



FLORIDA GENETICS 2012

THE EIGHTH ANNUAL SYMPOSIUM OF THE UF GENETICS INSTITUTE

NOVEMBER 28-29

Cancer and Genetics Research Complex
University of Florida
Gainesville, FL

PLATINUM SPONSORS

Department of Molecular
Genetics and Microbiology
Interdisciplinary Center for
Biotechnology Research

GOLD SPONSORS

Center for Epigenetics
College of Liberal Arts and Sciences
Emerging Pathogens Institute
Graduate Program in Plant Molecular
and Cellular Biology
Health Science Center Libraries
UF Genetics Institute

SILVER SPONSORS

Animal Molecular and Cellular Biology
Graduate Program
Evelyn F. and William L. McKnight Brain Institute

Florida Genetics 2012 Organizing Committee

Co-Chairs: Connie Mulligan and Michele Tennant
Members: Charlie Baer, Henry Baker, John Davis, Rob Ferl,
Douglas Soltis, Thomas Yang

FG2012 Sponsors

Platinum Level

Department of Molecular Genetics and Microbiology,
Interdisciplinary Center for Biotechnology Research

Gold Level

Center for Epigenetics, College of Liberal Arts and Sciences,
Emerging Pathogens Institute, Graduate Program in Plant Molecular and Cellular Biology,
Health Science Center Libraries, University of Florida Genetics Institute

Silver Level

Animal Molecular and Cellular Biology Graduate Program,
Evelyn F. & William L. McKnight Brain Institute

Special Thanks To

Matthew Daley, Taum Hanlon, Rolando Milian, Kristyn Minkoff, Tessa Mitchell, Hope Parmeter,
Connie Philebaum, Dustin Blanton, Bret Boyd, Tamar Carter, Mathew Citarella,
Ana Carolina Costa-Sa, Lauren Douma, Justin Fear, Terry Felderhoff, Ruli Gao, Lara Ianov,
Heather Rose Kates, Hye Won Lee, Guang-Yao Li, Lana McMillan, Aida Miro, Katie
O'Shaughnessy, Marcio Resende, Justyna Resztak, Tatiana Salazar, Andrew Smith, Jared Stees,
Ming Tang, Shaojun Tang, Carl Xiao, Yuanqing Yan

University of Florida Genetics Institute

Interim Director: Henry Baker

Associate Directors: Donald McCarty and Connie Mulligan

Executive Board: William Allen, Henry Baker, Brad Barbazuk, Jorg Bungert, Sixue Chen,
John Davis, Robert Ferl, Curt Hannah, William Hauswirth, Julie Johnson, Donald McCarty,
Michael Miyamoto, Connie Mulligan, Nick Muzyczka, Douglas Soltis, Pamela Soltis,
Michele Tennant, Marta Wayne, and Thomas Yang

Scientific Advisory Board:

Rebecca W. Doerge, PhD, Professor, Departments of Agronomy and Statistics,
Purdue University, West Lafayette, IN
Yoram Groner, PhD, Barnet Berris Professor of Cancer Research, Department of Molecular
Genetics, Weizmann Institute of Science, Rehovot, Israel
Peter M. Howley, MD, George Fabyan Professor of Comparative Pathology and Chair,
Department of Pathology, Harvard Medical School, Boston, MA
Thomas J. Kelly, MD, PhD, Director, Sloan-Kettering Institute, New York, NY
Ronald L. Phillips, PhD, Regents Professor, Agronomy and Plant Genetics,
University of Minnesota, St. Paul, MN
Ron Sederoff, PhD, Distinguished University Professor of Forestry, North Carolina State University
Forest Biotechnology Group, Raleigh, NC
Thomas E. Shenk, PhD, James A. Elkins, Jr. Professor in the Life Sciences, Molecular Biology,
Princeton University, Princeton, NJ

UFGI Strategic Plan

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

2012 Florida Genetics Symposium Schedule

Florida Genetics 2012 is in honor of Kenneth I. Berns, MD, PhD, pioneer in the use of adeno-associated virus (AAV) in gene therapy.

Wednesday, November 28, 2012

11:00 a.m. – 12:00 p.m.: Check-in and poster set-up at the CGRC, posters no. 1-65

12:00 p.m. – 12:15 p.m.: Opening Remarks: **Connie Mulligan*** and **Henry Baker***

Session I

Gene Therapy
Chair: Henry Baker*

12:15 p.m. – 12:45 p.m., Nick Muzyczka*:
"Gene therapy, 1974-2012: are we there yet?"

12:45 p.m. – 1:15 p.m., Mavis Agbandje-McKenna*:
"Tackling AAV gene delivery challenges with structural virology"

1:15 p.m. – 1:30 p.m.: Coffee break

1:30 p.m. – 2:15 p.m.: **Terence Flotte:**
"Developing gene therapy for genetic emphysema: from idea to phase 2 clinical trials"

2:15 p.m. – 3:00 p.m.: **Jude Samulski:**
"Hairpins to helper virus and the impact on AAV vectors"

3:00 p.m. – 3:30 p.m.: **Michael Linden:**
"Site-specific integration by AAV: back to the future"

3:30 p.m. – 5:00 p.m.: **Poster Session I:**
Posters no. 1-65, award presentation for the **Codified Art + Genetics competition**, and **reception** (for registered attendees)

****5:30 p.m. – 6:30 p.m., David Baltimore:**
"AAV to the rescue"
(Introduction by **Bert Flanagan**)

***= UF Genetics Institute Faculty**

****All activities will be at the Cancer/Genetics Research Complex except for Dr. Baltimore's presentation, which will be at 5:30 p.m. Wednesday in the auditorium of the HPNP building on the Health Science Center campus.**

Thursday, November 29, 2012

8:30 a.m. – 9:00 a.m.: Check-in, coffee, set up posters no. 66-130

Session II

Epigenetic Modification of Phenotype
Chair: Thomas Yang*

9:00 a.m. – 9:45 a.m., Michael Skinner:
"Environmentally induced epigenetic transgenerational inheritance of reproductive phenotypes and disease: ancestral ghosts in your genome"

9:45 a.m. – 10:15 a.m., James Resnick*:
"Imprinting mechanisms underlying Prader-Willi and Angelman syndromes"

10:15 a.m. – 10:30 a.m.: Coffee break

10:30 a.m. – 11:15 a.m., Karen McGinnis:
"Epigenetic gene regulation and phenotype in maize"

11:15 a.m. – 11:45 a.m., Connie Mulligan*:
"Epigenetic alterations and stress among new mothers and infants in war-torn Democratic Republic of Congo"

11:45 a.m. – 1:30 p.m.: **Poster Session II:**
Posters no. 66-130

11:45 a.m.: Lunch (for registered attendees)

Session III

Genetic Modification of Plants
Chair: John Davis*

1:30 p.m. – 2:15 p.m., Steve Strauss:
"Transgenic forests: A funny thing happened on the way to the revolution"

2:15 p.m. – 2:45 p.m., Curt Hannah*:
"ADP-glucose pyrophosphorylase: from bacterial selection to yield in the field"

2:45 p.m. – 3:15 p.m., Harry Klee*:
"The chemistry and genetics of tomato flavor"

Presentation Abstracts

(Listed in the order of presentation, external speakers followed by internal speakers)

Developing gene therapy for genetic emphysema: from idea to phase 2 clinical trials

Flotte TR

Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA
Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA
Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA

Alpha-1 antitrypsin (AAT) deficiency is a common single gene disorder caused by mutations in the SERPINA1 gene which encodes an abundant serum antiprotease, AAT. AAT is normally synthesized predominantly in hepatocytes, and is secreted for activity in the plasma and interstitial fluid, where it protects the interstitial elastin of the lung from degradation by neutrophil elastase. AAT deficiency is remarkably homogeneous genetically, with over 95% of patients possessing at least one copy of the specific mutant allele E342K (known as the PiZ allele). Z-AAT forms stable polymers which are trapped within hepatocytes, resulting in a deficiency state in plasma, which in turn results in emphysema in the vast majority of symptomatic individuals. Studies in compound heterozygotes indicate that a plasma level of 11 micromolar or 571 mcg/ml is protective against the development of emphysema, and protein replacement products requiring weekly infusion, have been FDA approved based on achieving that level. 12 to 15% of Z homozygotes also develop liver disease, which appears to be linked to the retention of the mutant protein, although the phenotypic variation in the liver phenotype among Z homozygotes remains unexplained. Our laboratory began studies of gene therapy for AAT deficiency, initially developing a series of rAAV2-AAT vectors utilizing different promoters and routes of administration. Targeting the lung disease initially, it was demonstrated that supra-therapeutic levels of serum AAT could be achieved in mice using rAAV2-AAT in muscle, with the AAT driven from a CMV enhancer/beta actin promoter/hybrid intron (CBA or CAG) expression cassette. The intramuscular (IM) route of administration was chosen because of its safety and relatively high efficiency. Formal preclinical and a phase 1 clinical trial of rAAV2-AAT were performed. Safety was excellent, but levels were low and transient. A switch to rAAV1-AAT was made because of data from mice indicating a 1000-fold potency advantage over rAAV2 with IM delivery. Again, formal preclinical toxicology studies were completed, and subsequent phase 1 and phase 2 clinical trials have been undertaken. These clinical studies have demonstrated robust expression from IM administration, persistent at levels at 3 to 5% of the target level for 12 months after a single IM injection. The immune response profile is complex, with both CD4 and CD8 responses to rAAV1 capsid epitopes, apparently not interfering with long-term transgene expression. In parallel, a strategy for long-term simultaneous allele-specific knockdown of Z-AAT and augmentation of wild-type M-AAT has been targeted to the liver using a bifunctional rAAV9 vector expressing both a synthetic miRNA targeting endogenous human AAT and an augmentation allele that has been altered in its codon usage to make it resistant to that miRNA while still expressing the wild-type amino acid sequence. This construct resulted in 80% knockdown and therapeutic augmentation levels for three months after a single systemic intravenous (IV) injection. Further studies of gene therapy for both the lung and liver disease of AAT deficiency are planned. Supported by grants from the NHLBI, NIDDK, Alpha-One Foundation, and AGTC.

Biography of Terence R. Flotte, MD

As Dean of the School of Medicine and Executive Deputy Chancellor of the University of Massachusetts Medical School, Terry Flotte, MD, serves UMMS as chief academic and administrative officer of the School of Medicine, overseeing all academic activities of the basic and clinical science departments, including education and research for the School of Medicine and the Graduate School of Biomedical Sciences. He was also recently appointed as the Celia and Isaac Haidak Distinguished Professor of Medical Education. Dr. Flotte joined UMMS from the University of Florida, where he was the Nemours Eminent Scholar and Chair of the Department of Pediatrics for the College of Medicine. He is a graduate of the University of New Orleans and the Louisiana State University School of Medicine. He served his residency in pediatrics at Johns Hopkins University, where he completed a pediatric pulmonary fellowship and postdoctoral training in molecular virology. Dr. Flotte's research has been funded by the National Institutes of Health, the Cystic Fibrosis Foundation, the Juvenile Diabetes Research Foundation and the Alpha One Foundation. He has received numerous honors and awards and is a member of many professional organizations. In 2005, Dr. Flotte received the Society for Pediatric Research's E. Mead Johnson Award for Outstanding Scientific Contributions. In 2010, he was elected to the Association of American Physicians and became a member of the Society for Clinical Investigation.

Hairpins to helper virus and the impact on AAV vectors

Samulski RJ

Department of Pharmacology, University of North Carolina, Chapel Hill, NC
Gene Therapy Center, University of North Carolina, Chapel Hill, NC

Adeno-associated virus (AAV) vector is demonstrating therapeutic effect in a number of phase 1 clinical trials. Although AAV has very broad tropism, most humans have neutralizing antibodies that restrict vector re-administration. To further enhance AAV vector tropism and identify capsid variants that escape neutralizing antibody, we have generated synthetic AAV2 capsid by replacing a hexapeptide sequence in a previously identified heparan sulfate receptor footprint with corresponding residues from AAV8. The AAV2/AAV8 chimera designated AAV2i8 has displayed an altered antigenic profile, readily traversed the blood vasculature, and selectively transduced muscle tissues including the heart with high efficiency in animal models. More remarkable, this capsid variant reduced hepatic tropism. In a parallel study, we demonstrated that a single amino acid (aa) insertion of Threonine into AAV2 capsid would convey high muscle transduction and changed the capsid immune profile as well. To explore whether the single aa insertion tropism in AAV2 was distinct from AAV2i8 capsid we inserted aa at 265 (AAV2i8D), and injected AAV2i8D vector encoding the firefly luciferase transgene into mice via the tail vein. Compared to parental AAV2i8, liver tropism was rescued and muscle transduction was preserved. To address whether unique pharmacokinetic, pharmacodynamic, and antigenic features of these synthetic AAV variants studied in mice translated across species, we screened thirty-three gravid nonhuman primates for AAV antibodies and identified six for study. Pregnancies were monitored sonographically during gestation and newborns were delivered at term by cesarean-section. Three AAV/luc vectors (AAV9, AAV2i8, and AAV2i8D) were administered intravenously at birth (N=2 per construct). Infants were raised in a nursery and transgene expression was monitored monthly by bioluminescence imaging (BLI) immediately after the intravenous injection of D-luciferin. Blood samples were also collected from a peripheral vessel monthly to monitor complete blood counts (CBCs) and clinical chemistry profiles. Animals remained healthy during the study period, to date, and two years post-vector administration health, growth, CBCs, and clinical chemistry panels were within normal limits for the age group. Vector transgene expression determined by BLI has persisted without significant decline over time. Our results suggest that rationally designed AAV variants can induce identical long-term transgene expression without safety concerns across various species (mice to nonhuman primates). Further studies comparing species-specific vector transduction tropism and immune profiles after systemic injection will be discussed.

