FLORIDA GENETICS 2009
THE FIFTH ANNUAL SYMPOSIUM OF THE UF GENETICS INSTITUTE

OCTOBER 28-29
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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.
2009 Florida Genetics Symposium Schedule

**Wednesday, October 28, 2009**

Noon – 1:00 p.m.: Check-in and poster set-up at the Cancer/Genetics Research Complex

1:00 p.m. – 1:15 p.m.:  
Opening Remarks:  
Indra Vasil and Kenneth Berns

Session I  
Translational Genomics  
Chair: Kenneth Berns

1:15 p.m. – 2:00 p.m., Terry Van Dyke:  
"Mechanistic discovery in murine cancer models: From basic discovery to clinical translation"

2:00 p.m. – 2:30 p.m., Rolf Renne: "KSHV-encoded miRNAs and their role in viral biology"

2:30 p.m. - 4:30 p.m.: Poster Session and Reception (for registered attendees)

*5:00 p.m. – 6:00 p.m., Leroy Hood: "Systems biology & systems medicine - catalyzing a transformation from reactive to proactive medicine”

*Note: All activities will be at the Cancer/Genetics Research Complex except for Dr. Hood’s presentation, which will be at 5 p.m. Wednesday in the auditorium of the HPNP building on the Health Science Center campus.

**Thursday, October 29, 2009**

8 a.m. - 8:30 a.m.: Check-in, coffee

Session II  
*Drosophila* Genetics  
Chair: Thomas Yang

8:30 a.m. – 9:15 a.m., Michael Levine:  
"Transcriptional precision in the *Drosophila* embryo”

9:15 a.m. – 9:45 a.m., Lei Zhou: "Epigenetic regulation controls cellular sensitivity to stress induced apoptosis”

Session III  
Human Evolution  
Chair: Connie Mulligan

9:45 a.m. – 10:30 a.m., Anna Di Rienzo:  
"Adaptations to local environments in humans”

10:30 a.m. – 11:00 a.m., David Reed: "Of lice and men: The inference of human evolutionary history from the perspective of its host-specific parasites”

11:15 a.m. – 1:30 p.m.: Poster Session

11:30 a.m. – Lunch (for registered attendees)

Session IV  
Genome Evolution  
Chair: Doug Soltis

1:30 p.m. – 2:00 p.m., Pam Soltis: "Whole-genome duplication in plant evolution: Case studies of ancient events and recent speciation”

2:00 p.m. – 2:30 p.m., Brad Barbazuk: "A conserved alternative splicing event in plants reveals an ancient exonization of 5sRNA”

2:30 p.m. – 3:15 p.m., John Doebley: "Darwin and domestication”

3:15 p.m. – 3:45 p.m., Karen Koch: "Maize domestication: Metabolic adaptations in grain evolution”
We have developed genetically engineered mice (GEM) to model many human cancers. Each model recapitulates major pathological features of the human disease. The tumor development stages in these models are associated with distinct genetic alterations in defined time windows, and as such the models provide us tools to identify potential biomarkers for therapy and early diagnosis and to develop therapeutic strategies to inform clinical trials. We have generated inducible GEM models of astrocytoma by targeting pathways (RB, KRAS and PTEN) frequently dysregulated in the human diseases. Alterations were induced specifically in adult GEM astrocytes via a tamoxifen-inducible, human GFAP promoter-driven CreERTM allele. GEM with astrocyte RB pathway inactivation alone developed low-grade astrocytomas (grade II). Neither constitutive KRAS activation nor PTEN inactivation alone produced detectable brain pathology. GEM harboring both inactivated RB and constitutively active KRAS in astrocytes developed high-grade astrocytomas (grade III); addition of PTEN inactivation produced tumors with histopathological features of GBM (grade IV). These results suggest roles for RB and KRAS-PTEN pathways in astrocytoma initiation and progression, respectively. Initial studies have begun to define the relative contribution to disease of each disrupted pathway, including potential downstream targets for therapeutic intervention. We have also developed a strategy for modeling serous epithelial ovarian cancer (EOC) using similar approaches. Thus far, no model of this disease suitable for preclinical testing has been developed. However, several basic studies have identified genetic aberrations required to drive disease in GEM, and we have shown that the combination of pRb, p53 and BRCA1 inactivation leads to EOC characteristic of the serous type. Our results thus far in developing an inducible EOC model will be presented. Finally, we have developed a prostate cancer model that develops heterogeneous cancers dependent upon impaired pRb, Pten and p53 function, including a complex co-evolution of stroma and carcinoma. Current studies are evaluating the cell of origin, the complex biology of tumor progression, specific pathways involved, and the role of inflammatory responses in disease.

Biography of Terry Van Dyke, Ph.D.

Terry Van Dyke, Ph.D., studies the mechanisms and pathways to cancer development at many levels, including genetic, molecular, cell and organ biology. Because cancer can develop in more than 100 distinct mammalian cell types, her lab has utilized genetically engineered mice as the foundation of its analyses. It has established several preclinical cancer models that have facilitated analyses of the tumor suppressors, including their contribution to normal growth control and the consequences of their inactivation to multistep tumorigenesis. The approach enables a detailed examination of the molecular and cellular events in developing tumors - studies that are not possible in humans. She received her Ph.D. in Medical Sciences from the University of Florida in 1981. As a postdoctoral fellow in Dr. Arnold Levine's lab in the early 1980s, she characterized one of the first transgenic cancer models. In 1993, she joined the University of North Carolina, where she is currently a Sarah Graham Kenan Distinguished Professor of Genetics with a joint appointment in Biochemistry and Biophysics. In 1998, she became the Facility Director of the UNC Animal Models Core Facility and, in 1999, she established the Carolina Mutant Mouse Regional Resource Center, one of four in the country. In the late 1990s, Dr. Van Dyke served as co-chair of an advisory committee to the Director of the National Cancer Institute on the use of mouse models to advance cancer research, which led to the establishment of the Mouse Models of Human Cancers Consortium (MMHCC). In this capacity she has provided significant leadership in initiatives, including the establishment of a national repository for GEM mice, an international database for mouse models of cancer, and a web site including summaries of human cancers and mouse cancer models. She continues to serve as a MMHCC PI, leading a multi-institutional collaboration on astrocytic cancers.
The challenge for biology in the 21st century is the need to deal with its incredible complexity. One powerful way to think of biology is to view it as an informational science. This view leads to the conclusion that biological information is captured, mined, and integrated by biological networks and finally passed off to molecular machines for execution. Hence the challenge in understanding biological complexity is that of deciphering the operation of dynamic biological networks across the three time scales of life - evolution, development and physiological responses. Systems approaches to biology are focused on delineating and deciphering dynamic biological networks and their interactions with simple and complex molecular machines. I will focus on our efforts at a systems approach to disease - looking at prion disease in mice. We have just published a study that lays out the principles of a systems approach to disease including dealing with the striking signal to noise problems of high throughput biological measurements and biology itself (e.g. polymorphisms).

I will discuss the emerging technologies (measurement and visualization) that will transform medicine over the next 10 years, including next generation DNA sequencing, microfluidic protein chips and single-cell analyses. I will discuss some of the computational and mathematical challenges that are fundaments to the revolution in medicine, both those that deal with medical sciences and those that deal in a general way with healthcare. It appears that systems medicine, together with pioneering changes in these emerging technologies and the development of powerful new computational and mathematical tools will transform medicine over the next five to 20 years from its currently reactive state to a mode that is predictive, personalized, preventive and participatory (P4). This will in turn lead to the digitalization of medicine, with ultimately a profound decrease in the cost of healthcare. It will also transform the business strategies for virtually every sector of the health care industry. These considerations have led the Institute for Systems Biology to begin formulating national and international strategic partnerships focused on accelerating the realization of P4 medicine. I will discuss some of these strategic partnerships and the implications for healthcare arising from P4 medicine.

Biography of Leroy Hood, M.D., Ph.D.

Leroy Hood, M.D., Ph.D., has focused on fundamental biology and on bringing engineering to biology through the development of five instruments that constitute the technological foundation for modern molecular biology and genomics - the DNA and protein sequencers and synthesizers and the ink-jet oligonucleotide synthesizer. Early in his career, he applied these technologies to the study of molecular immunology, discovering many of the fundamental mechanisms for antibody diversity, and neurobiology; he cured the first neurological disease by gene transfer in mice. In the late 1980s, he embarked on a systems biology approach to understand immunology. In 1992, he moved to the University of Washington as founder and Chairman of the cross-disciplinary Department of Molecular Biotechnology (MBT) where he initiated systems studies on cancer biology and prion disease. In 2000, he co-founded the Institute for Systems Biology in Seattle, Washington, to continue pioneering systems approaches to biology and medicine. Dr. Hood is now pioneering the idea that the systems approach to disease, the emerging technologies, and powerful new computational and mathematical tools will move medicine from its current reactive mode to a predictive, preventive, personalized and participatory mode (P4 medicine) over the next five to 20 years. Dr. Hood has been awarded numerous prizes - the Lasker Prize (1987), the Kyoto Prize in Advanced Technology (2002), the Lemelson–MIT Prize for Innovation and Invention (2003), the Association for Molecular Pathology Award for Excellence in Molecular Diagnostics (2003), the Biotechnology Heritage Award (2004), the Heinz Award in Technology, the Economy and Employment (2006), election to the Inventors Hall of Fame (2007) and the Pitcon Heritage Award (2008). Dr. Hood has received 17 honorary degrees from Institutions such as Johns Hopkins, Yale, UCLA, and Whitman College. He has published more than 650 peer-reviewed papers, received 15 patents, co-authored numerous textbooks, and co-authored a popular book on the human genome project ("The Code of Codes"). He is currently completing a textbook on systems biology. Dr. Hood is a member of the National Academy of Sciences, the American Philosophical Society, the American Association of Arts and Sciences, the Institute of Medicine and the National Academy of Engineering. He is one of only seven scientists elected to all three academies (NAS, NAE and IOM). Dr. Hood has also played a role in founding more than 14 biotechnology companies, including Amgen, Applied Biosystems, Systemix, Darwin and Rosetta. He has recently cofounded the company Integrated Diagnostics, which he expects to become a platform company for P4 medicine.
Transcriptional precision in the *Drosophila* embryo

Boettiger A, Hong J-W, Hendrix D, Levine M

Division of Genetics, Genomics, and Development, University of California, Berkeley, CA
Department of Molecular and Cell Biology, University of California, Berkeley, CA
Center for Integrative Genomics, University of California, Berkeley, CA

The post-genome analysis of dorsal-ventral (DV) patterning of the *Drosophila* embryo led to two unexpected findings. First, as many as half of the 60-70 target genes for the Dorsal regulatory gradient contain more than one enhancer for a single pattern (threshold) of gene expression. In some cases the duplicate enhancer (or “shadow” enhancer) maps within neighboring genes. Second, most of the Dorsal target genes contain stalled RNA Polymerase II (Pol II) prior to their induction in the early embryo. We have used a combination of comparative genome analysis and quantitative imaging methods to determine the significance of shadow enhancers and stalled Pol II in development and evolution. The identification of Dorsal target enhancers in divergent insects (flies, mosquitoes, beetles, and bees) helped distinguish between so-called primary and shadow enhancers. In some cases (e.g., the brinker locus) the presumed shadow enhancer (located in intron 2 of the neighboring Atg5 gene in *Drosophila*) is the one that is conserved in evolution.

Overall, DV enhancers tend to be located in fixed positions relative to the transcription units they regulate. Thus, it would appear that the evolution of novel patterns of gene expression depends on changing the sequence of old enhancers rather than inventing new ones. Some of the genes that are activated by low levels of the Dorsal gradient display erratic patterns of gene activation in *dl/+* embryos. These genes tend to lack shadow enhancers, whereas those genes containing shadows seem better buffered against genetic perturbation. Previous studies on stalled (paused) Pol II at the *Drosophila* hsp70 heat shock locus suggested that stalling helps accelerate gene induction in response to stress. Perhaps developmental control genes, like stress genes, are “poised” for rapid activation. The quantitative analysis of gene activation suggests that transcriptional synchrony is one manifestation of this poised induction. The analysis of 14 different genes in hundreds of early embryos suggests that there are two different classes of expression profiles, synchronous and stochastic. All nine genes with stalled Pol II display synchronous patterns of activation, while all five genes lacking stalled Pol II exhibit stochastic patterns of induction. Transcriptional synchrony might help ensure the orderly deployment of the complex gene networks that control embryogenesis. We propose that synchrony is a measure of population fitness.

Biography of Michael Levine, Ph.D.

Michael Levine, Ph.D., studies gene networks that control animal development and disease. With two colleagues, he discovered the “homeobox” genes, which turn certain DNA segments on and off in the fruit fly to control differences in body segments and, scientists were surprised to learn, have similar functions in humans. He now works with sea squirts, whose DNA more closely resembles human genetic material. After completing his bachelor's degree in genetics at the University of California-Berkeley in 1976, Levine went to Yale for his Ph.D. in the Department of Molecular Biophysics and Biochemistry, which he received in 1981. He held postdoctoral fellowships at the University of Basel (Switzerland) and at the University of California-Berkeley, before joining the faculty of Columbia University from 1984–1990, and then the University of California-San Diego from 1991–1996. He joined the faculty at University of California-Berkeley in 1996, where he is now F. Williams Professor and head of the Division of Genetics, Genomics, and Development and is co-director of the Center for Integrative Genomics. Levine was elected to the American Academy of Arts and Sciences in 1996 and the National Academy of Sciences in 1998. He received the Monsanto Prize in Molecular Biology from the National Academy of Sciences in 1996 and in 2009 he received the Wilbur Cross Medal, the highest honor bestowed by the Yale University Graduate School of Arts and Sciences.

Adaptations to local environments in humans

Di Rienzo A

Department of Human Genetics, University of Chicago

Humans have adapted to a remarkably diverse range of environments and show striking phenotypic variation among populations. The impact of these adaptations can still be detected in the geographic distribution of genetic variants that are beneficial in one environment, but deleterious or neutral in a different habitat. We use ecological information to search the human genome for correlations between the frequency of polymorphic variants in worldwide population samples and a set of environmental variables, including climate, subsistence, ecosystem, and diet. Variants with significant correlations with these environmental variables are strong candidates for genetic adaptations to local
environments. By using this approach, we find a striking enrichment of strong environmental correlations in genic and nonsynonymous SNPs relative to non-genic SNPs and for several biologically important sets of genes. This implies that climate, diet and subsistence played an important role in shaping variation in the human genome. In addition to genome-wide analyses, signals of adaptation to local environments at individual loci can be used to identify SNPs that may affect gene expression and function and that may be subjected to functional analyses. Such SNPs are likely to be important in gene-environment interactions.

**Biography for Anna Di Rienzo, Ph.D.**

Anna Di Rienzo, Ph.D., is a Professor of Human Genetics at the University of Chicago and a member of the Committees on Genetics, Genomics and Systems Biology, on Clinical Pharmacology and Pharmacogenomics, and on Molecular Metabolism and Nutrition. She received her bachelor's degree in Biological Sciences and her Ph.D. in Medical Genetics from the University of Rome “La Sapienza”. Her group is generally interested in characterizing the amount and patterns of variation at the DNA sequence level in human populations, and in elucidating the forces that shape and maintain this variation. She has combined empirical studies of sequence variation and modeling of population history to make inferences about human population size changes. In addition, her group has worked on the action of positive natural selection at specific loci and at the genome-wide level in humans. As greater attention is focused on dissecting the genetic bases of common diseases, she is interested in connecting signals of adaptations with genetic variants that influence human phenotypes and in developing evolutionary models of common diseases.

**Darwin and domestication**

Doebley J

Department of Genetics, University of Wisconsin, Madison, WI

In his book "On the Origin of Species", Charles Darwin used plant and animal domestication as a model to inform his theory on evolution under natural selection. Artificial selection during plant domestication is thought to have been largely unconscious, the inevitable product of a sowing-reaping cycle. Selection pressures placed by humans on crops are analogous to those placed by seed-dispersers such as birds on wild species. Nevertheless, Darwin’s use of domestication as a model for natural evolution has been controversial. Over the past 20 years, genetic and molecular research has begun to uncover the genetic basis of the changes involved in the evolution of plant form under both natural and artificial selection. In the case of domestication, approximately 15 genes involved in the changes in morphology have been isolated. For most of these genes, the nature of the alteration in the gene is understood. I will review what has been learned about change in form under domestication and whether any patterns are beginning to emerge.

**Biography of John Doebley, Ph.D.**

John Doebley, Ph.D., studies how genes control changes in plant morphology during domestication with a focus on maize. He is a Professor of Genetics and a member of the Plant Breeding Faculty at the University of Wisconsin-Madison. Doebley's work has earned him several awards and honors, including election to the National Academy of Sciences in 2002. Dr. Doebley has spent the past two decades examining the genetic differences and similarities between teosinte and maize (Zea mays). He and his laboratory members pioneered the use of quantitative trait locus mapping to successfully identify which regions of maize's genome are responsible for several “domestication traits,” features that separate maize from its undomesticated relatives. His team was one of the first to clone genes identified through QTL mapping, including a pivotal domestication gene known as teosinte branched1 (tb1), which affects kernel structure and plant architecture. In his “Inaugural Article” in the *Proceedings of the National Academy of Sciences*, he and colleagues determined that selecting for tb1 thousands of years ago did not affect genetic diversity at neighboring genes, clarifying how the maize genome was sculpted by past selective breeding and elucidating how the genomes of other organisms respond to selective pressure. He holds a bachelor's degree in anthropology from West Chester State College, his master's degree in anthropology from Eastern New Mexico University and a Ph.D. in botany from the University of Wisconsin-Madison.
KSHV-encoded miRNAs and their role in viral biology

Renne R

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
UF Shands Cancer Center, University of Florida, Gainesville, FL

MicroRNAs are small, non-coding RNAs that post-transcriptionally regulate gene expression by binding to 3'UTRs of target mRNAs. Kaposi's sarcoma-associated herpesvirus (KSHV), a virus linked to KS and primary effusion lymphoma (PEL), encodes 12 miRNA genes but only a few regulatory targets are currently known. Using ectopic expression of viral miRNAs in combination with gene expression profiling, we identified miRNA targets, including THBS, a strong anti-angiogenic factor, and several genes involved in regulation of apoptosis and proliferation. In addition, we found that KSHV-miR-K12-11 shares 100% seed-sequence homology with hsa-miR-155, a miRNA frequently found up-regulated in lymphomas and critically important for B-cell development. Based on this seed-sequence homology, we hypothesized that both miRNAs regulate an overlapping set of genes. Hence, KSHV-miR-K12-11 may mimic hsa-miR-155. To this end, we demonstrated that ectopic expression of either miRNA targets BACH-1 in PELs (Skalsky et al., 2007, J Virol 81(23):12836-45). Using bioinformatic approaches in combination with 3'UTR reporter assays, we found that CEBP/β and PU.1 are targeted by miR-K12-11/miR-155. Since both transcription factors play essential roles in B-cell differentiation, we propose that viral miRNA mimics miR-155 in the context of B-cell differentiation. Ongoing experiments to directly evaluate the effects of KSHV-miR-K12-11 expression on human hematopoiesis will also be discussed.

