-treated cultures in any given donor was selected and utilized in hierarchical agglomerative clustering. Mutual exclusive analysis

was performed to further identify genes detected from either virus- or mock-treated cultures alone. Functional analysis of gene expression during HIV-1 treatment identified complex genetic networks where crosstalk, synergy, and antagonism occur at multiple levels. Global analysis of intracellular proteins and secreted cytokines demonstrated evidence of an early shift, by day 2, to a predominant Th2-type milieu in primary macrophages induced by HIV-1. Network systems analysis identified altered expression of factors across several networking signal transduction pathways. The network analysis approach highlighted the uniqueness of the Toll-like receptor (TLR) pathway because few factors within the pathway were impacted by HIV-1. Viral escape of TLR recognition was confirmed using a bimodal approach. Collectively, this study emphasizes the utility of network analysis in high-throughput experiments that assimilates data across both the genome and the proteome.

7. Adeno-associated virus serotype 8 produces efficient gene delivery to widespread neuroendocrine and epithelial cell types

Tenace L[1], Cruz P[2], Conlon T[2], Clarke TJ[1,*], Foust K[2], Song S[3,*], Zolotukhin S[2,*], Flotte TR[2,*], Campbell-Thompson M[1,*]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL
[2]Department of Pediatrics, University of Florida, Gainesville, FL
[3]Department of Pharmaceutics, University of Florida, Gainesville, FL

Adeno-associated virus (AAV) serotypes have the potential to become important resources for clinical gene therapy by targeting specific cell populations. Among the novel AAV serotypes engineered for production of recombinant vectors, AAV8 has shown tremendous potential for *in vivo* gene delivery in rodents with nearly complete transduction of many tissues such as liver and heart after intravascular infusion. In this study, AAV8 carrying the green fluorescent protein (GFP) reporter gene was administered by intravascular infusion in mice and neuroendocrine cell populations examined for GFP expression to further define AAV8 transduction potential and efficiency. The ability to transduce cell types was evaluated by GFP positive cell percentage. Transduction of hepatocytes and cardiomyocytes was used as an index for maximum potential. Widespread GFP expression was found in gastric antrum, pancreas (acinar and islets), ovary (follicle, corpus luteum), and adrenal gland with the highest intensity found in the zona fasciculata in the adrenal gland. GFP positive cells were also found in epithelium in the gastrointestinal and reproductive tracts. These findings suggest that AAV8 could prove useful for neuroendocrine-directed systemic gene therapy and show potential for gene therapy of epithelial cells in the gastrointestinal and reproductive tracts as well.

8. Microarray profiling of *Persea americana* flowers: in search of organ-specific gene expression and developmental pathways.

Chanderbali A[1], Altman N[2], Leebens-Mack J[3], Soltis PS[4,*], Soltis DE[1,*]

[1]Department of Botany, University of Florida, Gainesville, FL
[2]Department of Statistics, Pennsylvania State University, University Park, State College, PA
[3]Department of Biology, Pennsylvania State University, University Park, State College, PA
[4]Florida Museum of Natural History, University of Florida, Gainesville, FL

As part of our goals to investigate the origin, conservation, and diversification of the genetic architecture of the flower, a microarray approach to study floral gene expression has been 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 to

bridge the gap between the plant models *Arabidopsis* and rice. The microarrays, printed by Agilent Technologies, contain in-situ synthesized 60-mer oligonucleotide 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 examined gene expression in young and medium floral buds, tepals, stamens, carpels, initiating fruit, and leaves using a double loop design. Using a cut-off of two-fold difference, we identified 1,354 floral transcripts that were up-regulated in at least one floral tissue compared to leaves. Among the florally up-regulated genes are homologues of AGAMOUS, APETALA3, PISTILLATA and SEPALLATA3, bona-fide floral genes in *Arabidopsis*. Hierarchical clustering identified several tissue-specific gene clusters. One cluster of ca. 60 genes was primarily expressed in stamens, another group of ca. 60 genes was restricted to tepals, and one of ca. 80 genes was up-regulated in both carpels and fruit. A single cluster of ca. 20 genes was up-regulated in fruit alone, and one of ca. 50 genes was up-regulated only early in floral development. Clustering of tissues suggested that tepals and stamens have similar expression profiles, while carpels and fruits formed a separate cluster. The ca. 20 *Persea* genes supporting the clustering of tepals with stamens includes several without *Arabidopsis* homologues or for which the function of *Arabidopsis* homologues is unknown. These genes are likely participants in a developmental pathway shared between stamens and tepals of *Persea*.

9. Regulation of β-globin expression through the recruitment of chromatin modifying enzymes by TFII-I and USF

Crusselle-Davis VJ[1-4], Anantharaman A[1-4], Dodev T[1-4], Bungert J[1-4]

[1]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
[2]Powell Gene Therapy Center, University of Florida, Gainesville, FL
[3]Center for Mammalian Genetics, University of Florida, Gainesville, FL
[4]UF Shands Cancer Center, Gainesville, FL

The human β-globin locus contains five functional genes which are arranged in the order of their developmental expression. Gene proximal cis-regulatory DNA elements and interacting proteins restrict expression of the genes to the embryonic, fetal, or adult stages of erythropoiesis. In addition, the relative order of the genes with respect to the locus control region also contributes to the temporal regulation of the genes. To more fully understand adult β-globin gene regulation, we examined the downstream promoter and found that transcription factors TFII-I and USF interact with elements within this region in erythroid cells. TFII-I was found to act as a repressor of β-globin expression while USF proteins were found to act as activators of β-globin expression. It is becoming increasingly clear that one role DNA binding proteins play is to recruit co-activators or co-repressors that modify histones or mobilize nucleosomes at regulatory sites. Therefore, to understand the mechanism behind the regulation of β-globin expression by TFII-I and USF we investigated the recruitment of chromatin modifying enzymes to the β-globin gene locus by these proteins in both an embryonic and adult environment. TFII-I was found to interact with HDAC3 exclusively in embryonic environment. Suz12, a component of the Polycomb group complexes 2,3, and 4 which contains histone methylase activity, was also found to interact at the β-globin promoter in an embryonic environment but not in an adult environment suggesting a role in repression. USF was found to interact with activators in an adult environment. The role of USF in β-globin expression was also further investigated in transgenic mice which express a dominant-negative protein to USF exclusively in erythroid cells.

10. A developmental interaction between the embryo and endosperm in maize is revealed by the rough endosperm 3 (rgh3) seed mutant.

Diego F[1], Elisa G[2], Joaquin R[2], Tesfaye B[1], Gregorio H[2], Settles AM[1,*]

[1]Horticultural Sciences Department, University of Florida, Gainesville, FL
[2]Universidad Alcala de Henares, Spain.

Higher plant seeds develop embryo and endosperm tissues from the two products of double fertilization. The embryo and endosperm interact during seed development. Maize seeds require a specific ratio of maternal to paternal chromosomes, and the embryo and endosperm are known to show tissue growth interactions. However, little is known about the molecular mechanisms of embryo-endosperm interactions. The rough endosperm3 (rgh3) mutation disrupts a locus that is involved in these interactions at a developmental level. We have shown that Rgh3 is required in the embryo for the normal development of endosperm tissues using marked genetic mosaic seeds. An analysis of cell type specific markers in recessive mutant seeds suggest that the primary endosperm defect occurs after cell type specification. The rgh3 endosperm developmental defects are then reflected in total seed composition. Mutant rgh3 seeds also germinate at a low frequency and result in lethal seedlings suggesting an additional function of Rgh3 after seed development is completed. We mapped the Rgh3 locus to the long arm of chromosome 5 (5L). Complementation tests with other seed mutants mapped to 5L indicated that Rgh3 is a novel locus. We have cloned a transposon-tag that is closely-linked to the rgh3 mutant. This tag will be used to identify the Rgh3 locus with the goal of understanding the molecular mechanism by which the embryo affects endosperm development.

11. Modeling adaptive point mutation mechanism of biofilm bacteria using phylogenetic models

Feng H[1], Joyce P[2]

[1]Department of Pharmacy Practice, University of Florida, Gainesville FL
[2]Department of Mathematics, University of Idaho, Moscow, ID

Biofilms are implicated in a significant amount of human bacterial infections. Biologic biofilm experiments provided evidences that biofilm bacteria have wider diversities of genotype and phenotype. The wider variation in biofilm bacteria might be explained by adaptive mechanism, in which point mutation could play an important role. In this study, stochastic functions were developed to understand the point mutation dynamics under the models of Jukes-Cantor and Kimura's 2-parameter. The simulation results reveled that 1) larger phenotypic variation results from higher proportion of non-synonymous mutation; 2) The ratio of non-synonymous differences (dN) to synonymous differences (dS) is positively related with the probability of fixation; and 3) the probability of at least one non-synonymous substitution at the end of a branch increases as the non-synonymous substitution rate per codon increases. Those developed models were applied to explain the findings in the artificial E.coli biofilm.

12. Stochastic simulation models of population size estimates in genetic sampling

Feng H[1], Joyce P[2]

[1]Department of Pharmacy Practice, University of Florida, Gainesville FL
[2]Department of Mathematics, University of Idaho, Moscow, ID

Recent advances in genetics provide a way to study wildlife populations by extracting DNA from animal samples of hair and scat without handling the animals. However, despite the promise of the new molecular approach to the non-invasive genetic sampling, there are several sources of errors associated with the molecular identification techniques during polymerase chain reaction (PCR) amplification. Those errors could significantly bias population estimates. Based on both of CAPWIRE estimator and Peter-Lincoln estimator, we use stochastic simulation algorithm to develop bias correction methods that correct for the effect of genotyping error and heterogeneous capturabilities. The methods greatly improve the accuracy of the population abundance estimates, allowing for the true benefits of non-invasive genetic sampling to be realized.

13. Transplastomic tobacco with expression of the L-aspartate-alpha-decarboxylase from *Escherichia coli* is more tolerant to high temperature stress

Fouad W, Altpeter F*

Department of Agronomy 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. The *Escherichia coli* L-aspartate-alpha-decarboxylase (AspDC), coded by the panD gene, catalyzes the decarboxylation of L-aspartate to generate beta-alanine and carbon dioxide. This is a pyruvoyl-dependent enzyme that undergoes limited self-processing required for its activities and it is unique to prokaryotes. 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 Prrn 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. Molecular and physiological data correlating transgene expression and plant performance under heat stress conditions will be presented.

14. Genetic diversity of the rare endemic plants from the Lake Wales Ridge, Florida using microsatellites

Germain-Aubrey C[1], Gitzendanner M[1,*], Weekley C[2], Menges E[2]

[1]Department of Botany, University of Florida, Gainesville, FL
[2]Archbold Biological Research Station, Sebring, FL

The Lake Wales Ridge is one of the most threatened endemic-rich ecosystems of the nation and remains a conservation priority. Understanding the genetic diversity of some of its unique plants will help us protect this quickly disappearing ancient sand dune.

15. ADP-glucose pyrophosphorylase subunits exhibiting vastly different rates of sequence divergence are equally sensitive to amino acid changes altering enzyme activity

Georgelis N, Shaw JR, Hannah LC*

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

It is generally thought that the rate of evolutionary change in an amino acid sequence reflects, to a great extent, the importance of the monitored amino acids to the function of the protein. In the case of the enzyme ADP-glucose pyrophosphorylase (AGPase) this premise has led to the hypothesis that the small subunit of the enzyme is more conserved than is the large subunit because it provides a function not shared with the large subunit. Accordingly, greater evolutionary constraints are placed on the amino acid sequence of the small subunit. Two independent approaches were used to test this hypothesis. First, the probability that a random, PCR-induced, missense mutation altered AGPase activity was calculated for each subunit of the major maize endosperm isoform. Both subunits exhibited the same probability. Second, functional maize endosperm large and small subunit genes from heavily mutagenized populations were sequenced and the ratio of missense to silent mutations was calculated. This ratio was the same for both subunits. Our results indicate that the small and the large subunit genes of AGPase are equally predisposed to activity-altering missense mutations. The subunits are equally robust to random amino acid changes affecting AGPase activity. Alternatively, we suggest that the small subunit is more evolutionarily conserved than is the large subunit because it has to form a functional enzyme complex with multiple large subunit partners in multiple cellular environments. We base this hypothesis on the fact that, in planta, the small subunit generally interacts with multiple large subunits. To test specifically whether this places additional constraints on amino acid changes in the small subunit, we expressed a collection of maize endosperm small subunit mutants with each of two different large subunits and monitored resultant enzyme activity. Approximately 25 percent of the small subunit mutants interacted differently with the two large subunits.