Biography for R. Jude Samulski, PhD

The primary focus of research in Dr. Samulski's laboratory is the development and use of viral vectors for human gene therapy. The major research effort utilizes adeno-associated virus (AAV) which is a non-pathogenic human parvovirus. In the absence of helper adenovirus, AAV establishes latency by integration into the host genome. The research from this laboratory has identified critical steps involved in adeno-associated virus (AAV) assembly and identified receptors for AAV type 1-6. These new findings, coupled with understanding vector persistence *in vivo*, have played a role in the development of the first clinical trial for Duchenne muscular dystrophy (DMD) in 2007 with Dr. Xiao Xiao. Efforts continue with important collaborations in the areas of: therapy for seizure (R01 NS035633), cystic fibrosis (P01 051818), pulmonary and hematologic disorders (P01 HL066973), and pharmacodynamics (P01 GM059299). Dr. Samulski graduated from the University of Florida from Dr. Nicholas Muzyczka's laboratory followed by a post doc with Tom Shenk at SUNY Stony Brook and Princeton. He established his research lab at the University of Pittsburgh's Department of Biology in 1986, as assistant professor. Dr. Samulski came to UNC in 1993 as an associate professor where he became the first Director of the UNC Gene Therapy Center as member of the Department of Pharmacology. During this time he has served on many NIH committees (e.g. RAC, and recently served as 2012 President of the American Society of Cell and Gene Therapy). Dr. Samulski's contributions in the field of gene transfer have been acknowledged by various societies including the National Hemophilia Foundation's Outstanding Scientist and the Gene Therapy Society's first awardee of Outstanding Achievement Award. In the last 14 years he has trained more than 20 graduate students and over 33 postdoctoral fellows. The laboratory continues to focus on how to engineer AAV for gene therapy applications, generate novel AAV mutant vectors and map the structural determinants and mechanisms of AAV capsid cell entry.

Site-specific integration by AAV: back to the future

Linden RM

Department of Infectious Diseases, King's College London School of Medicine at Guy's, King's and St Thomas' Hospitals, London, UK
Gene Therapy Consortium, University College London, London, UK

AAV is a virus that does not cause any known disease and has the unique ability to insert its genome into one specific site in the human genome. These features render this virus an ideal vehicle to shuttle corrective genes into the genome of people with genetic diseases. However, genetic engineering of cells for therapeutic purposes requires a thorough understanding of the viral mechanism of integration. We are testing the hypothesis that the mechanism underlying site-specific integration is based on the evolutionarily ancient bacterial parasite DNA replication mode and, as a consequence, whether AAV genome integration is a byproduct from biochemical activities of the AAV Rep proteins that are necessary for viral genome replication. The proteins responsible for this mode of replication, the rolling circle replication (RCR) initiators, are amongst the evolutionarily oldest proteins known. Their signature protein domain, the RCR domain, is present in the AAV Rep protein, which is the viral factor necessary and sufficient to mediate integration. In addition, we explore whether evolutionary adaptations have occurred to this ancient mechanism in order to secure stable integration within the complex chromosomal environment of the target site in the host.

Biography for R. Michael Linden, PhD

R. Michael Linden, Ph.D. is a Professor of Molecular Virology at King's College London and the Director of the Gene Therapy Consortium at University College London. After graduating in Biochemistry and Molecular Biology at the University of Zürich, Switzerland, Dr. Linden trained in molecular virology under the guidance of Dr. Kenneth I. Berns at Cornell University Medical College, N.Y. Since then Dr. Linden has used a multidisciplinary approach to understanding the molecular mechanisms and the potential applicability of site-specific genome integration by adeno-associated virus (AAV). His contributions to this field include the proposal for a mechanism of this potentially unique aspect of a viral strategy and the structural determination of the AAV Rep proteins, which orchestrate all aspects of this human parvovirus. During these studies Dr. Linden's laboratory has demonstrated the potential of using embryonic stem cells for gaining insights into complex viral mechanisms. More recently, Dr. Linden has engaged in the field of AAV-mediated gene therapy and, in his role as the Director of the UCL gene therapy consortium is responsible for clinical grade vector production and the support of further development of UK gene therapy efforts. In this context Dr. Linden is actively engaged in translational projects aimed at the development of therapies for Parkinson's disease, spinal muscular atrophy, lysosomal storage diseases and a variety of eye diseases using AAV-based vectors. During his career Dr. Linden has served on many scientific advisory committees including the Virology and Experimental Virology study sections and review panels evaluating human embryonic stem cell programs. Dr. Linden is currently an associate editor of *PLoS Pathogens*.

AAV to the rescue

Baltimore D

Division of Biology, California Institute of Technology, Pasadena, CA

Almost a decade ago, being convinced that standard routes to an HIV vaccine were unlikely to be effective, we committed ourselves to developing a new route to HIV prophylaxis. We believed, and have been vindicated in this belief, that broadly neutralizing anti-HIV antibodies (bnAbs) would be found. Then the best was b12, an antibody that was very effective against clade B HIV. We thus undertook to find a way to use b12 to protect against HIV infection. Our approach was to take advantage of the power of viral vectors to express Ab genes in human cells. We had the generous support of the Gates Foundation. Although we started with lentiviral vectors, when Alex Balazs joined my laboratory, he thought to use adeno associated virus (AAV) as a vector. All I knew about AAV was that my old friend, Ken Berns, had worked on it for many years. I was not aware of how versatile and how powerful it was. We realized that it had enough genetic space to encode one heavy and one light chain antibody gene with a little left over for regulatory elements. When an AAV vector encoding b12 was injected into the muscle of mice, up to 1 mg/ml of serum could be achieved and maintained for a year. Using immunocompromised mice with an adoptive human immune system, we could completely protect against HIV infection with the high levels of b12 we could achieve. More broadly reactive and more potent anti-HIV Abs then became

available. We found that our platform is versatile; we can express a variety of Abs and can protect against a variety of HIV strains delivered by both the intravenous and vaginal routes. We are now developing a clinical trail of this form of anti-viral prophylaxis, which we call VIP: Vecteded ImmunoProphylaxis. We are also extending VIP to other pathogens.

Biography for David Baltimore, PhD

David Baltimore, former president of the California Institute of Technology (1997-2006), President Emeritus and the Robert Andrews Millikan Professor of Biology, is an accomplished researcher, educator, administrator and public advocate for science and engineering and is considered one of the world's most influential biologists. He received his BA in Chemistry from Swarthmore College in 1960 and a PhD in 1964 from Rockefeller University. Awarded the Nobel Prize in 1975 in Physiology or Medicine for his research into viral replication that provided the key to understanding the life cycle of retroviruses, Dr. Baltimore has profoundly influenced national science policy on such issues as recombinant DNA research and the AIDS epidemic. Dr. Baltimore's numerous honors include the 1999 National Medal of Science and 2000 Warren Alpert Foundation Prize. He is Director of the Joint Center for Translational Medicine, a collaborative effort joining Caltech and UCLA in a program translating basic science discoveries into clinical realities. He is past-President and Chair of the American Association for the Advancement of Science and has published more than 650 peer-reviewed articles.

Environmentally induced epigenetic transgenerational inheritance of reproductive phenotypes and disease: ancestral ghosts in your genome

Skinner MK

Center for Reproductive Biology, Washington State University, Pullman, WA
School of Biological Sciences, Washington State University, Pullman, WA

Transgenerational effects of environmental toxicants significantly amplify the impact and health hazards of these compounds. One of the most sensitive periods to exposure is during embryonic gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation. Previous studies have shown that endocrine disruptors can cause an increase in a number of adult onset diseases in fertility, prostate, ovary, kidney and cancers. Interestingly, this effect is transgenerational (F1, F2, F3 and F4 generations) and hypothesized to be due to a permanent (imprinted) altered DNA methylation of the germ-line. The transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to alter the epigenetic (i.e. DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease development transgenerationally. Recently a variety of different environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease including: the fungicide vinclozolin, plastics BPA and phthalates, pesticides, dioxin and hydrocarbons. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational adult onset disease is a new paradigm in disease etiology that is also relevant to other areas of biology such as evolution.

Biography for Michael K. Skinner, PhD

Dr. Michael Skinner is a professor in the School of Biological Sciences at Washington State University. He did his BS in chemistry at Reed College in Portland Oregon, his PhD in biochemistry at Washington State University and his postdoctoral fellowship at the C.H. Best Institute at the University of Toronto. Recently he did a sabbatical in bioinformatics at Rosetta/Merck. He has been on the faculty of the Pharmacology Department at Vanderbilt University and the Reproductive Sciences and Physiology at the University of California at San Francisco. Dr. Skinner's research is focused on the area of reproductive biology and environmental epigenetics. Recent studies have elucidated several critical events in the initiation of male sex differentiation, testis development and ovarian primordial follicle development. His current research has demonstrated the ability of endocrine disruptors and environmental toxicants to promote epigenetic transgenerational inheritance of adult onset disease phenotypes, due to abnormal germ line epigenetic programming during gonadal development. Dr. Skinner has over 225 peer reviewed publications and has given over 220 invited symposia, plenary lectures and university seminars. Dr. Skinner established and was the founding Director of the Washington State University and University of Idaho Center for Reproductive Biology (CRB) since its inception in 1996. The CRB has over 90 faculty and is one of the largest reproductive sciences research centers in the world. Dr. Skinner also established and was the founding Director of the Center for Integrated Biotechnology (CIB). The CIB was established in 2002 and has over 170 active research faculty members. In 2008 he stepped down as Director of the

centers to focus his efforts on his research. His research has been highlighted in BBC and PBS documentaries and selected as top 100 discoveries in 2005 and 2007 by *Discover*. In addition, Dr. Skinner has been actively involved with the start-up of several biotechnology companies.

Epigenetic gene regulation and phenotype in maize

McGinnis KM

Department of Biological Science, Florida State University, Tallahassee, FL

Epigenetic gene regulation is crucial for normal growth and development in a broad range of organisms. It can be both responsive to environmental conditions, and heritable from one generation to the next. Thus, epigenetic gene regulation represents important mechanisms that bridge genotype to phenotype. In plants, one epigenetic regulatory pathway involves siRNA-directed DNA methylation and chromatin modifications. In recent years, several components of this pathway have been genetically identified and extensively studied in the agriculturally and economically important crop, *Zea mays*. These discoveries have facilitated the understanding of the mechanisms and impacts of RNA-directed epigenetic gene regulation in plants. Results of recent genetic, genomic and bioinformatic approaches to assess the impact of this pathway on gene expression and genome regulation will be described.

Biography for Karen M. McGinnis, PhD

Dr. Karen McGinnis is an assistant professor in the Department of Biological Science at Florida State University. Prior to joining the faculty in 2008, she held postdoctoral positions at the University of Arizona and with the USDA-ARS at Washington State University. Dr. McGinnis received her PhD in 2000 in Plant Biology from Arizona State University. Her research focuses on epigenetic regulation of gene expression in maize.

Transgenic forests: a funny thing happened on the way to the revolution

Strauss SH

Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR
Molecular and Cellular Biology Graduate Program, Oregon State University, Corvallis, OR

Because of their difficult genetic systems, transgenic methods had been touted as a revolutionary new means for conducting research and breeding of forest trees. The high heterozygosity, intolerance of inbreeding, and delayed onset of reproduction in forest trees makes conventional genetic approaches to genetic analysis nearly impossible. As a result, quantitative and whole-genome approaches, rather than Mendelian approaches, have dominated research and breeding. Transgenic methods could provide powerful new tools in bypassing the sexual cycle and enabling single-gene gain of function or loss of function phenotypes to be readily produced. RNA interference has been found to be an effective means for reduced function studies for numerous genes, which I will demonstrate from our studies of genetic control of flowering time in poplar by its TERMINAL FLOWER/CENTRORADIALIS homologs. Gain of function transgenic mutagenesis and gene traps have enabled the discovery of novel genes that control complex traits, which I will illustrate with our results from activation tagging of tree size and dormancy traits in poplar. Of course, transgenic approaches also enable novel traits, such as for pest or abiotic stress tolerance, to be inserted based on genes from other species that would be impossible via breeding. However, transgenic approaches have not been widely adopted in research or breeding of forest trees for a number of reasons that were not widely appreciated when they were first introduced in the 1980s. First, extending transformation/regeneration methods outside of model species has proven much more difficult and costly than first expected. The inflexible developmental system of trees poses obstacles that traditional *in vitro* horticulture and developmental biology have so far been unable to overcome, or are very costly to work around, for most species. Even species of immense economic importance, such as eucalypts and pines, remain highly recalcitrant, limiting transgenic studies to very few laboratories and companies worldwide. Second, anti-GMO campaigns have been far more widespread and influential than expected, leading to extremely onerous regulatory regimes and marketplace obstacles that treats all products of transgenic methods as putative environmental and food/feed safety threats, making essential field studies very difficult or impossible to carry out, including in the USA. Finally, the combination of extremely rapid DNA sequencing and computation methods, and lack of social resistance to its applications in molecular breeding, has moved the field of

biotechnology toward genomic analysis and selection applications. Thus, transgenics have been pushed to the sidelines for the foreseeable future, and there is a near absence of public funding and a declining capability for transgenic studies in academia. This is occurring despite the established power of transgenic methods to help modify traits such as pest and stress resistance, of direct and obvious value for helping forests cope with growing climate-associated stresses. A scientific, regulatory, and marketplace revolution is needed if transgenic tools are to make a significant contribution to forest health and productivity.