Epigenetic regulation controls cellular sensitivity to stress induced apoptosis

Zhou L, Zhang C, Lin N

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
UF Shands Cancer Center, University of Florida, Gainesville, FL

IAP-antagonists such as reaper and hid play pivotal role in mediating cell death in response to cytotoxic stimuli. A 33kb genomic region upstream of reaper, the irradiation responsive enhancer region (IRER), is required for mediating the induction of both reaper and hid following irradiation. Interestingly, IRER is subject to epigenetic regulation. While it is open in undifferentiated cells during early embryogenesis, chromatin in IRER become enriched for H3K27Me3 and H3K9Me3 and form facultative heterochromatin in late embryogenesis. This epigenetic modification of IRER blocks the irradiation-responsiveness of both reaper and hid and renders the cells resistant to irradiation. Several Polycomb group (PcG) proteins are required for this epigenetic regulation of IRER. Using an ubi-DsRed reporter knocked into IRER, we found that about 2% cells in Drosophila larvae are DsRed positive, i.e. have an open IRER. These cells have higher levels of reaper expression and are much more sensitive to stress-induced cell death. In addition, mosaic clone analysis showed that cells deficient for IRER developed hyperplasia, indicating that these cells are more resistant to environmental stress and/or cellular competition. Overall, our data indicated that epigenetic regulation of pro-apoptotic genes plays an important role in determining cellular sensitivity to stress-induced cell death.

Of lice and men: the inference of human evolutionary history from the perspective of its host-specific parasites

Reed D

Mammalogy, Florida Museum of Natural History, University of Florida, Gainesville, FL

Human history is written not only in our DNA, but also in the DNA of our parasites. This parascript of our history contains bits of human evolutionary history that may be obscured in host data. The ectoparasitic lice of primates have been coevolving with their primate hosts for at least 25 million years, and have already demonstrated their utility for inferring primate evolutionary history. Human and chimpanzee lice last shared a common ancestor 5-7 million years ago and diverged in tandem with their hosts. We also know that human head lice show a population expansion dated around 100,000 years ago that likely corresponds with the out-of-Africa expansion of their human hosts. However, these two examples merely confirm events in human history that are well known from human fossil and molecular data. The utility of studying the parasites is that they may shed light events that are unknown or potentially unknowable from human evidence. Research in my lab has shown that the lice on modern humans contain greater genetic diversity than can be
explained by a history restricted solely to modern *Homo sapiens*. DNA simulation studies show that lice arose on archaic hominids and switched to modern humans within the last 35,000 years, which would require direct physical contact between modern and archaic hominids. I'll provide other examples of how parasitic lice can be used to examine pivotal questions in human evolution, such as when humans began using rudimentary clothing.

**Whole-genome duplication in plant evolution: case studies of ancient events and recent speciation**

Soltis PS*

Laboratory of Molecular Systematics and Evolutionary Genetics, Florida Museum of Natural History, University of Florida, Gainesville, FL

The relatively small genome of the diploid flowering plant *Arabidopsis thaliana* shows signatures of multiple, ancient whole-genome duplications, and the genomes of other derived flowering plants are likewise duplicated. Analyses of new genomic resources for phylogenetically early lineages of flowering plants now suggest that perhaps all flowering plants have undergone ancient whole-genome duplication, explaining in part the generally large size and complexity of plant genomes. This process of whole-genome duplication – polyploidy - has long been recognized as an important speciation mechanism in flowering plants, but its long-term impact had been questioned. Studies of recently formed natural polyploid plants, such as those in the genus *Tragopogon*, coupled with analysis of synthetic polyploids, are demonstrating that new polyploid genomes are dynamic. Polyploid species of *Tragopogon* do not simply combine the genomic complements of their diploid parental species; instead, their genomes have undergone chromosomal rearrangements, loss of genetic loci, and changes in gene regulation - all in less than 80 years since their formation. Likewise, some changes are evident in early synthetic generations. The processes leading to genome duplication, rearrangement, and modification via gene loss and silencing have likely been occurring throughout the ~130 million years of flowering plant evolution and continue to contribute to the complexity of plant genomes.

**A conserved alternative splicing event in plants reveals an ancient exonization of 5sRNA**

Barbazuk WB*

Department of Biology, University of Florida, Gainesville, FL

Alternative splicing (AS) creates multiple mRNA transcripts from a single gene. Recently available plant genome and transcript sequence data sets are enabling a global analysis of AS in many plant species, and results are revealing differences between animals and plants in the frequency and preferred mechanisms of alternative splicing. Plant genome and transcript comparisons are also identifying conserved alternative splicing (AS) events among evolutionarily distant species, and these analyses can prioritize AS events for functional characterization and help uncover relevant cis- and trans-regulatory factors. A genome-wide search for conserved cassette exon AS events in higher plants revealed the exonization of 5S ribosomal RNA (5S rRNA) within the gene of its own transcription regulator, *TFIIIA* (transcription factor for polymerase III A). The 5S rRNA-derived exon in *TFIIIA* gene exists in all representative land plant species, but not in green algae and non-plant species, suggesting it is specific to land plants. *TFIIIA* is essential for RNA Polymerase III-based transcription of 5S rRNA in eukaryotes. Integrating comparative genomics and molecular biology revealed that the conserved cassette exon derived from 5S rRNA is coupled with nonsense-mediated mRNA decay. Most significantly, this study provides the first evidence of ancient exaptation of 5S rRNA in plants, suggesting a novel gene regulation model mediated by the AS of an anciently exonized non-coding element.

**Maize domestication: metabolic adaptations in grain evolution**

Koch KE*

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

Recent studies show that developing corn kernels and other young grains have very different interior environments than previously recognized. Little to no oxygen is detectable in their endosperms. This is centrally important, because it indicates that sites of starch and protein deposition in grains are likely controlled by fundamentally different metabolic processes than envisioned earlier. Consistent with this suggestion is the endosperm expression of genes for low-oxygen metabolism (e.g. specific sucrose synthases, glycolytic enzymes, alcohol dehydrogenases, and sorbitol dehydrogenase). Evolution of a low-oxygen, starch-storing endosperm preceded origin of grasses, but related changes in structure and
function continued within the caryopses. Unlike most grains, Zea and related Andropogoneae developed a more compact zone of vascular transfer, elaborated projections at the base of the endosperm, and more extensive regions of low-oxygen endosperm. Young maize kernels also differ from those of most other grains in lacking capacity for daytime oxygen production via photosynthesis in outer layers of the green grain (developing kernels are non-green). Adaptations in maize and related Andropogoneae may well include additional adjustments for anaerobic metabolism and non-vascular transport.
1. Generation and characterization of transgenic, fall armyworm resistant turfgrass (Paspalum vaginatum Swartz)

Altpeter F1,2,* Neibaur I1,2, Zhang H1,2, Meagher RL3, Gallo M1,2,*

1Agronomy Department, University of Florida, Gainesville, FL
2Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
3Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL

Seashore paspalum (Paspalum vaginatum Swartz) is a salt tolerant, fine textured turfgrass used on golf courses in coastal, tropical and subtropical regions. Fall armyworm (Spodoptera frugiperda (J. E. Smith)) is a devastating pest of seashore paspalum. Therefore, insect resistance is a prime target for genetic engineering of seashore paspalum. However, a genetic transformation protocol for seashore paspalum was lacking. Here we report the development of a genetic transformation protocol for this commercially important turfgrass species as well as the generation of fall armyworm resistant seashore paspalum expressing a synthetic, codon optimized Bt cry gene. Biologic gene transfer of embryogenic callus with a linearized plasmid carrying constitutive hygromycin and Bt cry expression cassettes was followed by selection with hygromycin and regeneration of plants. Transgene integration and expression of the regenerated plants was confirmed by PCR, Southern blot, RT-PCR, and ELISA respectively. Resistance of transgenic plants to fall armyworm was evaluated in comparison to wildtype and correlated well to Bt cry expression levels.

2. Improving turf and forage properties in apomictic bahiagrass (Paspalum notatum Flugge) by mutagenesis

Lomba P1,2, Altpeter F1,2,*

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

Bahiagrass (Paspalum notatum Flugge) is the predominant forage grass and a popular turf in the southeastern United States. Bahiagrass’ popularity is attributed to its strong persistence under low input conditions. However, the quality of bahiagrass is limited due to its open growth habit and prolific production of tall seedheads, coarse leaf texture, light green color and poor nutritive value. Improvement of bahiagrass cultivar ‘Argentine’ by conventional breeding is very difficult due to its apomictic reproduction mode. Our objective was to explore the potential of chemical and tissue culture derived mutagenesis for genetic improvement of apomictic bahiagrass for generation of uniform mutagenized seed progeny with improved turf and forage quality. Scarified and surface sterilized bahiagrass seeds were treated with the mutagen sodium azide at various concentrations and exposure times. Callus was induced from these seeds and regenerated via somatic embryogenesis to obtain uniformly mutagenized plants. Independently mutagenized lines with reduced stem length, higher tiller density or reduced or delayed seedhead formation were established under field conditions for further evaluation of density, leaf texture, tiller length, color, growth pattern, biomass, seedhead and seed production. Greenhouse and field data from selected mutagenized lines will be presented in comparison to non-mutagenized bahiagrass.

3. Lipopolysaccharide induced activation of circulating inflammatory molecules exert a negative influence on hippocampal progenitor cell differentiation in the adult brain

Asokan A, Ormerod BK*

J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL

Although the function of adult neurogenesis has not been elucidated completely, a strong correlation is emerging between performance on spatial memory tasks and optimal levels of neurogenesis. Previous reports have shown that neuroinflammation, induced by lipopolysaccharide (LPS) ablates adult hippocampal neurogenesis resulting in latent memory impairment, which can be treated with non-steroidal anti-inflammatory drug (NSAID) treatment; however, chronic NSAID treatment is associated with adverse side effects. Here we attempt to evaluate hippocampal neurogenesis and identify components of the neuroinflammatory response that are malicious to neuronal differentiation by quantifying cytokine levels in the blood and brain of LPS-treated mice using a multiplex ELISA strategy. As expected, within 5 hr, LPS treatment increased all pro-inflammatory cytokines, with robust elevations observed in TNF-α, IL-6, IL-1β, IFN-γ, IL-17 and MCP-1 levels in serum and hippocampus (p<0.01) and returned to baseline levels by 96 hr. However, levels of IL-12 (p40), KC and LIX overshot baseline levels and, were significantly lower in LPS- versus vehicle-treated mice 96 hr after treatment. Interestingly circulating levels of IL-12(p40), KC and LIX correlated negatively with new neuron (BrdU/DCX+ve) number (p<0.03). Our data suggest that these factors may modulate the pathways responsible for regulating cell fate decisions among hippocampal progenitor cells.
4. Functional pseudogene usage in natural infections with *Anaplasma phagocytophilum*

Barbet AF1,*, Foley JE2, Nieto NC3, Gabriel M4, Al-Khedery B5,*, Foley P4

1 Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL
2 Department of Medicine and Epidemiology, University of California, Davis, CA
3 Veterinary Genetics Laboratory, University of California, Davis, CA
4 Department of Biological Sciences, California State University, Sacramento, CA

The tick-borne rickettsiales *Anaplasma phagocytophilum* causes persistent infections with disease severity ranging from asymptomatic to fatal. The organisms express a major surface protein, MSP2/P44 from a genomic expression site into which duplicated cassettes from different donor pseudogenes recombine to generate expressed variants. There is a repertoire of ~100 pseudogenes in the genome, preferentially clustered close to the origin of replication, although some pseudogenes are more widely spread over other regions of the genome. We have determined that the structure of expression site MSP2/P44 variants from diverse hosts and locations in the U.S. and Europe (n=268) and investigated if this database might give information about the frequency of individual genomic pseudogenes. The most frequently used sequence donors were located between the expression site and the origin of replication. Usage was infrequent for pseudogenes located in map positions 0-1,000,000 bp. These data suggest that genome position of pseudogenes relative to the expression site influences their frequency of use as sequence donors. Many pathogens are now known to use cassette mechanisms for expression of antigenic variants, but it is less clear what structural features influence the probability of selection of an individual sequence donor. *Anaplasma phagocytophilum* should provide a useful system to evaluate these structural features.

5. Vitamin D binding protein serum levels and genotype in those with type I diabetes

Bierschenk L1, Blanton D1, Han Z1, Wasserfall C1, Haller M2, Schatz D2,*, Atkinson M1,*

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

Type 1 diabetes (T1D) is a complex autoimmune disease and a role for vitamin D has come under scrutiny recently. While our own studies have not specifically associated serum 25OH-vitamin D levels with T1D, others have found polymorphisms in the vitamin D hydroxylase enzyme loci to be associated with T1D. Here we sought to investigate the role of vitamin D binding protein (VDBP) both by ELISA measurement of circulating levels and restriction fragment length polymorphism (RFLP) analysis of the VDBP locus. A cross-sectional study was performed utilizing serum samples collected from 153 controls, 203 T1D subjects, and 116 first-degree relatives of subjects with T1D. A subset of these samples consisting of 53 controls, 81 T1D subjects, and 38 first-degree relatives were genotyped. VDBP levels (mean µg/mL; 95% CI) were highest in healthy controls (528.2; 467.3-589.0), intermediate in first-degree relatives (496.9; 410.3-583.4), and lowest in T1D subjects (424.8; 403.6-446.0). A significant difference in VDBP serum concentrations was found between the control and T1D groups (p=0.0028). RFLP analysis of sites at codons 416 and 420 of exon 11 revealed no association between VDBP genotype, disease state or serum levels, implying circulating concentrations of this analyte may be controlled by other factors. A much larger prospective study needs to be undertaken in order to increase the power of the study and to evaluate our observed lower VDBP levels in relation to T1D development.

6. Spatial variation in the prevalence of sigma virus, a vertically transmitted pathogen that infects *Drosophila melanogaster*

Blohm GM, Regan K, Wayne ML*

Department of Biology, University of Florida, Gainesville, FL

Many emerging pathogens (e.g. West Nile virus, Dengue, equine encephalitis) are transmitted vertically. Theory suggests that dispersal among populations can maintain vertically transmitted pathogens by increasing the genetic variability of the pathogen at a local scale. Using distance as a proxy for the timing and magnitude of dispersal among populations, we aim to estimate the effect of dispersal on the persistence of sigma virus, a vertically transmitted pathogen on *Drosophila melanogaster*. During the summer of 2009, we collected individuals from 7 locations along a 70-mile transect near Athens, GA. We also estimated temporal variability by sampling at five different times. We determined the prevalence of sigma virus in males and females from each location through time using a CO2 exposure assays. We then propagated the field-collected adults in the laboratory to measure rates of transmission. Our results indicate that the prevalence of sigma virus is highly variable among populations, ranging from 0% to 60% (mean: 25%), with no spatial trends in total prevalence. The results of this study contribute to our understanding of host-pathogen coevolution while potentially informing management strategies to reduce the incidence of vertically transmitted pathogens in humans.
7. An evolutionary basis for hypospadias: molecular development of external genitalia in the turtle Trachemys scripta elegans

Boger L1, Cohn MJ1,2,3,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Department of Biology, University of Florida, Gainesville, FL
3Howard Hughes Medical Institute, University of Florida, Gainesville, FL

Hypospadias is a birth defect of the penis in which the urethral tube fails to close. From an evolutionary perspective, a closed urethra is a derived character of mammals, in that other tetrapods possess genitalia with an open urethral groove or sulcus. The phallus of the turtle Trachemys scripta elegans (the red-eared slider) has fibrovascular structures and a collagen fiber arrangement homologous to that of mammals; however, like other non-mammalian tetrapods, the phallus has an open sulcus rather than an enclosed urethra. Comparison of external genital development in turtles and mammals has the potential to reveal a genetic basis for the origin of the urethra, and may uncover novel pathways involved in urethral tubulogenesis. We collected male T. scripta elegans embryos at various developmental stages and examined the expression patterns of genes known to be involved in the development of mammalian genitalia. Here we show expression patterns of such genes, including Shh, Mxs2, Bmp4, and Twist. Further analysis of these genetic networks will not only help to answer the larger evolutionary question of which developmental processes are conserved and which are variable between turtles and mammals, but may also give a better understanding of the etiology of hypospadias.

8. KSHV miR-K12-11 expression in human progenitors during in vivo hematopoiesis induces B-cell expansion in NOD/LtSz-scid IL2Rγnull mice

Boss IW1, Nadeau PE2, Abbott JR2, Mergia A2,* Renne R1,3,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL
3University of Florida Shands Cancer Center, Gainesville, FL

MiRNAs are small non-coding RNA molecules that regulate gene expression. Kaposi’s sarcoma-associated herpesvirus (KSHV), a lymphotropic virus, expresses 12 miRNAs during latent infection. Previous work by our lab and others found that one of these viral miRNAs, miR-K12-11, shares 100% seed sequence homology with hsa-miR-155, an oncogenic miRNA involved in B-cell activation and differentiation (Skalsky et al., 2007, J Virol 81(23):12836-45; Gottwein et al., 2007, Nature 450(7172):1096-9). Experimental analysis found that both miRNAs regulate an overlapping set of gene targets and that KSHV infected primary effusion lymphoma (PEL) cell lines do not express miR-155. These data suggested that miR-K12-11 may mimic the function of miR-155 in PEL cells, causing dysregulated B-cell differentiation. In this study, we examined the effects of miR-K12-11 expression and its ability to mimic miR-155 in human cord blood progenitors during hematopoiesis in NOD/LtSz-scid IL2Rγnull mice. Using foamy virus we forced expression of miR-K12-11 or miR-155 in progenitor cells transplanted into sub-lethally irradiated mice. Following reconstitution, we analyzed cell lineage differentiation with FACS and histological staining. Results show that forced expression of either miRNA leads to increased B-cell expansion in the spleen. This is the first in vivo study examining the role of a KSHV miRNA in human cells and further promotes the hypothesis that miR-K12-11 is a functional mimic of miR-155. In summary, these data suggest a role for miR-K12-11 in KSHV lymphomagenesis.

9. Recombination of adaptive alleles in introduced populations

Bouchard F1, McBride G1, Marcus C1, Wayne ML2,*

1Graduate Program in Genetics and Genomics, University of Florida, Gainesville, FL
2Department of Biology, University of Florida, Gainesville, FL

During introductions to new environments, populations have an increased probability of extinction due to a severe genetic bottleneck and a suite of selective pressures to which they are not adapted. Multiple introductions of genetically divergent populations followed by interbreeding can produce recombinants with adaptive alleles from each source that are better adapted to the introduced environment and more likely to survive than a single introduction. It has been proposed that this complementary gene action may be an important evolutionary force in the establishment of exotic invasive species. Testing the effect of multiple introductions in the field is difficult due to problems in sampling, lack of replication, and lack of proper controls. Using several geographically divergent lines of Drosophila melanogaster and ethanol concentrated food as a novel environment; the effects of multiple introductions were investigated in an artificial evolution experiment. Egg to adult viability was used as a measure of fitness in the novel environment. Results show that having multiple introductions increases fitness for some populations. This could have implications in the study of invasive species and their management.

10. Trade-off hypothesis and evolution of mycovirus virulence

Brusini J1, Robin C2

1Department of Biology, University of Florida, Gainesville, FL
2INRA, UMR1202 BIOGECO, Equipe de Pathologie Forestière, Villeneuve d’Ornon Cedex, France
All viruses that infect fungi are avirulent (i.e. host fitness does not decline in response to infection). These mycoviruses can be transmitted vertically or horizontally, with horizontal transmission occurring during somatic fusion. Here we present a rare example of a virulent mycovirus, *Cryphonectria hypovirus 1* (CHV-1), which infects the fungal pathogen *Cryphonectria parasitica* in Europe. In a cross-inoculation experiment, we found important differences in the degree of host specialization between the two sub-types studied, including a strong positive correlation between virulence and horizontal transmission for one viral sub-type. The low diversity of genes controlling fusion in European populations of *C. parasitica* suggests that horizontal transmission of the virus is common and, as such, our results suggest that the virulence of at least one sub-type of CHV-1 is driven by the trade-off hypothesis of virulence evolution. We therefore suggest that the general avirulence of mycoviruses is the direct consequence of the high diversity of genes controlling fusion in fungal-host populations.