16. Metabolic rate controls genotypic and phenotypic evolution

McCoy M, Gillooly J*

Department of Zoology, University of Florida, Gainesville, FL

Determining the relative importance of neutral versus adaptive processes in shaping rates of phenotypic evolution is a central aim of evolutionary biology. Many evolutionary biologists, while agreeing that most changes to the genotype are neutral, would argue that most changes to the phenotype are adaptive. However, the relative importance of neutral mutation versus natural selection in driving phenotypic change has been difficult to quantify. Here we apply the body size- temperature model of molecular evolution proposed by Gillooly et al. (2005) to address the importance of neutral processes in controlling rates of phenotypic evolution. Gillooly et al. (2005) showed that neutral rates of DNA evolution are proportional to mass-specific metabolic rate. In this study we show that for both mitochondrial and nuclear genes, non-neutral rates of DNA evolution, as well as rates of amino acid (i.e., phenotypic) evolution show the same size-and temperature dependence as neutral molecular evolution. This indicates that rates of phenotypic evolution, like neutral evolution, are directly proportional to mass-specific metabolic rate. More generally, these results suggest that the primary controls on individual metabolic rate (i.e., size and temperature) may also constrain the overall rate of evolution in organisms through their effects on mutation rate. Ultimately, our hope is that this work can be extended to better understand rates of speciation and patterns in biodiversity.

17. Analysis of LAT promoter deletion mutant 17DPst for altered histone modifications during latency and reactivation in rabbit trigeminal ganglia

Giordani NV[1], Neumann DM[2], Bhattacharjee PS[2], McAnany PK[1], Hill JM[2], Bloom DC[1,*]

[1]Department of Molecular Genetics & Microbiology, University of Florida, Gainesville, FL

[2]Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA

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. Since promoter deletion mutant 17dPst is highly restricted for reactivation in the rabbit, and to a much lesser degree in the mouse, we examined transcriptional permissiveness of the LAT locus using the rabbit eye model to determine whether the greater restriction in reactivation in the rabbit (*in vivo*) compared to the mouse (*in vitro*) lies in the establishment of a specific chromatin profile. Trigeminal ganglia (TG) from rabbits latently-infected with either HSV-1 wild-type strain 17syn+ or 17dPst were assessed for histone H3 K9, K14 acetylation and H3 K4 dimethylation during latency. Similar to results obtained from the mouse model (13), analyses in the rabbit reveal a transcriptional repression of lytic genes relative to LAT region targets. In contrast to latency in the mouse, the viral genome in the rabbit appears to display a higher level of H3 K9, K14 acetylation and H3 K4 dimethylation at the region just upstream of the Pst-Pst deletion than at the 5'exon/enhancer. Analyses of TG following epinephrine induction will determine whether 17dPst's defect in reactivation results from an inability of the LAT enhancer or ICP0 to be remodeled during the initial stages of reactivation.

18. Genetic analysis of BMP2 and BMP4 in the limb apical ectoderm ridge

Choi KS, Maatouk DM, Harfe BD*

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

During early limb development the apical ectodermal ridge (AER), a specialized epithelium on the distal tip of the limb bud, expresses a number of signaling proteins including bone morphogenetic proteins (BMPs) 2, 4 and 7. BMPs in the limb are expressed in both the limb mesoderm and ectoderm and have been proposed to play a role in digit patterning. To date, it has not been possible to directly examine the role BMPs play in limb development since mice deficient in BMP2 or BMP4 die before digit condensation occurs. Previous studies have circumvented this problem by conditionally removing a BMP receptor (BMPR-IA) or expressing a BMP antagonist (noggin) in the AER. These studies concluded that BMPs are essential for normal limb development. Because each of the previous studies blocked all BMP signaling, the functions of each individual BMP remains unknown. Our studies take advantage of an Msx2-cre ectoderm-specific transgene to delete BMPs in the limb AER. We have shown that removable of BMP2 from the limb ectoderm has no affect on digit patterning. Loss of ectodermal BMP4 typically causes formation of a posterior "nubbin". Double knockout of BMP2 and BMP4 in the ectoderm results in limb polydactyly and syndactyly. Using whole mount in situ hybridization we show that the expression domains of FGF4 and FGF8 in the AER were expanded along the anterior and posterior axis in double mutants. There was also a decrease in cell death in interdigital tissue, which resulted in webbing between the digits. Further in situ analysis shows the expression domains of Hoxd11, Hoxa13, and Hoxd13 were extended anteriorly in the limb mesenchyme. These results demonstrate that ectodermal BMPs are partially functional redundant in the limb ectoderm and play an important role in cell death and anteroposterior patterning of the developing limb.

19. Phase I clinical trials of intramuscular injection of rAAV2 and rAAV1-pseudotyped versions of an alpha-1 antitrypsin (AAT) vector in AAT-deficient adults.

Brantly ML[2,4,*], Spencer LT[1], Humphries M[1,4], Conlon TJ[1,4], Spencer CT[1,4], Poirier A[1,4], Garlington W[1], Baker D[1], Song S[4,5,*], Berns KI[3,4,*], Muzyczka N[3,4,*], Snyder RO[3,4,*], Washer S[6], Byrne BJ[1,3,4,*], Flotte TR[1,3,4,*]

[1]Department of Pediatrics, University of Florida, Gainesville, FL
[2]Department of Medicine, University of Florida, Gainesville, FL
[3]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
[4]Powell Gene Therapy Center, University of Florida, Gainesville, FL
[5]Department of Pharmaceutics, University of Florida, Gainesville, FL
[6]Applied Genetic Technologies Corp., Alachua, FL

A phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 (rAAV2) alpha-1 antitrypsin (AAT) vector (utilizing a CMV enhancer/beta actin promoter/hybrid intron [CBA] expression cassette) was performed in 12 AAT-deficient adults, 10 of whom were male. All subjects were either homozygous for the most common AAT mutation (a missense designated PI*Z) or compound heterozygous for PI*Z and another mutation known to cause disease. There were four dosage cohorts, ranging from 2.1x1012 vector genomes (vg) to 6.9x1013 vg, with 3 subjects per cohort. Subjects were injected sequentially in a dose escalating fashion with a minimum of 14 days between patients. Subjects who had been on AAT protein replacement discontinued that therapy 28 days prior to vector administration. There were no vector-related serious adverse events in any of the 12 participants. Vector DNA sequences were detected in the blood between 1 and 3 days after injection in nearly all patients receiving doses of 6.9x1012 vg or higher. Anti-AAV2 capsid antibodies were present and rose after vector injection, but no other immune responses were detected. One subject who had not been on protein replacement exhibited low-level expression of wild-type M-AAT in the serum (82nM), which was detectable 30 days after receiving an injection of 2.1x1013 vg. Unfortunately, residual but declining M-AAT levels from the wash-out of the protein replacement elevated background levels sufficiently to obscure any possible vector expression in that range in most of the other individuals in the higher dose cohorts. Based on a desire to increase the potency for M-AAT expression per vector genome, we further pursued pseudotyping of this same vector genome into AAV serotype 1 capsids (rAAV1-AAT). Preclinical data indicated that the potency per vector genome was approximately 500-fold higher in mice with this cassette. Toxicology and biodistribution studies in mice and rabbits indicated that IM injection of rAAV1-AAT posed some risk of injection-site inflammation and biodistribution to distant sites, including gonads and semen, both of which were dose-related, but neither of which were significant within the dose range (per kg) planned for the rAAV1 clinical trial. Based on this, a phase I trial has now been initiated with the pseudotyped vector. Three subjects have now been injected with 6.9x1012 vg of rAAV1-AAT. One of the three showed a level of total AAT peaking at 11microM 30 days after injection, but it is unclear whether this was due to vector expression or to an intercurrent bacterial epididymitis, with its associated fever and acute phase reaction, which could have induced endogenous Z-AAT to be transiently elevated. To date, there are no vector-related adverse events noted. Nine additional subjects are anticipated to receive doses ranging up to 1x1014 vg. Supported by grants from NHLBI, NCRR, and Applied Genetic Technologies Corporation.

20. The three endothelin receptors in the killifish, *Fundulus heteroclitus*: physiological and phylogenetic relationships

Hyndman KA, Miyamoto MM*, Evans DH

Department of Zoology, University of Florida, Gainesville, FL

The purpose of this study was to sequence and characterize the endothelin receptors (ETRs) from the gill of the killifish, *Fundulus heteroclitus*, where the ET signaling cascade is hypothesized to be involved in control of local blood flow and ion transport. In mammals there are two ETRs termed ETA and ETB. Interestingly, in non-mammalian vertebrates there are three ETRs: ETA, ETB1, and ETB2 (ETC in frogs). Using standard cloning and sequencing, we have sequenced the three ETRs from killifish gill cDNA. Our phylogenetic analysis supports previous findings that the three ETRs are produced by separate genes, and are not simply splice variants. To further characterize these receptors, tissue distribution and quantitative PCR mRNA analyses were performed. Given that animals from fishes to birds have three ETRs while mammals have only two, we hypothesize that mammals have lost the ETB2 gene.

21. Fingerprinting cultivars: DNA polymorphisms of bermudagrass (*Cynodon* spp.) microsatellite loci

Kamps TL[1], Ortega VM[1], Williams NR[2], Chamusco KC[1], Scully BT[3], Chase CD[1,*]

[1]Department of Horticultural Sciences, University of Florida, Gainesville, FL
[2]Monsanto Corp., Soybean Trait Integration Group, Des Moines, Iowa
[3]UF/IFAS Indian River Research and Education Center, Fort Pierce, FL

Bermudagrass is a complex of interbreeding *Cynodon* species that includes cultivars developed specifically for diverse utilizations ranging from close-cut golf greens to pastures. Breeding programs have produced high value seeding and strictly vegetatively propagated turf varieties designed for specific uses and responses to environmental conditions. Fast and reliable molecular marker fingerprinting is necessary to overcome the inherent difficulties of cultivar verification based on subtle morphological characteristics that can also be confounded by environmental influences. By developing an SSR based marker system that is technically simple, highly reproducible, and amenable to high-throughput analysis, we have made a strong improvement above the previous, cumbersome AFLP and DAF methods for genetically fingerprinting bermudagrasses. Primer pairs for PCR were designed from sequences flanking SSRs of clones from a $(CA)_n$ enriched genomic library of Tifway 419. Utility of the resulting set of 20 markers is demonstrated by genetic characterization by gel electrophoresis and/or capillary analysis of the PCR products generated from the DNA of a diploid (2N) and a tetraploid (4N) plant introduction (PI) lines used in turf breeding, and 12 named turf type bermudagrasses. PCR amplification identified 2 -13 alleles for the genotypes included in this study. Polymorphism information content (PIC) is high, ranging from 0.42 – 0.86 with an average of 0.75. Degree of revealed genetic diversity between accessions was consistent with expectations of the breeding method employed and this type of genetic marker system. Genetic relationships between accessions and purported progenitors were also analyzed and are reported.