Biography for Steven H. Strauss, PhD

Dr. Steven H. Strauss is a Distinguished Professor of Forest Biotechnology in the Department of Forest Science at Oregon State University, and has a joint appointment in the Molecular and Cellular Biology Graduate Program. He is Director of the Tree Biosafety and Genomics Research Cooperative at OSU, a university-public agency-industry consortium formed in 1994. It conducts research on the biosafety and physiology of genetically engineered trees used in plantation forestry and horticulture. Strauss directs the OSU Program for Outreach in Resource Biotechnology, aimed at promoting public understanding, and facilitating science-based public debates, in food and natural resources biotechnology (<http://agsci.oregonstate.edu/orb/>). Strauss received the Barrington-Moore Memorial Award from the Society of American Foresters in 2001, which recognizes outstanding achievement in biological research leading to the advancement of forestry. He was recognized as a Fellow of the American Association for the Advancement of Science in 2009, for advancing of the science and policy regarding forest biotechnologies. He was the Forest Biotechnology Partners Forest Biotechnologist of the Year in 2009 recognizing his outstanding contributions to science, dialogue, and stewardship. In 2005 he was recognized as a Leopold Leadership Fellow, taking part in a program aimed at training eminent environmental scientists to be more effective at influencing public policy and presenting science to news media. Dr. Strauss has earned degrees in biological sciences from Cornell, Yale, and the University of California at Berkeley. He has published 200 scientific papers, given more than 200 invited lectures on biotechnology and genetics of trees, and obtained more than 17 million dollars of competitive grant support. His laboratory has trained 22 graduate students, 26 postdoctoral scientists, and more than 40 professional and technical staff. He has served on panels at the United States National Research Council, National Science Foundation, and Department of Agriculture. Dr. Strauss' current research focuses on genetic engineering of flowering, stature, and transformation-based "functional genomics" using poplar trees as model organisms. He has also advised governments and written in many scientific journals about national and international regulations on field research and commercial development of genetically engineered crops and trees. He was an editor for the highly cited plant science journal *New Phytologist* for nine years.

Gene therapy, 1974-2012: are we there yet?

Muzyczka N*

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

Almost 40 years ago gene therapy was widely believed to be the next new therapeutic modality in medicine, but we have yet to have a single drug certified by the FDA. We now appear to be on the verge of success. We will discuss the issues that had to be resolved in the last 40 years, the major lessons learned, and the medical applications that are likely to emerge in the next decade.

Tackling AAV gene delivery challenges with structural virology

Agbandje-McKenna M*

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

The recombinant AAV gene delivery system is entering a crucial and exciting phase with the promise of more than 20 years of intense research now realized in a number of successful human clinical trials that report therapeutic efficacy. However, significant challenges have been highlighted in studies using animal models and in the human clinical trials, including difficulties in delivering genes efficiently to certain target cells/tissues and avoiding the detrimental effects of the pre-existing host immunity to AAV capsids. Adopting a multidisciplinary approach, utilizing biochemistry, molecular genetics, structural biology, and *in vivo* studies, we are "mapping" the antigenic structures of the AAVs as they relate to capsid determinants of tissue tropism and transduction (gene expression). Such studies are aimed at obtaining information for rational capsid engineering of AAV vectors to achieve escape from host antibody neutralization while retaining their natural or targeted tropisms. Our latest results will be presented.

Imprinting mechanisms underlying Prader-Willi and Angelman syndromes

Resnick JL*

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

Approximately 150 human genes are subject to genetic imprinting, the selective transcriptional silencing of one parental allele. Only a few diseases are known to result from deficits in imprinted genes. A 2 Mb region at 15q11-q13 harbors both paternally and maternally expressed genes that are responsible for both Prader-Willi and Angelman syndromes (PWS / AS), two clinically distinct neurobehavioral disorders. PWS results from the lack of genes normally expressed from the paternal allele while AS results from the absence of maternal specific gene expression. Imprinted genes are mostly located in clusters and allele specific expression at each cluster is controlled by an imprinting center. The PWS-AS imprinting center contains two parts separated by 35 kb, the PWS-IC and the AS-IC. The PWS-IC activates expression of genes transcribed from the paternal allele. The AS-IC epigenetically modifies the PWS-IC in oocytes, so that the PWS-IC does not activate genes on the future maternal allele. Thus the AS-IC functions in female germ cells to establish the imprint and the PWS-IC functions in somatic cells to effect allele specific gene expression. We have developed mouse models to explore imprinting defects arising from the lack of PWS-IC or AS-IC function. Surprisingly, we have found that presence of the PWS-IC is required only transiently during embryonic development, but has durable effects even in its continued absence. We also found that AS-IC activity resides in several oocyte specific promoters located upstream of the PWS-IC, and that the establishment of imprinting at the locus requires transcription transiting the PWS-IC in oocytes.

Epigenetic alterations and stress among new mothers and infants in war-torn Democratic Republic of Congo

Mulligan CJ*

Department of Anthropology, University of Florida, Gainesville, FL

Early life experiences, including those *in utero*, have been linked to increased risk for adult-onset chronic disease. The current study is the first to test the idea that extreme maternal psychosocial stressors, as observed in the war-ravaged Democratic Republic of Congo, may modify epigenetic marks in newborns resulting in altered health outcomes. Maternal and umbilical cord blood samples were collected from 25 mother-infant dyads. Detailed ethnographic interviews and perinatal trauma surveys were administered to all mothers in order to develop three emic (culturally relevant) measures of prenatal stress. Both locus-specific and genome-wide methylation data were collected. The glucocorticoid receptor, *NR3C1*, has been previously implicated in newborn birthweight. In a locus-specific study, we observed that increased methylation at the promoter for *NR3C1* in newborns was significantly correlated with maternal stress and newborn birthweight. This increased methylation may constrain plasticity in subsequent gene expression and restrict the range of stress adaptation responses possible in affected individuals, thus increasing their risk for adult-onset diseases. Our genome-wide methylation analysis began with a pilot study using reduced representation bisulfite sequencing (RRBS) and Illumina GAIIX technology. One lane of data, for four indexed technical replicates, yielded coverage for ~1.5 million CpGs per library. However, after estimating a minimum necessary coverage for accurate epi-genotyping and identifying library overlaps, the number of informative sites was reduced to ~0.2 million. Observing that each additional library would further reduce the number of informative sites we opted to switch to Illumina's HumanMethylation Bead Chip technology. Preliminary results indicate that data for almost 0.5 million CpG sites were acquired for 72 samples, exceeding what could be acquired from RRBS at a similar price. Further analyses are ongoing.

ADP-glucose pyrophosphorylase: from bacterial selection to yield in the field

Hannah LC*

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

Historically, the cereal seed has played a critical role in feeding humans and animals. The need for even greater cereal yields is necessitated by population growth, shrinking agricultural acreage, global climate change and the use of cereals for biofuel production. Accordingly conventional breeding efforts have been intense and yield increases of ~ 1% per year have been realized from maize (corn) breeding programs. We have taken a biochemical approach to this problem and focused on a rate limiting step in starch synthesis. Starch constitutes 70% of the seed weight and hence is the major contributor to yield. The rate limiting target is ADP-glucose pyrophosphorylase, an allosterically-controlled, heat-labile, heterotetrameric enzyme. Using initially *in planta* transposon mutagenesis and subsequently an *E. coli* expression system in which enzyme levels in bacterial colonies can be monitored by a glance of the eye, we selected variants that enhanced heat stability and reduced inhibition by allosteric effectors. One such variant produces a 38% yield increase in wheat, a 23% increase in rice and up to a 64% yield increase in maize, depending on growth conditions. Surprisingly this variant increases yield by enhancing the probability that an ovary will produce a seed rather than by increasing individual seed size. Other unexpected insights into the function of this gene as well as targets for breeding under elevated temperatures will be discussed.

The chemistry and genetics of tomato flavor

Klee H*

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

Flavor is a conscious appreciation of the total sensation derived from a food involving integration of multiple sensory inputs. We use tomato as a model to understand the chemical interactions that drive human preferences, exploiting the large range of flavor chemicals produced in heirloom cultivars. Many cultivars spanning a broad range of biochemical diversity have been evaluated by consumers, generating a subjective sensory profile of perceptions. Our models of human preferences provide hypotheses about the chemical contributions to flavor. While sugar is a major driver of flavor intensity, sweetness and liking, additional volatile chemicals contribute to these perceptions. Several volatiles widely accepted as being important contributors to flavor do not contribute to flavor intensity, liking or sweetness. Knowing how the components of flavor contribute to human preference constitutes a new direction in the chemistry of flavor preferences for tomato and, more broadly, food products. In parallel, we are developing a comprehensive understanding of the pathways and regulation of synthesis of the most important volatiles. We have identified a large number of quantitative trait loci affecting volatile synthesis and are systematically identifying the most important "regulatory" genes in each pathway. The ultimate goal is to build a toolbox for the molecular breeder to use in restoring great flavor into elite commercial varieties of tomato.

Poster sessions

*= UF Genetics Institute Faculty. Presenters are underlined.

Poster Session I, Posters no. 1 – 65
Wednesday, November 28, 3:30 p.m. – 5:00 p.m.

1. Genome-wide genetic diversity and demographic events shaping the evolutionary history of two *Pinus* species

Acosta JJ¹, Neves LG², Fahrenkrog A², Davis JM^{1,2,*}, Holliday J³, Kirst M^{1,2,*}

¹School of Forest Resources and Conservation, University of Florida, Gainesville, FL

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

³Department of Forest Resources and Environmental Conservation, Virginia Polytechnic Institute and State University, Blacksburg, VA

Recent advances in genome complexity reduction and sequencing make possible the genome-wide analysis and identification of genes under natural selection. We applied DNA sequence-capture to the analysis of two conifer species, *Pinus taeda* and *Pinus elliottii*, that are widely distributed in the south-southeastern US. Haploid DNA from megagametophytes was extracted from seeds of 24 trees of each species, collected from native populations in 11 states representing their natural range. For genotyping, libraries were prepared by sequence-capture of a small fraction (0.03%) of the *Pinus* genome that represents 14,729 genes. Six multiplexed pools, of eight barcoded DNA samples each, were hybridized to sequence-capture oligonucleotides and sequenced in a high-throughput sequencer (Illumina's HiSeq). More than 30,000 SNPs were identified from the sequence data, and for each individual on a gene level, DNA sequence alignment references were reconstructed. For each pine species, population statistics were calculated, and coalescent simulations were made assuming three demographic models (bottleneck, growth and neutral equilibrium). Using approximate Bayesian computation simulation techniques, posterior probabilities for each demographic model given the observed population data were calculated. For *Pinus elliottii*, results indicate population size expansion due to a bottleneck event (posterior probability 0.998966). *Pinus taeda* analyses are underway. The availability of data from both species will also allow the analysis of the relationship between within-species diversity and between-species divergence, which has not yet been possible at this scale.

2. Nuclear genetic diversity in human lice (*Pediculus humanus*) reveals continental differences and high inbreeding among worldwide populations

Ascunce MS¹, Toups M¹, Kassu G¹, Fane J¹, Scholl K¹, Toloza AC², Picollo MI², González-Oliver A³, Reed DL^{1,*}

¹Mammalogy, Florida Museum of Natural History, University of Florida, Gainesville, FL

²Centro de Investigaciones de Plagas e Insecticidas (CONICET-CITEDEF), Buenos Aires, Argentina

³Facultad de Ciencias, Universidad Nacional Autónoma de México, México Distrito Federal, México

The ability to control human parasites has been hampered by the scarce knowledge of parasite population dynamics and structure. This is the case for human lice, where despite growing knowledge about their ancient coevolution with humans, little is known about their current genetic structure. Pediculosis is a prevalent infectious disease caused by the two ecotypes: the head louse (*Pediculus humanus capitis*), and the clothing (body) louse (*Pediculus humanus humanus*). Hundreds of millions of head louse infestations affect children every year, and this number is on the rise in part because of increased resistance to insecticides. Here, we present the first assessment of the genetic structure of human lice by analyzing the nuclear genetic variation at 15 microsatellite loci in 93 human lice from 11 sites worldwide. Bayesian clustering analyses assigned lice to four genetic clusters that were geographically structured. Clothing lice clustered within or close to the Asian head louse cluster. Although overall gene flow values were low, some pairwise comparisons between clothing and head lice showed more similar Nem values than between two populations of head lice, reflecting a consistent pattern with cluster assignments. The geographical and ecological structure among human louse populations could affect the evolution of insecticide resistance as well as disease transmission, thus our results are relevant to strategies for the control of human lice. Furthermore, our panel of microsatellite markers provides powerful data to elucidate not only ecological and evolutionary processes in lice, but also may elucidate those processes in humans because of their long-term coevolution.

3. Correlation between development time and virus titer in *Drosophila melanogaster*

Atkinson E¹, Brusini J², Wayne ML^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA

The “trade-off” hypothesis for the evolution of virulence, which states that pathogens will tend to evolve toward an intermediate level of virulence, relies heavily on the assumption that there exists a causal relationship between the number of pathogens in a host (titer) and the pathogen’s virulence. Using the Sigma virus-*Drosophila melanogaster* system, we performed an artificial selection experiment to test the reliability of this assumption. When we selected on lines for high and low titer, we saw a correlated response for host development time, such that flies with high titer had longer development time than flies with low titer. We now test the generality of this result by performing the inverse experiment: selecting on high and low development time, and testing for a correlated response in terms of titer. Lines responded quickly to selection on development time. Analysis of titer by qPCR is in progress.

4. Map-based cloning and anatomical analysis of a maternal-effect seed mutant *mre*^{*}-594

Bai F¹, Bagadion A¹, Evans M², Settles AM^{1,*}

¹Horticultural Sciences Department, University of Florida, Gainesville, FL

²Department of Plant Biology, Carnegie Institution for Science, Stanford, CA

Genomic imprinting in plants is an epigenetic phenomenon by which a subset of genes is expressed in a parent-of-origin-dependent manner. Imprinted gene expression primarily occurs in the endosperm and is thought to influence seed size and embryo development. We identified a maternal-effect seed mutant, named as maternal rough endosperm 594 (*mre*^{*}-594). When inherited from the female parent, *mre*^{*}-594 seeds show a rough, etched, or potted endosperm surface as well as a reduced seed size and weight. Transmission of the *mre*^{*}-594 allele through the male does not affect seed development. The severity of *mre*^{*}-594 embryo defects is correlated to seed size with the least severe mutants developing near normal embryos and seedlings.