### 11. Expansion of a direct shoot organogenesis system in peanut to include U.S. varieties

Burns S1, Gallo M1-2,*, Tillman B1,3

1Agronomy Department, University of Florida, Gainesville, FL  
2Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL  
3North Florida Research and Education Center, University of Florida, Marianna, FL

An alternative to lengthy bombardment and regeneration protocols is *Agrobacterium*-mediated transformation employing direct shoot organogenesis, which allows for transgenic plants to be obtained quickly (3 - 4 months). Peanut cultivars, ‘Florida-07’ (Runner), ‘Georgia Green’ (Runner), ‘Georgia Brown’ (Spanish), ‘New Mexico-A’ (Valencia), and ‘VC2’ (Virginia), were selected to represent all four market types. Two types of cotyledonary explants were examined, those that previously had an attached embryo-axis upon cotyledon separation (explant A) and those that were embryo-axis-free upon separation (explant B). Explants were classified as having no growth, callus-like growth, adventitious bud formation or small plantlet development (1, 2, 3 and 4 respectively). Differential shoot induction was observed for the cotyledon explants examined (Pr>[t]=<0.0001). Explant A had greater shoot induction with a visual rating of 1.75, while explant B had a rating of 1.64 (Pr>[t]=<0.0001). Cultivars responded to the culture conditions differently with Georgia Green on 40 µM BA producing the most shoot buds (31.2%) and the highest visual rating (2.22), followed by VC2 on 10 µM BA (17.3%, 1.84), New Mexico-A on 640 µM BA (15.9%, 1.84), Georgia Brown on 80 µM BA (9.1%, 1.73), and Florida-07 on 40 µM BA (5.6%, 1.82). Georgia Green, New Mexico-A and VC2 appear to be the best suited for future transformation experiments based on their shoot bud production.

### 12. An analytical methodology for the in situ visualization of cellulases in maize cell wall mutants

Caicedo HM1, Ladisch M2, Mosier N2, Vermerris W1,*

1Agronomy Department, University of Florida, Gainesville, FL  
2Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN

The current interest in alternative fuels from renewable resources has been triggered by the increasing global demand for energy along with environmental concerns. Corn stover, the vegetative residues remaining after the grain harvest, is an abundant feedstock (lignocellulosic biomass) for the production of fermentable sugars (glucose) which can be used for the production of ethanol. The composition and structure of the lignocellulosic biomass has a big impact on the efficiency of enzymatic saccharification. We have studied the composition of lignin in corn stover through genetic approaches. The brown midrib1 (bm1), bm3 and near-isogenic bm1-bm3 mutations affect lignin subunit composition and each increase the yield of glucose per gram dry stover relative to the wild-type control. Here, we present an analytical methodology to investigate the basis of the enhanced hydrolysis in the bm mutants by assaying the binding of cellulases to stover, using recombinant proteins consisting of the cellulose binding module (CBM) isolated from *Trichoderma reesei* endoglucanases labeled with green-fluorescent protein (GFP). Because of lignin autofluorescence, this approach cannot be performed in situ, but instead has to rely on a fluorescence subtraction assay. The optimized assay showed that the increased rate of hydrolysis in the bm mutants was due to enhanced binding relative to the wild-type control, which effectively increases the titer of the cellulases.

### 13. Rapid microsatellite development in organisms without sequenced genomes using 454 sequencing

Chaffee CL, Braun EL*, Osenberg CW

Department of Biology, University of Florida, Gainesville, FL

Until recently, computational methods for identifying candidate microsatellite loci were only available for those organisms with a sequenced genome. New, low cost DNA sequencing technology (454-Titanium shotgun sequencing), however, makes it possible to use these computational methods for organisms that do not have such genomic resources available. We used such an approach to identify candidate loci for two coral reef organisms for which little is known about their population genetics: Christmas tree worms (*Spirobranchus giganteus*) and vermetid gastropods (*Dendropoma maximum*). Although superficially similar, the species are quite divergent phylogenetically, have different larval durations (and hence dispersal potential) and interact with corals in opposite ways. By using ICBR’s 454 sequencing capabilities, we were able to identify hundreds of candidate loci, without the need to build costly and time-
Anthrax lethal toxin (LT), produced by the Gram-positive bacterium *Bacillus anthracis*, is a potent zinc dependent metalloprotease that cleaves the N-terminus of MAPKKs and is known to play a major role in impairing the host immune system during an inhalation anthrax infection. Here, we present the transcriptional responses of lethal toxin treated human monocytes in order to further elucidate the mechanisms of LT induced host immune system collapse. Using 2D-DIGE in combination with microarray analysis, we identify over 820 probe sets differentially regulated after LT treatment at a 0.001 significance, interrupting the normal transcription of over 60 known pathways. As expected, the MAPK signaling pathway was drastically affected by LT, while the p38 pathway was also highly impacted. Numerous genes involved in actin transduction of over 60 known pathways. As expected, the MAPK signaling pathway was drastically affected by LT, including genes involved in actin transduction, transcriptional regulation and cytokine signaling. Using these results, we can further understand how anthrax LT impairs normal human monocyte function by focusing on the unique transcriptional responses and their contribution to host immune system dysfunction.

**14. Microarray analyses of the transcriptional responses of human peripheral monocytes to *Bacillus anthracis*’ lethal toxin**

Chauncey KM\(^1\), Lopez MC\(^2\), Szarowicz SE\(^1\), Baker HV\(^2\,*\), Southwick FS\(^1\)

\(^1\)Department of Medicine, University of Florida, Gainesville, FL
\(^2\)Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Anthrax lethal toxin (LT), produced by the Gram-positive bacteria *Bacillus anthracis*, is a potent zinc dependent metalloprotease that cleaves the N-terminus of MAPKKs and is known to play a major role in impairing the host immune system during an inhalation anthrax infection. Here, we present the transcriptional responses of lethal toxin treated human monocytes in order to further elucidate the mechanisms of LT induced host immune system collapse. Using Affymetrix Human Genome U133 Plus 2.0 Arrays, we identify over 820 probe sets differentially regulated after LT treatment at a 0.001 significance, interrupting the normal transcription of over 60 known pathways. As expected, the MAPK signaling pathway was drastically affected by LT, while the p38 pathway was also highly impacted. Numerous genes outside the well-recognized pathways were also influenced by LT, including genes involved in actin regulation, signal transduction, transcriptional regulation and cytokine signaling. Using these results, we can further understand how anthrax LT impairs normal human monocyte function by focusing on the unique transcriptional responses and their contribution to host immune system dysfunction.

15. **Proteomics and mass spectrometry applications in biomedical research**

Diaz C, Chow M, Zheng R, Chung A, Chen S*  
Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

Proteomics and mass spectrometry have provided unprecedented tools for fast, accurate, high throughput biomolecular separation and characterization, which are indispensable towards understanding the biological and medical systems. Studying at the protein level allows researchers to investigate how proteins, their dynamics and modifications affect cellular processes and how cellular processes and the environment affect proteins. Here we present our capabilities in proteomics and other analytical services. The tools include a gel-based 2D-DIGE (Two Dimensional Difference Gel Electrophoresis) and gel-free iTRAQ (Isobaric Tags for Relative and Absolute Quantitation). Along with our capacity of separating thousands of proteins and characterizing differential protein expression, we have a suite of state-of-the-art mass spectrometers available for biomedical sciences and advanced technology research. These instruments are mainly used for protein identification, posttranslational modification characterization and protein expression analysis (e.g., Mass Western). Our facility is also set up to provide Edman *de novo* N-terminal protein sequence analysis and Biacore biomolecule interaction analysis. We are fully set up to synthesize and purify peptides and have a good track record with this service as well. Proteomics and mass spectrometry are useful in large-scale survey of proteome for hypothesis generation as well as in detailed analysis of target proteins for hypothesis testing.

16. **A redox active isopropylmalate dehydrogenase functions in the biosynthesis of glucosinolates and leucine in Arabidopsis**

He, Y\(^1,2\), Mawhinney T\(^3,4\), Preuss M\(^5\), Schroeder AC\(^5\), Chen B\(^1\), Abraham L\(^1\), Jez J\(^5\), Strul J\(^1\), Chen S\(^1,2,6,*\)

\(^1\)Department of Biology, University of Florida, Gainesville, FL
\(^2\)Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
\(^3\)Department of Biochemistry, University of Missouri, Columbia, MO
\(^4\)Department of Child Health, University of Missouri, Columbia, MO
\(^5\)Department of Biology, Washington University, St. Louis, MO
\(^6\)Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

We report a detailed functional characterization of an Arabidopsis isopropylmalate dehydrogenase (AtIPMDH1) that is involved in both glucosinolate biosynthesis and leucine biosynthesis. AtIPMDH1 shares high homology with enzymes from bacteria and yeast known to function in leucine biosynthesis. In plants, AtIPMDH1 co-expresses with nearly all known genes in aliphatic glucosinolate biosynthesis. Mutation of AtIPMDH1 leads to a significant reduction in the levels of free leucine and glucosinolates with side-chains of four carbons or longer. Complementation of the mutant phenotype by ectopic expression of AtIPMDH1, together with the enzyme’s substrate specificity, implicates AtIPMDH1 in both glucosinolate and leucine biosynthesis. This functional assignment is substantiated by the subcellular localization of the protein in the chloroplast stroma and the gene expression patterns in different...
17. A novel transcriptional pathway regulating xenobiotic detoxification genes in Caenorhabditis elegans as a target for multidrug resistance
Choe KP*
Department of Biology, University of Florida, Gainesville, FL

Anthelmintics have been used to control parasitic nematodes for decades and many species are evolving multidrug resistance. In diverse organisms, multidrug resistance is mediated by the increased expression of enzymes that detoxify xenobiotics. Unfortunately, the molecular and genetic mechanisms of detoxification and their roles in multidrug resistance are poorly defined in nematodes. Transcription factors that control the expression of xenobiotic detoxification genes are promising, but largely unexplored, multidrug resistance targets. The transcription factor SKN-1 regulates the expression of xenobiotic detoxification genes in the nematode C. elegans. We recently used genome-wide RNAi screening to identify a principal pathway regulating SKN-1. Genetic, molecular, and biochemical data support a model in which the WD40 repeat protein WDR-23 regulates SKN-1 abundance. Importantly, the homologous mammalian transcription factor Nrf2 is regulated by a distinct mechanism. Therefore, the WDR-23/SKN-1 pathway is a promising new target for drugs that inhibit xenobiotic detoxification and drug resistance in nematodes without affecting analogous pathways in mammals. The small size, simple culturing characteristics, and genetic tractability of C. elegans make it an ideal system in which to screen for pharmacological inhibitors of SKN-1 that would provide tools for studying the role of SKN-1 in parasitic species and could greatly increase the useful life of anthelmintics.

18. Cell-autonomous role of hedgehog signaling in notochord and intervertebral disc development
Choi KS, Harfe BD*
Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

The vertebrate notochord is a transient embryonic structure that serves as a signaling center in the midline of the developing early embryo. During later mouse embryogenesis the notochord derives all cells found within the nucleus pulposus, which is located in the center of each intervertebral disc. Although hedgehog signaling has been well studied over the past decades, the role of hedgehog signaling in the notochord and nucleus pulposus is still unclear. Here we show that hedgehog signaling is required not only for maintaining notochord structure but for forming the nucleus pulposus. Upon loss of hedgehog signaling, notochord formation was initiated but failed to differentiate to make the notochord sheath, which normally surrounds the notochord. Failure to form a notochord sheath resulted in aberrant nucleus pulposus formation and downregulation of T, Noto, and Foxa2 expression in caudal mutant notochords. Interestingly, Shh expression was also decreased in the entire notochord and floorplate during early embryonic development. In later development, Shh expression in the notochord became discontinuous in the caudal mutant notochord. Our data indicates that hedgehog signaling plays a role in maintaining Shh expression in both the notochord and floorplate. In addition, these results demonstrate that hedgehog signaling is required for formation of the notochord sheath and intervertebral discs.

19. A noncoding RNA found during gonadogenesis in a turtle that expresses temperature-dependent sex determination
Chojnowski JL, Braun EL*
Department of Biology, University of Florida, Gainesville, FL

Noncoding RNAs (ncRNAs) have been spotlighted in the past few years as more than just regulatory machinery and wasted space. They have been implicated in major biological processes such as cancer development, gene expression regulation, and nuclear trafficking. MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is a recently discovered long ncRNA found to be upregulated in breast cancer. It is conserved among mammals and it may regulate gene expression, possibly after cleavage yielding a smaller tRNA-like cytoplasmic RNA (~61 nt). MALAT1 may have a more permanent role in development than previously thought since it accumulates at high levels in mammalian ovaries and shows high levels of accumulation in male turtles during gonadogenesis. Determining local expression patterns and establishing patterns of hormonal regulation in the developing turtle gonad will reveal the contribution of MALAT1 to turtle gonadogenesis and facilitate understanding the role of this ncRNA in sexual development across vertebrates.

20. Generation of early AMD mouse model by induction of oxidative stress
Seo S1, Cohen ZP1, Yun L2, Hauswirth WW3,* Lewin AS1,*
1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Generation of early AMD mouse model by induction of oxidative stress
Purpose: Oxidative stress in the retinal pigment epithelium (RPE) complex is thought to be a leading cause in the development of age-related macular degeneration. Our hypothesis is that reduction of RPE specific MnSOD2 will cause increased levels of reactive oxygen species in the retina/RPE/choroid complex leading to pathogenesis of the early signs of AMD. Methods: We introduced Cre through a subretinal injection of VMD2-CRE virus into the floxed-SOD2 mice. Alternatively, we bred mice to introduce a Tet-On RPE65Cre+/- allele into SOD2f/f mice. The expression of MnSOD2 was measured by immunohistochemical staining for MnSOD2 in RPE flat mounts. The disease phenotypes were characterized by fundus, dihydroethidium, 4-HNE staining and histological analysis and fundus. Results: In the reporter mouse line, YFP expression was detected in the RPE layer. Reduced expression of sod2 was confirmed by staining of SOD2 in RPE-flat mounting, immunohistochemical staining and western blot. The increased oxidative stress in the RPE was measured by DHE, DCFDA and 4-HNE staining. The histology of retinas showed shortened outer photoreceptors, thinning of outer nuclear layer and RPE damages. Fundus was performed and showed significant increased number of yellowish deposits that appeared drusen. Conclusion: RPE-specific down-regulation of SOD2 leads to increased oxidative stress in the RPE and histological changes.

21. Functional interchangeability of rod and cone transducin α subunits

Deng W-T1, Sakurai K2, Liu J1, Dinculescu A1, Li J1, Pang J1, Chiodo VA1, Boye SL1, Chang B2, Kefalov VJ2, Hauswirth WW1,*

1Department of Ophthalmology, University of Florida, Gainesville, FL
2Department of Ophthalmology and Visual Sciences, Washington University, St. Louis, MO
3The Jackson Laboratory, Bar Harbor, ME

Rod and cone photoreceptors use similar but distinct sets of phototransduction proteins to achieve different functional properties, suitable for their role as dim and bright light receptors, respectively. The role of the structural differences between rod and cone transducin α subunits (Tα) in determining the functional differences between rods and cones is unknown. To address this question, we studied the translocation and signaling properties of rod Tα expressed in cones and cone Tα expressed in rods in three mouse strains: rod Tα knockout, cone Tα GNAT2cpf3 mutant, and rod and cone Tα double mutant rd17 mouse. Surprisingly, although the rod/cone Tα are only 79% identical, exogenously expressed rod or cone Tα localized and translocated identically to endogenous Tα in each photoreceptor type. Moreover, exogenously expressed rod or cone Tα rescued electroretinogram responses (ERGs) in mice lacking functional cone or rod Tα respectively. Ex vivo transretinal ERGs and single-cell recordings from rd17 retinas treated with rod or cone Tα showed comparable rod sensitivity and response kinetics. These results demonstrate that cone Tα forms a functional heterotrimetric G-protein complex in rods and that rod and cone Tα couple equally well to the rod phototransduction cascade. Thus, rod and cone transducin α-subunits are functionally interchangeable and their signaling properties do not contribute to the intrinsic light sensitivity differences between rods and cones.

22. Genetic connectivity, phylogeography and demographic history of the West Indian topshell Cittarium pica (Arqueogastropoda: Trochidae): implications for management and conservation

Diaz-Ferguson E1, Haney RA2, Silliman B1, Wares J3

1Department of Biology, University of Florida, Gainesville, FL
2Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL
3Department of Genetics, University of Georgia, Athens, GA

We examined the phylogeographic structure, genetic diversity, demographic history and connectivity patterns of the the Caribbean Top Shell, along its distributional range using mtDNA sequence variation (COI and 16S). Genetic diversity patterns determined using values of nucleotide and haplotype diversity exhibited a longitudinal gradient increase from South Western areas to Eastern and North Western regions. Tajima’s DT and Fu’s Fs neutrality tests were significant (p<0.04) and negative on Eastern and North Western sites as evidence for population expansion. Phylogeographic structure and genetic connectivity was tested using AMOVA, Fst per wise and migration rates. Spatial distribution of haplotypes was examined using median-joining haplotype networks, parsimony trees and Monmonier’s algorithm. Phylogeographic structure among regions and within populations was elevated and five regions were detected: 1. North Western (BSS) 2. North Eastern (WPR and USVI) 3. South Eastern (BN) 4. Central Panama (BV) and 5. South Western (CR, PC and BO). The observed regional structure was concordant with previous regions established for corals and fishes in four out of five cases (1, 2, 4, 5). Connectivity was restricted to specific areas and coherent with current patterns also in four out of five cases (<300Km of separation; 1. South Western sites (CR, PC, BO) 2. North Eastern sites (USVI and WPR) 3. Bonaire-South Western sites 4. Bahamas-North Eastern 5. Bahamas-Buenaventura).

23. Reducing lignin content in bahiagrass by down-regulation of 4-coumarate-CoA ligase

Fouad WM1,2, Martin L1,2, Vermerris W1,2,* Altpeter F1,2,*

1Agronomy Department, University of Florida, Gainesville, FL
2Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
Bahia grass is one of the most important warm season forage grasses. In Florida alone it is grown on more than 5 million acres. However, the high lignin content in the bahia grass biomass significantly reduces its forage quality. A key enzyme in the lignin biosynthetic pathway is the 4-coumarate-CoA ligase (4CL); it catalyzes the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates. We cloned four 4CL cDNAs from tetraploid bahia grass cv. Argentine and an RNAi construct targeting a highly conserved domain was constructed using 200 bp of the coding sequences. The 4CL-RNAi construct was introduced to bahia grass callus by biolistic gene transfer under transcriptional control of three alternative promoters: the constitutive e35S promoter, OsC4H promoter for xylem specific expression and the ZmdJ1 promoter for expression in the green tissue. Following regeneration of plants their transgenic nature was confirmed using PCR and Southern blot analysis. Significant reduction of 4CL gene expression was detected in several transgenic lines by Northern blot analysis. RNAi suppression of 4CL was more effective under transcriptional control of the xylem specific OsC4H promoter than under control of the e35S or ZmdJ1 promoters. Data describing the effect of 4CL suppression on Klasson lignin in transgenic lines will be presented.