22. Program for the isolation, identification, and characterization of pheromones from *C. elegans*

Alborn H[1], Bruschweiler R[2], de Bono M[3], Dossey A[4], Edison AS[4], Jorgensen E[5], Kaplan F[4], O'Brian N[4], Rocca JR[4], Srinivasan J[6], Stadler M[4], Sternberg P[6], Teal P[1], Truong L[5], White J[5], Zhang F[2]

[1]USDA Laboratory, Gainesville, FL
[2]National High Magnetic Field Laboratory, Tallahassee, FL
[3]MRC Laboratory of Molecular Biology, Cambridge, UK
[4]Department of Biochemistry & Molecular Biology, University of Florida, Gainesville, FL
[5]Department of Biology, University of Utah, Salt Lake City, UT
[6]Department of Biology, Cal Tech, Pasadena, CA

The small soil nematode *Caenorhabditis elegans* is one of the best-studied and understood animals. Despite its wealth of genetic, cellular, and anatomical information, very little is known about the chemistry that *C. elegans* uses to communicate between individuals and its environment. Members of this research team and other scientists have identified mating and aggregation behaviors that are also likely mediated through chemical communication between animals. We hypothesize that *C. elegans* utilizes an extensive chemical "language" that allows individuals to communicate and regulates basic behaviors such as feeding, mating, and population density control. Our goal is to identify several different molecules produced by the worm in order to begin to decipher its chemical language. This information will not only add to the extensive scientific knowledge of *C. elegans* but may provide important new clues for the biological control of other nematode parasites of plants or animals. We are currently pursuing the problem from two independent directions. First, we are using a "traditional" forward approach of growing large amounts of culture, separating compounds by various chromatographic methods, testing for biological activity, further purification, and identification by mass spectrometry and nuclear magnetic resonance (NMR). Second, we are experimenting with a reverse approach by using NMR to compare mixtures of cultures produced both with and without worms. From the mixture of compounds, we are attempting to identify resonances that are unique to worms and not present in bacteria. We will use these unique resonances to guide HPLC purification, test the compounds for biological activity, and identify them with NMR and MS. Updates from both directions will be presented.

23. Silencing of Ant4 gene expression by the E2F6 repressor complex

Kehoe S[1], Oka M[2], Gaubatz S[3], Terada N[1,*]

[1]Department of Pathology, University of Florida College of Medicine, Gainesville, FL 32610, USA
[2]Department of Frontier Biosciences, Osaka University, Osaka, Japan
[3]Department of Physiological Chemistry, University of Wuerzburg, Wuerzburg, Germany

Adenine nucleotide (ADP/ATP) translocase 4 (Ant4), is a gene with testis-specific expression recently discovered by our lab. Its critical role in spermatogenesis and male fertility is currently being elucidated. Ant4 is initially expressed in embryonic stem (ES) cells but is downregulated upon ES cell differentiation. Here we focus on the mechanisms by which Ant4 repression occurs during ES cell differentiation. We have previously shown that DNA methylation of the Ant4 promoter by DNA methyltransferase 3 (Dnmt3) is required for Ant4 repression. We now aim to determine how Dnmt3 is recruited to the Ant4 promoter. More specifically, we show using gel mobility shift and luciferase assays that E2F6, a known repressor of male germ cell-specific genes, binds to the Ant4 promoter and represses its transcription. Similarly, using site-directed mutagenesis, we demonstrate that mutation of an E2F6 binding site at the Ant4 promoter prevents E2F6-induced repression of Ant4 transcription. Furthermore, we show that in E2F6

knockout mouse embryonic fibroblasts, Ant4 is no longer repressed. Our findings suggest the existence of an E2F6 repressor complex whereby E2F6 recruits Dnmt3 to the Ant4 promoter to repress its transcription.

24. Human and Florida manatee (*Trichechus manatus latirostris*) comparative genome maps generated by cross-species fluorescence in-situ hybridization (Zoo-FISH)

Kellogg ME[1], Dennis TR[2], Burkett S[3], Bonde RK[4], Zori RT[2], McGuire PM[5], Stanyon R[6]

[1]Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL
[2]Department of Pediatrics, Division of Genetics, University of Florida, Gainesville, FL
[3]Comparative Molecular Cytogenetics Core, National Cancer Institute - Frederick, Frederick, MD
[4]U.S. Geological Survey, Florida Integrated Science Center, Sirenia Project, Gainesville, FL
[5]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
[6]Laboratorio di Antropologia, Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, Firenze, Italy

Although anatomical and molecular sequencing studies have arranged the 18 extant placental (Eutherian) orders into four super-orders, Afrotheria, Xenarthra, Euarchontoglires and Laurasiatheria, unresolved questions remain. There is still no consensus for the assembly and evolutionary position of the Afrotheria in which morphologically dissimilar African placentals were united by corresponding genetic sequences. In addition, nuclear DNA evidence places Afrotheria as the most ancient mammalian clade, whereas recent comparative cytogenetic information does not rule out the possibility that Xenarthra is basal. Chromosome painting can provide an alternative source of data to test hypotheses of placental mammalian phylogeny and taxonomy. Sirenia (manatee and dugong) and Hyracoidea (hyrax) are the two Afrotherian orders not yet examined using comparative painting techniques. Presented here are the human and Florida manatee (*Trichechus manatus latirostris*) comparative genome maps. Cross-species fluorescence in-situ hybridization (Zoo-FISH) investigations into the taxonomic assemblage will define chromosomal homologies and identify evolutionary chromosome breakpoints. Human (HSA) chromosome painting probes were constructed by degenerate oligonucleotide-primed PCR amplification of flow-sorted chromosomes. A comparative genome map is presented based on the hybridization pattern. These maps identified 41 evolutionarily conserved segments on 21 (of 23) autosomal manatee chromosomes and the X. Nine human chromosomes demonstrated conserved synteny, retaining complete and exclusive hybridization to a single chromosome pair in the Florida manatee. Eight human chromosomes delineated whole chromosome homology in the manatee karyotype. The unique Afrotherian syntenic associations of HSA 1/19 and 5/21 were found in manatee and phylogenetically support the Afrotherian super-order assemblage. These chromosome painting data offer non-DNA sequence support for the Afrotherian clade. Comparative human and manatee genomic maps provide new insights into the karyotype organization of the proto-afrotherian, and the ancestral Eutherian karyotype. These studies also facilitate high-resolution genetic mapping of the manatee genome, as well as intraspecific and interspecific comparisons to other non-human species.

25. The evolution of the *AGL6*-like MADS-box genes

Kim S[1], Zahn LM[2], Chanderbali A[1], Zheng Z[1], Oppenheimer DG[1,*], Ma H[2], Soltis DE[1,*], Soltis PS[3,*]

[1]Department of Botany, University of Florida, Gainesville, FL
[2]Department of Biology, Huck Institute of the Life Sciences and Institute for Molecular Genetics and Evolution, The Pennsylvania State University
[3]Florida Museum of Natural History, University of Florida, Gainesville, FL

AGL6-like genes form one of the major subgroups of the MADS-box gene family and are closely related to the *SEP* (E-class) and *SQUA* (A-class) subfamilies of MADS-box genes. In *Arabidopsis*, *AGL6* and *AGL13* have been reported from the *AGL6* subfamily, but detailed expression studies of these genes have not yet been performed. We identified several new *AGL6*-like genes from pivotal taxa in angiosperm phylogeny and analyzed them together with previously reported *AGL6*-like genes. Structural analyses showed 1) a one-aa gap in the I-domain in all *AGL6*-like genes relative to *SEP*-like and *SQUA*-like genes, 2) a seven-aa insertion in the C-domain of genes from asterids, and 3) a one-aa insertion in the C-domain of genes from gymnosperms. Phylogenetic analyses showed that *AGL6*-like genes are sister to *AGL2*-like genes, and *SQUA*-like genes are sister to these two groups. These relationships were strongly supported in both amino acid and DNA analyses. The phylogenetic tree of *AGL6*-like genes generally tracks organismal phylogeny as inferred from multigene data sets. Unlike other subgroups of MADS-box genes (e.g., *DEF*, *SQUA*, and *SEP*), no major duplication of *AGL6*-like genes occurred in the common ancestor of core eudicots. However, several recent gene duplications were detected (e.g., within *Magnoliaceae*). We investigated the expression of *AGL6* genes from *Arabidopsis*, *Eschscholzia*, *Magnolia*, *Liriodendron*, *Persea*, *Nuphar*, and *Amborella* using relative-quantitative RT-PCR and *in situ* hybridization, and consider the expression patterns in a phylogenetic context.

26. Bayesian mapping QTL for dynamic quantitative traits.

Li JH[*], Yang RQ[1]

[1]School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai, China

Quantitative traits whose phenotypic values change with time are called dynamic quantitative traits. Genetic analysis of dynamic traits is usually conducted in one of two ways. One is to treat phenotypic values collected at different time points as repeated measurements of the same traits, which are analyzed in the framework of multivariate theory. Alternatively, a growth curve may be fitted to those observations, and inference can be made through the parameters of the growth trajectories. The latter has been used in QTL mapping for dynamic quantitative traits. We demonstrate that one can fit dynamic traits with the Legendre polynomials, which are sufficiently general for any shape of trajectories by selecting different orders of the Legendre polynomials. Then we take a Bayesian approach implemented via the Markov Chain Monte Carlo (MCMC) algorithm to estimate the positions and effects of multiple QTL. The entire genome is divided into a finite number of regions, and QTL positions and effects of all regions are evaluated simultaneously. With this method, regions with no actual QTL will have negligible estimated QTL effects. We present this method using simulated data as well as data on the growth of leaf age collected from published experiments of DH population of rice.

27. Sequence-indexed mutations in maize using the UniformMu transposon-tagging population

Li L[1], Holding DR[2], Tan BC[1], Latshaw SP[1], Liu J[3], Suzuki M[1], O'Brien BA[1], Fajardo DS[1], Wroclawska E[1], Tseung CW[1], Lai J[4], Hunter III CT[1], Avigne WT[1], Baier J[1], Lonon D[1], Messing J[4], Hannah LC[1,*], Koch KE[1,*], Becraft PW[3], Larkins BA[2], McCarty DR[1,*], Settles AM[1,*]

[1]Horticultural Sciences Department, University of Florida, Gainesville, FL
[2]Department of Plant Sciences, University of Arizona, Tucson, AZ
[3]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA
[4]Waksman Institute, Rutgers University, Piscataway, NJ

Gene knockouts are a critical resource for functional genomics. In plant species such as *Arabidopsis* or rice, gene knockout collections have been generated by amplifying and sequencing genomic DNA flanking insertion mutants. These Flanking Sequence Tags (FSTs) map each mutant to a specific locus within the genome. In maize, FSTs have been generated using DNA transposons. Transposable elements can generate unstable insertions that are difficult to analyze for simple knockout phenotypes. Transposons can also generate somatic insertions that fail to segregate in subsequent generations. Transposon insertion sites from >100 UniformMu FSTs were tested for inheritance by locus-specific PCR. We confirmed 89% of the FSTs to be germinal transposon insertions. We found no evidence for somatic insertions within the 11% of insertion sites that were not confirmed. Instead, this subset of insertion sites had errors in locus-specific primer design due to incomplete or low-quality genomic sequences. We conclude from these data that the FSTs from the UniformMu population identify stable, germinal insertion sites in maize. We also infer that the larger collection of 1,882 non-redundant insertion sites from UniformMu provide a genome-wide resource for reverse genetics to generate hypotheses about gene function.

28. Expression of a synthetic *Bacillus thuringiensis* endotoxin to enhance resistance against fall armyworm in bahiagrass

Luciani G[1], Yactayo-Chang J[1], Altpeter F[1,*], Zhang H[1], Gallo M[1,*], Meagher R[2], Wofford D[1,*]

[1]Department of Agronomy, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
[2]USDA, ARS, CMAVE, Gainesville, FL

Bahiagrass is a low input, drought tolerant and disease resistant warm season turfgrass used for forage and turf in the Southeastern US and other subtropical regions of the world. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a destructive migratory pests of many tropical and subtropical grasses including bahiagrass. The objective of this study was to improve the insect resistance of bahiagrass by constitutive expression of an optimized, synthetic *Bacillus thuringiensis* endotoxin. A gene encoding an optimized, synthetic *Bacillus thuringiensis* endotoxin (Bt toxin) was sub-cloned under the control of the constitutive ubiquitin promoter. Co-transfer of constitutive nptII and Bt-toxin expression cassettes into seed derived callus cultures from turf-type, apomictic bahiagrass (cv. 'Argentine') was carried out by biolistic gene transfer. Transgenic nature of the regenerated plants was confirmed by PCR, Southern blot analysis, RT-PCR and immunoassays. Data on an initial screening for resistance of transgenic and wildtype bahiagrass leaves against laboratory reared neonate larvae of the fall armyworm will be presented.