Microscopic examinations of developing seeds from 4 days after pollination (DAP) to 10 DAP revealed developmental delay of basal endosperm transfer cell layer (BETL) as well as starchy endosperm defects. The BETL in normal 4 DAP kernels is a single cell layer of elongated cells with extensive secondary cell wall ingrowths. The mutants did not develop BETL cells until 8 DAP. Moreover, *mre*^{*}-594 starchy endosperm cells failed to accumulate starch granules. *mre*^{*}-594 maps to chromosome 4S, and we are in the process of positional cloning this gene. Based on *mre*^{*}-594 inheritance patterns, we expect the mutant to

encode an imprinted gene required for normal cell differentiation and grain-fill.

5. Evolution of alternative splicing across land plants

Mei W[#], Chamala S[#], Walts B, Barbazuk WB^{*}

Department of Biology, University of Florida, Gainesville, FL

[#]These authors contributed equally to this work

Alternative splicing (AS) is the process by which a pre-mRNA is processed into several alternate isoforms. AS contributes to transcriptome and proteome diversity, and also involves in gene regulation. Alternative splicing has been extensively investigated in human and mouse, but its role in plants has not been extensively studied. We are interested in examining the evolution of alternative splicing in the land plants by taking a comparative genomics approach to identify conserved alternative splicing between *Amborella*, *Arabidopsis thaliana*, *Oryza sativa* and *Physcomitrella patens*. By identifying AS isoforms in all four genomes we are able to compare the distribution of alternative splicing, detect AS that is specific to angiosperms, eudicots, monocots, and enable examination of how AS is changed by whole genome duplication. Our analysis uses RNA-Seq to identify splice isoforms within four species, and then examines the alternative splicing potential of each member of a set of orthologous genes. In terms of *Amborella*, there are 26,846 genes in the current annotation, 17,089 of these contain multi-exons. Considering only five major types of alternative splicing events (alternative donor, alternative acceptor, exon skipping, intron retention and alternative exon), 6,407 (37.5%) of intron containing genes produce AS transcript. The total number of predicted AS transcripts is 17,095. Intron retention is the most prevalent and occurs within 7,860 (46.0%) of the isoforms, while alternative donor, alternative acceptor, exon skipping, and alternative exon events contribute to 4,673, 6,206, 2,540 and 4,096 isoforms, respectively. The AS analysis in three other species is currently underway.

6. How does obligate symbiosis shape bacterial genomes? Comparative genomics of closely related bacterial symbionts of insects provides clues

Boyd BM^{1,2}, Allen JM³, de Crecy-Lagard V^{4,*}, Reed, DL^{1,*}

¹Mammalogy, Florida Museum of Natural History, University of Florida, Gainesville, FL

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

³Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Champaign, IL

⁴Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

Candidatus Riesa is one of the fastest evolving and youngest known heritable bacterial symbionts of insects. Found in symbiosis with human, chimpanzee, and gorilla lice, these recently acquired symbionts represent either a symbiont replacement or novel symbiosis within lice. Why louse symbionts have multiple origins remains unknown, but presents a unique opportunity to study how bacterial genomes evolve in symbiosis. We assembled the genome of the symbiont of the chimpanzee louse, *Candidatus* Riesa pediculischaeffi, from genomic DNA extracted from whole lice. Comparisons between the genomes of *C. Riesa pediculischaeffi* and the closely related *C. Riesa pediculicola*, symbiont of the human body louse, revealed gene loss, duplicated regions, and numerous unique short coding sequences of unknown function. In contrast genome synteny and the majority of identified genes are maintained across the two taxa. Tests for selection showed that all but two shared genes are under strong purifying selection. We conclude that despite being young and rapidly evolving, gene content and order is largely static and maintained by purifying selection.

7. Fruit-specific genes in strawberry: physiology and protein-protein interactions

Brunings A, Chambers A, Wang Y, Folta KM*

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

Strawberry (*Fragaria x ananassa*) is an excellent model plant to study the function of novel, fruit-specific genes. Strawberry transcriptome data yielded 50 genes whose expression is greatly enriched in fruit. RNAi and over-expressor lines were developed in our transgenic system and their phenotypes analyzed. These genes include a mini-zinc finger protein, a number of hypothetical proteins, and others providing only inferred function. One gene encoding a putative F-box protein was used as bait in a yeast-2-hybrid screen to identify potential interaction partners, and interacting candidates were cloned, and sequenced. Most importantly, the new genes characterized in strawberry may be applied to other members of the Rosaceae family (like apple, rose and peach) that are not as easily genetically manipulated and studied because of their longer generation times and large size.

8. Detection of sickle hemoglobin in febrile patients in Léogâne, Haiti

Carter TE¹⁻³, Von Fricken M^{4,5}, Mulligan CJ^{1,*}, Memnon G⁶, Romain JR⁶, Okech BA^{4,5}

¹Department of Anthropology, University of Florida, Gainesville, FL

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

³Graduate Program in Genetics and Genomics, University of Florida, Gainesville, FL

⁴Emerging Pathogens Institute, University of Florida, Gainesville, FL

⁵Department of Environmental and Global Health, University of Florida, Gainesville, FL

⁶Hopital Sainte Croix, Léogâne, Haiti

Sickle cell disease and trait are common erythrocyte disorders that are caused by a mutation in the hemoglobin beta gene. Sickle cell trait (i.e. heterozygous for the sickle cell allele) is selectively advantageous against severe malaria. Haiti is a malaria-endemic country, yet little is known about the prevalence of sickle cell disease and trait in this country. The purpose of our study was to detect the presence of sickle cell disease and trait in Haiti, as part of a larger epidemiological study on malaria in Haiti. Sixty-two individuals at Hopital Sainte Croix were first screened for sickle hemoglobin using a solubility-based rapid diagnostic test (RDT), SickleHeme (Michlone Associates, INC). RDT solution turbidity, as an indicator of sickle hemoglobin, was assessed visually and with spectrophotometry. Samples were also genotyped for hemoglobin beta gene mutations. Of the 62 individuals screened for sickle hemoglobin using the RDT kit, 11 (17.6%) were positive. Of the 11 RDT positive samples, only six actually carried the sickle cell mutation, and as a heterozygote only. All other samples carried the wild type genotype. Additionally, we observed "clumping" behavior in some RDT samples after sitting for eight hours, but only in sickle hemoglobin samples that were confirmed by genotyping. We conclude that genotyping is the most accurate method to estimate the frequency of sickle hemoglobin. However, genotyping is expensive and requires specialized equipment. Thus, the "clumping" observation in the RDT assay may be the best option for detecting sickle hemoglobin in Haiti and similar resource-limited regions.

9. Assembly and validation of an evolutionary reference genome for flowering plants (*Amborella trichopoda*)

Chamala S¹, Walts B¹, Chanderbali A¹, Albert V², Ayyampalayam S³, Burnette J^{4,5}, dePamphilis C⁶⁻⁸, Der J⁶⁻⁸, Estill J³, Lan T², Leebens-Mack J³, Lee S⁹, Ma H⁶⁻⁸, Moore R¹⁰, Palmer J¹¹, Ralph P⁶⁻⁸, Rice D¹¹, Rounsley S^{12,13},

Schuster S¹⁴, Tomsho L¹⁴, Wessler S^{4,5}, Wing R^{9,12,13}, Yu Y⁹, Jiao Y⁶⁻⁸, Xiao N¹⁰, Soltis DE^{1,15,*}, Soltis PS^{15,*}, Barbazuk WB^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY

³Department of Plant Biology, University of Georgia, Athens, GA

⁴Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA

⁵Center for Plant Cell Biology, University of California, Riverside, Riverside, CA

⁶Intercollege Graduate Degree Program in Plant Biology, Pennsylvania State University, University Park, PA

⁷Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, PA

⁸Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA

⁹Arizona Genomics Institute, University of Arizona, Tucson, AZ

¹⁰OpGen, Inc., Gaithersburg, MD

¹¹Department of Biology, Indiana University, Bloomington, IN

¹²School of Plant Sciences, University of Arizona, Tucson, AZ

¹³BIO5 Institute, University of Arizona, Tucson, AZ

¹⁴Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA

¹⁵Laboratory of Molecular Systematics and Evolutionary Genetics, Florida Museum of Natural History, University of Florida, Gainesville, FL

Amborella trichopoda, as the sister to all other extant angiosperms, occupies a crucial phylogenetic position, and its genome sequence is therefore an important reference for comparative genomic studies across the angiosperms. A complete genome sequence of *Amborella* will help in understanding the evolution of key angiosperm traits and provide a baseline to examine genome organization throughout the angiosperms. We are using a whole-genome shotgun strategy to sequence the ~731-Mb *Amborella* genome. We assembled over 48 Gb of DNA sequence, comprising single-end (SE) 454-FLX, SE 454-FLX+, 11-kb paired-end (PE) 454-FLX, and 3-kb PE Illumina HiSeq, and 69,466 BAC-end reads. These sequences were extensively screened and filtered for organellar contaminants, short read lengths, poor quality, artificial duplicates, and chimeras. *De novo* sequence assembly was performed on these filtered sequence sets (~23 Gb) using Newbler. This resulted in 5,745 scaffolds totaling 706 Mb with a mean scaffold size of 123 kb and N50 size of 4.9 Mb covering over 96% of the *Amborella* genome. Significantly, about 90% of our assembled sequence resides within 155 scaffolds, each greater than 1.1 Mb in length. In the absence of genetic and physical

maps for *Amborella*, we employed a combination of chromosomal FISH analysis and optical mapping (OpGen) to evaluate the fidelity and the chromosomal positioning of our current scaffolds. Using this approach, our assembly continuity increased by 2X (N50 from 4.9 Mb to 9.3 Mb and N90 from 1.2 Mb to 2.9 Mb). We are further evaluating the potential of this approach to serve as a surrogate to using long-range PE libraries to increase the contiguity of an assembly.

10. The role of USF2, BRG1 and GATA-2 in the early activation of the β -globin locus control region

Chamales P¹, Morton S¹, Liang S¹, Stees J^{1,2}, Fan X¹, Varn F¹, Huang S^{1,*}, Deng C¹, Bungert J^{1,*}

¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

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

Expression of the β -globin genes is regulated by a locus control region (LCR). The LCR is composed of several DNase I hypersensitive sites that function together to mediate high-level expression of the linked β -globin genes during development and differentiation. Previous work demonstrated that the LCR HS form before globin gene expression during differentiation. We found that LCR HS2 and HS3 recruit RNA polymerase II (Pol II) transcription complexes early on during differentiation of erythroid cells before the genes are expressed. Furthermore, efficient recruitment of Pol II required the function of transcription factor USF. Subsequent studies demonstrated that USF interacts with the nucleosome remodeling factor BRG1. We thus hypothesized that USF recruits BRG1 to the LCR and that the re-organized nucleosome structure leads to HS formation and Pol II recruitment. However, we found that in transgenic embryos expressing dominant negative USF (AUSF) the association of BRG1 with LCR HS2 is increased. Furthermore, preliminary data suggest that expression of GATA-2 is enhanced in A-USF expressing d10.5 yolk sac cells. Previous studies have shown that GATA-1 interacts with BRG1. We thus propose that GATA-2 recruits BRG1 to the LCR in undifferentiated erythroid cells. This allows USF mediated recruitment of Pol II. Transcription of HS2 and HS3 keep these regions in an open configuration and during subsequent differentiation steps erythroid-specific protein complexes associate with the LCR establishing a domain that mediates efficient transcription of the globin genes.

11. Identifying genes for strawberry flavor with a breeding-genetics-genomics approach

Chambers A¹, Pollard H¹, Bai J², Plotto A², Whitaker VM^{1,3,4}, Folta KM^{1,4,5,*}

¹Horticultural Sciences Department, University of Florida, Gainesville, FL

²Gulf Coast Research and Education Center, University of Florida, Balm, FL

³Agricultural Research Service, U.S. Department of Agriculture, Fort Pierce, FL

⁴Plant Innovation Program, University of Florida, Gainesville, FL

⁵Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Cultivated strawberry (*Fragaria x ananassa*) is prized for complementation of desserts and adding attractive color to foods. However, commercial strawberries have been bred for shipping, disease resistance and product size, leaving flavor and aroma as areas for improvement. Several flavor volatiles have been recognized as desirable for consumers, namely methyl anthranilate (grape-like) and gamma-decalactone (peachy) as well as other fruity, tropical terpenes and esters. We have taken an approach leveraging breeding, genetics and genomics to identify the genes that control the production of these volatiles. First, a candidate gene approach was used. An uncharacterized alcohol acyl transferase was overexpressed and suppressed by RNAi. Analysis of fruits shows a decrease in several esters and methyl anthranilate, suggesting a role for this enzyme in the process, and ability to use several substrates. In a second approach a non-commercial variety containing several important volatiles was crossed with a Florida variety possessing a different set. Progeny segregated for increased volatiles. Bulk RNAseq on progeny fruit expressing and not expressing different volatiles has identified several candidates with intuitive connections to flavor volatile synthesis that are now being validated in transgenic plants.