24. The rough endosperm3 locus encodes a predicted splicing factor required for plant development and regulation of cell proliferation

Fouquet R, Fajardo D, Martin F, Policht T, Tseung CW, Settles AM
Horticultural Sciences Department, University of Florida, Gainesville, FL

Plant development requires controlled cell proliferation and cell differentiation. The rough endosperm3 (rgf3) mutant is essential for maize seed and seedling development. Our phenotypic characterization of rgh3 suggests that this locus is required to regulate both cell differentiation and proliferation. Rgh3 seedlings germinate at a low rate and seedling leaves are typically adherent. Mutant rgh3 plants are lethal within 2-4 weeks of planting; however, the Rgh3 locus is not essential for cell viability. Mutant endosperm tissues are far more proliferative than normal endosperm tissues when grown in vitro. These data suggest that the Rgh3 locus has an essential developmental role. We cloned a Mu1 insertion that is tightly-linked to the rgh3 mutant. The Mu1 element disrupts a U2AF35 Related Protein that we named ZmUrp. We identified a second rgh3 allele from a directed EMS tagging experiment that suggests the loss of ZmURP protein causes the rgh3 phenotype. ZmUrp produces multiple spliced products with only one variant encoding a predicted protein. A GFP fusion with ZmURP is localized to the nucleolus and nuclear speckles when transiently expressed in Arabidopsis protoplast or in tobacco epidermal cells. These data are consistent with ZmURP having a function in RNA splicing and suggest a role for RNA splicing in regulating cell proliferation and cell differentiation.

25. Using Saccharomyces cerevisiae as a model to study the genetic interaction between Arabidopsis 14-3-3s and G-box binding factor 3 (GBF3)

Gokirmak T1, Laughner BJ2, Paul AL2*, Ferl RJ1,2,*
1Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
2Horticultural Sciences Department, University of Florida, Gainesville, FL

G-box binding factor 3 (GBF3) is a member of a bZIP transcription factor family that recognizes a cis acting element called G-box (5'-CCACGTGG-3'). G-box is present in many environmentally regulated plant gene promoters (Lu et al., 1996, Plant Cell 8(5):847-57; Mallappa et al., 2008, J Biol Chem 283(51):35772-82; Menkens et al., 1995, Trends Biochem Sci 20(12):506-10; Shinozaki et al., 1997, Plant Physiol 115(2):327-34). Microarray and RT-PCR studies showed that GBF3 is regulated both developmentally and environmentally. In plants, 14-3-3s were first discovered to be part of transcriptional G-box DNA binding complexes in Arabidopsis Adh promoter (Lu et al., 1992, Proc Natl Acad Sci U S A 89(23):11490-4). This same study showed that the 14-3-3 proteins did not directly interact with G-box. However, it is proposed that 14-3-3s can be part of the G-box multiprotein complex through interaction with GBF3. In this study, we showed that there is a genetic and direct interaction between 14-3-3s and GBF3. Expression of GBF3 in Saccharomyces cerevisiae causes cellular toxicity. This toxicity can be eliminated by deletion of N-terminal proline rich domain or the C-terminal uncharacterized domain. This suggests that these two domains are essential for GBF3 function. Furthermore, co-expression of 14-3-3s in yeast rescues GBF3 mediated cellular toxicity through direct 14-3-3/GBF3 interaction.

26. Comparative proteomics of redox regulated proteins

Alvarez S1, Zhu M1, Goldsmith J1, Chen S1,2,*
1Department of Biology, University of Florida, Gainesville, FL
2Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

We report the dynamic changes of redox proteins in response to oxidative stress induced by methyl jasmonate (MeJA) in Arabidopsis using a 2D gel based comparative proteomics approach. Monobromobimane (mBBr), a fluorescent dye, was used to label the thiol groups of proteins obtained after alkylation of free thiols groups and reduction of disulfide bonds. The labeled proteins were separated on 2D gels. After visualization of disulfide proteins, total proteins were stained with SyproRuby to compare the signal intensity of the thiol groups labeled with
mBBr and the protein expression levels. A comparative map of potential redox regulated proteins from MeJA-treated and control Arabidopsis shoots and roots was established. Proteins involved in the sulfur and the antioxidant metabolisms (e.g., cysteine synthase, dehydroascorbate reductase), the lipid metabolism (e.g., mosaic death 1), the jasmonate biosynthesis pathway (e.g., allene oxide cyclase 2) and several stress responsive proteins were identified using LC-MS/MS. Collectively, the comparative proteomics approach allowed us to differentiate potential redox proteins from structural disulfide proteins. Novel disulfide proteins were identified, and the cysteines involved in the formation of disulfide bonds were mapped. The functional significance of the redox proteins will be discussed.

27. Regulation of cysteine synthesis in soybean

Gordon C¹², Kirst M²⁻, Vyas D²⁻, Harmon A¹².*

¹Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
²Department of Biology, University of Florida, Gainesville, FL
³Indian Institute of Integrative Medicine (CSIR), Jammu, India

Cysteine biosynthesis is the terminal step of sulfur assimilation, and it lies at the junction between sulfur and nitrogen metabolism. Cysteine is synthesized from serine by the enzymes serine acetyltransferase (SAT) and O-acetylserine (thiol) lyase (OASTL). In Arabidopsis, cysteine synthesis is regulated by metabolites which control the reversible formation of a complex between SAT and OASTL, called the cysteine synthase complex (CSC). Our results for three SATs and two OASTLs from soybean show that, as in Arabidopsis, formation of CSC enhances SAT activity while it inhibits OASTL activity. However, the plastidic/cytosolic SAT (GmSerat2;1) was less able than the cytosolic SAT to inhibit OASTL. GmSerat2;1 has an isoform unique phosphorylation site, and the mutant GmSerat2;1S378D mimics constitutive phosphorylation in that it is insensitive to feedback inhibition by cysteine. GmSerat2;1S378D did not inhibit OASTL activity to the same degree as wild type isoforms. In addition, in assays of the ability of CSC to synthesize cysteine from serine, complexes containing GmSerat2;1S378D showed the highest activity relative to those containing wild type enzyme. These experiments suggest that phosphorylation supports higher production of cysteine, by relieving feedback inhibition of GmSerat2;1 and by lowering inhibition of OASTL in the CSC. (Supported by USDA NRI CREES Award 2006-35318-17392)

28. Evolutionary patterns of HCV infection are independent of clinical phenotype

Gray RR¹, Santos LA², Veras NMC², Goodenow MM¹⁺, Salemi M¹⁺

¹Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL
²Laboratorio Avancado de Saude Publica, Centro de Pesquisa, Goncalo Moniz, Fundacao Oswaldo Cruz, Salvador, BA, Brazil
³Instituto de Biologia, Universidade de Brasilia, Brasilia, DF, Brazil

Background: Hepatitis-C virus (HCV) infection is associated with development of hepatocellular cancer (HCC), chronic hepatitis and liver cirrhosis. The high evolutionary rate of HCV results in the rapid accumulation of mutations over time. Although previous studies have attempted to define a correlation between evolutionary patterns and in vivo viral pathogenicity, the relationship remains unclear. Methods: Previously published sequence datasets from 22 HCV-infected subjects were re-analyzed individually using powerful phylogenetic techniques. Datasets were derived from serially sampled plasma (n=15) or multiple tissues (n=7) and included sequences from the core gene (n=7), the hypervariable region (HVRI, n=9) and the envelope region (n=6). Results: The HVRI dataset contained too little phylogenetic signal to analyze with confidence. Inferred phylogenies for the longitudinal envelope sequences indicated strong temporal evolution, purifying selection, and constant population diversity. Patterns were consistent between the subjects with mild and severe disease. In the datasets containing core sequences amplified from multiple tissues, several analyses detected significant anatomical compartmentalization and population subdivision in a subset of patients with HCC. Conclusions: Previously reported characteristics of HCV evolution potentially linked with liver disease stage and development of HCC could not be replicated in this study using robust phylogenetic methods.

29. Regulatory divergence in Drosophila melanogaster and D. simulans, a genome-wide analysis of allele-specific expression

Graze RM¹, McIntyre LM¹⁻²⁻, Main BJ¹, Wayne ML¹⁺*, Nuzhdin SV¹⁻⁵

¹Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
²Department of Statistics, University of Florida, Gainesville, FL
³Section of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA
⁴Department of Biology, University of Florida, Gainesville, FL
⁵Department of Evolution and Ecology, University of California, Davis, CA

Species-specific regulation of gene expression contributes to the development and maintenance of reproductive isolation and to species differences in ecologically important traits. A better understanding of the evolutionary forces which shape regulatory variation and divergence can be developed by comparing expression differences among species and interspecific hybrids. Once expression differences are identified, the underlying genetics of regulatory variation or
divergence can be explored. With the goal of associating cis and/or trans components of regulatory divergence with differences in gene expression, overall and allele-specific expression levels were assayed genome-wide in female adult heads of *D. melanogaster*, *D. simulans* and their F1 hybrids. A greater proportion of cis differences than trans differences were identified for genes expressed in heads and, in accordance with previous studies, cis differences also explained a larger number of species differences in overall expression level. Regulatory divergence was found to be prevalent among genes associated with defense, olfaction, and among genes downstream of the *Drosophila* sex determination hierarchy. In addition, two genes, with critical roles in sex determination and micro RNA processing, *Sxl* and *loa*, were identified as misexpressed in hybrid female heads, potentially contributing to hybrid incompatibility.

30. Tissue-specific roles of FgfR2 in urethral tube closure

Gredler ML1, Seifert AW1, Ornitz DM2, Cohn MJ1,3,4,*

1Department of Biology, University of Florida, Gainesville, FL
2Department of Developmental Biology, Washington University, St. Louis, MO
3Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
4Howard Hughes Medical Institute, University of Florida, Gainesville, FL

Hypospadias is a malformation of the penis that is characterized by incomplete closure of the urethral tube and affects approximately 1 in 125 live male births. Patterning of the mammalian external genitalia requires coordination of proximal to distal outgrowth with urethral tubulogenesis. Reciprocal signaling between the urethral epithelium and mesenchyme of the genital tubercle - the embryonic precursor of the penis and clitoris - maintains the expression of key genes necessary for development of the external genitalia. Fibroblast growth factor 10 (*Fgf10*) is expressed in the genital tubercle mesenchyme and signals through the receptor *FgfR2* that is expressed in two adjacent cell populations, the urethral epithelium and the ectoderm. Null mutations in *FgfR2* result in failure of urethral tube closure (hypospadias) and loss of mature urethral epithelium. In this study, we explore the distinct roles of *FgfR2* in the urethra and in the ectoderm using tissue-specific knockouts in mice. We find that the loss of *FgfR2* from the ectoderm results in hypospadias, although the resulting urethral epithelium maintains relatively normal character. In contrast, deletion of *FgfR2* from urethral endoderm inhibits its maturation into a complex epithelium, although gross hypospadias does not occur. Our results demonstrate that *FgfR2* plays distinct roles in these two cell populations, and that both are necessary for proper patterning of the genital tubercle and maturation of the urethra.

31. The role of Nlr1 in leaf and plant development

Gustin JL, Fajardo DS, Tseung CW, Black J, Settles AM*

Horticultural Sciences Department, University of Florida, Gainesville, FL

The developmental program that produces a flat leaf blade requires specification of polar domains including establishment of the adaxial and abaxial (top/bottom) axis. Several developmental mutants suggest transcript processing mechanisms are likely to have important roles in leaf morphogenesis. We recovered a maize mutation that impacts development in multiple tissues including distinct narrow leaf and rough endosperm (nlr1) phenotypes. The nlr1 mutant displays characteristic markers of altered adaxial/abaxial domain specification including reduced blade expansion, reduced number of macrohairs, and irregular lateral vein organization. In addition, nlr1 alters transcript accumulation of adaxial leaf polarity genes in developing leaves. These data suggest Nlr1 is involved in adaxial domain specification in maize. We identified a transposon insertion from a Robertson’s Mutator (Mu) flanking sequence tag that is tightly linked to nlr1. The transposon disrupts a Type C, J-domain protein, named DjC78. J-domains activate Hsp70 ATPase activity and proteins containing these domains have diverse functions in the cell. DjC78 also contains an arginine/serine (RS) rich domain, which is found in pre-mRNA splicing factors. Transient expression of ZmDjC78 indicates localization to the nucleus in punctate sub-domains consistent with a hypothesis that ZmDjC78 is involved in transcriptional processing. Based on these data, we hypothesize Nlr1 has a role in developmental gene expression.

32. A subset of the diverse COG0523 family of putative metal chaperones is linked to zinc homeostasis in all kingdoms of life

Haas CE1, Rodionov DA2,3, Kropat J4,5, Malasarn D4,5, Merchant SS4,5, de Crécy-Lagard V1,*, 1Department of Microbiology and Cell Science, University of Florida, Gainesville, FL
2Burnham Institute for Medical Research, La Jolla, CA
3AA Kharkevich Institute for Information Transmission Problems, Moscow, Russia
4Department of Chemistry and Biochemistry, University of California, Los Angeles, CA
5Institute for Genomics and Proteomics, University of California, Los Angeles, CA

COG0523 proteins are, like the Ni chaperones of the UreG family, part of the G3E family of GTPases linking them to metalocenter biosynthesis. Even though the first COG0523-encoding gene, *cobW*, was identified almost 20 years ago, little is known concerning the function of this ubiquitous family. Based on a combination of comparative genomics, literature and phylogenetic analyses and experimental validations, the COG0523 family can be separated into at
least fifteen subgroups. The CobW subgroup involved in B12 synthesis represents only one small sub-fraction of the family. Another, larger subgroup, is suggested to play a predominant role in the response to Zn limitation based on the presence of the corresponding COG0523-encoding genes downstream from putative Zur binding sites. We have also predicted a link between COG0523 and regulation by Zn in Archaea and show that two COG0523 genes are induced upon Zn depletion in a eukaryotic reference organism, Chlamydomonas reinhardtii. This work lays the foundation for the pursuit by experimental methods of the specific role of COG0523 in metal trafficking. Based on phylogeny and comparative genomics, both the metal specificity and the protein target(s) might vary from one subgroup to another. Additionally, Zur-dependent expression of COG0523 may represent a mechanism for hierarchal Zn distribution in the face of inadequate Zn nutrition.

33. A phylogenomic survey of avian transposons

Han K-L, Braun EB, Kimball RT*
Department of Biology, University of Florida, Gainesville, FL

Transposon insertions are rare genomic changes that are thought to have a low probability of multiple independent insertions at any single location, making them useful for phylogenetics. We searched for transposons and determined their distribution among loci and clades by examining non-coding sequences from a large-scale dataset representing the diversity of birds. The majority of transposons identified were CR1 (chicken repeat 1) elements, which were even more frequent relative to other transposon types than expected based upon the chicken genome. Most large insertions in the aligned intron data were attributed to transposable elements, suggesting that transposons have a major effect on genome size variation among species. Less than half of the insertions were synapomorphic, and these united clades that were also well supported by nucleotide analyses. Although most transposon insertions were homoplasys-free, several appeared to exhibit homoplasys or presented other potential problems for phylogenetic analyses due to independent insertions at the same site or precise deletion of an insertion. These results suggest that transposons, while a valuable tool in phylogenetics, need to be examined rigorously, and conclusions based upon these insertions treated with caution unless corroborated by congruence among multiple insertions or independent types of data.

34. Polymorphisms in KSHV microRNA sequences observed in clinical samples from KS and MCD patients cause miRNA maturation and expression differences

Han S*, Marshall V**, Whitby D*, Renne R1,2

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Viral Oncology Section, AIDS and Cancer Virus Program, SAIC-Frederick, NCI-Frederick, Frederick, MD

MicroRNAs (miRNAs) are 22-24 nt single-stranded RNA molecules that post-transcriptional regulate gene expression. So far 12 miRNAs have been identified in Kaposi's sarcoma-associated herpesvirus (KSHV), which causes Kaposi's sarcoma (KS), Multicentric Castleman Disease (MCD), and Primary effusion lymphoma (PEL). Recently, we sequenced the KSHV miRNA-encoding region in clinical samples from KS and MCD patients of different geographical origins and several PEL cell lines. Our analysis revealed single or multiple nucleotide polymorphisms within pre-miRNA sequences in several miRNAs (Marshall et al., 2007, J Infect Dis 195(5):645-59). Because miRNA processing by microprocessor and Dicer depends on primary miRNA secondary structure, effects of polymorphisms on miRNA maturation cannot be deduced. To ask whether naturally occurring polymorphisms affect miRNA expression, we analyzed polymorphisms occurring in several pre-miRNA for their expression. Using in vitro maturation assays, we determined the maturation affect by single and multiple polymorphisms within pre-miRNAs in miR-12-2, -4, -5, -7, and -9. Transient transfection assays and miRNA array were performed as further experiments. Our results demonstrate that naturally occurring polymorphisms in KSHV miRNA genes translate into maturation differences. Since, KSHV-encoded miRNAs can regulate genes in angiogenesis, apoptosis and cell survival pathways, mutations in miRNA genes may be associated with clinical variants or phenotypes of KS, PEL, and MCD.

35. The role of genetics in vitiligo susceptibility

Herbstman DM1, Hou W2, Garvan CW3, McCormack WT4,5, Wallace MR1-2

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Department of Epidemiology and Health Policy Research, University of Florida, Gainesville, FL
3Office of Educational Research, University of Florida, Gainesville, FL
4Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL

Vitiligo is an autoimmune pigment disorder of skin, which is seen on patients as depigmented areas that may enlarge. It affects about 0.5-1% of all ethnic groups worldwide, and is associated with an increased risk for other autoimmune diseases. The cause of vitiligo is unknown, but is thought to involve genetic and environmental factors. Our hypothesis is that vitiligo pathogenesis is caused in part by genetic susceptibility to both autoimmune and autotoxic events, due to polymorphisms in genes involved in the regulation of the immune response and melanin production. Human genomic DNA samples from vitiligo patients, their family members, and healthy controls with no autoimmune diseases were genotyped for a number of different single
nucleotide polymorphisms (SNPs) in COMT, TYR, TYRP1, DCT, and PAH, genes involved in melanin biosynthesis, and the immunoregulatory gene AIRE. Using case/control and family-based genetic association studies, as well as haplotype analysis, susceptibility to vitiligo was linked to the AIRE gene; significant results were also found in the melanin biosynthesis genes. These results support a possible role for genes involved in immune system regulation, as well as for genes involved in melanin synthesis, in vitiligo susceptibility. Our aim was to identify genes involved in vitiligo susceptibility so that in the future, therapies that might prevent or ameliorate vitiligo may be developed based on our understanding of genetic causes of vitiligo.

36. Circadian cycle-dependent effects on neural progenitor cell proliferation, differentiation and survival in adult mice

Hoang-Minh LB1, Kelly P1, Palmer TD2, Ormerod BK1,2,*

1J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL
2Department of Neurosurgery, Stanford University, Stanford, CA

Previous work suggests that adult hippocampal neurogenesis increases during the dark cycle in mice, when they are most active. We tested whether hippocampal neurogenesis is increased during the dark (active) phase of the light:dark cycle, and whether nigral neurogenesis may become detectable if we looked during the dark cycle, when levels would potentially be increased. Adult (8 week-old) female C57Bl/6 mice that were exposed to a freely moving or immobilized running wheel were injected with either a single i.p. injection or a series of 6 injections of the cell synthesis marker BrdU (50 mg/kg) either 2h after lights off (0700) or 2h after lights on (2100). Mice receiving a single BrdU injection were perfused 2h later to measure cell proliferation and mice receiving 6 daily or nightly injections were perfused 1 week or 4 weeks after the first BrdU injection to measure neurogenesis and new cell survival (n=5 per group). ANOVAs revealed that cell proliferation in both the hippocampus and substantia nigra was increased in runners and during the dark cycle (p<0.05). Although more new neurons were produced in the hippocampus of mice during the dark cycle versus light cycle (p<0.05) no new neurons were found in the substantia nigra. In addition, cell survival was increased among cells produced during the dark versus light cycle in both regions (p<0.05). Here, we uncover an interesting model for examining the mechanisms that govern progenitor cell behavior.