29. Rapid and accurate pyrosequencing of angiosperm plastid genomes

Moore MJ[1], Dhingra A[2], Soltis PS[3,*], Shaw R[4], Farmerie WG[4,*], Folta KM[2,*], Soltis DE[1,*]

[1]Department of Botany, University of Florida, Gainesville, FL
[2]Horticultural Sciences Department, University of Florida, Gainesville, FL
[3]Florida Museum of Natural History, University of Florida, Gainesville, FL
[4]Interdisciplinary Center for Biotechnology Genome Sequencing Service Laboratory, University of Florida, Gainesville, FL

Plastid genome sequence information is vital to several disciplines in plant biology, including phylogenetics and molecular biology. The past five years have witnessed a dramatic increase in the number of completely sequenced plastid genomes, fuelled largely by advances in conventional Sanger sequencing technology. Here we report a further significant reduction in time and cost for plastid genome sequencing through the successful use of a newly available pyrosequencing platform, the Genome Sequencer 20 (GS 20) System (454 Life Sciences Corporation), to sequence the whole plastid genomes of eight species of angiosperms, representing many of the basal lineages of eudicots. More than 99.3% of each plastid genome was obtained during GS 20 sequence runs, to an average depth of coverage of ~20×. To assess the accuracy of the GS 20 sequence, over 45 kilobases of sequence was generated for two of the eight species, *Nandina domestica* (Berberidaceae) and *Platanus occidentalis* (Platanaceae) using conventional sequencing. Overall error rates of 0.043% and 0.031% were observed in GS 20 sequence for *Nandina* and *Platanus*, respectively. More than 97% of all observed errors were associated with homopolymer runs, with ~60% of all errors associated with homopolymer runs of 5 or more nucleotides and ~50% of all errors associated with regions of extensive homopolymer runs. No substitution errors were present in either genome. Error rates were generally higher in the single-copy and noncoding regions of both plastid genomes relative to the inverted repeat and coding regions. The high accuracy of GS 20 plastid genome sequence is particularly important because it was generated for a significant reduction in time and cost over traditional shotgun-based genome sequencing techniques. The GS 20 System should be broadly applicable to most plastid genome sequencing projects, and therefore promises to expand the scale of plant genetic and phylogenetic research dramatically.

30. SNP discovery of Voltage-gated calcium channel beta 2 subunit

Niu Y, Moss J, Langaee TY, Gong Y, Johnson JA[*]

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

The gene encoding the beta 2 subunit of the Voltage-gated calcium channels (CACNB2) affects multiple channel properties such as voltage-dependent activation, inactivation rates, G-protein modulation, drug sensitivity, and cell surface expression. We undertook a single nucleotide polymorphism (SNP) discovery effort to characterize polymorphisms in CACNB2 using genomic DNA from 60 ethnically diversed individuals by direct DNA sequencing. We identified a total of 25 SNPs in CACNB2 of which 7 were population-specific SNPs. Fourteen of the polymorphisms (56%) are novel SNPs not previously reported. The coding block contained no non-synonymous SNP and only 3 synonymous SNPs, located in the last coding exon. The other 22 SNPs were located in promoter, intronic, or untranslated regions. Allele frequencies ranged from less than 1.7% to 42.5% 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 groups.

31. Phylogenetic analysis to identify the most informative regions of the human mitochondrial genome

Non AL, Kitchen A, Mulligan CJ*

Department of Anthropology, University of Florida, Gainesville, FL

Analysis of complete mitochondrial genome sequences is becoming increasingly common in genetic studies. The availability of full genome datasets enables an analysis of the information content distributed throughout the mitochondrial genome in order to optimize the research design of future evolutionary studies. The goal of our study was to identify informative regions of the human mitochondrial genome using two criteria: 1) accurate reconstruction of a phylogeny and 2) consistent estimates of time to most recent common ancestor (TMRCA). We created two series of datasets by deleting individual genes of varied length and by deleting 10 equal-size fragments throughout the coding region. Phylogenies were statistically compared to the full-coding-region tree, while coalescent methods were used to estimate the TMRCA and associated credible intervals of defined clades. In general, deletion of equal-size fragments had a greater effect on the phylogenetic analysis than deletion of genes. Individual fragments important for maintaining a phylogeny similar to the full-coding-region tree encompassed bp 577-2122 and 11399-16023, including all or part of 12S rRNA, 16S rRNA, ND4, ND5, ND6, and cytb. The control region-only tree was the most poorly resolved with the majority of the tree manifest as an unresolved polytomy. Coalescent estimates of TMRCA were less sensitive to removal of any particular fragment(s) than reconstruction of a consistent phylogeny. Overall, we discovered that half the genome, i.e. bp 3669-11398, could be removed with no significant change in the phylogeny (pAU=0.077) while still maintaining overlap of TMRCA 95% credible intervals.

32. Intermittent fasting diet improves motor performance of hereditary neuropathic mice and is associated with alterations in peripheral nerve proteins

Madorsky I[1], Waber A[1], Dunn Jr. WA[2], Notterpek L[1,2,*]

[1]Departments of Neuroscience, University of Florida, Gainesville, FL
[2]Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Intermittent fasting (IF) is known to increase longevity and protect neurons against degenerative processes in experimental models. In the CNS, the underlying mechanisms appear to involve the stimulation of chaperones (heat shock proteins, HSPs) and neurotrophins. In this study we asked whether IF is an effective inducer of HSPs and autophagy in the PNS, and if it could alleviate the neuropathic phenotype of Trembler J (TrJ) mice, carrying a Leu-16-Pro mutation in peripheral myelin protein 22 (PMP22). Nine-week old, male wild type (wt) and heterozygous TrJ mice were maintained on the IF regimen for 4 months (food provided or removed at 12 noon each day). Littermates received food ad libitum (AL). The body weight of wt and TrJ animals was monitored at regular intervals, and decreased <10% on the IF regimen. After 4 months on the IF diet, TrJ mice exhibited improved motor performance on the accelerated rota-rod, when compared to AL littermates. Biochemical analysis revealed alterations in several protein groups, including HSPs, myelin proteins and markers of degradative pathways. The levels of HSP70, and of the autophagy-related proteins, beclin, Gsa7 and LC3, were elevated. Significantly, the expression of myelin proteins (PMP22, myelin basic protein) was increased in nerves of diet restricted mice. The differences between IF and AL mice were usually more pronounced in TrJ, as compared to wt. These results suggest that the chaperone and autophagic pathways are potential therapeutic targets for the neural disorders involving protein misfolding. Supported by funds from the MDA and NIH-NINDS.

33. Development of animal model for pulmonary arterial hypertension (PAH)

Hong K-H, Lee Y-J, Park SO, Jang D-S, Seki T, Oh SP*

Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL

Idiopathic Pulmonary Arterial Hypertension (IPAH) is a rare but life-threatening pulmonary vascular disorder characterized by the sustained elevation of mean pulmonary artery pressure. Genetic linkage analyses indicated that two TGF-β superfamily receptors activin receptor-like kinase-1 (ALK1) and bone morphogenetic protein receptor type II (BMPR2) predispose PAH. Heterozygous mutation of BMPR2 is responsible for about 70% and 40%of cases of familial PAH and of sporadic PAH, respectively. However, low disease penetrance of heterozygous mutation suggests that the loss of one allele of BMPR2 gene is not sufficient to cause pathogenesis of PAH. Recent studies raise the possibility that additional environmental and/or genetic factor(s) along with heterozygous mutation of BMPR2 (or ALK1) are involved in the development of PAH. We have previously demonstrated that Alk1 is predominantly expressed in arterial endothelium during embryonic and neonatal stages. In adult mice, Alk1 expression is greatly diminished in systemic vessels, yet persistent in pulmonary vasculature. We also showed that 9.2kb Alk1 promoter/enhancer is sufficient for spatiotemporal expression of Alk1 in transgenic mice. By using the 9.2kb Alk1 promoter/enhancer, we have generated a novel Cre mouse line showing a strong and consistent Cre activity in the pulmonary vascular endothelial cells (ECs) during development and postnatal life. Because heterozygous Bmpr2 mice display only mild elevation of pulmonary blood pressure, we investigated whether the second genetic mutation in the Bmpr2 gene only within pulmonary ECs is one of the aforementioned "additional factors" for triggering the pathophysiology of PAH. We generated homozygous Bmpr2loxp/loxp mice containing the ALK1-Cre transgene. The Bmpr2loxp/loxp;Alk1-Cre mice develop severe pulmonary hypertension with right ventricular hypertrophy and significant increase in the thickness of smooth muscle layers of pulmonary vessels. This result indicates that Bmpr2loxp/loxp;Alk1-Cre mice are an unique animal model for PPH which will provide valuable resources to enhance our understanding of the etiology and pathogenesis of PPH.

34. *In vivo* assessment of the ALK1-ALK5 balance for mediating TGF-β signal in endothelium during vascular development in mice

Park SO, Seki T, Hong K-H, Lee Y, Fliess N, and Oh SP*

Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL

TGF-β transmits signals through a hetero-tetrameric cell-surface complex of type II (Tgfbr2) and type I (Alk5) serine/threonine kinase receptors. We have shown that another type I receptor, Alk1, can also mediate TGF-β signals via BMP-activated Smads in vascular endothelial cells (ECs), and had proposed a hypothesis that two TGF-β signaling pathways via Alk1 and Alk5 in vascular ECs may play a balancing role for controlling angiogenesis. Mounting evidence has clearly shown that such a balance exists and functions in cultured ECs, and that Alk5 is required for Alk1 signaling. In contrast to these *in vitro* data, our *in vivo* expression mapping studies using lacZ-knockin reporter strains showed that Alk1 is predominantly expressed in arterial endothelium, whereas Alk5 expression is localized in the medial and adventitial layers of large blood vessels but undetectable in the intimal layer of blood vessels. These mutually exclusive expression patterns of Alk1 and Alk5 in blood vessels suggest that each type I receptor has its own unique function in vascular development. We hypothesize that Alk1 is the sole TGF-β type I receptor in the vascular endothelium. Therefore, Tgfbr2, but not Alk5, is required for TGF-β signaling in the endothelium during vascular development. To test this hypothesis, we have generated Alk1-conditional knockout mice and a transgenic mouse line which expresses Cre recombinase in a

subset of vascular endothelium. Using this Cre expressor, we have deleted Alk1, Alk5, or Tgfbr2 in the subset of endothelium. Blood vessels where Alk1 gene is deleted showed extensive dilation and arteriovenous malformation (AVM), recapitulating typical HHT vascular lesions, indicating that it will be served as an outstanding animal model for studying pathogenesis of HHT. On the other hand, mice harboring the Alk5 deletion by the same Cre expressor appeared to be viable and show no apparent vascular abnormality. These results strongly support our hypothesis and demonstrate that Alk1, but not Alk5, is essential in endothelium for the vascular development and function.