12. Characterization of phosphoproteins in signaling pathways using phosphopeptide enrichment mass spectrometry

Silva-Sanchez C¹, Zhu M², Diaz C¹, Chen S^{1,2,*}

¹Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

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

Protein phosphorylation and dephosphorylation constitutes one of the reversible post-translational mechanisms that control gene expression, signal transduction, cell cycle, apoptosis and cytoskeletal regulation. Due to its dynamic nature, stoichiometry of phosphorylation is usually low. This presents a challenge for mass spectrometry analysis of phosphoproteins. To overcome the challenge, a phosphopeptide enrichment step becomes necessary prior to mass spectrometry analysis. The goal of this work was

to develop an effective methodology for phosphopeptide enrichment and phosphorylation site mapping through comparing the capabilities of different TiO₂, ZrO₂ and Phos tag resins. The protocol for each media was tested with β -casein to determine the efficiency. Then, we used a phosphorylated SnRK to optimize the protocol for detecting low phosphorylation stoichiometry in biological samples. The protein samples (SnRK and SnRK-pho) were separated in a gel. After Coomassie staining and in-gel digestion, the phosphopeptides were enriched using different media. All phosphopeptide samples were run on a nanoflow UPLC coupled to a LTQ-Orbitrap mass spectrometer. The results led to an optimized procedure for analyzing phosphoproteins in signaling pathways.

13. Aberrantly activated AREG-EGFR signaling is required for the growth and survival of CRTC1-MAML2 fusion-positive mucoepidermoid carcinoma

Chen Z, Wu L*

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

Salivary gland tumors (SGT) are a group of highly heterogeneous head and neck malignancies with widely varied clinical outcomes and no standard effective treatments. The CRTC1-MAML2 gene fusion, encoded by a specific chromosomal translocation t(11;19)(q14-21;p12-13), is a highly specific genetic alteration in more than 50% of mucoepidermoid carcinomas, the most common malignant SGT. To date, it remains poorly elucidated regarding the importance of the CRTC1-MAML2 fusion gene in supporting the growth and survival of MEC and its critical downstream mediators. In this study, we showed that depletion of the CRTC1-MAML2 fusion oncoprotein reduced the growth and survival of fusion-positive human MEC cancer cells *in vitro* and the *in vivo* growth of human MEC xenograft tumors. Since CRTC1-MAML2 is a nuclear protein with no known enzymatic activity, we then investigated critical target genes and pathways downstream of the fusion oncoprotein for targeting opportunities. We performed gene expression profiling analyses and functional studies and identified that an autocrine AREG (amphiregulin)-EGFR signaling is a downstream mediator of the CRTC1-MAML2 fusion oncogene and is essential for fusion-positive MEC cells. Excitingly, inhibition of EGFR signaling with an anti-EGFR antibody, Cetuximab, significantly blocked human fusion-positive MEC cell growth *in vitro* and *in vivo*. Therefore, our study revealed a critical role of aberrantly activated AREG-EGFR signaling axis in mediating oncogenic functions of the CRTC1-MAML2 fusion. Our study provides a molecular justification for the clinical evaluation of anti-EGFR therapeutic agents in treating CRTC1-MAML2 positive mucoepidermoid carcinoma.

14. MALDI mass spectrometry of nematode surfaces

Clendinen CS¹, Menger R², Yost R², Edison AE¹

¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

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

Like many other organisms, nematodes synthesize and use small molecules to communicate within and between species. In *Caenorhabditis elegans*, ascarosides have been found to control dauer formation (Butcher et al., 2007, *Nat Chem Biol* 3(7):420-2), mating (Srinivasan et al., 2008, *Nature* 454(7208):1115-8), aggregation (Macosko et al., 2009, *Nature* 458(7242):1171-5), and olfaction (Yamada et al., 2010, *Science* 329(5999):1647-50). Several behaviors, like aggregation, may be mediated or enhanced by chemical cues on the cuticle surface of the nematodes. Using a combination of MALDI MS and principal component analysis (PCA), we present an alternative approach for *C. elegans* chemical biology studies that uses small numbers of individual animals. To demonstrate the potential for this approach, we have used multivariate analysis to compare MALDI-MS data from several mutants that were predicted to produce different compositions of small molecule metabolites.

15. Phenotyping a segregating pseudo F2 population of elephantgrass for biomass related traits

Corsato CE^{1,2}, Sinche M¹, Kannan B¹, Vilarinho AA^{1,3}, Altpeter F^{1,*}

¹Agronomy Department, University of Florida, Gainesville, FL

²Departamento de Ciências Agrárias, Universidade Estadual de Montes Claros, Janaúba, Brazil

³Embrapa Roraima, Empresa Brasileira de Pesquisa Agropecuária, Boa Vista, Brazil

Elephantgrass (*Pennisetum purpureum* Schum.) is one of the best adapted and highest biomass yielding perennial C4 grasses in the costal SE. Due to the very high biomass yields the emerging biofuels industry is currently preparing to grow elephantgrass as feedstock for their new biofuel conversion facility. To enhance biosafety of elephantgrass we are developing elephantgrass accessions that combine late flowering and high biomass yields. Genetic distance of elephantgrass accessions can be estimated with the help of molecular markers and allows the identification of two contrasting parents for flowering and biomass yield. Biomass related traits (e.g. stem diameter, number of tillers, stem length) are quantitative traits, expressed as a

continuous distribution between maximum and minimum levels, and are normally under the control of a larger number of minor genes, each with small additive effects. Positions on the genome that can be attributed to detectable differences in phenotype due to gene effects are known as quantitative trait loci (QTL). To identify and locate possible QTL associated with these traits there is a need to identify co-segregation of phenotypic traits in the mapping population and markers on the genetic map. Hence accurate phenotyping of the mapping population is a crucial prerequisite for marker development and for locating putative QTLs. Therefore, we generated an F1 population (segregating pseudo F2 population) from contrasting parents. This F1 population was vegetatively propagated and phenotyped under replicated field conditions at the UF-PSREC in Citra, FL. The statistical evaluation and distribution of the data describing the flowering time, flowering frequency and biomass related traits (e.g. stem diameter, length of tillers and number of tillers, biomass yield) within the mapping population will be presented.

16. Decreased survival following combined exposure to heat and oxidative stress is correlated with attenuated expression of glutathione S-transferase

Crombie TA, Julian D

Department of Biology, University of Florida, Gainesville, FL

We investigated the combined effects of heat stress and oxidative stress on survival and induction of the oxidative stress response in the nematode model *Caenorhabditis elegans*. To generate oxidative stress, nematodes were exposed to the redox cycling compound juglone at six concentrations (0–100 μ M). Heat stress was administered by increasing the incubation temperature from 20 to 25, 30 or 35° C. We found that heat stress and oxidative stress, when administered simultaneously for 4 h, interacted synergistically. This synergy caused a marked reduction in survival above 30° C at all juglone concentrations. Glutathione S-transferase-4 (*gst-4*) helps detoxify juglone by conjugating it with glutathione, increasing its solubility and aiding in its excretion. Induction of *gst-4* is via the SKN-1 transcription factor. We examined whether heat stress impairs the response to juglone-induced oxidative stress in a reporter strain containing GFP fused to a SKN-1 promoter fragment upstream of *gst-4*. We found that induction of *gst-4* by juglone was attenuated at temperatures greater than 30° C. We validated this finding using qPCR, which showed a 3-fold loss in *gst-4* induction capacity at 33° C. These findings suggest that temperature-dependent inhibition of *gst-4* induction is at least one mechanism by which heat

and oxidative stress synergize to reduce nematode survival.

17. Membrane localization of a novel regulator of actin depolymerizing factor

Cuddy KK¹, Grey PH¹, Zhang X², Oppenheimer DG^{1,3,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Section of Cell and Developmental Biology, Division of Biological Sciences, University of California San Diego, San Diego, CA

³Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Actin filament turnover is required for many actin-dependent cellular processes including cell motility and membrane trafficking. Members of the actin depolymerizing factor/cofilin (ADF) family of actin binding proteins are essential for severing/depolymerizing actin filaments, and thus regulating actin dynamics. We recently identified a new regulator of ADF in *Arabidopsis* named IRREGULAR TRICHOME BRANCH 3 (ITB3). Our *in vitro* analysis of ITB3 function showed that it interacts with ADF and inhibits actin binding to ADF. ITB3 is one of 22 family members in *Arabidopsis*. Interestingly, about half of the ITB members possess a putative signal sequence (SS). Given that signal sequences direct proteins for secretion, we have to reconcile the fact that ADF, which interacts with ITB3, is not secreted. We thus hypothesize that the putative signal sequences are novel N-terminal transmembrane anchors. To test this hypothesis, we constructed YFP fusions to the ITB3L-10 protein, which contains a putative SS, and used particle bombardment to transiently transform *Arabidopsis* epidermal cells. Results show putative localization to the endoplasmic reticulum. We are also conducting *in vitro* transcription/translation of ITB3L-10 in the presence of eukaryotic microsomes to test for co-translational membrane insertion.

18. Cross immunity in *Drosophila melanogaster* for bacteria and viruses

Culbreth E¹, Harshman L², Wayne ML^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE

Is the response of the host to a parasite specific to the given parasite, or a more general function of the host? Host responses include tolerance or resistance. There are a number of genetic models suggesting that tolerance and/or resistance are parasite specific. One also finds terms like "immunocompetence", which suggests a qualitative immune

response that might translate to a spectrum of parasites. We attempt to distinguish between these two ideas by evaluating infection rates by sigma virus of lines of *Drosophila melanogaster* artificially selected for increased resistance to *Bacillus cereus*. Also, two sets of controls exist, one a "wounding" control such that animals were wounded exactly as the selected lines; and an unselected control, such that animals were not treated at all. If response to selection has been in the form of "immunocompetence", we expect the selected lines to be more difficult to infect with sigma virus than either of the control lines. If response to selection has been in the form of a parasite-specific genetic change, we do not expect to see any difference in our ability to infect the selected lines rather than the control lines. Preliminary data suggest that there is no difference between treatments, and thus that response to selection for resistance to *B. cereus* is specific. Further experiments, both challenging these selection lines against parasites other than sigma and evaluating other selected lines, are needed to draw conclusions.

19. Investigating the role of the WICH chromatin remodeling complex in maintaining facultative heterochromatin at the human inactive X chromosome

Culver-Cochran AE, Chadwick BP

Department of Biological Science, Florida State University, Tallahassee, FL

X chromosome inactivation (XCI) is the mammalian form of dosage compensation that balances the levels of X-linked gene expression between the sexes. Early in female development, transcription from one X is shut down as the chromosome is repackaged into facultative heterochromatin. Once established, the same X is maintained as the inactive X chromosome (Xi) throughout subsequent somatic cell divisions. While much is known about the initiation of XCI, our understanding of how the Xi chromatin is faithfully maintained through each cell cycle is limited. We have identified the multifunctional Williams syndrome transcription factor (WSTF) as a protein that transiently associates with the Xi as it is replicated in late S-phase, and, therefore, is a prime candidate for maintaining XCI. We have previously shown that WSTF acts at the Xi as a component of the WSTF-ISWI chromatin remodeling complex (WICH). Interestingly, we have found that WICH and two other proteins implicated in heterochromatin maintenance, γ -H2A.X and BRCA1, sequentially associate with the Xi, suggesting that each plays a unique role in maintaining the chromosome's epigenetic signature. In order to assess the role of WICH at the Xi, we have compared the localization of WICH with the spatially distinct types of heterochromatin at the Xi. We have characterized the localization of WICH in relation to XIST RNA as well as to

X-linked BAC clones. Additionally, we have determined the temporal order in which WICH, γ -H2A.X, and BRCA1 associate with the Xi. We are actively investigating the precise function of WICH at the Xi.

20. DNA content increases with cell size in humans

Gillooly JF*, Hein A, Damiani R, Verster K

Department of Biology, University of Florida, Gainesville, FL

Endopolyploidy has been observed in many human cell types, but still is often viewed as exceptional, tissue-specific, or indicative of pathology. Here we show that nuclear DNA content varies by over two orders of magnitude across 19 different human cell types, and that it is positively correlated with cell volume. We also show that this increase in DNA content with cell size is similar to intraspecific relationships between DNA content and cell size in other species, and interspecific relationships between diploid genome size and cell size. Our results therefore suggest that endopolyploidy is a general, size-dependent feature of cells, which may have important consequences for cell function.

21. Characterizing a lncRNA associated with the X-linked macrosatellite DXZ4

Darrow EM, Tremblay DC, Moseley SC, Chadwick BP

Department of Biological Science, Florida State University, Tallahassee, FL

DXZ4, a macrosatellite located at Xq23, is one of at least three extensive tandem repeats located on the human X chromosome that adopt a euchromatic chromatin state on the otherwise heterochromatic inactive X chromosome. We have identified several DNA sequences located both proximal and distal to DXZ4 that are candidate regulatory elements for the epigenomic organization of the macrosatellite. A luciferase reporter assay revealed strong promoter activity for one of these sequences, located approximately 80 kb distal to DXZ4. The promoter drives transcription of a long non-coding RNA (lncRNA) toward DXZ4. Numerous alternatively spliced transcripts are generated, which remarkably include variants that transcribe across the entire macrosatellite, terminating on the proximal edge. RT-PCR of pooled tissue samples and human embryonic stem cells suggests that expression of the DXZ4 traversing transcript is tissue-specific and independent of monomer copy number. Active transcription of the lncRNA across the array may contribute to chromatin organization and suggests that DXZ4 adopts an alternate chromatin configuration in different tissues and cell types. In order to assess the role of the distal promoter in establishing and maintaining DXZ4 chromatin, we are generating TAL effector nucleases to induce removal of the promoter sequence via

homology-mediated genome editing, thereby disrupting lncRNA transcription.