37. Involvement of SSRP1 in latent replication of Kaposi’s sarcoma-associated herpesvirus

Hu J1,2, Liu E1,2, Renne R1,2,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2University of Florida Shands Cancer Center, Gainesville, FL

Kaposi’s sarcoma-associated herpesvirus (KSHV) is a γ-herpesvirus that undergoes both lytic and latent infection. During latent infection, two viral elements are required for DNA replication: the latency-associated nuclear antigen (LANA), which functions as an origin binding protein, and the origin, which resides within the terminal repeats (TRs) of the viral genome. Previously, we have identified two cis-elements within TR which are required for replication: two LANA binding sites (LBS1/2) and a GC-rich RE element upstream of LBS1/2. To further characterize RE, we constructed a 71 bp minimal replicon (MR) and performed a detailed mutational analysis. It indicated that the first 8 nts within RE are critical for replication. Changing the position and the distance between RE and LBS1/2 can affect ori activity, suggesting that RE may function as a loading pad for cellular proteins involved in replication. Using biotinylated DNA fragments of wt or mutant MR as probes, we identified several cellular origin-interacting proteins putatively involved in LANA-dependent replication. Thirty proteins were found to preferentially bind to MR. Among these proteins, SSRP1, a subunit of the FACT complex, and TRF2 formed complexes with LANA at the MR region. SiRNA-based knock-down of SSRP1, but not dominant negative-based knock-down of TRF2, significantly decreased the efficiency of TR replication. These results indicate SSRP1 as a novel cellular protein involved in LANA-dependent DNA replication.

38. Characterization of sugarcane caffeic acid 3-O-methyltransferase (COMT) and 4-coumarate-CoA ligase (4CL)

Jung JH1,2, Kim JY1,2, Fouad W1,2, Vermerris W1,2,*, Gallo M1,2,*, Altpeter F1,2,*

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

Sugarcane is the highest yielding biomass producer. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning. Fuel grade ethanol can be made from sugarcane leaf litter residue following acid hydrolysis pre-treatments to remove lignin which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. In the lignin pathway, 4-coumarate-CoA ligase (4CL) and Caffeic acid 3-O-methyltransferase (COMT) are key enzymes that catalyze
the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates or the methylation of 5-
hydroxyconiferaldehyde to sinapaldehyde, respectively. However, sugarcane has a complex polyploid genome and these
genes belong to a large gene family. Their broad
substrate specificities have made it difficult to identify
orthologs that are specifically involved in lignin biosynthesis.
We have isolated two 4CL genes and two COMT genes from
a commercially important sugarcane cultivar by a PCR-based
strategy. Results from RT-PCR expression analysis in
different tissues will be presented. In vitro substrate
preferences will be determined from purified enzymes. RNAi
suppression of selected target genes will allow validation of
their relative importance in lignin biosynthesis of sugarcane.

39. Transgenic flowering locus T expression: a
bypass to long term juvenility

Kamps TL, Pajon M, Moore GA*

Horticultural Sciences Department, University of Florida,
Gainesville, FL

Citrus plants display a very long period of growth and
development (up to 10 years) prior to flowering and fruiting
when grown from seed or from tissue culture regenerants of
juvenile transformation. This developmentally regulated
delay severely impedes utilizing genetic based strategies to
improve fruit quality, disease resistance, and responses to
abiotic environmental parameters. Our goal is to overcome
this barrier using genes that promote precocious flowering.
Ectopic expression of certain genes behind a constitutive
promoter has been shown in many species to promote
precocious flowering. One of these genes, FT (Flowering
Locus T) encodes a small, globular protein which is
transported from phloem companion cells in leaves to shoot
apical meristems, where it functions in promoting flowering.
Importantly, in some species the FT protein has been shown
to be transferable across graft unions. Transgenic citrus
over-expressing FT will be used with grafting strategies to
induce precocious flowering of breeding genotypes, and
possibly for manipulating flowering of commercial citrus. To
date, Agrobacterium-mediated transformation of juvenile
tissue of the citrus hybrid Carrizo citrange and tobacco has
been carried out with genomic clones of the three citrus FT
orthologs, cifT1, cifT2, and cifT3. Surprisingly, flowering
has been observed during tissue culture of some citrus
materials transformed with the 34FMV-cifT3-GUS construct.

40. Developmental stage, reproductive tissue, and
cytotype effects on transcriptional and post-
transcriptional regulation of mitochondrial genes

Kamps TL, Siripant MN, Chamusco KC, Chase CD*

Horticultural Sciences Department, University of Florida,
Gainesville, FL

Cytoplasmic male sterility (CMS) is maternally inherited and
results from the interaction of nuclear and mitochondrial
expressed gene products. We have profiled transcripts and
protein products of mitochondrial genes in developmentally
staged pollen and developing female reproductive structures
(immature ears) from isogenic NB and CMS-S maize
cytotypes that do not have CMS-S nuclear fertility restoring
alleles. Western blots showed the accumulation of
mitochondrial ATP synthase and respiratory complex
subunits were clearly reduced in both CMS-S and normal
microspores as compared to the immature ears. We
examined RNA editing, a post-transcriptional feature of plant
mitochondrial gene expression, to test a possible mechanism
for producing the observed tissue and developmental stage
specific phenotypes. We report on the editing patterns
determined from sequence analysis of RT-PCR products of
atp4, atp6, atp8, and atp9 for microspores and immature
ears of our isogenic Mo17 CMS-S and NB cytotypes. In
addition, the comparison of these results to publicly
available data of NB editing, and results from RNA editing
prediction software will be presented.

41. Generation and characterization of interspecific
hybrids between elephantgrass (Pennisetum
purpureum Schum.) and pearl millet (Pennisetum
glaucum L.)

Kannan B1,2, Sollenberger L1,2, Altpeter F1,2,*

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

Napiergrass (Pennisetum purpureum Schum.) has been
introduced to all tropical and subtropical areas of the world
because of its ability to produce large amounts of high
quality forage biomass. Napiergrass is also considered one
of the best adapted perennial feedstocks for biofuel
production in the southern U.S. Napiergrass causes less
environmental problems than several other potential
biomass crops. However, napiergrass is listed as invasive in
Southern Florida by the Florida Exotic Pest Plant Council.
Plant propagation and establishment of new napiergrass
plantings occurs through vegetative plant parts. Therefore,
unlike seeded crops, flowering of napiergrass is not
necessary for crop production and its suppression will
significantly reduce its potential for invasiveness. We
produced triploid, interspecific hybrids between napiergrass
(tetraploid) and pearl millet (diploid) to introduce male and
female sterility. Tall, stress tolerant parents were chosen
with the goal to generate interspecific hybrids with good
productivity and persistence as well as male and female
sterility. Pearl millet (AA genome) multiline population with
A4 CMS represented the female parent and was crossed
with allopolyploid napiergrass (A’A’BB) genotypes Merkeron
or N 51. We will present data describing the phenotypic
variability in these hybrids which allowed selecting lines with
excellent vigor and sterility. Further experiments will
evaluate the persistence of the interspecific hybrids.
42. Induction of a novel putative trypsin inhibitor gene in *Fortunella margarita* upon canker infection

Khalaf A<sup>1,2,3</sup>, Moore G<sup>1,2,4</sup>, Gmitter FG<sup>1,2,3</sup>

<sup>1</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL  
<sup>2</sup>Horticultural Sciences Department, University of Florida, Gainesville, FL  
<sup>3</sup>Citrus Research and Education Center, University of Florida, Lake Alfred, FL

Asiatic citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*) has been considered one of the most severe diseases of citrus species and cultivars. Previously we have shown that *Fortunella margarita* exhibits a hypersensitive response to *Xac* indicative of an incompatible interaction. In addition, a microarray expression analysis was done to confirm visual results suggestive of the hypersensitive response; 352 genes were identified to be significantly differentially expressed after infection. The above study identified the components of the incompatible interaction, reactive oxygen species (ROS) production, and programmed cell death (PCD). In addition, a number of common defense mechanisms and a number of resistance genes were identified. In this study we show the differential expression of a novel putative protease inhibitor in *F. margarita* post canker infection. Further characterization of the gene will be presented.

43. Biolistic gene transfer of minimal expression cassettes into sugarcane

Kim JY<sup>1,2</sup>, Gallo M<sup>1,2,4</sup>, Altpeter F<sup>1,2,4</sup>

<sup>1</sup>Agronomy Department, University of Florida, Gainesville, FL  
<sup>2</sup>Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Sugarcane (*Saccharum* sp. hybrids) is a highly productive C4 grass used as the main source of sugar and recently to produce ethanol, a renewable transportation fuel. Biolistic gene transfer is superior to alternative methods in genetic engineering, when multiple expression cassettes need to be co-expressed. However, biolistic gene transfer is sometimes associated with complex transgene integration that may cause gene silencing. This study investigated the integration complexity following biolistic gene transfer of minimal transgene expression cassettes without vector backbone and the effects on transgene expression. Cross sections of immature inflorescences from sugarcane (cv. CP-88-1762) were placed on callus induction medium and subcultured biweekly to induce embryogenic callus. Embryogenic callus was used as a target for biolistic gene transfer of an expression cassette consisting of nptII under the control of the constitutive 3SS promoter with HSP70 intron and NOS 3’ UTR. Prior to gene transfer, the vector backbone was removed by restriction digestion. The minimal nptII expression cassette was precipitated on 1.0 µm gold. Transgenic plants were regenerated following selection on geneticin or paromomycin containing media. PCR screening suggested that 83% of the regenerated plants were transgenic. Ten plants were randomly selected per experiment (total of 60 plants) and analyzed for transgene expression by NPTII - ELISA and transgene integration by Southern blot analysis.

44. Epigenetic bases of neuronal diversity and plasticity in *Aplysia californica*: toward single neuron epigenome

Kohn AB<sup>1</sup>, Bobkova Y<sup>1</sup>, Moroz LL<sup>1,2,4</sup>

<sup>1</sup>Whitney Laboratory for Marine Biosciences, University of Florida, St. Augustine, FL  
<sup>2</sup>Department of Neuroscience, University of Florida, Gainesville, FL

What are the genomic bases of unique neuronal phenotypes? Epigenetics refers to inheritable modifications in phenotypes that do not involve changing the underlying DNA sequences. These mechanisms include DNA methylation of the cytosine residues in CpG dinucleotides, covalent and non-covalent modifications to the histone proteins, chromatin remodeling by the exchange of histone variants, chromatin dynamics involving the switching of active and silent chromatin, non-coding RNA including siRNA, and regulation of transcription. We identified and cloned 13 canonical histones and their variants expressed in the CNS of *Aplysia californica* including a unique molluscan H3.4 as well as the H2Macro only described in vertebrates. We also identified 15 major histone modifying enzymes as well as more than 50 other components involved in static and dynamic chromatin remodeling. Using 454/SOLiD sequencing from single neurons and *in situ* hybridization, we show that the histones, histone modifying enzymes and many other chromatin associated proteins revealed a high level of differential expression: nearly all central neurons in *Aplysia* have their own unique expression profiles. This work also opens unprecedented opportunity to study the epigenome of individual neurons at a resolution difficult to achieve elsewhere.

45. Population genomics using high-throughput platforms in *Drosophila*

Kulathinal RJ<sup>1</sup>, Sackton TB<sup>2</sup>, Clark AG<sup>2</sup>, Hartl DL<sup>2</sup>, Barbazuk WB<sup>4,5</sup>, McIntyre LM<sup>1,4</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL  
<sup>2</sup>Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA  
<sup>3</sup>Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY  
<sup>4</sup>Department of Biology, University of Florida, Gainesville, FL
Genomics is transforming the field of population genetics by offering novel approaches to estimate population diversity at the genome level. The challenges are to identify questions that such high-throughput data can successfully address in addition to developing sound analytical and statistical methods. Here, we evaluate the utility of two platforms that will advance population genomics inference. First, we apply the Roche/454 platform to survey, at shallow coverage, natural variation in Drosophila melanogaster from two populations. Reads were aligned to the reference D. melanogaster genomic assembly, SNPs identified, and nucleotide variation was quantified genomewide. Simulations and empirical results suggest that nucleotide diversity can be accurately estimated from sparse data with as little as 0.20x coverage per line. Such unbiased genomic sampling demonstrates that short-read sequencing methods provide an efficient means to quantify variation in genome organization and content. As a second approach, we explore the use of a population-based, allele-specific SNP chip (Affymetrix custom array) that was recently developed in our lab. Hybridization levels of gDNA against array features were compared between F1 hybrids and their parents from two divergent populations of D. simulans to evaluate the power to detect heterozygosity. The valuation of these two platforms is both instructive and critical as we enter a new era in population genomics.

46. Analysis of the role of transforming growth factor-beta (TGF-b) on the proteolytic processing of connective tissue growth factor (CTGF)

Kuznia PM1, Lewin AS1,*, Schultz GS2,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Department of Obstetrics and Gynecology, University of Florida, Gainesville, FL

Purpose: The TGF-β system which includes CTGF has been determined to play a key role in the formation of scar tissue. CTGF has the ability to stimulate two opposing functions, proliferation and differentiation. These diverse functions may be associated with proteolytic processing of CTGF.

Methods: Human corneal fibroblasts (HCF) were serum starved for 48 hours then stimulated with 5ng/mL of TGF-β. At different time points, cell extracts and conditioned media were collected with the addition of a protease inhibitor cocktail. In a second experiment, serum starved HCF cultures had differing amounts of protease inhibitor cocktails with or without EDTA and then stimulated with TGF-β at 48 hours. After 24 hours, cell extracts and conditioned media were removed. All collected samples were separated by SDS-PAGE and analyzed using western blots. Results: The addition of TGF-β to the HCF increased levels of ~38kDa CTGF in both the cell extracts and conditioned media. In serum starved media, ~75 and 150kDa molecular weight bands were observed, but with the addition of TGF-β these bands decreased. Also, the addition of TGF-β caused the level of the ~18kDa fragment to decrease, whereas the~20kDa fragment remained constant. Finally, the addition of the any protease inhibitor cocktail reduced levels of~20kDa fragment in serum starved media. Conclusion: These data indicate that TGF-β influences the production and proteolytic processing of CTGF.

47. Polycomb group protein Bmi1 binds to the latent HSV-1 genome and maintains repressive marks during latency

Kwiatkowski DK1, Thompson HW2, Bloom DC1,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2Section of Biostatistics, Louisiana State University Health Sciences Center, New Orleans, LA

The mechanism by which Herpes Simplex Virus type 1 (HSV-1) establishes latency in sensory neurons is largely unknown. Recent studies indicate that epigenetic modifications of the chromatin associated with the latent genome may play a key role in the transcriptional control of lytic genes during latency. In this study, we found both constitutive and facultative types of heterochromatin to be present on the latent HSV-1 genome. Deposition of facultative marks trimethyl H3K27 and histone variant macroH2A varied at different sites on the genome whereas the constitutive marker trimethyl H3K9 did not. In addition, we show that in the absence of the LAT, the latent genome shows a dramatic increase in trimethyl H3K27, suggesting that expression of the LAT during latency may act to promote an appropriate heterochromatic state that represses lytic genes but is still poised for reactivation. Due to the presence of the mark trimethyl H3K27, we examined whether polycomb-group proteins, which methylate H3K27, are present on the HSV-1 genome during latency. Our data indicates that Bmi1, a member of the PRC1 maintenance complex, associates with specific sites in the genome, with the highest level of enrichment at the LAT enhancer. To our knowledge, this is the first example of polycomb-mediated repression of a viral genome leading to the establishment of latency.

48. H4R3 methylation facilitates beta-globin transcription by regulating histone acetyltransferase binding and H3 acetylation

Li X1, Hu X1,2, Patel B1, Zhou Z1, Liang S1, Ybarra R1, Qiu Y3, Felsenfeld G1,5, Bungert J1,5, Huang S1,3,4

1Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2Edmond H. Fischer Signal Transduction Laboratory, Jilin University, Changchun, China
3Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL
4Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD
Histone modifications play an important role in the process of transcription. However, how certain histone modifications are functionally linked to transcriptional activity remains unclear. The globin genes are regulated by a highly organized chromatin structure that juxtaposes the locus control region (LCR) with downstream globin genes. We report here that the targeted recruitment of asymmetric dimethyl H4R3 catalyzed by PRMT1 facilitates histone H3 acetylation on Lys 9/Lys14 and is important for efficient interactions between the LCR and the bmaj-promoter during transcription. We show that dimethyl H4R3 provides the binding surface for PCAF and facilitates histone H3 acetylation in vitro. Furthermore, knock down (KD) of PRMT1 by RNA interference in erythroid progenitor cells prevents histone acetylation, enhancer and promoter interaction, as well as the recruitment of transcription complexes to the active beta-globin promoter. Reintroducing rat PRMT1 into the PRMT1 KD MEL cells rescues PRMT1 binding and beta-globin transcription. Taken together, our data suggest that PRMT1-mediated dimethyl H4R3 facilitates histone acetylation and enhancer/promoter communications, which lead to the efficient recruitment of transcription preinitiation complexes to active promoters.

49. Defective erythropoiesis in transgenic mice expressing dominant negative upstream stimulatory factor

Liang SY1, Moghimi B1, Crusselle-Davis VJ1, Lin I-J1. Rosenberg MH1, Li X1, Strouboulis J3, Huang S1,3,*, Bungert J1,3,4,*

1Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2Institute of Molecular Oncology, BSRC Alexander Fleming, Varkiza, Greece
3Center for Epigenetics, University of Florida, Gainesville, FL
4Powell Gene Therapy Center, University of Florida, Gainesville, FL

Transcription factor USF is a ubiquitously expressed member of the helix-loop-helix family of proteins. It binds with high affinity to E-box elements and, through interaction with co-activators, aides in the formation of transcription complexes. Previous work demonstrated that USF regulates genes during erythroid differentiation, including HoxB4 and β-globin. Here we show that erythroid-specific expression of a dominant negative mutant of USF, A-USF, in transgenic mice reduces expression of all β-type globin genes and leads to diminished association of RNA polymerase II with locus control region element HS2 and with the β-globin gene promoter. We further show that expression of A-USF reduces expression of several key erythroid-specific transcription factors, including EKLF and Tal-1. We provide evidence demonstrating that USF interacts with known regulatory DNA elements in the EKLF and Tal-1 gene loci in erythroid cells. Furthermore, A-USF-expressing transgenic mice exhibit a defect in the formation of CD71(+) progenitor and Ter-119(+) mature erythroid cells. In summary, the data demonstrate that USF regulates globin gene expression indirectly by enhancing expression of erythroid transcription factors, and directly by mediating the recruitment of transcription complexes to the globin gene locus.

50. Identification and characterization of a barrier/insulator flanking an enhancer region in Drosophila

Lin NW, Zhang YP, Zhang C, Zhou L1*

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

In our previous work, we identified an irradiation-responsive enhancer region (IRER), a ~33 kb evolutionarily conserved intergenic region upstream of the pro-apoptotic gene reaper (rpr), that is required for the induction of pro-apoptotic genes rpr and hid in response to irradiation. During developmental stage 12, this IRER turns into a facultative heterochromatin structure refractory to DNase I, accompanies with the enrichment of repressive chromatin marks, such as H3K27me3 and H3K9me3, as well as several PcG proteins. We have proven that this switch of chromatin structure is responsible for the sensitive-to-resistant transition of pro-apoptotic genes’ responsiveness to irradiation during embryogenesis. The epigenetic modification in the late embryos is limited in the rpr upstream regulatory region, without affecting the rpr promoter and basic enhancer region. Using a reporter assay, we showed that a 9kb region at the IRER left boundary contains the barrier function that prevents the propagation of heterochromatin associated with PcG-mediated silencing. Efforts have been taken to identify the essential barrier/insulator element and the cis-factors required for the barrier activity.