35. Centromere mapping in sweet orange, *Citrus sinensis*

O'Malley DM, Chaparro JX

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

As part of a collaborative effort in citrus genomics, we developed 32 EST-based microsatellite markers for a large interspecific Citrus sinensis x Poncirus trifoliata family, the mapping population. These markers and more microsatellites were located on a genetic map at University of California, Riverside. A fundamental goal of genomics is alignment of genetic, cytological, and physical maps. Microsatellite markers developed from ESTs can be readily integrated with physical maps. The major cytological feature of chromosomes is the centromere. Centromere DNA contains highly repetitive satellite DNA, with flanking middle repetitive DNA sequences. Gene density is lower and recombination is suppressed near centromeres. Although centromeres are stably inherited, they evolve rapidly so are difficult to locate from DNA sequence alone. The mapping population included 40 – 50 spontaneous triploid progeny. To recognize triploids that received two identical sweet orange alleles, gene dosage was interpreted from peak height differences in microsatellite trace files from an automated fluorescent capillary DNA sequencing instrument (Megabase 1000). Our analysis of the triploid microsatellite marker genotypes provides strong evidence that the spontaneous triploids arose from 2N ovules in sweet orange through meiotic diplospory and second division restitution (SDR). We used half tetrad analysis to estimate marker:centromere map distances for 18 microsatellite markers from sweet orange (C. sinensis). Knowledge of centromere locations provide useful information on the completeness of the genetic map and triploids has implications for seedless cultivar breeding. We have obtained preliminary gene:centromere distances from 58 additional UCR microsatellite markers and are working towards locating centromere regions on the consensus map.

36. A novel regulator of actin organization in plants

Zhang X, Oppenheimer DG*

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

We use *Arabidopsis trichomes* (epidermal hairs) as a model to probe the molecular and genetic mechanisms that control plant cell shape. By identifying mutants that show altered trichome shape and then cloning the genes identified by the mutations, we are dissecting the pathways that control plant cell shape. *Arabidopsis trichomes* are single cells that have three or four branches on the end of a stalk. We isolated a new class of mutants, the irregular trichome branch (itb) mutants, that show dramatic changes in trichome branch position and length. These mutations therefore identify genes that participate in the branch formation step of trichome morphogenesis. We cloned the ITB genes using a map-based approach. One of these genes, ITB3, encodes a novel protein of unknown function that is conserved in land plants, but is not found in algae, fungi, or animals. Because the plant cytoskeleton plays a central role in

controlling plant cell expansion, and hence shape, we used indirect immunofluorescence and confocal microscopy to examine the microtubule and actin cytoskeletons in developing itb3 mutant trichomes. We found that the microtubule cytoskeleton was normal, but actin organization was profoundly disrupted in developing trichomes of itb3 mutants. In addition to a preponderance of thick, bundled actin cables in the mutant, many actin rings of various sizes were prominent. Fusions of ITB3 with GFP were constructed and used to transform itb3 mutant plants. Rescue of the mutant trichome phenotype showed that the ITB3-GFP fusions were functional. The ITB3-GFP fusion proteins were found in the cytoplasm, consistent with the hypothesis that ITB3 functions as a regulator of the actin cytoskeleton. This work was supported by a grant from NSF (IOB 0352916)

37. A preliminary analysis of gene flow and population structure of the Florida manatee (*Trichechus manatus latirostris*)

Pause KC[1], Clark AM[2], Bonde RK[3], McGuire PM[1]

[1]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
[2]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL
[3]U.S. Geological Survey, Florida Integrated Science Center, Sirenia Project, Gainesville, FL

Previous studies with allozymes and mitochondrial DNA have demonstrated low genetic diversity, and failed to resolve the population structure for Florida manatees. Microsatellite libraries have been developed and screened to identify loci for population studies, pedigree analysis, and individual identification using genetic fingerprints. Eight previously published microsatellite primers and 10 additional unpublished primers are being screened on approximately 400 Florida manatees. Six of the 8 previously published loci and eight of the ten newly developed loci appear to be in Hardy-Weinberg equilibrium. Average heterozygosities for the established primers range from 0.23 to 0.60, whereas the new primers range from 0.31 to 0.73. Primers from closely related species were also tested on the Florida animals. Although products of the expected sizes were amplified, no polymorphism was detected. Additional loci from our manatee-specific libraries are being optimized and tested for polymorphism to help increase the power of the estimates. The polymorphic loci will be instrumental in examining the genetic appropriateness of the current management units and estimating gene flow within the state of Florida. Preliminary analyses suggest no clear genetic subdivision in the Florida population.

38. Liver X receptor-α; (LXRA) genotype and response to intensive lipid-lowering therapy with statins

Price ET[1], Arant CB[2], Wessel TR[2], Schofield RS[2], Zineh I[1]

[1]Department of Pharmacy Practice and Center for Pharmacogenomics, University of Florida, Gainesville, FL
[2]Division of Cardiovascular Medicine, University of Florida, Gainesville, FL

Intensive lipid lowering with statins is a preferred treatment strategy in certain patient populations. The benefit of high-dose treatment is thought to be due to both greater low-density lipoprotein (LDL) and C-reactive protein (CRP) reduction. However, variability in LDL and CRP responses exist, and genetic factors may contribute. We investigated whether a single nucleotide polymorphism (SNP) in the LXRA gene, a potential nuclear site of statin action, is associated with either LDL or CRP responses to atorvastatin 80 mg. Subjects were eligible if they were at least 18 years old without CHD, CHD risk equivalents, or contraindications to statins. Subjects received atorvastatin 80 mg daily for 8 weeks. Baseline and 8-week lipids and CRP were obtained from the university hospital clinical laboratory. Genotype determination of the LXRA rs12221497 G/A

SNP was performed by pyrosequencing. Biomarker changes were tested by t-test and multivariate analysis. A total of 61 subjects (59% women; 79% white) were analyzed. Baseline age, total cholesterol, LDL, HDL, triglycerides, and CRP were 32±13 years, 178±38 mg/dl, 98±31 mg/dl, 62±18 mg/dl, 97±54 mg/dl, and 1.8±2.9 mg/L, respectively. The variant A allele frequency was 16%. There were no differences in lipid changes by genotype. However, wild-type homozygotes (G/G) had an 11% reduction in CRP compared with a 25% increase in variant carriers (p= 0.047). In multivariate analysis, age (p=0.01), baseline CRP (p=0.02), and LXRA genotype (p=0.04) were significant predictors of CRP response (model p=0.002; r2=0.23). LXRA genotype did not affect LDL response to 8 weeks of high-dose atorvastatin. However, LXRA genotype was associated with atorvastatin-mediated CRP changes. This is the first study to demonstrate a genetic association with the CRP statin response and should be further evaluated.

39. Rearranged MEF2D and DAZAP1 functional domains exhibit transforming properties in the variant t(1;19)(q23;p13) reciprocal translocation products

Prima VI, Hunger SP

Department of Pediatrics, University of Florida, Gainesville, FL

We analyzed the TS-2 acute lymphoblastic leukemia (ALL) cell line that contains a t(1;19)(q23;p13.3) but lacks E2A-PBX1 fusion typically present in leukemias with this translocation. We found that the t(1;19) in TS-2 fuses the 19p13 gene DAZAP1 to the 1q23 gene MEF2D, leading to expression of reciprocal in-frame DAZAP1/MEF2D and MEF2D/DAZAP1 transcripts. MEF2D is a member of the MEF2 family of DNA binding proteins that activate transcription of genes involved in control of differentiation and survival of lymphoid, neural and muscle cells. DAZAP1 is a novel RNA binding protein involved in mRNA processing/trafficking and expressed most abundantly in the testis. We demonstrate that MEF2D/DAZAP1 binds avidly and specifically to DNA in a manner indistinguishable from that of native MEF2D and is a substantially more potent transcriptional activator than MEF2D. We also show that DAZAP1/MEF2D is a sequence-specific RNA-binding protein. MEF2D has been identified as a candidate oncogene in murine retroviral insertional mutagenesis studies. Our studies of cultured cells transduced with retroviruses expressing DAZAP1/MEF2D and MEF2D/DAZAP1 show transforming properties of the fusion proteins. These data implicate MEF2D in human cancer and suggest that MEF2D/DAZAP1 and/or DAZAP1/MEF2D contribute to leukemogenesis by altering signaling pathways normally regulated by wild-type MEF2D and DAZAP1.

40. Asynchronous teams for probe selection problems

Meneses CN[1], Pardalos PN[2,3,4,*], Ragle MA[2]

[1]Department of Transportation Engineering, University of Sao Paulo, Brazil
[2]Department of Industrial and Systems Engineering, University of Florida, Gainesville, FL
[3]Biomedical Engineering Department, University of Florida, Gainesville, FL
[4]Computer & Information Science & Engineering Department, University of Florida, Gainesville, FL

We propose the use of the Asynchronous Team (A-Team) technique to try to select good oligonucleotide probe sets from large sets of candidate probes. An A-Team is comprised of several different heuristic algorithms that communicate with each other via shared memories. We designed A-Teams for two probe selection problems called the Maximum Distinguishing Probe Set (MDPS) and the Minimum Cost Probe Set (MCPS), and developed a C++ program with graphical user interface (GUI) to run instances of the problems. When compared to other methods, our approach produced better or comparable results in a small fraction of the time.

41. Poxvirus-encoded tumor necrosis factor-binding proteins: variation in ligand binding specificities

Rahman MM[1], Barrett JW[2], Brouckaert P[3], McFadden G[1,*]

[1]Emerging Pathogens Initiative, Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
[2]BioTherapeutics Research Group, Robarts Research Institute, London, ON
[3]Molecular Pathophysiology and Experimental Therapy Unit, Department of Molecular Biomedical Research, Ghent University and VIB, Ghent, Belgium

The Yatapoxviruses encode a distinct class of secreted TNF-binding protein (TNF-BP) that resembles an MHC class I heavy chain but distinct from any other known TNF inhibitor. Characterization of these viral TNF inhibitors from Tanapox virus, Yaba monkey tumor virus and a closely related version from Swinepox virus revealed dramatically differential TNF binding specificities for different mammalian species. The Tanapox virus 2L protein (TPV-2L) formed inhibitory complexes with human TNF, and interacted with monkey and canine TNF with high affinity but rabbit TNF with low affinity. On the other hand, YMTV-2L bound human and monkey TNF with high affinity but rabbit TNF with only low affinity. The TNF-BP from swinepox virus (SPV003/148) only interacted with porcine TNF with high affinity. The observed TNF binding analysis mirrored the biological activity of these TNF- binding protein to block TNF-induced cellular cytolysis. TPV-2L and YMTV-2L also inhibited the human TNF-mediated signaling in cells but TPV-2L exhibited higher affinity for human TNF (KD=43pM) compared to monkey (KD=120pM) whereas for YMTV-2L, the affinities were reversed (human TNF KD=440 pM; monkey TNF KD=230 pM). The interaction domain of human TNF with TNF-binding proteins is significantly different from that of TNFRs, as determined using human TNF mutants. We conclude that these poxvirus TNF-binding proteins represent a new class of TNF inhibitors and are distinct from the viral TNF-receptor homologues characterized to date.

42. Inhibitors of Hsp90 modulate the formation of peripheral myelin protein 22 aggregates

Rangaraju S, Madorsky I, Go J, Notterpek L[*]

Department of Neuroscience, University of Florida, Gainesville, FL

The correct expression of peripheral myelin protein 22 (PMP22) is required for normal Schwann cell function and myelination. Duplication (C22 mouse), deletion or point mutations (TrJ mouse) in PMP22 are associated with a range of demyelinating peripheral neuropathies. Neuropathic Schwann cells exhibit abnormal protein trafficking with decreased turnover rate of PMP22 resulting in intracellular accumulation and subsequent aggregate formation. To aid the folding and trafficking of the wild type PMP22 in neuropathic models, we modulated the levels of heat shock proteins (Hsps), Hsp90 and Hsp70, by activating the heat shock response. In this study, we screened 15 novel Hsp90 inhibitor compounds (analogs of geldanamycin, GA) provided by Conforma Therapeutics. These novel GA-analogs at 50-500 nM show no significant cellular toxicity, as determined by MTT assays. Sixteen hour exposure of cells to these concentrations induces Hsp70 levels. To explore the influence of elevated Hsps on the formation of PMP22 aggregates, Schwann cells were treated simultaneously with the proteasome inhibitor, lactacystin (10 μM) and Hsp90 inhibitor compounds (50 nM) for 16 h. Biochemical analyses of total cell lysates revealed a modest reduction in slow-migrating polyubiquitinated proteasome substrates in response to combined treatment, as compared to Lc alone. The aggregate reducing effect of these compounds (50 nM) upon spontaneous aggregate containing neuropathic cells was also investigated and was associated with a modest reduction in polyubiquitin. Significantly, improved

trafficking and processing of PMP22 in neuropathic mouse Schwann cells correlated with increased myelin production in dorsal root ganglion explant cultures. These results demonstrate that Hsp90 inhibitors are potential therapeutic agents for PMP22 neuropathies.