22. Novel regulators of cellular antioxidation and detoxification genes identified by RNAi screening in *Caenorhabditis elegans*

Deonarine A, Choe KP*

Department of Biology, University of Florida, Gainesville, FL

Animal cells share a common inducible transcriptional response to oxidative stress that is mediated by cap 'n' collar (CNC) transcription factors. CNCs play a central role in stress defense, drug metabolism, redox homeostasis, tumorigenesis, neurodegeneration, and longevity. Regulation of CNC factors is highly complex and coordinated with numerous physiological and developmental processes. Understanding CNC factor regulation will yield fundamental insights into understanding how animal cells detect changes to environmental conditions and lay the foundation for therapeutic modulation. We take advantage of the genetic and molecular tractability of *C. elegans* to identify and characterize novel regulators of CNCs. *C. elegans* has one functional CNC named SKN-1 that plays a central role in longevity and resistance to oxidants. A genome wide RNAi screen yielded eleven novel regulators of *gst-4*, a SKN-1 target, in response to an oxidant. Four are thought to be required for general transcription or translation and are thus unlikely to play a specific role in CNC regulation. The remaining seven *gst-4* regulators are predicted to function in transcription, ubiquitin labeling of proteins, and inositol-1,4,5-trisphosphate signaling. We are now testing these genes for SKN-1 regulation, stress resistance, and longevity. Characterization of these will define novel mechanisms for regulation of a conserved stress defense and longevity pathway.

23. Role of multiple quorum sensing signals for regulation and synchronization of group behaviors in *Sinorhizobium meliloti* populations

Dilanji GE¹, Madhavan R¹, McLeod LC¹, Teplitski M^{2,*}, Hagen SJ^{1,*}

¹Department of Physics, University of Florida, Gainesville, FL

²Soil and Water Science Department, University of Florida, Gainesville, FL

Quorum sensing (QS) bacteria use small molecule signals to regulate and synchronize collective behaviors. *Sinorhizobium meliloti* synthesizes several *N*-acyl-L-homoserine lactones (AHLs) as QS signals that differ in their carbon side chain length (C8-C18) and molecular

structure (i.e. saturated vs. un-saturated or keto substituted). These signals differ in diffusivity and solubility. *S. meliloti* uses these signals to coordinate complex behaviors necessary for invasion of plant roots. We have studied the regulation of exopolysaccharide (EPS) production utilized in swarming colonies of *S. meliloti*. We hypothesized that *S. meliloti* exploits the physical and chemical properties of AHLs to segregate behaviors among cells within a population and swarm more efficiently. To test this hypothesis we studied the activation kinetics of the EPS genes in the presence of AHLs, investigated the single cell EPS gene activation in well-mixed AHL environments, and measured the spatial and temporal range of the diffusing signal in a spatially extended system. We found only three AHLs regulated EPS production, each inducing different kinetic responses of the EPS genes. The different AHLs elicit different distributions of EPS expression levels (e.g. broad vs. narrow) from populations of cells. Moreover, in a spatially extended colony, diffusing AHLs readily activate EPS genes over time scales of ~20 h and over distances of ~0.5 cm (oxoC14 and C16:1-HSL) or ~1 cm (oxoC16:1-HSL). Our results suggest that *S. meliloti* uses multiple AHLs to generate differences in population-wide expression profiles. Furthermore, variation in AHL diffusivity may allow for control of long range synchronization.

24. Effect of colony stimulating factor 2 (CSF2) during the morula-blastocyst stages of development on gene-specific methylation in inner cell mass (ICM) and trophoctoderm (TE) cells of the bovine blastocyst as determined by high resolution melting analysis

Dobbs K^{1,2}, Sudano M¹⁻³, Hansen PJ^{1,2,*}

¹Department of Animal Sciences, University of Florida, Gainesville, FL

²DH Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL

³Universidade Estadual Paulista, Campus de Botucatu, Sao Paulo, Brazil

CSF2 can enhance the competence of the bovine embryo for establishment and maintenance of pregnancy. In particular, treatment of *in vitro* produced embryos with CSF2 from day 5-7 post-insemination (pi) increased ICM cell number, conceptus survival and the proportion of pregnancies at day 35 that developed to term. One possible mechanism by which CSF2 improves post-transfer survival is alterations in DNA methylation of the embryo. Here we used ICM and TE cells isolated from bovine blastocysts at day 7 after *in vitro* fertilization to test the hypothesis that exposure to CSF2 alters the methylation status of specific genes in a cell-type specific manner. Pools of blastocysts from embryos treated with 0 or 10 ng/ml CSF2 from day 5-7 pi were harvested on day 7 pi,

and subject to magnetic-activated cell sorting to separate ICM and TE. Genomic DNA was extracted, bisulfite-converted, and subjected to methylation-sensitive high resolution melting analysis (MS-HRM) to examine CpG islands within IGF2, DNMT3B and SNRPN. There was no effect of cell type or interactions of CSF2 and cell type on degree of methylation for any gene. CSF2 reduced methylation for IGF2 (2.8 + 0.3% vs. 1.6 ± 0.3%). There was no effect of CSF2 on methylation of DNMT3B or SNRPN. In conclusion, exposure to CSF2 during the morula-blastocyst stages of development can alter methylation of specific genes in the bovine embryo. The functional significance of the change in IGF2 methylation needs to be determined by examining whether transcription is affected. It was also demonstrated that MS-HRM could be used with low amounts of ICM and TE cells to examine DNA methylation.

25. B and T cell immunomodulation attenuates anaphylaxis in a novel mouse model of Pompe disease

Doerfler P^{1,2}, Nayak S^{1,3}, Herzog RW^{4,*}, Byrne BJ^{1,3,*}

¹Department of Pediatrics, University of Florida, Gainesville, FL

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

³Powell Gene Therapy Center, University of Florida, Gainesville, FL

⁴Division of Cellular and Molecular Therapy, Department of Pediatrics, University of Florida, Gainesville, FL

Absence of acid α -glucosidase (GAA) causes cardiac, respiratory, and skeletal muscle dysfunction in Pompe patients. Enzyme replacement therapy (ERT) using recombinant human-GAA (rhGAA) can lead to life threatening immune reactions. We utilized a novel human CD20 (hCD20) Gaa^{-/-} mouse to evaluate immunomodulatory therapies using rituximab (Ritux) concomitant with rapamycin (rap) or methotrexate (MTX). hCD20Gaa^{-/-} mice received Ritux one week prior to ERT to deplete B cells; MTX (10 mg/kg) or Rap (4 mg/kg) were gavage fed daily for a month with once/week IV rhGAA (20 mg/kg). Controls received rhGAA only. After six weeks, mice were re-challenged with ERT. Controls developed high Ab titers (IgG1 1.2x10⁵ ng/mL by four weeks P<0.01). The Rap and MTX cohorts had ~18-fold reduction in anti-rhGAA IgG1 that increased on secondary challenge. Pulse oxymetry revealed that without immune suppression, arterial oxygen saturation, pulse distention, breath distention, body temperature and heart rate decreased significantly (P<0.0001) within 5 minutes of IV rhGAA. MTX group had decreased survival despite low anti-rhGAA titers. Ritux cohorts had a reduction in B cell numbers and activation compared to controls. MTX cohort showed increased CD4⁺ and CD4⁺CD69⁺ cells (P<0.05),

Rap cohort showed an expansion of CD4+CD25+ T cells compared to MTX ($P < 0.05$). Overall, Ritux and Rap administration reduced anaphylactic response, T cell activation and maintained prolonged low anti-rhGAA titer while expanding CD4+CD25+ T cells more effectively than Ritux-MTX cohort. Although preliminary, these results suggest Pompe immunomodulatory therapy should include Ritux and Rap to preclude the adverse effects of ERT.

26. Examining the roles of bacterial clamp loaders in DNA replication and repair

Douma LG^{1,2}, Bloom LB^{1,*}

¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

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

To increase DNA polymerase processivity during replication, sliding clamps anchor DNA polymerase to the DNA template. These sliding clamps are ring-shaped proteins that are loaded onto the DNA by clamp loaders which harness the power of ATP hydrolysis. In *E. coli*, the two clamp loaders are γ complex and τ complex, named for the γ or τ subunits in the complex that perform the required ATP hydrolysis. Both subunits are products of the same gene, *dnaX*. The smaller γ subunit is produced through a programmed ribosomal frameshift that results in a premature stop codon, while τ subunit is the full gene product. The τ subunit is essential for viability, coordinating interactions at the replication fork by its C-terminal end (absent in γ). It has been debated if γ complex has any real role. Temperature-sensitive cells that contained mutations in the *dnaX* gene were found to have defects in template-switching repair. Plasmids expressing γ , not full-length *dnaX*, restored the template-switching repair phenotype. Recombinant proteins harboring the mutations were expressed, and gel filtration analysis of these proteins show that the subunits aggregate to form a large, stable oligomer (equivalent to 16 γ and 13 τ subunits), whereas the wild-type DnaX subunits forms a tetramer in solution. Oligomerization may reduce the efficiency of DnaX2016 subunits assembling into clamp loader complexes *in vivo*. These mutants will serve as useful tools to define cellular functions of γ and τ complex.

27. Cancer progression is linked to altered chromatin structure at multiple scales

Druliner BR¹, Fincher JA¹, Sexton BS¹, Roche M^{2,3}, Lyle S^{2,3}, Dennis JH¹

¹Department of Biological Science, Florida State University, Tallahassee, FL

²Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA

³Department of Pathology, University of Massachusetts Medical School, Worcester, MA

Chromatin structure plays a fundamental role in regulating nuclear processes, and is a critical point of reference in the development and progression of cancer. We used nucleosome distribution and chromatin sensitivity assays to analyze the genome-wide chromatin structure of patients with different grades and stages of lung adenocarcinoma as compared to matched normal tissue. Here, we report that low-grade adenocarcinoma displays widespread nucleosome alterations compared to matched normal tissue at 50% of 886 genes studied. These altered nucleosome architectures are consistent among patients indicating that they may serve as important early cancer markers. We demonstrate that the nucleosomal alterations are driven by the underlying DNA sequence. Conversely, substantial disruptions in chromosomal sensitivity were seen in a patient with an aggressive tumor, while low grade and stage tumors are nearly identical to normal. Importantly, these chromatin sensitivity results are concordant with folding principles of the human genome determined by long-range interactions (Hi-C). Our data show that chromatin structure changes during the progression of lung adenocarcinoma. We have developed a model in which low-grade lung adenocarcinoma is linked to changes in nucleosome distributions, whereas aggressive tumors are linked to high order chromosomal changes. Moreover, we identified the underlying signals that explain altered nucleosome distribution profiles as features intrinsic to the DNA sequence itself. These results provide a foundation to identify a new class of chromatin-based diagnostic, prognostic, and therapeutic markers in cancer progression.

28. Studying the interaction between actin depolymerizing factor (ADF) and a novel ADF regulator

Emmanuel M¹, Grey PH¹, Oppenheimer DG^{1,2,*}

¹Department of Biology, University of Florida, Gainesville, FL

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

The actin cytoskeleton is required by all eukaryotic cells to carry out key functions such as cellular motility and intracellular trafficking of cellular components. The actin-binding proteins are important in controlling actin dynamics: the ability of the actin cytoskeleton to remodel by polymerization and depolymerization in response to cellular signals. One of the most important actin-binding proteins responsible for actin remodeling is actin depolymerizing factor (ADF). Earlier work in our lab led to the identification of a novel regulator of ADF in plants, called IRREGULAR TRICHOME BRANCH 3 (ITB3). In

addition to ITB3, *Arabidopsis* has 21 additional ITB3-like genes (ITB3L1-ITB3L21). In this study we will conduct tests on one of the ITB3-like family members, ITB3L04, to quantify binding to ADF. To do this, we will first construct multiple gene fusions, express and isolate the proteins of interest and then have the option of performing various tests of interaction such as pull-down assays and bio-layer interferometry. Studying the interaction between ITB3L04 and ADF will allow us to confirm its effect as a regulator of ADF and quantify its binding properties. This is the first step in characterizing this new pathway that contributes to actin dynamics.

29. Exome resequencing in a *Populus deltoides* association population

Fahrenkrog AM¹, Neves LG¹, Barbazuk WB^{1,2,*}, Kirst M^{1,3,*}

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

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

³School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Populus deltoides (eastern cottonwood) is a model species to study the biology and genetics of wood formation and biomass growth in woody, perennial plants. The availability of a reference genome and ease of genetic transformation and vegetative propagation make poplars especially suited to conduct association genetics studies to identify the genes determining bioenergy traits. In this study we are resequencing the coding fraction of the genome in 579 individuals of a *P. deltoides* association population. This "whole-genome" genotyping approach is based on the generation of genomic DNA libraries where each individual is identified by a barcode sequence. The exome is captured by hybridization of 12 multiplexed genomic DNA libraries with 204,108 oligonucleotide probes designed to capture 18,153 genes. This highly multiplexed, genome complexity reduction by sequence capture allows high-throughput characterization of a large number of individuals, and reduces the sequencing cost significantly. The information generated will be used to identify polymorphisms such as single nucleotide polymorphisms (SNPs) and copy number variants (CNVs), which will be used for population genetics and association studies.