51. Defensive reaper-induction of michelob_x(mx) in mosquito midgut cells following virus infection

Liu B1,2, Becnel J3, Zhang Y1,2, Zhou L1,2,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2University of Florida Shands Cancer Center, Gainesville, FL
3Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL

Apoptosis is an important cellular response to viral infection. Genetic analysis in Drosophila revealed that a group of IAP-antagonists, including reaper, play a pivotal role in regulating cell death during development and in response to environmental stimuli. However, there is a lack of empirical evidence as to whether reaper-like pro-apoptotic genes are involved in regulating cell death during pathogen infection of insect vectors. In this study, we cloned the reaper ortholog,
**52. The role of Foxa genes in intervertebral disk formation**

Maier J, Harfe B*

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

The intervertebral disk (IVD) is composed of a tough, outer annulus fibrosus, and an inner, gel-like nucleus pulposus (NP). The NP is derived from the notochord in mice. Degeneration of the NP results in back pain. Despite its prevalence, effective treatments for chronic back pain are limited. Little is known about the mechanisms of IVD development and degeneration; this information could lead to improved treatments. The forkhead box (Fox) genes are expressed in many tissues and function in development and post natal life. Foxa1 and Foxa2 genes are expressed in all three germ layers of the early embryo. They have been well-studied in the endoderm, but not in the notochord. Foxa2 null mice die in utero lacking a notochord. Cre alleles have been used to ablate Foxa2 in the endoderm. These conditional alleles have also been used with a Foxa1 null allele to make double knockouts. We used these alleles with an inducible ShhERT2cre line to remove Foxa2 in tissues where Sonic hedgehog is expressed in E7.5 mouse embryos. Histology and fate-mapping with the Rosa26 reporter allele were done. Mice null for Foxa1 and lacking Foxa2 in Shh-expressing cells appear to have a severely deformed NP and a shortened tail. Fate-mapping in these mice suggests defects in the migration of notochord cells to the NP in mice heterozygous for Foxa1 and missing Foxa2. Study of the role of Foxa family action in IVD development may provide insight into new treatments for disk degeneration.

**53. Adeno-associated virus-vectored gene therapy with wild-type rhodopsin gene for treatment of autosomal dominant retinitis pigmentosa (ADRP)**

Mao H¹, Gorbatyuk M¹, Hauswirth WW¹,²,*, Lewin AS¹, *

¹Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

²Department of Ophthalmology, University of Florida, Gainesville, FL

Autosomal dominant retinitis pigmentosa (ADRP) is frequently caused by mutations in the RHO gene, which codes for the opsin of rod photoreceptor cells. We are engaged in development of gene therapies for ADRP using adeno-associated virus (AAV), which efficiently infects and transduces photoreceptor cells. In the present set of experiments, we designed and constructed a “hardened” form of the rhodopsin (RHO) gene that is specifically resistant to degradation by the siRNA 301. We had previously demonstrated that siRNA301 degrades both mutant and wild-type mouse and human RHO mRNA. The “hardened” RHO gene (RHO301) was generated by introducing silent mutations to eliminate the siRNA cleavage site. We are testing gene therapy in a mouse model of ADRP that expresses a human transgene with a prevalent ADRP mutation: proline 23 substituted by histidine (P23H). With delivery of RHO301 in AAV5 to P23H transgenic mice in a background of mouse RHO+/-, the retinal degeneration of injected eyes was slower compared with that of uninjected eyes or of control-injected eyes. The finding that a gene encoding wild-type rhodopsin could moderate the retinal degeneration in this model suggests that P23H rhodopsin causes a dominant negative effect, but not by gain of a toxic function, since increased production of normal rhodopsin can suppress the effect of the mutation. Our finding implies that some RHO mutations leading to ADRP can be treated by gene transfer of normal RHO.

**54. Subsets of SSR markers enable rapid bulk-segregant analyses in multiple maize inbred backgrounds**

Martin F¹,², Dailey S¹, Settles AM¹,²,*

¹Horticultural Sciences Department, University of Florida, Gainesville, FL
²Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Map-based cloning is a robust approach to identify the genetic causes of mutant phenotypes. The recent completion of the maize genome has made map-based cloning practical. Maize has high levels of nucleotide diversity further enabling positional cloning. The maize community has developed mutagenized populations that are primarily within a few inbred genetic backgrounds. A core set of molecular markers that can be used for cloning within these backgrounds will be useful. Simple sequence repeats (SSRs) are short motifs of 2 to 6 bases in length that are repeated in tandem arrays. SSRS can give sequence length polymorphisms that are rapid and cheap to use. Currently, the maize genetic map is composed of a vast array of marker types with many markers having SSRs but only being characterized for allelic variation in restriction fragments or single nucleotide polymorphisms. We screened
AAV hepatic gene transfer induces immune tolerance to several protein antigens and has been exploited in animal models for systemic delivery of therapeutic proteins. Adequate levels of transgene expression in hepatocytes induce an immune suppressive T cell response. We tested whether AAV gene transfer can induce tolerance to a cytoplasmic protein. AAV2 hepatic gene transfer for expression of β-galactosidase (β-gal) was performed in mice, followed by a secondary β-gal gene transfer with an adenoviral vector to provoke a severe immunotoxic response. Transgene expression from AAV2 in ~2% of hepatocytes almost completely protected from inflammatory responses against β-gal, eliminated antibody formation, and significantly reduced adenovirus-induced hepatotoxicity. Consequently, ~10% of hepatocytes continued to express β-gal 45 days after secondary Ad-LacZ gene transfer, while control mice had lost all Ad-LacZ expression. A combination of adoptive transfer studies and flow cytometric analyses demonstrated induction of Tregs that actively suppressed CD8+ T cell responses to β-gal and that were amplified in liver and spleen upon secondary Ad-LacZ gene transfer. These data demonstrate that tolerance induction by hepatic AAV gene transfer does not require systemic delivery of the transgene product and that expression of a cytoplasmic neo-antigen in few hepatocytes can induce Treg and provide long-term suppression of inflammatory responses and immunotoxicity.
describe our recent experiments with the axolotl using arrays to investigate spinal cord and limb regeneration and tissue transplantation from transgenic animals to investigate limb regeneration and wound healing.

58. Prevention and treatment of inhibitor formation in gene therapy for hemophilia B

Nayak S1, Cao O2, Herzog RW2,*

1Advanced Concentration in Immunology and Microbiology, Interdisciplinary Program in Biomedical Sciences, University of Florida, Gainesville, FL
2Division of Cellular and Molecular Therapy, Department of Pediatrics, University of Florida, Gainesville, FL

Immune responses to therapeutic proteins limit treatment of inherited protein deficiencies. Rapamycin (Rap)/IL-10/specific antigen epitope depletes antigen-specific effector T cells and induces CD4+CD25+FoxP3+Treg. This strategy prevented inhibitor formation in muscle-directed gene transfer in hemophilia B mice (F9-/-), indicating the potential for development of a prophylactic immune tolerance protocol. Optimization via different routes of administration of the drug combination Rap/IL-10/specific peptide in ova transgenic mice showed that Treg induction is more efficient in intraperitoneal, IP (4.7x), followed by subcutaneous, SQ (3.3x) and tail vein, IV (2.8x) delivery. Deletion of Teff was more pronounced via IP (2.7x) followed by SQ (2.3x) and IV (1.7x reduction) compared to control mice. This data shows differential route dependant effectiveness. We are testing additional routes (such as oral) and alternatives to IL-10 in the drug cocktail (such as IL-2). We tested the protocol for treatment of inhibitors in an ongoing immune response. Hemophilia B mice injected IM with 1x1011 vg of AAV1-CMV-hFIX formed anti-hF.IX (6 µg IgG/ml) at 1 and 2 months after gene transfer. However, animals that received the tolerance protocol, starting at 1 month after the onset of the immune response.

59. Molecular detection of rAAV in blood from non-human primates

Ni W1, Le Guiner C2, Bello-Roufai M3, Moullier P1,2, Snyder RO1,4,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2INSERM UMR649, Nantes Cedex, France
3Center of Excellence for Regenerative Health Biotechnology, University of Florida, Gainesville, FL
4Department of Pediatrics, University of Florida, Gainesville, FL

Gene transfer of therapeutic genes has shown the potential to treat human diseases, however, an emerging issue of this promising technology is misuse by athletes looking for an advantage. Gene doping is the transfer of genes to enhance athletic performance. Following IM injection of rAAV vectors into non-human primates, our group has reported that vector DNA can be detected in serum, urine, feces, saliva, and nasal fluid for several weeks post-injection. In developing a test to screen athletes, the use of less invasive sampling and sensitive detection techniques is imperative to detect gene doping. We are determining the smallest dose of a rAAV vector injected IM that can be detected in blood from non-human primates. We also aim to discern the relationship between vector dose and longevity in blood. The test we are developing involves the collection of blood and the analysis of inhibitor formation by qPCR. As a first target, we have optimized qPCR conditions to detect at least 5 copies of the Epo transgene in the background of endogenous sequences. Data generated in the non-human primate is the basis for developing a legally defensible commercial qPCR assay. Given that gene transfer technology encompasses a variety of vectors, routes of administration, as well as injection formulations, the vector biodistributions can vary widely, thus an outcome of our work will be to better define assays that can be utilized to elucidate vector distribution for legitimate gene therapy applications.

60. Epigenetic regulation of pro-apoptotic genes in Drosophila during pupae development

Novo M1,2, Zhang C1,2, Pang J1,2, Zhou L1,2,*

1Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
2University of Florida Shands Cancer Center, Gainesville, FL

IAP-antagonists reaper and hid play a pivotal role in mediating cell death during development and in response to cytotoxic stimuli. The two genes have a synergistic effect on cell death induction and are often co-regulated. A 33kb genomic region upstream of reaper, the IRER, is required for mediating the induction of both reaper and hid following irradiation. Interestingly, IRER is subject to epigenetic regulation. Although it is open in most cells during early embryogenesis, chromatin in IRER becomes enriched for H3K27Me3 and H3K9Me3 and forms facultative heterochromatin in differentiating and differentiated cells post-embryonic stage 12. This epigenetic modification of IRER blocks the irradiation-responsiveness of both reaper and hid. To monitor the epigenetic status of IRER in individual cells and live animals, we knocked into IRER an ubiquitin-DsRed reporter through homologous recombination. DsRed expression is suspected to reflect the epigenetic status of IRER. In this study, we monitored its expression during the pupae stage, when apoptosis is triggered by stage-specific pulses of the steroid hormone ecysdsone that activate a transcriptional cascade resulting in the expression reaper and hid. We are also in the process of generating a novel transgenic fly that will allow us to manipulate cells with an open IRER. The data provide a
more detailed understanding of stage-specific apoptosis and future routes for research.

61. A new method for gene prediction: application to comparative analysis of *Pseudomonas aeruginosa* genomes

Oden SM, Brocchieri L*

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

We developed new scoring methods and a novel computational gene predictor for the identification of genes based on scoring overall nucleotide-base usage asymmetries between codon positions as a function of local sequence content. Since our scoring scheme is based on global compositional propensities it does not suffer from over-parametrization and can be applied without detailed knowledge or assumptions on codon or dicodon usage. We applied our method to the analysis of the genomes of different strains of *P. aeruginosa*, an opportunistic pathogen of great nosocomial importance. In an effort to correlate gene content with pathogenicity, the genomes of several different pathogens of *P. aeruginosa* have been sequenced since publication of the first *P. aeruginosa* genome, PAO1. Currently, among all predicted protein-coding genes of *P. aeruginosa* PAO1, only 30% are functionally characterized and only 10% have been experimentally verified. For meaningful comparative-genomics analyses it is highly relevant to obtain improved characterizations and measures of reliability of gene predictions. We identified several new non-annotated genes in the genomes of *P. aeruginosa* PAO1, PA7 and PA14. However, several hypothetical genes previously annotated in *P. aeruginosa* PA7 are not characterized by compositional properties that distinguish them from random sequences. Our analysis provides statistically supported sets of genes and a more reliable classification of common or strain-specific genes.

62. The relationship between mutation rate and mating system

Ostrow DG, Joyner-Matos JA, Upadhyay A, Blanton D, Rosenbloom J, Izhar K, Hong J, Chik V, Grigaltchik V, Cadavid F, Baer CF*

Department of Biology, University of Florida, Gainesville, FL

Theory predicts that the strength of natural selection to reduce the deleterious mutation rate will be much stronger in asexual and selfing taxa than in outcrossing taxa. If asexual and/or selfing lineages can be shown to have substantially lower mutation rates than their outcrossing relatives, it would demonstrate differential selection on the mutation rate and cast doubt on the generality of deleterious mutation-based arguments for the evolution of sex. Rhabditid nematodes provide an opportunity to directly address the question of how mating system influences the mutation rate. The common ancestor of the Rhabditidae is inferred to have been gonochoristic (outcrossing), but there have been at least six independent evolutionary transitions to hermaphroditism (selfing). We initiated a new mutation accumulation experiment using two gonochoristic species from the genus *Caenorhabditis*, *C. remanei* and CB5161, and a gonochoristic sister species to *C. briggsae* (strain JU787) as well as an inbred strain of the *C. elegans* mutant *fog-2* as a control. Descendant populations were compared to unmutated ancestral control stocks to assess genomic mutation rates. Comparisons between selfing taxa and congeneric gonochoristic taxa will be discussed.

63. Long term rescue following AAV-mediated cone targeting gene therapy to Cpf15 mouse, a model of human achromatopsia with CNGA3 mutation

Pang J1, Lei B2,3, Mao S1, Everhart D4, Liu L1, Deng W1, Li Q1, Chang B5, Barlow R4, Hauswirth WW1,2*

1Department of Ophthalmology, University of Florida, Gainesville, FL
2Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO
3Department of Ophthalmology, University of Missouri, Columbia, MO
4Department of Ophthalmology, SUNY Upstate Medical University, Syracuse, NY
5The Jackson Laboratory, Bar Harbor, ME

Purpose: To test if cone targeted CNGA3 gene delivery can restore the cone system function in cpf15 mice, a natural model of human achromatopsia 2 with CNGA3 mutation. Methods: At postnatal day 14, 1 µl of AAV5-PR2.1-CNGA3 vector (1 x 10¹³ genome containing viral particles/ml) was injected subretinally into one eye of 20 cpf15 mice. The other eye was used as control. Dark- and light-adapted ERGs were recorded periodically starting. Ten months later, visual function was assessed with routine ERGs and 10 Hz flicker ERGs. Behavioral tests were also tested, followed by histochemical studies. Results: In treated eyes, restored light-adapted ERGs were observed at 3 weeks after injections and remained stable for at least 10 months; the amplitudes were about 50% of those of the normal eyes. The dark-adapted flicker ERGs also showed significantly improved cone-driven responses. No cone-driven ERGs were recorded in untreated eyes. Behavioral tests showed nearly normal cone-driven visual acuity and contrast sensitivity in the treated eyes, but not in the untreated eyes. In the treated cpf15 retinas, immunohistochemistry showed CNGA3 staining in the inner and outer segments of the cones. However, no CNGA3 expression was observed in the untreated eye from the same mouse. Conclusions: 1. AAV mediated gene therapy corrects CNGA3 deficiency in a naturally occurring mouse model of human achromatopsia 2. The genetic intervention restores and maintains the cone function for at least 10 months.
64. Comparative proteomics of salt tolerance between Arabidopsis thaliana and Thellungiella halophila

Pang Q1,2, Chen S2,3,*, Dai S1, Wang Y1, Yan X1

1College of Life Science, Northeast Forestry University, Harbin, China
2Department of Biology, University of Florida, Gainesville, FL
3Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

The majority of plants under salt stress exhibit slow growth, severe shrinkage or even death. To survive the stress, plants respond and adapt with complex mechanisms. In recent years there have been many reports on salt tolerance, most studies have been focused on Arabidopsis thaliana. However, the outcomes of such work are limited by the fact that A. thaliana is actually a true glycophyte. More recently, the halophytic plant species Thellungiella halophila has been proposed as an ideal model for studying molecular mechanisms of salinity tolerance in plants because of its ‘extremophile’ characteristics manifested by extreme tolerance to high salinity. To explore proteome changes, proteins from control and NaCl treated A. thaliana and T. halophila leaf samples were extracted and separated by two-dimensional gel electrophoresis. Differentially expressed proteins were identified by LC Q-Trap MS/MS and Mascot database searching. As expected, most of the identified proteins were involved in ion transport, stress response, photosynthesis, and energy metabolism in A. thaliana and T. halophila. With the use of iTRAQ tagging and two-dimensional liquid chromatography mass spectrometry, it was possible to identify differentially expressed membrane proteins involved in salinity responses in A. thaliana and T. halophila.

65. C3′H mutant expression study in maize

Parker J1, Vermerris W2,*

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

Lignin is a hydrophobic plant cell wall polymer that provides structural integrity to the plant but also reduces the efficiency of agro-industrial processing. Use of knockout alleles to examine the synthesis of lignin has provided insights in the relationship between cell wall chemical composition and function. The C3′H-118::Mu allele in maize represents an insertion event in the p-coumaroyl shikimate/quinate 3′ hydroxylase (ZmC3′H1) gene (kindly provided by Pioneer Hi-Bred). A preliminary analysis of lignin content in the mutant indicates a strong reduction in guaiacyl (G) and syringyl (S) residues and an increase in p-hydroxyphenyl (H) residues. The fact that some residual G- and S- residues are present suggests that maize has a second C3′H gene. A BLAST search of the maize genome sequence indeed revealed the presence of ZmC3′H2. The alignment of the two deduced amino acid sequences reveals a high degree of similarity. RT-PCR will be performed to study gene expression. We predict that the expression of ZmC3′H2 will be higher in the mutant than in the wild type, reflecting a compensation for the defective ZmC3′H1 gene. Cell wall compositional analyses will be performed to study overall effects of the ZmC3′H1 knockout. By studying ZmC3′H1 and ZmC3′H2 expression and effects on lignin and cellulose in maize, we will not only further enhance the knowledge of lignin biosynthesis, but discover other ways to further engineer maize to create more viable energy sources.

66. PRMT1-mediated dimethyl H4R3 cross-talks with H3K4 methylations

Patel B1, Li X1, Huang S1,2,*

1Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL
2University of Florida Shands Cancer Center, Gainesville, FL

Covalent modifications of histones regulate a number of processes essential for normal cellular functions, including gene transcription. Whereas, each of these modifications has a specific function, how they are communicated and cross-regulated remains unclear. Previous work demonstrated that asymmetric dimethylation of H4R3 residues by protein arginine methyltransferase PRMT1 potentiates histone acetylation and is essential both in vitro and in vivo for the establishment or maintenance of the active histone acetylation patterns. We report here that PRMT1-mediated dimethyl H4R3 cross-talks with histone PTMs dictates a specific biological outcome. SET1 in maintaining active chromatin configuration will provide insight into how the combinatorial regulation of histone PTMs dictates a specific biological outcome.