43. Episodic selection drives the *in vivo* evolution/amplification of HIV-1 X4 co-receptor switch mutants associated with disease progression

Salemi M[1,*], Burkhardt BR[1], Pomeroy S[1], Lowe A[1], Ghaffari G[2,*], Sleasman KW[3], Goodenow MM[1,2,*]

[1]Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL
[2]Department of Pediatrics, Division of Immunology, Rheumatology, and Infectious Diseases, University of Florida, Gainesville, FL
[3]Department of Pediatrics, Division of Allergy, Immunology, and Rheumatology, University of South Florida and All Children's Hospital, St. Petersburg, FL

During the course of HIV-1 infection the phenomenon of coreceptor switch from viruses using CCR5 as coreceptor (R5) to viruses using CXCR4 as coreceptor (X4), which are characterized by high charge amino acid residues in the V3 loop of the envelope gp120 protein, is associated with disease progression. Despite the importance of X4 viral strains emergence for pathogenesis, little is know on the *in vivo* evolution of coreceptor switch mutants. We used high-resolution phylodynamic analysis to investigate the emergence of X4 virus populations in longitudinal peripheral blood mononuclear (PBMC) samples, and lymphoid and non-lymphoid tissues collected post mortem from therapy naïve patients. Reconstruction of ancestral HIV-1 sequences showed that HIV-1 quasispecies undergo several population bottlenecks, driven by episodic positive selection, and that the X4 strains *in vivo* naturally emerge, in the absence of anti-retroviral therapy, as a fitter population surving the bottleneck. The evolution of X4 strains is, in fact, a multi-step temporally structured process that requires the initial fixation of positively selected mutations in V1, V2 and C2 domains of the gp120, without which high charge V3 mutants cannot survive. Moreover, migration analysis based on coalescent theory showed that the thymus constitutes a viral reservoir where the X4 quasispecies is primarily amplified. The key-role of the thymus in the emergence of X4 variants during the course of HIV-1 infection has important consequences for the development of effective therapeutic strategies, based on both conventional antiretroviral regimens and receptor-blocking drugs, aiming at the eradication the virus. R5 coreceptor-blocking agents may be extremely efficient, during the early stage of the disease, in avoiding the emergence of the X4 quasispecies associated with disease progression, while the development of novel drugs targeting the export of X4 infected naïve T-cells from the thymus could be critical to control HIV-1 infection in advanced disease stages.

44. Understanding genetic variation in *Rhabditid* nematodes

Salomon M[1], Blanton D[1], Bour W[1], Brown J[1], Custer A[1], Keller T[1], Levy L[1], Ostrow DG[1], Phillips N[2], Rosenbloom J[1], Baer C[1,*]

[1]Department of Zoology, University of Florida, Gainesville, FL
[2]Department of Biology, Arcadia University, Glenside, PA

Over the past several years our lab has been investigating the properties of new mutations in a model nematode system. Mutations have been allowed to accumulate in the (relative) absence of natural selection, thus allowing us to estimate the genetic variance introduced by new mutation (VM) for two species of *Rhabditid* nematodes, *Caenorhabditis elegans* and *C. briggsae*. However this begs the question, what is the relationship of VM to the standing genetic variance (VG) and

what can this relationship tell us about the nature and magnitude of selection acting on these species? To complement our previous studies and generate an estimate of VG within *C. elegans* and *C. briggsae*, estimates of VG in "wild" worms are needed. We assayed 40 wild strains of *C. elegans* and 8 wild strains of *C. briggsae* for fecundity and body size at 20°C. The primary goal of this study is to characterize the extent of standing life-history variation in these two species. Comparisons of VM to VG between our mutation accumulation lines and the natural isolates allow us to infer the magnitude and pattern of constraint on phenotypic evolution, as well as provide valuable information about the forces responsible for the genetic diversity within and between these two species. Our results suggest that the standing genetic variance in *C. briggsae* is much greater than in *C. elegans*, with *C. briggsae* displaying approximately twice the variance of *C. elegans*. The data also suggests evidence for Mutation Selection-Balance acting on the life history trait of fitness, with a VG to VM ratio much smaller than 4Ne. Also, the persistence time of a new mutation was found to be longer in *C. briggsae* than in *C. elegans*.

45. Assessment of pollen mediated, intraspecific gene transfer from apomictic, transgenic bahiagrass cultivar "Argentine"

Sandhu S[1,*], Altpeter F[1,*], Blount A[2]

[1]Department of Agronomy, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
[2]Department of Agronomy, North Florida Research and Education Center, University of Florida, Marianna, FL

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. The tetraploid cytotypes, including the commercially important cultivar 'Argentine' are considered obligate apomicts, whereas, the sexual types are cross pollinating. Argentine bahiagrass is being genetically modified in our laboratory to increase its stress tolerance, turf and forage quality. Compared to sexually reproducing grasses apomictic bahiagrass might reduce the risk of unintended transgene spread. Thus, it is important to evaluate pollen mediated intraspecific gene transfer from apomictic, transgenic bahiagrass. Glufosinate resistant 'Argentine' bahiagrass lines were generated by biolistic gene transfer and herbicide resistance was used as a marker to study the intraspecific gene flow from transgenic apomictic 'Argentine' bahiagrass to wild type diploids under field and greenhouse conditions. Data on herbicide resistance of seed progeny and intraspecific gene transfer frequencies under greenhouse and field conditions will be presented.

46. An essential role for Dicer and microRNAs in skeletal muscle development

Seay HR[1], O'Rourke JR[1], Goldhamer DJ[2], McManus MT[3,*], Swanson MS[1,*], Harfe BD[1,*]

[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
[2]Department of Molecular and Cell Biology, University of Connecticut, Stors, CT
[3]Diabetes Center, Department of Microbiology and Immunology, University of California, San Francisco, CA

MicroRNAs (miRNAs) are a class of evolutionary conserved, small (~22nt) and non-coding RNAs that regulate gene expression post-transcriptionally. miRNAs originate as long primary transcripts that are cleaved by the endonucleases Drosha and Dicer. Dicer is responsible for the production of the functional miRNA, and loss of Dicer results in the accumulation of the non-functional precursors and depletion of miRNAs. Dicer is ubiquitously expressed in mice and humans. However, miRNAs exhibit tissue-specific expression patterns, suggesting that these molecules

function in tissue differentiation. Three miRNAs, miR-1, miR-133 and miR-206, have been shown to be specifically enriched in skeletal muscle. To gain insight into the roles of Dicer and miRNAs in skeletal muscle, we deleted Dicer and functional miRNAs in mouse skeletal muscles by crossing mice containing Dicer conditional alleles to those expressing a MyoDcre transgene. The resulting Dicer cond/cond; MyoDcre animals die perinatally. Mutant embryos have an increased number of apoptotic cells and reduced skeletal muscle mass in the limb buds. Our results indicate that miRNA are required for normal myogenesis and proper fiber formation.

47. Cellular origin of the penile urethra and temporal fate of the urethral epithelium

Seifert AW[1], Harfe B[2,*], Cohn MJ[1,*]

[1]Department of Zoology, University of Florida, Gainesville, FL
[2]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Hypospadias are a congenital malformation of the penis, affecting 1:125 live male births. As hypospadias affects both proper location of the urethral opening and correct formation of the tubular urethra, a detailed understanding of the embryonic origins and fate of the urethral epithelium (UE) is vital to a correct model of genital tubercle (GT) organogenesis. The prevailing view is that the distal (glandar) urethra arises from ectodermal cells invaginating from the distal tip of the glans. We tested the hypothesis that the glandar urethra originates from endoderm, by using Sonic Hedgehog (Shh), a marker of hindgut endoderm, to genetically label and follow UE cells during mouse GT development. We used a gfp-cre fusion cassette knocked into the Shh locus (Shhgfpcre) to activate the Rosa26 reporter in endodermal cells to permanently label all cells that express Shhgfpcre and their descendants. Our detailed fate-map revealed an endodermal origin for the glandar urethra, based on lacZ expression in newborn mice. In addition to fate-mapping the entire urethra, we investigated the temporal development of the urethra to determine if different parts of the urethra are laid down at different stages. We utilized a tamoxifen-inducible allele of cre knocked into the Shh locus (ShhcreErt2) to label UE cells expressing Shh at daily intervals. Our results indicate that the entire urethra is determined prior to developmental stage E14.5, suggesting that development of the urethra does not follow a proximal to distal progression like that found in the limb.

48. A haplotype of ß2-adrenoceptor is associated with the increased risk for transplant or death in heart failure patients

Shin J[1], Lobmeyer M[1], Gong Y[1], Zineh I[1], Langaee TY[1,*], Yarandi H[1], Pauly DF[2], Aranda Jr. JM[2], Schofield RS[2], Hill JA[2], Johnson JA[1,*]

[1]Center for Pharmacogenomics/Department of Pharmacy Practice, University of Florida, Gainesville, FL
[2]Division of Cardiovascular Medicine/Department of Medicine, University of Florida, Gainesville, FL

BACKGROUND: Heart failure (HF) is characterized by neurohormonal activation of sympathetic nervous and renin-angiotensin systems. We tested whether genetic polymorphisms in these systems are associated with death or heart transplantation in patients with HF.
METHODS: 227 HF patients were enrolled from a tertiary care HF clinic and followed for outcomes every 6 months for up to 4 years. We genotyped 8 polymorphisms in 6 genes including: ß1 adrenergic receptor (ADRB1, S49G and R389G), ß2 adrenergic receptor (ADRB2, G16R and Q27E), α2c adrenergic receptor (ADRA2C, Insertion/Deletion (I/D) 322-325), angiotensinogen (AGT, M235T), angiotensin receptor type 1 (AGTR1, A1166C), and angiotensin converting enzyme (ACE, I/D in intron 16). HAP software was used for haplotype assignment for

ADRB1 and ADRB2. Cox proportional hazard regression was performed to model the outcomes with genotypes/haplotypes, adjusting for non-genetic predictors of HF.
RESULTS: During a median 2.5 year follow-up period, 78 patients had an adverse outcome. Ninety five percent and 81% of the patients received an ACE inhibitor/angiotensin receptor blocker and a ß-blocker at baseline, respectively. Significant predictors are shown in the Table. The ADRB2 RQ haplotype was the only genotype/haplotype associated with adverse outcomes.

Variable	Hazard ratio	95% confidence interval	P-value
NYHA class	2.56	1.84-3.56	<0.0001
Creatinine clearance	0.99	0.98-0.99	0.0016
Sodium	0.91	0.86-0.97	0.0019
Male gender	2.33	1.33-4.10	0.0032
ADRB2 RQ haplotype	1.40	1.01-1.93	0.047

CONCLUSIONS: In addition to other traditional predictors, ADRB2 haplotype may have a significant effect on risk for heart transplant/death in HF patients who receive contemporary HF pharmacotherapy.