30. *In utero* caffeine exposure alters DNA methylation patterns in adult hearts

Fang X¹, Xue H², Rivkees SA¹, Wendler CC¹

¹Child Health Research Institute, Department of Pediatrics, University of Florida, Gainesville, FL

²Division of Genetics, Department of Medicine, Brigham

and Women's Hospital, Harvard Medical School, Boston, MA

Our previous research demonstrated that caffeine exposure on embryonic day (E) 8.5 increased body weight, altered cardiac morphology and function in adult male mice. However, these adverse effects were not observed in adenosine A1 receptor knockout (A1AR^{-/-}) mice. Our hypothesis is that A1AR action mediates changes in DNA methylation patterns in mice exposed *in utero* to caffeine and that these changes in methylation lead to long-term effects in adult mice. To test this hypothesis, DNA Methylation 2.1M Deluxe Promoter Arrays (NimbleGen) were used to examine the methylation patterns of DNA isolated from left ventricles of the A1AR knockout line exposed to 20 mg/kg caffeine at E8.5 *in utero*. In A1AR^{+/+} mice, 4,896 hypermethylated and 2,823 hypomethylated regions were discovered in the left ventricles of the caffeine group compared to the normal saline group. In A1AR^{-/-} mice, 1,024 hypermethylated and 1,757 hypomethylated regions were found in the caffeine group. The differentially methylated regions (DMRs) in A1AR^{+/+} were mapped to 6,148 genes, 4,853 promoters, 4,111 primary transcripts, 816 CpG islands, and 98 miRNAs. Functional annotation clustering of genes revealed that many genes were involved in the development of hypertrophic cardiomyopathy and other heart diseases, which may explain our earlier findings of thickening of the left ventricle after *in utero* caffeine treatment. The methylation changes in several DMRs, *mef2c*, *ins2*, *tnnt2*, and *myh6*, were validated by bisulfite sequencing. In summary, *in utero* caffeine exposure caused DNA methylation changes in adult left ventricles and that these changes were possibly mediated by A1ARs.

31. Transcriptome-wide profiling of gene expression using single molecule real time (SMRT) sequencing on the Pacific Biosciences RS

Zhang Y¹, Panayotova N¹, Shanker S¹, Moraga D¹, Underwood J², Farmerie W^{1,*}

¹Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

²Pacific Biosciences Inc., Menlo Park, CA

Alternative RNA splice forms create dynamic blends of gene transcripts derived from a single gene locus. Microarrays, and more recently, highly parallel NGS DNA sequencing methods (RNA-Seq) are the most common methods for discovering alternative splicing events. Transcriptome characterization by DNA sequencing requires no prior knowledge of gene structure, and as such can be used to discover unknown gene expression motifs. Massively parallel, short read (ca ~ 100-150 bp) DNA sequencers (Illumina, Applied Biosystems, and Ion Torrent) use statistical analysis of reads mapped to a

reference genome to infer alternative exon usage and minor transcript frequency. While extremely powerful, short DNA sequence reads cannot infer the complete end-to-end structure of single, full-length mRNA transcripts. Pacific Biosciences (Pacbio) Single Molecule Real Time (SMRT) DNA sequencing technology generates DNA sequencing reads having a median length of 3,000-4,000 bp, which is sufficient to fully characterize the bulk of mature gene transcripts. Hence Pacbio SMRT cDNA sequencing can be used to examine the complexity of gene expression in ways heretofore not possible. Here we describe methods for cDNA preparation and sequence analysis using the exceptionally long reads of the Pacbio SMRT system. The UF ICBR study is part of a larger comprehensive multi-center project being conducted by the Association of Biomolecular Resource Facilities (ABRF) with support from the ABRF and Pacific Biosciences.

32. Identifying the activity of male-specific isoforms of *Drosophila fruitless*

Fear JM^{1,2}, Dalton JE^{3,4}, McIntyre LM^{2,*}, Arbeitman MN³

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

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

³Program in Neuroscience, Biomedical Science Department, Florida State University, Tallahassee, FL

⁴Department of Biological Sciences, University of Southern California, Los Angeles, CA

In *Drosophila melanogaster*, the transcription factor *fruitless* (*fru*) produces male-specific isoforms (Fru^M) required for courtship behaviors. *fru* is part of the somatic sex determination hierarchy. Fru^M isoforms are produced by alternative splicing at the 5' end of the transcript, while alternative splicing at the 3' end results in isoforms with different DNA binding domains. To understand differences in activity, each Fru^M isoform was overexpressed or perturbed in *fru*-expressing neurons. We identified differences in expression downstream of each Fru^M isoform and distinct differences in Fru^M activity between males and females. Genes that are significantly induced (repressed) by Fru^M are highly enriched (depleted) on the X chromosome in males, but not in females. Different isoform activities in *fru* may be explained by differences in DNA binding. Each Fru^M isoform has different DNA binding specificities. We examined enrichment of these motifs in genes induced (repressed) by different Fru^M isoforms. In males there was an enrichment of Fru^M binding motifs in genes that were significantly induced by overexpression of Fru^M , while genes repressed by Fru^M overexpression showed no such enrichment. This pattern indicates that Fru^M isoforms likely induce gene expression by acting as a transcription factor, while repressing gene expression indirectly through other factor(s). This pattern did not

exist in females, indicating a distinct difference in Fru^M activity between males and females.

33. Monitoring chromatin organization and regulation of the X-linked macrosatellite DXZ4 in human embryonic stem cells

Figueroa DM, Chadwick BP

Department of Biological Science, Florida State University, Tallahassee, FL

DXZ4 is an epigenetically regulated X-linked macrosatellite that adopts alternate chromatin states in primates, in response to X chromosome inactivation (XCI), the mammalian dosage compensation process. On the female inactive X chromosome (Xi) DXZ4 is arranged into euchromatin, whereas on the active X chromosome (Xa) the macrosatellite is packaged into heterochromatin. Most female human embryonic stem cells (hESCs) have already undergone XCI and possess a Xa and Xi. Thus, in female hESC, the DXZ4 chromatin is expected to resemble that of terminally differentiated female somatic cells. However, ChIP in female hESCs failed to show heterochromatin marks characteristic of DXZ4 on the Xa. Nevertheless, bisulfite sequencing revealed both DXZ4 alleles are extensively hypermethylated at CpG dinucleotides in independent female hESC. It is possible that 5-hydroxymethylcytosine (5-hmC), an epigenetic modification often associated with regulatory elements in hESCs, may be partly responsible for the CpG methylation detected at DXZ4. Preliminary data suggests this is indeed the case. Immediately proximal to DXZ4 is a putative promoter, IMPX, which drives expression of a long noncoding RNA (lncRNA) towards the array in hESCs, but is shut down by Polycomb on differentiation and remains silent in somatic cells. Polycomb signals and slight reactivation of IMPX activity is observed in male cancers that shift DXZ4 packaging from a heterochromatic to euchromatic state. IMPX silencing coincident with dynamic differentiation associated chromatin change at DXZ4 combined with transformation-associated deregulation make this lncRNA a good candidate for regulating DXZ4 chromatin states.

34. Escape from siRNA-induced silencing in an orthobunyavirus, Tensaw virus

Fitzpatrick DM, Maruniak JE*

Department of Entomology and Nematology, University of Florida, Gainesville, FL

The infectivity of populations of short-interfering RNA (siRNA) induced virus escape mutants from Vero cells was assessed in both vertebrate and mosquito cell cultures,

using a model orthobunyavirus, Tensaw virus (TSV). RNA interference is a major component of antiviral immunity in dipteran insects, including mosquitoes. Virus-specific siRNAs are being considered as a potential therapy to provide resistance to viral infection *in vivo*. However, due to the high mutation rate of RNA viruses, antiviral siRNA treatments often result in the emergence of escape mutants and a delayed reestablishment of infection. Yet, few studies have examined the infectivity profiles of viral escape populations, especially in arboviruses, where host alternation during arboviral transmission cycles constrains major genomic shifts. SiRNAs targeting the overlapping nucleocapsid/nonstructural protein coding regions of TSV significantly reduce viral titer in Vero cells for 48 h. However, viral escape is seen at 72 h, and this viral population establishes infection. According to tissue culture infective dose assays, viral supernatants collected at 120 hpi from siRNA-treated, TSV-infected Vero cells were significantly less infective in human HeLa cells than parent virus but did not show significantly different growth curves in Vero and mosquito (C6/36) cells compared to parent virus. Further experiments will utilize short-hairpin RNA expressing plasmid constructs comparable to siRNA treatments to prolong antiviral RNA expression, and examine escape mechanisms by quantitative PCR of immune genes, changes to particle-to-infectivity ratio, and genomic mutations associated with small RNA-induced selection.

35. EMOs - environmentally-modified organisms: controlling consumer-desired traits with light

Childers KS¹, Knitter E¹, Evans S¹, Pate D¹, Carone C¹, Folta KM^{1,2,*}

¹Horticultural Sciences Department, University of Florida, Gainesville, FL

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

Plants are anchored to the earth and have limited capacity to move. To meet this challenge plants have evolved intricate sensory networks that carefully monitor the ambient environment and guide adjustments in physiology as necessary. The light-sensing networks that regulate growth and development have been well defined in the model plant *Arabidopsis thaliana*. The effects of light on pigmentation, stature and composition are well understood. In this work we test the hypothesis that non-natural light conditions may be applied to expose latent genetic programs in plants that increase traits with significant horticultural value. This study analyzes 'microgreens', juvenile plants marketed as sprouts, subjected to a range of narrow-bandwidth light treatments. The results show that significant differences in pigmentation, leafiness, size and nutraceutical production may be achieved with simple, progressive treatments that

excite molecular and biochemical pathways leading to enhancement of consumer-desired traits. These techniques increase their attractiveness and nutritional quality for the consumer, adding value for the grower. Here we bridge basic and applied biology, implementing the well-established rules of photomorphogenesis to design the next generation of consumer produce.

36. Role of the proteasome in the phytopathogenic bacterium *Streptomyces scabies*

Francis I, Loria RL

Plant Pathology Department, University of Florida, Gainesville, FL

Unlike most prokaryotes, streptomycetes and other actinobacteria contain a proteasome similar to the 20S core particle found in eukaryotes. Proteasomes are essential to eukaryotic life but, since bacteria have specialized proteases and peptidases to degrade unneeded or damaged proteins, their function in bacteria remains to be elucidated. Nevertheless, mutation of the *mpa* gene, that encodes the proteasome-associated ATPase, renders *Mycobacterium tuberculosis* more susceptible to nitric oxide (NO) and reactive oxygen species (ROS) produced as a defense response by the macrophages, leading to an attenuated virulence phenotype in mice. The primary virulence determinant of the plant pathogen *S. scabies* is a family of nitrated dipeptide phytotoxins called thaxtomins; these novel molecules inhibit cellulose biosynthesis in expanding plant cell tissue in both monocots and dicots, at nanomolar concentrations. Thaxtomin A, the predominant form, is synthesized from L-phenylalanine and 4-nitro-L-tryptophan by the non-ribosomal peptide synthases TxtA and TxtB. A nitric oxide synthase (NOS) generates NO, some of which is incorporated into the tryptophan moiety specifically at position 4 of the indole ring by a unique cytochrome P450, TxtE. We hypothesized that the proteasome would have a role in defending the bacterium against the deleterious effects of NO on proteins. Experimental results show that a deletion mutant in the *mpa* homolog showed reduced growth and thaxtomin production under thaxtomin, and therefore NOS, inducing conditions, or when challenged with NO.

37. Deep sequencing of maize seedling transcriptomes to understand the impact of the ROUGH ENDOSPERM3 splicing factor

Gault CG¹, Mei W², Martin F¹, Fouquet R¹, Barbazuk WB^{1,2,*}, Settles AM^{1,3,*}

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

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

³Horticultural Sciences Department, University of Florida, Gainesville, FL

More acres of maize were harvested in 2011 than any other crop in the United States (USDA NASS, 2012). Because maize is such an economically important crop, a deeper understanding of the molecular mechanisms that govern maize development can lead to great benefits, such as improvements in maize yield and grain quality. We study the maize *rough endosperm3* (*rgh3*) mutant, which exhibits defective kernel and seedling development, in order to gain insight into maize development. *rgh3* is a hypomorphic allele of the U2AF³⁵-related protein (ZmURP). Prior characterization of the *rgh3* mutant indicates that the gene is required to repress cell proliferation and promote cell differentiation. In humans, URP is associated with the minor and major spliceosomes, but its role in maize unclear. To test whether *rgh3* affects mRNA splicing, we sequenced the *rgh3* and normal seedling transcriptomes. Twelve cDNA libraries were constructed from the roots and shoots of *rgh3* and normal seedlings, and the libraries were sequenced on the HiSeq 2000 platform. The run produced 149 million paired-end 100 bp reads that mapped to more than 35,000 genes. mRNA splicing in *rgh3* and normal tissues was analyzed at the mRNA isoform level using the Mosaik2, GSNAP, and Cufflinks software programs. Reverse transcription PCR validation of altered splicing in *rgh3* seedlings reveals that the *rgh3* mutant fails to efficiently splice introns from a select group of pre-mRNA transcripts.

38. How does *Pectobacterium carotovorum* promote proliferation of *Salmonella* in tomatoes?

George A, Noel J, Teplitski M*

Soil and Water Science Department, University of Florida, Gainesville, FL

In tomatoes infected with a soft rot plant pathogen *Pectobacterium*, *Salmonella* can grow to numbers 10 fold higher than in intact tomatoes without *Pectobacterium*. There are three hypotheses under investigation which may adequately explain this phenomenon. The first hypothesis is that pectinolytic activities increase nutrient availability allowing for increased growth of *Salmonella*. The second hypothesis is that rotting of the tomato increases the pH of the fruit thereby reducing the acid stress on *Salmonella* which may allow it to grow to higher final densities. Lastly, we also tested whether cell-to-cell signaling between *Salmonella* and *Pectobacterium* promotes increased proliferation of *Salmonella* within soft rots. A series of one week-long *in vitro* experiments have indicated that *Salmonella* grows to higher numbers in the presence of both polygalacturonic acid (PGA) and GA. Further experiments conducted with a deletion mutant of the

metabolic repressor KdgR, indicated a possible increase in growth when the repressor is non-functional. The role of *Pectobacterium* in this is not yet clear, leading us to the second hypothesis. These hypotheses are being tested using co-inoculation and fold change experiments. The co-inoculation experiment compares the fitness of type strain *Salmonella enterica* sv Typhimurium 14028 with the mutants that lack genes with functions in the acid tolerance response (ATR) or are involved in the use of PGA monomers. Rotting by *Pectobacterium* tends to increase fruit pH, which should allow the mutants to be more competitive due to a decrease of acid stress. Furthermore, this same process should make PGA oligomers and monomers more readily available.