67. FoxA1 and FoxA2 regulate the Hedgehog pathway and are required for division of the embryonic cloaca in mice

Patterson SE1, Seifert AW1, Gray S1, Cohn MJ1,2,3,*

1Department of Biology, University of Florida, Gainesville, FL
2Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
3Howard Hughes Medical Institute, University of Florida, Gainesville, FL

Abnormal development of the anogenital system underlies several congenital birth defects. These include hypospadias,
a common malformation characterized by ectopic or supernumerary urethral openings, and persistent cloaca, in which there is a single opening of the urinary and alimentary canals. Despite this, the genetic mechanisms of anogenital development remain poorly understood. Using tissue-specific transcriptional profiling and in situs hybridization, we identified two Forkhead box genes, FoxA1 and FoxA2, expressed in epithelial cells of the developing urethra and cloaca. To determine the roles of these genes, we examined the effects of inactivating FoxA1 and FoxA2 in the mouse anogenital system. Removal of either FoxA1 or FoxA2 does not disrupt anogenital development, however deletion of both genes results in persistent cloaca, suggesting that FoxA1 and FoxA2 regulate cloacal septation. In addition, expression of Sonic Hedgehog (Shh) pathway members, crucial for external genital development, is reduced in mice deficient for FoxA1 and FoxA2. This suggests that FoxA1 and FoxA2 are upstream regulators of the Shh pathway and are required for normal development of the anogenital system.

68. Noise in the quorum-sensing system of the Vibrio fischeri marine bacterium

Pérez PD, Young J, Johnson E, Hagen SJ

Department of Physics, University of Florida, Gainesville, FL

Vibrio fischeri is a luminescent marine bacterium that naturally colonizes several marine macroscopic life forms. Their luminescence is controlled by the LuxIR quorum-sensing (QS) mechanism, which has an architecture similar to those of many other proteobacteria including pathogens to humans. The objective of our research is to see how noise in the gene regulatory system affects the QS communication. QS in V. fischeri has been extensively studied in bulk cultures, but little is known about how it is affected by gene noise, or the intercell variability in gene expression. We are measuring the light output of individual cells in time and under different conditions. We are using dark field microscopy imaging to find and focus the cells, and an intensified CCD (iCCD) to collect the bioluminescence light of individual cells (or small clusters) in time. By acquiring single cell luminescence data, we observe individual cell signals vs. time, histogram distributions of small groups of cells and clusters, inhibition by rich growth media, and intercell variations under different QS autoinducer concentrations. The analysis of the individual cell luminescence signal and its noise can eventually lead to a better understanding of how stochastic noise affects QS.

69. Cloning and characterization of somatic embryogenesis receptor kinase1 (SERK1) gene from sugarcane (Saccharum spp. hybrid)

Petefish MR, Jain M, Izquierdo A, Gallo M

1Agronomy Department, University of Florida, Gainesville, FL

2Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

Somatic Embryogenesis Receptor Kinase1 (SERK1) is an LRR-RLK proposed to output a signal transduction cascade implicated in early embryonic development, somatic embryogenesis, reproductive development and immune responses. Full-length cDNA and genomic clones (1869 and 4752 bp, GenBank acc. nos. GQ283907 and GQ457454, respectively) encoding a putative SERK1 were isolated from an embryogenic cell culture line of sugarcane (Saccharum spp. hybrid cv. CP88-1762). The ScSERK1 gene shares a highly conserved exon/intron structure with other members of the SERK gene family. The deduced ScSERK1 amino acid sequence is 622 residues with a predicted mass of 68.53 kDa, and a 25 residue signal peptide for secretory pathway targeting. The mature peptide contains all the hallmark features of LRR-RLKs, namely, a leucine zipper, five LRR branches of the archaeal phylogenetic tree, and in recent years the biosynthetic steps through the formation of the precursor base preQ0, and its incorporation into tRNA have been elucidated; however, the enzyme (or enzymes) responsible for the conversion of preQ0-tRNA to archaeosine-tRNA have remained elusive. Comparative genomic analysis revealed that most archaeal genomes sequenced to date contain two gene families annotated as tgt, tgtA1 and tgtA2, and that these genes cluster together in a number of genomes. TgtA1 encodes the experimentally characterized TGT enzyme, which catalyzes the insertion of the precursor preQ0 into tRNA. Archaea kingdom is divided mainly into two main phyla Euryarchaea and Crenoarchaeaa.

70. Identification of the enzymes involved in the last steps of archaeosine synthesis


1Department of Microbiology and Cell Science, University of Florida, Gainesville, FL
2Department of Chemistry, Portland State University, Portland, OR
3Department of Basic Medical Sciences, Western University of Health Sciences, Pomona, CA

Guanosines at position 15 of most archaeal tRNAs are modified to the 7-deazaguanosine derivative archaeosine (G*). The archaeosine modification has been found in all branches of the archaeal phylogenetic tree, and in recent years the biosynthetic steps through the formation of the precursor base preQ0, and its incorporation into tRNA have been elucidated; however, the enzyme (or enzymes) responsible for the conversion of preQ0-tRNA to archaeosine-tRNA have remained elusive. Comparative genomic analysis revealed that most archaeal genomes sequenced to date contain two gene families annotated as tgt, tgtA1 and tgtA2, and that these genes cluster together in a number of genomes. TgtA1 encodes the experimentally characterized TGT enzyme, which catalyzes the insertion of the precursor preQ0 into tRNA. Archaea kingdom is divided mainly into two main phyla Euryarchaea and Crenoarchaeaa.
While Euryarchaeae contains homologs of tgtA2, Crenoarchaea does not. Additional comparative genomic analysis revealed that these organisms possess either a QueC enzyme fused to a glutamine amidotransferase type II protein domain (GAT-QueC) or a QueF like enzyme, suggesting that G^+ might be synthesized before being inserted into tRNA. Genetic and biochemical studies were conducted to test the hypothesis that TGTA2 catalyzes a late step in archaeosine biosynthesis in euryarchaeal organisms whereas GAT-QueC and QueF catalyzes the last step in archaeosine formation in crenoarchaeal organisms.

71. Insights into the evolutionary history of singing mice, genus Scotinomys

Pino JL, Campbell P, Pasch B, Reed D, Phelps SM*

1Department of Biology, University of Florida, Gainesville, FL
2Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ
3Mammalogy, Florida Museum of Natural History, University of Florida, Gainesville, FL

The genus Scotinomys is restricted to elevations >1000m in Mesoamerica. Two species, Scotinomys teguina and S. xerampelinus, are recognized since last revision of the genus, 35 years ago. According to the literature, S. teguina is distributed from southern Mexico to western Panama with a major population disjunction due to the Nicaraguan lowlands-depression and S. xerampelinus occurs only south of this depression; distribution of both species ends in western Panama where they segregate elevationally (S. xerampelinus >2100m). This research examined the relationships of populations of both species throughout their distributional range by analyzing mitochondrial/nuclear DNA sequences. Samples were provided by museum and field collections. Our analyses confirm haplotypes of what was thought to be S. teguina in populations to the north and south of the Nicaraguan lake, showing geographic structure among populations. Results suggest an interesting demographic history with evidence of a bottleneck in the southernmost populations of S. teguina and a recent expansion in northern populations. Haplotype of former S. xerampelinus (Costa Rica-Panama) were present in populations to the north of Lake Nicaragua, suggesting the possible presence of disjunct populations that would greatly expand the previously suggested distributional range. The mtDNA tree generated suggests other species level relationships, but more nuclear data must be added to the existing dataset to test this prediction thoroughly.

72. Effects of sigma virus on female fecundity of Drosophila melanogaster

Regan KL, Wayne ML*

Department of Biology, University of Florida, Gainesville, FL

Effects of sigma virus on female fecundity of Drosophila melanogaster indicate that these viruses can affect reproduction. In this study, four replicates of each line for a total of 32 vials of flies for each of the four assays. We found that the infected flies produced fewer eggs than the uninfected flies. However, both groups had the same percent of fully emerged adult offspring in the egg viability experiment. Development time also did not differ between infected and uninfected flies.

73. In search of genetic determinants of a bioenergy sorghum ideotype

Saballos A, Caicedo H, Vermerris W*

Agronomy Department, University of Florida, Gainesville, FL

Sorghum is a promising source of biomass that can be produced as a multi-purpose crop. Sorghum can supply food, feed, fodder, energy and feedstocks for novel applications even in harsh conditions such as hot and dry climates, poor soils, and limited inputs. We are working towards the improvement of sorghum as a bioenergy crop by exploiting the rich genetic diversity of the species, including sweet and grain sorghums and high biomass sorghums with altered lignin composition. Genomic and genetic approaches are being used to study of the basis of sugar accumulation and its relationship with grain and biomass production, the resistance to drought stress via the study of the root system, and the effects of modifications in the lignin biosynthesis pathway. We have recently identified two genes underlying two of the brown midrib (bmr) lignin mutants. Mutations in these genes result in increased saccharification of the plants stover, an important advantage for ethanol production. The chemical characteristics of lignins from additional bmr mutants are being investigated for their potential utilization in the production of novel polymers. The combination of QTL identification, mutant characterization and gene expression studies will ultimately allow us to identify genes underlying traits for successful bioenergy production. This knowledge will facilitate the creation of lines with high yield and good bioprocessing characteristics, adapted to the stresses of their area of production.

74. The ER stress transcription factor XBP1s protects against amyloid-β neurotoxicity
Sanchez-Garcia J1, Casas-Tinto S2, Zhang Y2, Rincon-Limas DE2, Fernandez-Funez P1,*

1Department of Neurology, University of Florida, Gainesville, FL
2Department of Neurology, University of Texas Medical Branch, Galveston, TX

Background: Alzheimer’s disease (AD) is a neurodegenerative brain disorder for which there is no cure. The most prominent pathologic hallmark in the AD brain is the abnormal accumulation of the amyloid beta1-42 (Aβ) peptide, but the exact pathways mediating Aβ neurotoxicity are virtually unknown. For instance, ER stress is activated in AD; however, mostly indirect evidence suggests that ER stress plays a role in Aβ pathogenesis. Objectives: To examine the role of the ER stress in the Aβ neurotoxicity and to understand the mechanisms of the protective activity. Methods: We used transgenic flies expressing Aβ to characterize XBP1 and confirmed the results in human neuroblastoma treated with Aβ oligomers. Results: We report that Aβ activates the ER stress response factor X-box binding protein 1 (XBP1) in transgenic flies and in human neuroblastoma, yielding its active form, the transcription factor XBP1s. Remarkably, XBP1s is neuroprotective in flies expressing Aβ and in human neuroblastoma treated with Aβ oligomers. We also demonstrate that XBP1s prevents the accumulation of free Calcium in the cytosol, thus explaining its protective activity. XBP1s seems to regulate intraluminal Ca release by downregulating the expression of Ryanodine receptors that are elevated by Aβ. Conclusions: Together, these results highlight the functional relevance of XBP1s in the ER stress pathways triggered by Aβ, and uncover the potential of XBP1s as a therapeutic target for AD.

75. Site-specific recombination to improve transgenic medfly strains

Schetelig ME1, Scolari F2, Handler AM1,*, Kittelmann S3, Gasperi G2, Wimmer EA3

1Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL
2Dipartimento di Biologia Animale, Università di Pavia, Pavia, Italy
3Department of Developmental Biology, Georg-August-University Göttingen, Göttingen, Germany

The Sterile Insect Technique (SIT) is an environmentally friendly bio-control method used in area-wide pest management of the Mediterranean fruit fly (medfly) Ceratitis capitata (Wiedemann). Recently, we generated new transgenic strains to improve reproductive sterility and species-specific marking of medfly. All the DNA constructs, which were used to generate these strains, carried an attachment P site (attp) - a short DNA sequence for site-specific recombination. The efficiency of the reproductive sterility and marking systems and the fitness of the transgenic flies were highly influenced by position effects of the transgenes. Strains that were successfully tested and evaluated as “beneficial” for the functionality of the transgenic system were then studied for further improvements by using site-specific recombination via their attp site. A two-step modification was tested in these transgenic medfly strains: 1) the combination of two transgenic systems at a positively evaluated genomic position and 2) the increase of transgene stability by the deletion of transposon ends with newly developed medfly jumpstarter strains. Such new possibilities in modifying medfly transgenes will enhance the development of new and safe applications for SIT programs as well as functional studies in Tephritid species.

76. Evolving spiking neural networks for the prediction of transcription factor binding sites

Sichtig H, Riva A*

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

Our understanding of complex biological adaptive systems, from the cellular to the molecular level, can be used to develop valuable computational tools for interdisciplinary research in bioinformatics and biomedical engineering. We propose the use of spiking neural networks, able to realistically model the neurological system, to address challenging problems in computational biology, and of artificial evolutionary processes, such as genetic algorithms, to tune the network parameters. These tools can be extremely useful for interdisciplinary research because of their generality and their applicability to any complex system of interest. We will present work in progress using artificial spiking neurons applied to the well-known problem of predicting transcription factor binding sites (TFBSs) in DNA sequences. The system is trained using real TFBS data from the TRANSFAC database, and uses a top-down modeling approach to simulate biological information processing based on neurological coding. The goal of our work is to reduce the number of false positives in the predicted TFBSs through a more accurate modeling of the information contained in the alignments in the training data. We will present an evaluation of our system’s performance for the detection of TFBSs and compare it to alternative methods.

77. Genetic characterization of the murine Angelman syndrome imprinting center


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

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurological disorders resulting from improper gene expression from the imprinted domain on chromosome 15q11-q13, the PWS/AS locus. This locus is controlled by a
bipartite imprinting center consisting of the PWS-IC and the AS-IC. The most widely accepted model of IC function proposes that the PWS-IC promotes gene expression from the paternal allele while the AS-IC acts to epigenetically silence the PWS-IC on the maternal allele, thus silencing the paternally expressed genes. The PWS/AS locus is well conserved from human to mouse but a murine AS-IC remains uncharacterized. As in humans, the mouse paternally expressed genes. The PWS/AS locus is well silenced the PWS-IC on the maternal allele, thus silencing the paternal allele while the AS-IC acts to epigenetically silence the maternal allele. We have taken a transgenic approach to study the potential regulatory role of these alternative exons. To do so, we utilized a bacterial artificial chromosome (BAC) containing Snrpn and three alternative upstream exons. This BAC transgene displayed proper imprinted expression and epigenetic imprinting marks at the Snrpn DMR, thus demonstrating the presence of a functional AS-IC. Upon deletion of the three upstream exons, Snrpn was expressed after both maternal and paternal transmission of the transgene and there was a loss of the epigenetic imprint at the Snrpn DMR. Thus, through our innovative transgenic system, we have identified a functional murine AS-IC contained within the Snrpn upstream exons.

78. Using historical museum specimens to reconstruct the evolutionary history of the southeastern pocket gopher (Geomysinae)

Barlow L1,2, Reed DL1,*, Allen JM1, Soto-Centeno JA1

1Mammalogy, Florida Museum of Natural History, University of Florida, Gainesville, FL
2Department of Biology, Florida State University, Tallahassee, FL

The evolutionary history and taxonomy of the southeastern pocket gopher (Geomys pinetis) has been notoriously elusive. We collected mitochondrial cytchrome-b sequence data from modern and historical museum specimens to determine whether G. pinetis subspecies occurring west of the Apalachicola River warrants species status relative to the eastern subspecies, whether the Apalachicola-Chattahoochee-Flint River drainage serves as a geographic barrier, and whether rare subspecies of conservation concern are genetically distinct from widespread subspecies of G. pinetis. Maximum-likelihood and Bayesian analyses indicate two major taxonomic units within G. pinetis (average uncorrected sequence divergence = 8.5%), possibly separated by the Apalachicola-Flint Rivers as also evidenced by ecological niche models. Using cyt-b sequences from additional pocket gophers and a fossil calibration point, we estimate divergence between the eastern and western clades of G. pinetis at roughly 1.5 million years ago. This is consistent with the age of the Apalachicola drainage system and suggests that these taxa have remained isolated for a considerable time, and certainly since the last glacial maximum. This study points out the need for revision of the taxonomy and distribution of G. pinetis, suggests that geology, climate, and habitat structure in the southeastern U.S. have influenced this taxon, and highlights the utility of museum specimens for investigating rare or extinct populations.

79. Construction of an RNAi-based mouse model of Barth syndrome

Soustek M1, Partono S2, Lewin A 2,*, Byrne B1,*

1Department of Pediatrics, University of Florida, Gainesville, FL
2Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Barth syndrome (BTHS) is an X-linked mitochondrial disease that is associated with mutations within the Tafazzin (TAZ) gene. BTHS is characterized by dilated cardiomyopathy, neutropenia, muscle weakness, and growth retardation. Studies of BTHS pathogenesis and development of therapies have been hindered due to the lack of a mammalian model for the disease. Here we report progress in the beginning stages of creating a tissue specific TAZ knockdown model in mouse using RNA interference (RNAi) techniques. We have screened several siRNAs that effectively knockdown exogenous mouse Taz target in tissue cultures. In addition, we have created a retroviral-siRNA construct that resulted in knockdown of endogenous Taz in mouse NIH 3T3 cells. Furthermore, we have packaged one of the siRNAs (siRNA9) into an AAV9 serotype vector, and have tested the infection and knockdown efficiency of this viral vector in mouse cardiac tissue. Preliminary data did not demonstrate knockdown of Taz mRNA, although high infection rate was observed. Our data suggest that in future research TAZ-specific siRNAs can be delivered as transgenes to establish a systemic model of Barth syndrome.

80. Transcriptomics of queen conch (Strombus gigas) testis in the Florida Keys: possible role of metals in reproductive failure

Spade DJ1, Feswick A1, Glazer RA2, Barber DS1, Denslow ND1,7

1Department of Physiological Sciences, University of Florida, Gainesville, FL
2Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Marathon, FL

Queen conchs (Strombus gigas) in the near-shore Florida Keys fail to reproduce, while offshore conchs reproduce successfully. While gonad development is hindered near-shore, the responsible stressors and their modes of action are unknown. Using a custom queen conch microarray, we observed differences in gene expression of near-shore versus offshore conch gonads. 257 transcripts were differentially regulated (ANOVA, p<0.01, FDR=5%). Gene Ontology (GO) term enrichment analysis (Fisher’s exact test, p<0.05) found significant enrichment of the terms “spermatogenesis”, “proton transport”, and “mitochondrial
transport”, with the majority of these genes down-regulated near-shore, corresponding with the observed decrease in testis development near-shore. Additionally, small GTPase-mediated signal transduction was induced, with most genes being down-regulated. This disruption indicates a possible role of Ras-related signaling molecules in conch testis development. Inductively-coupled plasma mass-spectrometry (ICP-MS) analysis indicates that tissue burdens of Cu, Zn, and Sn may be elevated in near-shore conch tissues, in particular Cu in the gonads and Zn and Sn in the digestive gland. Therefore, metal accumulation in near-shore conch tissues may have a role in the observed reproductive failure. Understanding the factors contributing to reproductive failure of near-shore conchs could aid in management efforts toward re-establishing a healthy queen conch population in the Florida Keys.

81. Using biomarkers to predict successful versus unsuccessful aging in rats

Speisman RB1, Kumar A2,3, Foster TC2,3, Ormerod BK1,*

1J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL
2Evelyn F. and William L. McKnight Brain Institute, University of Florida, Gainesville, FL
3Department of Neuroscience, University of Florida, Gainesville, FL

Chronological age does not predict cognitive success across senescence, which can vary from “successful” with minimum impairment to “unsuccessful” with significant impairment despite no identifiable pathology. Senescent rats can be characterized as memory unimpaired (MU) and memory impaired (MI) using the spatial water maze (SWM) and inhibitory avoidance tasks (IAT), which exhibit a concordance in their sensitivity to age-related memory impairments. Interestingly, senescent rats categorized as MI begin to show impaired cognition in middle age. We use this observation to investigate whether either hypothalamic-pituitary-adrenal axis or inflammatory biomarkers for “unsuccessful aging” emerge in middle age. To test this hypothesis young (8 mo), middle aged (14 mo), and aged (20 mo) male Fischer 344 rats were tested on the SWM and IAT, where some animals received mild foot shock. Analytes in blood serum and brain tissue samples were quantified using a multiplex ELISA strategy. Profiles were created for each analyte to diagram the natural influence of age. Multiple pro-inflammatory and recruitment/trafficking cytokines showed significant correlation with memory impairment in the cortex and hippocampus suggesting a possible mechanism relating age and cognition. A significant difference was also noted in middle-aged MU and MI serum levels of MIP-1α and GRO-KC (p=0.0163 and 0.0333 respectively) which may be possible biomarkers for predicting old-age memory impairment in middle-aged animals.