49. Accurate breast cancer prognosis through the combination of clinical and genetic markers

Sun Y[1], Goodison S[2], Li J[3], Li L[1], Farmerie W[1,*]

[1]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL
[2]Department of Surgery, University of Florida, Gainesville, FL
[3]Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL

Motivation: Accurate prognosis of breast cancer can spare a significant number of breast cancer patients from receiving unnecessary adjuvant systemic treatment and its related expensive medical costs. Recent studies have demonstrated the potential value of gene expression signatures in assessing the risk of post-surgical disease recurrence. However, these studies all attempt to develop genetic marker-based prognostic systems to replace the existing clinical criteria, while ignoring the rich information contained in established clinical markers. Given the complexity of breast cancer prognosis, a more practical strategy would be to utilize both clinical and genetic marker information that may be complementary.
Methods: A computational study is performed on publicly available microarray data which has spawned a 70-gene prognostic signature. The recently proposed I-RELIEF algorithm is used to identify a hybrid signature through the combination of both genetic and clinical markers. A rigorous experimental protocol is used to estimate the prognostic performance of the hybrid signature and other prognostic approaches. Survival data analyses are performed to compare different prognostic approaches.
Results: The hybrid signature performs significantly better than other methods, including the 70-gene signature, clinical makers alone and the St Gallen consensus criterion. At the 90% sensitivity level, the hybrid signature achieves 69% specificity, as compared to 47% for the 70-gene signature and 48% for the clinical makers. The odds ratio of the hybrid signature for developing distant metastases within five years between the patients with a good prognosis signature and the patients with a bad prognosis is 23.0 (95% CI: 7.0-75.0), far higher than either genetic or clinical markers alone.

50. A role in arsenic resistance for a novel glutaredoxin from hyperaccumulator fern *Pteris vittata L.*

Sundaram S, Rathinasabapathi B*

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

Chinese brake fern *Pteris vittata L.* has an unusual ability to tolerate and hyperaccumulate arsenic and hence is valued for phytoremediation of arsenic-contaminated soil and water. In a functional genomics project to identify arsenic resistance genes from this fern, PVGrx5-6, a cDNA for a glutaredoxin (GRX) was isolated based on its ability to confer arsenic resistance upon expression in *Escherichia coli*. Deduced amino acid sequence of PVGrx5-6 showed high homology with an *Arabidopsis* GRX and protein kinase C-interacting cousin of thioredoxin homology domain (PICOT-HD) proteins. PVGRX contained two domains – the N-terminus region (1-128 aa) that was unique, and the C-terminus region (128-214 aa) similar to glutraredoxins known in many different organisms. Each domain had a CXXS catalytic motif. Purified recombinant PVGRX exhibited classical glutaredoxin activity which was activated 1.8-fold by the addition of 10 mM arsenate in the assay. To understand the nature of arsenic resistance conferred by PVGrx5-6, it was expressed in *E. coli* mutants lacking the arsenic resistance genes of the ars operon (AW3110Δ), a glutathione-deficient strain (JTG10Δ), an arsenate reductase-deficient strain (WC3110Δ), and an aquaglyceroporin-deficient (OSBR1Δ) strain. Except in OSBR1, expression of PVGrx5-6 increased cellular arsenic tolerance, suggesting that PVGrx5-6 had a role in arsenic tolerance independent of the arsenic operon genes. Cells expressing PVGrx5-6 had significantly lower levels of arsenite compared to vector controls when cultured in medium containing 2.5 mM arsenate. Our results are consistent with PVGRX having a role in regulating intracellular arsenite levels directly or indirectly through modulating an aquaglyceroporin previously known in arsenite transport.

51. Application of suppression subtractive hybridization (SSH) to cloning differentially expressed cDNA in nematode resistant and susceptible cultivars of peanut (*Arachis hypogaea* L.) under nematode attack

Varma TSN[1], Gallo M[1,2,*]

[1]Department of Agronomy and University of Florida, Gainesville, FL
[2]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Annual economic losses in cultivated peanut (*Arachis hypogaea* L.) due to nematodes are estimated at $102 million. Root-knot nematodes (*Meloidogyne* spp.) are the most important nematode pathogens of peanut, and the predominant pathogenic species in the southern United States is *M. arenaria*. Resistance to root-knot nematodes from wild peanut species has been introgressed into cultivated peanut. Following a backcrossing program using Florunner, the nematode resistant, nearly isogenic cultivar, NemaTAM, was released. To identify possible candidate genes conferring resistance to this pest, we have constructed a cDNA library enriched with differentially expressed sequences obtained by Mirror Orientation Selection PCR from root tissues of the nematode resistant cv. NemaTAM (Ne) and the nematode susceptible cv. Florunner (FL) through suppression subtractive hybridization. Randomly picked clones (480) from the tester Ne-specific library and the driver FL-specific library were used for differential screening. From this screen, 140 Ne -specific clones and 123 FL -specific clones were obtained. Virtual northern blot analysis was performed to confirm the differential screening results, and verified clones were sequenced. Sequence analysis revealed that a majority of the identified NemaTAM-specific sequences had pathogenesis-related and stress-related functions, and the Florunner-specific

sequences were involved in signal transduction and cellular communication. This work provides the foundation for the identification and study of genes involved in the molecular defense mechanism of nematode resistance in peanut.

52. Analysis of 6-phosphogluconate dehydrogenase enzyme activity during seed development in maize

Tseung CW, Nguyen R, Settles AM*

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

The 6-phosphogluconate dehydrogenase enzyme (6PGDH) has a central role in the oxidative pentose phosphate pathway and carbon metabolism. 6PDGH carries out the oxidation and decarboxylation of glucose-6-phosphate to produce ribulose-5-phosphate and NADPH. Ribulose-5-phosphate is used for the synthesis of metabolic intermediates such as nucleotides, and NADPH is used for reductive biosynthesis reactions within the cell. In plants, 6PGDH is found in both the cytosol and the plastid. There are three known loci that are predicted to encode 6PGDH enzymes in maize. Pgd1 and Pgd2 encode cytosolic enzymes, and the PGD3 protein is predicted to be chloroplast-localized. Homozygous mutants in both pgd1 and pgd2 show normal plant and seed development. Here we show that a pgd3 mutant is linked to a rough endosperm (rgh) kernel phenotype. The rgh/pgd3 mutant shows reduced grain-fill as well as embryo morphogenesis defects suggesting that PGD3 is an important enzyme for seed development. Native gel activity assays with pgd1, pgd2, and pgd3 mutants showed that PGD3 is an active 6PGDH enzyme and that PGD3 is induced during seed development. These results suggest that chloroplast-localized 6PGDH is necessary during seed development and that cytosolic 6PGDH enzymes cannot complement the pgd3 mutant.

53. Proteolytic mapping of AAV serotypes

Van Vliet K[1], Brument N[1,5], Blouin V[1,5], Mohiuddin Y[1], Moullier P[1,5], Agbandje-McKenna M[2,*] Snyder RO[1,3,4,*]

[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
[2]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
[3]Department of Pediatrics, University of Florida, Gainesville, FL
[4]Powell Gene Therapy Center, University of Florida, Gainesville, FL
[5]Laboratoire de Therapie Genique, Nantes Cedex 44035, France

Adeno-associated virus (AAV) is a very promising gene delivery system that is being tested in the clinic. In excess of 100 AAV serotypes have been identified to date. Unique viral surface features are associated with different serotypes that provide different efficiencies and tissue tropism. AAV2 is the most studied of the AAV serotypes; however, clinical trials for other AAV serotypes with different tissue tropism have begun. AAV2 vectors have been shown to elicit a neutralizing antibody response after the first administration that render subsequent treatments with AAV2 ineffective. AAV pseudotyping, a process in which the therapeutic transgene is flanked by the AAV2 ITR's and packaged into the capsid of another serotype is common. Pseudotyping allows comparison of different AAV serotypes expressing the same transgene cassette when evaluating gene transfer efficiency to specific organs. Pseudotyping may also be useful to provide an effective treatment for patients needing repeat dosing. AAV manufactured in compliance with current Good Manufacturing Practices (cGMP) regulations must undergo product identity testing before being released for use in the clinic. Currently, identity testing involves sequencing the packaged vector DNA and verifying the sequence against the GenBank database. Assays to verify the capsid serotype are not common and rely on immunological reagents that can cross react

with multiple serotypes. In the present study, we utilize proteolytic mapping as a tool for AAV capsid serotype identification. Treatment of AAV capsids with a variety of proteases results in unique fragmentation patterns for different AAV serotypes, and we are using these reagents to develop standardized identity tests for AAV. By characterizing different serotypes with different proteases, we are developing a fingerprint database that can be used to identify several AAV serotypes. These assays and databases provide better product characterization and protection of patients.

54. Characterization of photoreceptor genes regulating apothecial morphogenesis of *Sclerotinia sclerotiorum*

Veluchamy S, Rollins JA*

Department of Plant Pathology, University of Florida, Gainesville, FL

Apothecial development is a particularly important step in the developmental life cycle of *Sclerotinia sclerotiorum*, as ascospores are the primary source of initial inoculums and each apothecium is capable of producing millions of ascospores. This morphogenesis is light dependent requiring light in the UV-A wavelengths. We have identified a number of putative photoreceptor encoding genes from our collaborative *S. sclerotiorum* Whole Genome Sequencing Project. In an attempt to identify a UV-A photoreceptor we been studying a gene homology to cryptochromes (scry1) in *S. sclerotiorum*. The predicted scry1 protein sequence shows a high degree of sequence identity (50%) to N. crassa cry1. scry1 transcript accumulates only after exposure to UV-A and not in other wavelengths of light including constant dark, white light, blue, red or green. Northern blot analyses revealed the accumulation of scry11 transcript in all stages of apothecia and vegetative mycelia but not in sclerotia Different UV-A light fluence rates (from 1.2 to 14 μmoles/m^2/s) were tested to study transcript accumulation kinetics in mycelia and apothecia. In apothecia, scry1 transcript was significantly increased by two and six hours of UV-A light treatment at 3.5 μmoles/m^2/s). We are currently working to functionally disrupt the scry1 gene to determine its involvement in light dependent apothecial development.

55. The regulation of peripheral myelin protein 22 expression by micoRNAs

Verrier JD[1], Lau P[2], Hudson LD[2], Notterpek L[1,*]

[1]Departments of Neuroscience and Anatomy and Cell Biology, University of Florida, Gainesville, FL
[2]Section of Developmental Genetics, National Institute of Neurological Disorders and Stroke, National Institutes of Health

MicroRNAs (miRNAs) are small ~22 nucleotide RNA molecules that have been shown to regulate gene expression at the post-transcriptional level primarily through interaction with the mRNA's 3'UTR region. Recently miRNAs have been shown to be involved in many cellular processes, including development and apoptosis, as well as disease states. Using several miRNA prediction models (TargetScan, miRBase, and miRanda), we have identified two miRNAs, mir-9 and mir-29, that are likely to interact with Peripheral Myelin Protein 22 (PMP22). PMP22 is an essential myelin protein synthesized by Schwann cells in the peripheral nervous system. Under- and overexpression of this protein are linked to hereditary peripheral neuropathies. To investigate the potential roles of mir-9 and mir-29 in the regulation of pmp22 in rat Schwann cells, we first overexpressed the miRNAs through transfection of plasmids and analyzed PMP22 RNA levels by Northern blots. Using an emerald-green fluorescent protein (EmGFP) encoding plasmid, the transfection efficiency of the cells was ~30%. Twenty-four hours post-transfection, we observed a notable reduction in the levels of PMP22 mRNA with both miRNAs, when compared to control cells. Steady-state levels of PMP22 protein were similarly reduced, as compared to EmGFP-alone

expressing cells. In agreement, fluorescence microscopy revealed a reduction of PMP22-like immunoreactivity in miRNA-positive cells. Steady-state protein levels are more affected by mir-9, as compared to mir-29. These experiments reveal a new mechanism in the regulation of the PMP22 gene and identify a potentially novel therapeutic target for PMP22-linked diseases.