82. Dosage-dependent genes affecting seed composition or weight

Spielbauer G1,2, Armstrong P3, Baier J1,2, Richardson K6, Grijalba D1,2, Griffin M1,2, Kosick K1,2, Song B5, Kahveci T5,*, Settles AM1,2,*

1Horticultural Sciences Department, University of Florida, Gainesville, FL
2Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
3Grain Marketing and Production Research Center, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, KS
4Department of Biology, Florida Agricultural and Mechanical University, Tallahassee, FL
5Department of Computer and Information Science and Engineering, University of Florida, Gainesville, FL

Kernel composition is an important target for developing improved grain for food, feeds, and various industrial processes. In addition, genes affecting individual seed weight may have an impact on grain yield. Our goal is to identify maize mutants that have significant effects on the chemical composition or weight of seeds. We are screening the UniformMu transposon-tagging population using single kernel near-infrared spectroscopy (NIR) and seed weights. NIR spectroscopy determines chemical composition and seed weight phenotypes of individual maize kernels non-destructively and at high-throughput. We built an automated single-kernel NIR grain analyzer and developed partial least square (PLS) calibration models to predict the individual seed starch, protein, oil, and weight. We have developed a seed spectra and weight database as well as novel statistical tools to identify maize ears segregating for differences. We are focusing on phenotypes that show dosage-dependent or parent-of-origin changes to the kernels based on the hypothesis that these genes are the best targets for modifying the seed with transgenes. Finally, we have developed 454 sequencing protocols to identify the transposon insertion sites in UniformMu mutants. We are screening these insertion sites for linkage to dosage-effect mutants.

83. Spontaneous network activity of fetal and adult cortical cells is altered by the addition of adult neural progenitor cells

Stephens CL, DeMarse TB, Ormerod BK*

J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL

Research has targeted neural progenitor cell (NPC) transplantation as a promising treatment for neurodegenerative disease. Although new neurons can potentially integrate into adult neural circuits as they do in the mammalian hippocampus, we do not fully understand how their transplant would affect brain function and in turn
how local cell activity would influence NPC fate. Employing microelectrode arrays (MEAs) we tackled these questions in a biologically relevant in vitro model of NPC transplant onto fetal or adult cortical cells. The MEA can detect and record extracellular potentials long term to observe spontaneous network activity, a well-characterized phenomenon. Action potentials appear within days of cell plating and network bursts begin in 7-10d. Bursts gradually change pattern, becoming modulated at 20-30d with rates of 0.78±0.08Hz intermittent with 37.5±6.35s of quiescence known as an “immature” state. Then at 35-40d the bursts “mature” into a steady pattern (0.25±0.04Hz) that remains stable (>90d). We demonstrate that a mature neural population reverts its burst pattern to an “immature” state about 14d after NPC addition with bursts at 0.55±0.04Hz and quiescent periods of 24.0±4.64s. Concurrently, NPCs express neural or glial markers at 14d. These data suggest that NPC maturation alters the existing activity and therefore could alter brain function.

84. The a-maize-ing mini-me: an evolutionary switch that led to the divergence of cane and bunch grasses and potential tool in crop modification?

Koch KE1,2,* , McCarty DR1,2,* , Vermerris W1,3,* , Tan S1

1Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
2Horticultural Sciences Department, University of Florida, Gainesville, FL
3Agronomy Department, University of Florida, Gainesville, FL

A better understanding of the mechanisms controlling plant growth and development can ultimately be used to increase crop yield, enable adaptation to variable environments, and allow novel uses for existing crops. The mini-me mutant of maize (Zea mays L.) is a dwarf mutant, that, unlike any other known maize dwarf mutants, forms many basal branches and ears (female reproductive organ). The mini-me mutant hence resembles a bunch grass. We hypothesize that the Mini-me gene represents a key switch that led to the evolutionary divergence of cane grasses (maize, sorghum, sugar cane, bamboo, etc.) and bunch grasses (rye grass, fescue grass, bahia grass, etc.). The mutant was identified in the UniformMu population and is likely the result of a Mutator transposon insertion. Using the PCR-based MuTail method, we have identified the APETALA2 (AP2) transcription factor gene as a candidate gene for Mini-me. The AP2 transcription factor is unique to plants and plays a major role in regulating plant growth, such as formation of floral organs, regulating epidermal cell differentiation, and response to environmental stress. Once cloned and characterized, the Mini-me gene may be used as a tool for crop modification, leading to the development of grasses that are storm resistant, have improved biomass yields, and are able to tolerate stressful environments.

85. Development of a computer pipeline in analyzing transcriptome sequencing (RNA-seq) data

Tang S, Riva A*

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

Next generation transcriptome sequencing (RNA-seq) has significantly impacted biomedical and bioinformatics researches by producing high resolution poly(A) transcriptome sequencing (Shendure, 2008, Nat Methods 5(7):585-7). It also has gained increased popularity because it addresses many shortcomings in microarray technology, such as prior knowledge dependency and problems with hybridization (Mortazavi et al., 2008, Nat Methods 5(7):621-8; Shendure, 2008, Nat Methods 5(7):585-7; Sultan et al., 2008, Science 321(5891):956-60). Furthermore, RNA-seq will enable us to identify novel transcripts and alternative splice variants. Therefore, the application of this new technology will shed light on complex disease study. Currently, there are very few computer pipelines that systematically analyze RNA-seq data (Deneou et al., 2008, Genome Biol 9(12):R175; Trapnell et al., 2009, Bioinformatics 25(9):1105-11). In our study, we are interested in developing a computer pipeline to assemble RNA-seq data. The development of this pipeline will automate RNA-seq data analysis ranging from identification of splice junctions to construction of global alternative splicing variants. The application of this computer pipeline to alternative splicing will facilitate more in depth research of complex diseases for the biomedical and/or bioinformatics scientists.

86. Regeneration response of sugarcane leaf-roll explants to different growth regulators

Taparia Y, Fouad WM, Gallo M*, Altpeter F*

Agronomy Department, University of Florida, Gainesville, FL

In vitro culture plays a crucial role in the conservation, creation and utilization of genetic variability of sugarcane, including cryopreservation, in vitro selection, genetic engineering and commercial mass production of disease-free sugarcane. Young meristematic tissues such as immature leaf, immature inflorescence or basal shoot meristems are required in sugarcane to induce regenerable tissue cultures. In this study, the culture response of sugarcane leaf-roll cross sections from the commercially important sugarcane cultivar CP-88-1762 was examined on media differing in auxin type and cytokinin concentration. Auxins 1-naphthalenacetic acid (NAA; 10 µM); 2,4-dichlorophenoxyacetic acid (2,4-D; 22.6 µM), 4-amino-3,5,6-trichlorophyridine-2-carboxylic acid (Picolram; 40 µM), 4-chlorophenyx acetic acid (CPA; 10 µM) plus naphthalenacetic acid (NAA; 10 µM) were evaluated alone or in combination with the cytokinin 6-benzylaminopurine (6-BAP) at 0.4 or 4.0 µM in a 3x4 factorial design with 10
replications. Differences in callus induction, callus morphology, necrosis and regeneration pathways were observed. Data on the quantity and quality of callus and the plant regeneration efficiency of the explants on the different media will be reported.

87. Is there a deeply conserved genetic program for cartilage development? Fibrillar collagens in the cuttlefish Sepia pharaonis

Tarazona OA1, Cohn MJ2,3,*

1Department of Biology, University of Florida, Gainesville, FL
2Howard Hughes Medical Institute, University of Florida, Gainesville, FL
3Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Collagen-based cartilage was long considered to be unique to jawed vertebrates, but recent work has revealed this to be a shared character of the vertebrate crown group. Phylogenetically fibrillar collagens (FCs) form three major clades (A, B and C). Clade A FCs is the most abundant and 1 Kb and 1.29 Kb (SphColAa and SphColAb) correspond to the C-propeptide and part of the triple helix domain. Bayesian phylogenetic analysis places them within the clade A FCs. We examined their expression patterns in cephalopod embryos and find that they are expressed during cartilage development. The results raise the possibility of a deeply conserved genetic program for chondrogenesis in the bilateria.

88. 5-HTTLPR genotype and drinking in bar patrons

Thombs DL1,*, O’Mara R3, Hou W3, Wagenaar AC3, Dong HJ3, Merves ML4, Goldberger BA4, Weiler RM2

1Department of Behavioral Science and Community Health, University of Florida, Gainesville, FL
2Department of Health Education and Behavior, University of Florida, Gainesville, FL
3Department of Epidemiology and Health Policy Research, University of Florida, Gainesville, FL
4Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL

The serotonin transporter promoter polymorphism (5-HTTLPR) has been linked to a number of human behavioral traits and disorders. The variants of 5-HTTLPR are commonly reported in three forms, L/L, S/L, and S/S, with the latter most often associated with emotional distress and/or behavioral dysfunction. No previous research has examined event-level associations between 5-HTTLPR and risk behavior in natural drinking settings. This study reports associations between 5-HTTLPR, alcohol intoxication, and intention to drive among patrons exiting on-premise drinking establishments. Self-report measures, breath alcohol concentration (BrAC) readings, and oral fluid samples for DNA analysis were collected at night. Multivariate analyses were performed on 225 patrons likely to be near their peak intoxication level for the night. 5-HTTLPR genotype was associated with exitig patron BrAC after adjusting for potential confounders. An interaction effect involving 5-HTTLPR and drink specials had an independent association with BrAC, suggesting that selection of price-discounted drinks increased intoxication in patrons with an L allele. In addition, patrons with the S/S genotype were 3 times more likely to intend to drive a motor vehicle (after drinking on the night of study participation) compared to those with the L/L genotype. The 5-HTTLPR genotype may play an important role in the etiology of problems associated with on-premise drinking establishments.

89. Highly-stable extracellular archaeal glyco-laccase from Haloferax volcanii

Uthandi S, Maupin-Furlow JA*

Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

Laccases couple the oxidation of phenolic compounds to the reduction of molecular oxygen and, thus, span a variety of applications. While laccases of eukaryotes and bacteria are well characterized, these enzymes have not been described in archaea. Here we report the identification and characterization of a laccase (LccA) from the halophilic archaeon Haloferax volcanii. H. volcanii was engineered to over-produce LccA into the milieu of cells grown to high density with a peak of activity (2.48 U•ml⁻¹) at 2.9 × 10⁹ CFU per ml. LccA was readily purified from culture broth to electrophoretic homogeneity with an overall yield of up to 96% and specific activity as high as 59.27 U•mg⁻¹. The enzyme purified as a 65.4-kDa monomer with an absorbance spectrum typical of blue multicopper oxidases. The mature LccA was glycosylated to 6.9 % carbohydrate content and oxidized a variety of organic substrates including bilirubin, syringaldazine (SGZ), 2,2',-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and dimethoxyphenol (DMP). Optimal oxidation of ABTS and SGZ was at 45 ºC and pH 6 and pH 8.4, respectively. The apparent Km values for SGZ, bilirubin and ABTS were 35, 236 and 670 µM with corresponding kcat values of 22, 29 and 10 s⁻¹, respectively. The ability of haloarchaeon to thrive
in environments of unusually low water activity (high salt, solvent and/or desiccation) has made these organisms and their enzymes ideal candidates for biotechnology advancements.

90. Altering lignin content in sugarcane by RNAi suppression of 4-coumarate-CoA ligase

Xiong Y, Steeves C, Fouad WM, Sandhu S, Gallo M¹, Vermerris W¹, Altpeter F²

Agronomy Department, University of Florida, Gainesville, FL

Sugarcane (Saccharum sp. hybrids) is a highly productive C4 grass used as the main source of sugar and more recently to produce ethanol, a renewable transportation fuel. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning. Fuel grade ethanol can be made from sugarcane leaf litter residue. However, a major constraint for economic ethanol production from hemicellulosic sugarcane residues is lignin which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. Therefore, the objective of this study is to reduce lignin content in sugarcane by altering 4-coumarate-CoA ligase (4CL), a key enzyme in lignin biosynthesis. Two 4CL partial sequences were isolated from the genome of sugarcane. Two RNAi constructs targeting a conserved region in the two genes were constructed using 200 bp from each of the two genes. One or both Sc4CL-RNAi constructs, under the control of the xylem specific OsC4H promoter, were introduced into sugarcane callus along with a selectable nptII expression cassette by biolistic gene transfer. Following selection on medium containing genetin and regeneration, 49 independent transgenic lines were generated. The transgenic nature of these lines was confirmed using NPTII ELISA analysis. Data describing the level of 4CL suppression will be presented.

91. Epigenetic regulation of an enhancer region controls stress-induced apoptosis in Drosophila

Zhang Z, Wang H, Zhou L¹

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

Epigenetic regulation plays an important role in stem cell maintenance, cellular differentiation, and the aging process. Our lab recently demonstrated that during Drosophila embryogenesis, the cellular response to ionizing radiation (IR)-induced apoptosis is also under the control of epigenetic regulation of an irradiation responsive enhancer region (IRER). Embryonic cells before stage 11 contain an open IRER and are extremely sensitive to DNA damage-induced cell death. However, chromatin in IRER form a heterochromatin structure after stage 12 and consequently cells lose their sensitivity to stress-induced apoptosis. The goal for this study is to examine the functional significance of IRER during development and stress response. Mosaic clones deficient for IRER (IRER/-/-) contain more cells than simultaneously generated twin spots (IRER+/+) in the imaginal discs, and have reduced level of cell death upon IR. These results indicate that loss of function of IRER reduces cellular sensitivity to stress-induced cell death and promotes cell survival. A newly generated transgenic fly carrying an ubiquitin-DsRed reporter within IRER allows us to monitor the accessibility of IRER in the individual cell. Following 40 Gy IR, more cells in the imaginal discs exhibit increased DsRed signal, suggesting an epigenetic response of IRER to stress. Our data indicated that epigenetic regulation plays a significant role in determining the cellular sensitivity to stress induced cell death.

92. Genetic basis of sexual dimorphism in external genitalia and brain development

Zheng Z¹, Evans K¹, Cohn MJ¹,²,³,*

¹Department of Biology, University of Florida, Gainesville, FL
²Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
³Howard Hughes Medical Institute, University of Florida, Gainesville, FL

Sexual dimorphism is the difference in structure or physical characteristics between males and females of the same species. Dimorphisms are particularly pronounced in areas regulated by endocrine signaling, including differentiation of gonads, genitalia, breasts, muscle mass, hair pattern, and brain regionalization. Male and female external genitalia develop from the same embryonic precursor, the genital tubercle, starting at E10.5 in mice. The tubercle is then masculinized to form the penis or feminized to form the clitoris. Sexual differentiation of the brain has been proposed to occur around this same period, causing dimorphism in specific brain nuclei and total size. Androgens have been proposed to drive differentiation of the genitalia and brain, but little is known about the genetic targets of androgen signaling during organogenesis. To address this question, we produced spatial, temporal and quantitative analysis of gene expression in the external genitalia and brain of mouse embryos. The bone morphogenetic proteins Bmp4 and Bmp7, and the Wnt antagonist Dkk2 are expressed at higher levels in female external genitalia and brains at E18.5. Ptc1, which is a receptor of Hedgehog, is expressed at higher levels in male external genitalia, but in female brains. Fgf receptors are also expressed in dimorphic patterns in external genitalia and brains. Our results suggest that androgens may direct sexual dimorphism of the external genitalia and brain through a common suite of genetic targets.
93. USF and NF-E2 cooperate to regulate the recruitment and activity of RNA polymerase II in the beta-globin gene locus

Zhou Z, Li X, Deng C, Huang S*, Bungert J*

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

The human beta-globin gene is expressed at high levels in erythroid cells and regulated by proximal and distal cis-acting DNA elements, including promoter, enhancer, and a locus control region (LCR). Transcription complexes are recruited not only to the globin gene promoters but also to the LCR. Previous studies have implicated transcription factors USF and NF-E2 in the recruitment of transcription complexes to the beta-globin gene locus. Here we demonstrate that while USF is required for the efficient association of RNA polymerase II (Pol II) with LCR hypersensitive site 2, USF and NF-E2 together regulate the association of Pol II with the adult beta-globin gene promoter. Recruitment of Pol II to the LCR occurs in undifferentiated cells but phosphorylation of globin gene locus associated Pol II at the C-terminal domain (CTD) is mediated by erythroid differentiation and requires the activity of NF-E2. Furthermore, we provide evidence showing that USF interacts with NF-E2 in erythroid cells. The data demonstrate that ubiquitous and tissue-restricted transcription factors collaborate to regulate the recruitment and activity of transcription complexes in a tissue-specific chromatin domain.

94. Identification of thiol-based redox regulated proteins in guard cell ABA signaling

Zhu M1, Zhu N1, Simons B2, Chen S1,3,4,*

1Department of Biology, University of Florida, Gainesville, FL
2MDS Analytical Technologies (SCIEX), Ontario, Canada
3Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

As a central control mechanism in cell metabolism, redox regulation is employed to adjust the plant antioxidant defense system to the prevailing environment. Cysteine is one of the most important amino acids with the capacity to be oxidized (e.g., to form disulfide bonds when oxidized) or to keep sulfhydryl groups in reduced states. The oxidation and reduction of sulfhydryl groups has been found to be an essential regulatory switch in a spectrum of physiological processes in plants. Previous studies suggest that ABA can induce reactive oxygen species production and potentially oxidative stress of plant cells. And a large part of ABA responsive proteins in guard cells are redox-related. However, thiol-based redox switches remain largely unknown in plant stomatal opening and closing processes. Here we employ two complementary redox proteomics methods, isotope coded affinity tag (ICAT) and saturation differential gel electrophoresis (DIGE) to identify thiol-based redox regulated proteins under ABA treatment of guard cells. Most of the proteins with disulfide bond formation or breakage in the course of the treatment belong to the energy, stress and metabolism functional groups. This analysis not only creates a most comprehensive inventory of components in the redox regulation system underlying ABA function in guard cells but also highlights some interesting candidates in the ABA signal transduction for future investigation.

95. Quantification of plant metabolites by multiple reaction monitoring mass spectrometry

Zhu N1, Pang Q1, Zhu M1, Jin X2, Assmann SM2, Chen S1,3,4,*

1Department of Biology, University of Florida, Gainesville, FL
2Biology Department, Pennsylvania State University, University Park, PA
3Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
4Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

The analysis of different plant metabolites is essential for understanding the molecular networks underlying plant functions. Because of the low abundance, high complexity and dynamics of many metabolites, accurate measurement is often challenging. We have developed a liquid chromatography multiple reaction monitoring (MRM) mass spectrometry (MS) method to capture the small molecules in plants. Plant extracts are separated by reverse phase and hydrophilic interaction chromatographic columns to enhance coverage. MRM MS on a linear ion trap mass spectrometer is used for absolute and relative quantification. With this technology, our aim is to establish a targeted plant metabolite database that includes MS spectra, MRM transitions, standard curve and quantities for each target metabolite in plants. The database has more than 400 chemicals that are divided into seventeen groups, e.g. organic acids, hormones, carbohydrates, and specialized metabolites. The establishment of this platform will enhance our capability in building molecular networks.