56. Functional characterization of myrosinases and interacting proteins in *Arabidopsis*

Wang Y-YE[*], Vijayvargiga K[*], Godsmith J[*], Chen S[*]

Department of Botany, University of Florida, Gainesville, FL

Plants are unique in their ability to produce a wide array of secondary compounds used for foods, flavors, medicines, and industrial materials. Glucosinolates are a group of such compounds, the degradation products of which are not only beneficial to humans but also play an important role in plant growth, development, and interactions with insect herbivores. Hydrolysis of glucosinolates is known to be catalyzed by myrosinase, the only known S-glucosidases in nature. In model plant *Arabidopsis*, there are 6 myrosinase genes and many genes encoding myrosinase associated proteins and binding proteins. The functions of these genes and their interactions are not well understood. In our lab, we took multidisciplinary approaches including molecular biology, biochemistry, proteomics, reverse genetics and physiology to study gene functions in glucosinolate metabolism. We have cloned genes of myrosinase TGG1 and TGG2, myrosinase associated protein MyAP1 and myrosinase binding protein MBP1 in *E. coli*. The genes were transformed into yeast *S. cerevisiae* for protein expression and interaction studies. Western blot analysis has shown positive protein expression. The heterologously expressed proteins are currently tested for enzyme activities, substrate profiles and interactions with each other. In the meantime, vacuolar proteomics studies in *Arabidopsis* have revealed vacuolar localization of these proteins. Myrosinase complex formation will be analyzed using purified vacuoles from *Arabidopsis*. Reverse genetics tools were also implemented to characterize *in vivo* protein functions. T-DNA knockout mutants including single and double mutants of myrosinase TGG1 and TGG2 were obtained. Recent results will be presented and discussed.

57. SLCO1B1 gene variation and apolipoprotein response to atorvastatin

Welder GJ[1], Arant CB[2], Wessel TR[2], Schofield RS[2,3], Langaee TY[1,*], Zineh I[1]

[1]Center for Pharmacogenomics/Department of Pharmacy Practice, University of Florida, Gainesville, FL
[2]Division of Cardiovascular Medicine, University of Florida College of Medicine, Gainesville, FL
[3]Department of Veterans Affairs Medical Center, Gainesville, FL

Studies suggest that apolipoprotein (apo) B, apoA1, and apoB/apoA1 ratio may be superior predictors of cardiovascular disease (CVD) risk over low-density lipoprotein (LDL) cholesterol. There are no pharmacogenetic studies evaluating variable statin effects on apos. The SLCO1B1 gene encodes a hepatic transporter responsible for statin uptake into their site of action. We investigated whether genetic variants in SLCO1B1 impact the apo response to atorvastatin. Forty-five subjects (64% women) without CVD or risk equivalents were analyzed. Fasting apos were measured at baseline and after 8 weeks of atorvastatin 80 mg daily. SLCO1B1 genotypes and haplotypes were determined by PCR and pyrosequencing. T-tests were used to test the hypothesis that 174Ala variant carriers and/or subjects with the *5 haplotype had diminished apo responses when compared to wildtype homozygotes (*1a/*1a), since it has been shown that these variants have diminished transporter activity. Baseline age, apoB, and apoA1 were: 34±15 yrs, 81±23 mg/dl, and 144±31 mg/dl respectively. There were no differences in baseline

characteristics between genotype groups. 174Ala carriers (N=18) had an apoB reduction of 33% compared with 41% in *1a/*1a subjects (p=0.10) and a 12% greater increase in apoA1 (p=0.08). Those with the SLCO1B1*5 haplotype (N=6) had an apoB reduction of 34% compared to *1a/*1a subjects (p=0.07). An apoB/apoA1 reduction of 32% was seen in this haplotype group compared to 41% in wild-type homozygotes (p=0.07). SLCO1B1 polymorphisms were associated with differences in the apo response to atorvastatin 80 mg daily. Further investigations into SLCO1B1 variations are warranted to confirm differences in the therapeutic effects of statin treatment.

58. The ankyrin repeat, host range protein of myxoma virus, M-T5, activates Akt and can be functionally replaced by PIKE-A

Werden SJ [1,2], Barrett JW[2], McFadden G[1,*]

[1]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, Fl.
[2]Department of Microbiology and Immunology, University of Western Ontario and Robarts Research Institute, London, Ontario, Canada.

The myxoma virus (MV) ankyrin-repeat host range factor, M-T5, has the ability to bind and activate cellular Akt, leading to permissive MV replication in a variety of diverse human cancer cell lines (Wang et al. Proc. Natl. Acad. Sci. U.S.A. 103:4640–4645, 2006). The susceptibility of permissive human cancer cells to MV infection is directly correlated with the basal or induced levels of phosphorylated Akt. When M-T5 is deleted from MV, the knockout virus, vMyxT5KO, can no longer productively infect a subset of the human cancer cells (designated Type II) that exhibit little or no endogenous phosphorylated Akt. In searching for a host counterpart of M-T5 we noted sequence similarity of M-T5 to a recently identified ankyrin-repeat cellular binding protein of Akt called PIKE-A. PIKE-A binds and activates the kinase activity of Akt in a GTP-dependent manner and promotes the invasiveness of human cancer cell lines. Here we demonstrate that transfected PIKE-A is able to rescue the ability of vMyxT5KO to productively infect Type II human cancer cells that were previously resistant to infection. As well, cancer cells that were completely non-permissive for both wild-type and vMyxT5KO infection (called Type III) were rendered fully permissive following ectopic expression of PIKE-A. We conclude that the MV M-T5 host range protein is functionally interchangeable with the host PIKE-A protein, and that the activation of host Akt by either M-T5 or PIKE-A is critical for the permissiveness of human cancer cells by MV.

59. Transgenic approaches to enhance abiotic stress tolerance of bahiagrass

Xiong X[1], James V[1], Lomba P[1], Halbig SM[1], Altpeter F[1,*], Blount A[2]

[1]Department of Agronomy, Plant Molecular and Cellular Biology Program, Gainesville, FL
[2]Department of Agronomy, North Florida Research and Education Center, Marianna, FL

Bahiagrass (*Paspalum notatum Flugge*) is an important turf and forage grass in the southern United States and subtropical regions of the world. The productivity and performance of bahiagrass is limited by abiotic stresses, such as drought, salt, and low temperature (chilling and freezing). Altering the transcriptional regulation of stress response genes is a successful strategy to enhance stress tolerance in plants. Stress inducible transcription factors like HvWRKY38 and OsMYB4 have the potential to enhance the expression of stress protective genes. We generated transgenic bahiagrass plants constitutively over-expressing HvWRKY38 or OsMYB4 via biolistic gene transfer. We will present molecular and physiological data allowing the correlation of transcription factor over-expression and plant performance under abiotic stress.

60. Characterization of a bipartite rAAV vector for AAVS1 site specific integration *ex vivo* therapy

Zhang C, Cortez N, Berns KI*

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

Wild type adeno-associated virus 2 (AAV2) can establish a latent infection in cell culture by integrating at a specific site (AAVS1) on the q arm of human chromosome 19. Requirements for site specific integration include a permissive intracellular milieu (e.g., absence of a helper virus co-infection), the AAV Rep 68/78 protein in trans, the AAV inverted terminal repeat (itr) in cis (this may not be essential if the P5 promoter, which contains a Rep binding site (RBS) is present), and AAVS1. AAV is currently a popular vector for human gene therapy. However, current vectors do not contain the rep gene or the P5 promoter and consequently do not undergo site specific integration. Development of an AAV vector which could integrate into AAVS1 might offer significant advantages in the engineering of progenitor cells and stem cells *ex vivo*. Appropriately engineered cells could be expanded, cloned, characterized and used for human gene therapy. In this report we describe the development of a bipartite AAV vector which can preferentially integrate the transgene at AAVS1 while the rep gene remains extrachromosomal. Our results demonstrated that with the optimal MOI and ratio of rAAVSVAV2 and rAAVUF11, the transgene integrated at AAVS1 specifically with high efficiency (83.3%). Most importantly, the cloned cell lines with the AAVS1 site specific integrated GFP were healthy and continuously and stably expressing GFP. In discussion we propose a model of AAVS1 site specific integration *ex vivo* gene therapy of rAAV.

61. Lamprey type II collagen and Sox9 reveal an ancient origin of the vertebrate collagenous skeleton

Zhang G, Miyamoto MM*, Cohn MJ*

Department of Zoology, University of Florida, Gainesville, FL

Type II collagen is the major cartilage matrix protein in the jawed vertebrate skeleton. Lampreys and hagfishes, by contrast, are thought to have non-collagenous cartilage.This difference in skeletal structure has led to the hypothesis that the vertebrate common ancestor had a non-collagenous skeleton, with type II collagen becoming the predominant cartilage matrix protein after the divergence of jawless fish from the jawed vertebrates, approximately 500 million years ago. Here we report that lampreys have two type II collagen (Col2a1) genes that are expressed during development of the cartilaginous skeleton. We also demonstrate that the lamprey skeleton is comprised of Col2a1 protein. Furthermore, we have isolated Sox9, a direct transcriptional regulator of Col2a1 in jawed vertebrates, and show that it is co-expressed with both Col2a1 genes during development of the lamprey skeleton. These results reveal a highly conserved genetic pathway for chondrogenesis in lampreys and gnathostomes, and suggest that a collagenous skeleton evolved surprisingly close to the origin of vertebrates.

62. Altering plant architecture of bahiagrass by constitutive expression of the *Arabidopsis* ATHB16 gene, a repressor of cell expansion

Zhang H, Lomba P, Altpeter F*

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

Bahiagrass (*Paspalum notatum Flugge*) is a popular low-input turfgrass in subtropical regions. However its turf quality and widespread use as residential turf is limited by up to 60 cm tall seedheads and its open, sparse looking growth habit. Due to rising mowing costs and the absence of a dwarf bahiagrass cultivar, plant growth retardants (PGR's) are being used increasingly to suppress seedheads and leaf growth in bahiagrass. However, PGRs have to be applied frequently and may result in phytotoxicity. Improvement of turf quality by genetic engineering represents a promising alternative. In this study, the ATH16 transcription factor gene was isolated from Arabidopsis, cloned in an expression cassette between the CaMV 35S promoter and nos terminator and genetically transferred to bahiagrass via particle bombardment. The integration of ATHB16 in the bahiagrass genome and its constitutive expression were confirmed by Southern and RT-PCR analysis respectively. Morphological characteristics of bahiagrass over-expressing ATHB16 under controlled environment conditions will be presented in comparison to non-transgenic bahiagrass.

63. Analysis of β-globin locus activation during erythroid differentiation of embryonic stem cells

Zhou Z, Crusselle-Davis V, I-Chun L, Levings P, Bungert J*

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

The β-globin gene locus consists of five developmentally regulated genes that are under control of a locus control region (LCR) located far upstream of the genes. The LCR is composed of several subregions exhibiting high sensitivity to DNaseI in erythroid cells, called HS sites, which function together to mediate high level globin expression. Recent studies have shown that individual HS sites recruit transcription complexes. We found that RNA polymerase II (RNA Pol II) is recruited to LCR HS elements prior to erythroid differentiation. In order to understand the temporal order of transcription factor recruitment and modification of chromatin structure, we continued our analysis of the globin locus during *in vitro* differentiation using chromatin immunoprecipitation. Several transcription factors, including the ubiquitously expressed helix-loop-helix protein USF are detectable at LCR elements prior to erythroid differentiation. We have recently developed an *in vitro* system to study the transfer of activities from the LCR to the β-globin gene. We found that RNA Pol II can be transferred from immobilized LCR constructs to a β-globin;-globin gene, that the transfer is sensitive to mutations in the basal promoter of the β-globin;-globin gene, and that the hematopoietic transcription factor NF-E2 facilitates the transfer from the LCR to the globin gene promoter. We continued this analysis and found that other proteins are transferred from the LCR to the globin gene promoter, including USF, Fog-1, and GATA-1. Our data suggest that NF-E2 decreases the affinity of RNA Pol II to the LCR and at the same time increases the binding affinity to the β-globin gene promoter. Taken together our data support the proposition that transcription complexes are first recruited to the LCR and subsequently transferred to the globin genes.