39. Differences in AAV serotype dependent distribution and tropism within the CNS of wild type and mutant mice in the MPSIIIB mouse model

Gilkes JA, Bloom MD, Kolarich AR, Heldermon CD*

Division of Hematology and Oncology, Department of Medicine, University of Florida, Gainesville, FL

Mucopolysaccharidoses IIIB (MPSIIIB) is an autosomal recessive lysosomal storage disease caused by mutations in the gene N-acetyl-glucosaminidase (NAGLU). Defective NAGLU activity results in aberrant retention of heparan sulfate within lysosomes and progressive central nervous system (CNS) degeneration. Treatment options are limited by the need to overcome the blood-brain barrier and gain successful entry to the CNS. Here, we assess the relative effectiveness of various intracranial delivery methods and several adeno-associated virus vectors for gene therapy based treatment of this disease. Using the MPSIIIB mouse model, we perform a comparative study between intracranial six site (IC6) injections and thalamic injections of AAV-GFP serotypes -5, -8, -9 and -10, to determine the best treatment modality as determined by tropism and distribution. In both wild-type and MPSIIIB animals, neurons were primarily transduced by all serotypes, while microglia were the least transduced. Interestingly, the broadest distribution patterns and tropism were exhibited by AAV-8 in both IC6 and thalamic treatment groups. However, a greater number of cells and cell types were transduced in the IC6 treatment group. Considerably fewer cells were transduced in the hippocampus of MPSIIIB compared to wild-type mice. Transduction and distribution patterns of AAV-8 and AAV-9 were comparable in the thalamus of wild-type and mutant animals. Importantly, this data suggests that AAV-8 exhibits the better distribution and tropism with the IC6 method for use in future gene therapy studies. Further, the data strongly suggest that distribution and tropism are altered in MPSIIIB compared to wild-type brains.

40. Daxx and USP7: novel regulators of mitosis, taxane sensitivity and genomic stability

Giovinazzi S^{1,2}, Morozov VM^{1,2}, Reinhold WC³, Ishov AM^{1,2,*}

¹Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

²University of Florida Shands Cancer Center, Gainesville, FL

³Genomics and Bioinformatics Group, Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, MD

Daxx is a multifunctional protein that plays a pivotal role in both physiological and pathological cellular processes. We previously demonstrated that cells with low levels of Daxx have reduced sensitivity to taxanes, powerful chemotherapeutic agents, by persisting in a pro-metaphase block that allows cells to escape taxane-induced cell death. In this study we dissected the mechanisms of Daxx-dependent taxane resistance that also suggests function of this protein in mitotic progression. We show that Daxx interacts and cooperates with ubiquitin specific processing protease-7 (USP7) to regulate mitosis. We demonstrate that depletion of USP7 promotes stabilization of cyclin B, aneuploidy and mitotic anomalies, as it was previously observed for Daxx. We further demonstrate that USP7 depletion results in reduced stability of the mitotic E3 ubiquitin ligase Checkpoint with Forkhead and RING finger (CHFR). Consequently cells depleted by USP7 accumulate CHFR substrate, Aurora A kinase that has a crucial role in mitotic progression. We conclude that Daxx and USP7 are necessary to regulate proper execution of mitosis and their effects are at least partially mediated by CHFR and Aurora A kinase. Results from colony formation assay and *in silico* analysis show that USP7 expression negatively correlates with response to taxanes in cancer cell lines indicating that this protein can be used as a predictive factor for taxane response in cancer patients.

41. Fasting glucose loci associated with glucose response to antihypertensives – results from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study

Gong Y^{1,*}, Moore MJ¹, Karnes JH¹, McDonough CW¹, Wang Z², Langaee TY^{1,*}, Beitelshes AL³, Turner ST⁴, Chapman AB⁵, Gums JG^{1,6}, Bailey KR⁴, Boerwinkle E², Johnson JA^{1,6,*}, Cooper-DeHoff RM^{1,6} for the PEAR investigators

¹Department of Pharmacotherapy and Translational Research, University of Florida, Gainesville FL

²IMM Center for Human Genetics, University of Texas at Houston, Houston, TX

³Division of Endocrinology, Diabetes and Nutrition,

University of Maryland, Baltimore, MD

⁴Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN

⁵Renal Medicine Division, Department of Medicine, Emory University, Atlanta, GA

⁶Division of Cardiovascular Medicine, Department of Medicine, University of Florida, Gainesville FL

BACKGROUND: Genome-wide association studies have identified 36 loci to be associated with fasting glucose in nondiabetic white individuals. We assessed the association of these loci with plasma glucose response to atenolol (ATEN) and hydrochlorothiazide (HCTZ), antihypertensives that have been associated with adverse metabolic effects. **METHODS:** PEAR evaluated blood pressure and glucose response in hypertensive patients randomized to either ATEN or HCTZ monotherapy then the combination. Genotypes of 33 loci were obtained from Illumina 50K cardiovascular or Omni1M GWAS chips. Associations with ATEN or HCTZ induced glucose change was evaluated in 450 white patients using linear regression. Genetic risk scores based on number of glucose-increasing allele at each SNP were created to assess the collective genetic effect of nominally associated SNPs. Permutation tests were performed to validate the p values of the genetic risk scores.

RESULTS: Three SNPs (PROX1rs340874, ARAP1rs11603334 and PCSK1rs4869272) were nominally associated with ATEN-induced glucose increase. Two SNPs (MTNR1B rs10830963 and SLC2A2 rs11920090) were nominally associated with HCTZ-induced glucose increase. The corresponding genetic risk scores were strongly associated with glucose increase after ATEN monotherapy ($p=6.4E-6$, $\beta=2.1$) or HCTZ monotherapy ($p=8.3E-5$, $\beta=2.8$).

The permutation p values for both scores were < 0.0001 . **CONCLUSION:** Five fasting glucose loci were also associated with ATEN or HCTZ induced increase in plasma glucose. These data highlight that atenolol and HCTZ may constitute an environmental risk factor similar to those known to be associated with dysglycemia and diabetes.

42. Diverse roles of strigolactone signaling in maize architecture and the uncoupling of a branching-specific sub-network

Guan JC, Suzuki M, Klee HJ*, Koch KE*, McCarty DR*

Horticultural Sciences Department, University of Florida, Gainesville, FL

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

Strigolactones (SLs) control lateral branching in diverse species by regulating transcription factors orthologous to Teosinte branched1 (Tb1). In maize, however, selection for a strong central stalk during domestication is attributed primarily to the Tb1 locus, leaving the architectural roles

of SLs unclear. To determine how this signaling network is altered in maize, we first examined effects of a knock-out mutation in an essential SL biosynthetic gene that encodes Carotenoid Cleavage Dioxygenase 8 (CCD8), then tested interactions between SL signaling and Tb1. Comparative genome analysis revealed that maize depends on a single CCD8 gene (*ZmCCD8*), unlike other panicoid grasses that have multiple CCD8 paralogs. Function of *ZmCCD8* was confirmed by transgenic complementation of *Arabidopsis max4* (*CCD8*), and by phenotypic rescue of the maize mutant (*zmccd8::Ds*) using a synthetic SL (GR24). Analysis of the *zmccd8* mutant revealed a modest increase in branching that contrasted with prominent pleiotropic changes that include (1) marked reduction in stem diameter, (2) reduced elongation of internodes (independent of carbon supply), and (3) a pronounced delay in development of the centrally-important, nodal system of adventitious roots. Analysis of the *zmccd8*, *tb1* double mutant revealed that Tb1 functions in an SL-independent sub-network that is not required for the other diverse roles of SL in development. Our findings indicate that in maize, uncoupling of the Tb1 sub-network from SL signaling has profoundly altered the balance between conserved roles of SLs in branching and diverse aspects of plant architecture.

43. The role of flagella in virulence of *Vibrio vulnificus*

Gulig PA^{1,*}, Tucker M¹, Thiaville P^{1,2}, Rezaie N¹, Comiskey J¹

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

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

The bacterium *Vibrio vulnificus* is the leading cause of reported death from ingestion of seafood in the United States. It causes sepsis after ingestion of oysters and skin infection from contamination of wounds. Compromised patients can die within days of initial symptoms. The mechanisms by which this opportunistic pathogen causes such rapid death with extensive tissue destruction are mostly unknown, although some virulence factors have been identified. We used signature-tagged transposon mutagenesis to identify genes essential for virulence in a mouse model of infection. One insertion was in a gene involved with flagellar biosynthesis. *V. vulnificus* possesses six genes encoding the major protein subunit of flagella: flafBA and flacDE. Through deletional mutagenesis we determined that only flac and flae were necessary and sufficient to enable full virulence in our mouse model. Interestingly, when the bacteria encoded only flac, their growth was inhibited; they were non-motile, non-flagellated, and exhibited a more severe attenuation than if they expressed no flagella at all. This result suggests

that the Flac and Flae flagellins must interact to form intact and functional flagella. We also determined that flagellated bacteria that are non-motile or are defective at chemotaxis are attenuated for infection of mice. Ongoing studies are aimed at determining the structure/function relationships of the flagella in motility and virulence.

44. Advancing complex phenotype analyses through machine vision and computation

Gustin J¹, Settles AM^{1,*}, Miller N², Subramanian R², Yoshihara T², Baier J¹, Durham Brooks T³, Ferrier N², Spalding E²

¹Horticultural Sciences Department, University of Florida, Gainesville, FL

²Department of Botany, University of Wisconsin-Madison, Madison, WI

³Department of Biology, Doane College, Crete, NE

Phenotyping methods frequently limit functional genomic studies. Phenotypes are currently not studied with the same degree of sophistication or throughput as genomes or processes more proximate to the genome such as gene expression. Because phenotypes are such an important source of information about gene function, we are integrating multiple machine vision platforms to study seed and seedling phenotypes. Machine vision utilizes information contained in an image or other optoelectronic signal, such as reflectance spectroscopy, to collect quantitative measures of phenotypes. We are focusing on the interrelationships of maize kernel traits with seedling growth traits. We have developed a semi-automated pipeline to collect kernel weight, near infrared reflectance (NIR) kernel spectra, kernel color and 3D shape, and dynamic seedling root growth. Serial phenotyping on indexed kernels will provide greater statistical power to detect interrelationships that have a physiological basis. Computational workflows are being developed to automatically extract biologically relevant data from each phenotyping platform and to interrelate the machine collected data. The combination of high-throughput serial phenotyping and diverse genetic resources available in maize allow access to a wide variety of relationships between seed and seedling characteristics. As an example, preliminary studies show that aspects of the NIR spectra of a kernel are correlated with seedling root gravitropism response. We are also using this pipeline to identify quantitative trait loci (QTL) underlying the phenotypes and physiological relationships within the maize Nested Association Mapping (NAM) population.

45. The effects of silver nanoparticle administration on intestinal microbiota composition in C57/B7b16 mice

Gutalij A^{1,2}, Ukhanova M², Hinkley G³, Roberts S³, Yao J², Wang X², Mai V^{1,2,*}

¹Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

²Emerging Pathogens Institute, University of Florida, Gainesville, FL

³Clinical Toxicology Graduate Program, University of Florida, Gainesville, FL

Silver nanoparticles are currently being explored for their known antimicrobial properties and their potential as a novel medical tool. Several studies have been conducted using silver nanoparticles to investigate their effect on different organs such as the liver, brain, and kidneys. However, to date, few studies have analyzed their effects on the composition and diversity of gut microbiota. Here we investigate, in a mouse model, the hypothesis that administering silver nanoparticles will alter the microbiota composition.

46. The phytochrome gene family of strawberry (*Fragaria vesca*)

Hart JE¹, Brunings A¹, Folta KM^{1,2,*}

¹Horticultural Sciences Department, University of Florida, Gainesville, FL

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

Phytochromes are red/far-red light sensing pigments that inform plants of ambient light conditions. Phytochromes exist as a small multigene family in each plant species. Family members tend to have specific roles with some functional overlap. The phytochromes control horticulturally important processes such as control of flowering, plant stature and fruit/vegetable ripening. It is therefore of great interest to characterize the phytochromes of strawberry. Five phytochromes were identified in the diploid strawberry (*Fragaria vesca*) genome. One phytochrome closely resembles phyA, the far-red active photoreceptor in other species. Another resembles phyB, and another phyC. Two other similar sequences match best with phyE, and no phyD is present. Tissue-specific expression has been examined and shows that some family members are greatly enriched in specific plant parts. To test the functions of these molecules *in vivo* transgenic RNAi lines were created. Loss-of-function phenotypes reveal the roles for individual light sensors in the control of various biological processes. Currently we have many independent lines of the phyA RNAi plants. These plants exhibit differences in runnering, as phyA

RNAi plants produce more stolons than wild-type plants. After 16 h in intense far-red light wild-type plants show strong leaf inclination toward the light source. The phyA plants do not. These preliminary observations show some of the likely responses to far-red light. Future studies will examine seedling responses and the effects on flowering and fruit development, with the goal of understanding how strawberry light sensors contribute to these processes.

47. Optimization of a direct organogenesis system from the cotyledonary node explants of peanut (*Arachis hypogaea* L.)

Hsieh Y-F¹, Jain M², Wang J^{1,2,*}, Gallo M¹⁻³

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

²Agronomy Department, University of Florida, Gainesville, FL

³Current address: Molecular Biosciences and Bioengineering Department, University of Hawai'i at Mānoa, Honolulu, HI

Cultivated peanut (*Arachis hypogaea* L.) is the second most important food and oilseed legume in the world. Published direct regeneration protocols for transgenic improvement in peanut are highly genotype-dependent and have provided limited utility for peanut cultivar development. An alternative repetitive somatic embryogenesis system is suitable only for biolistic gene delivery and requires prolonged *in vitro* subculturing. We have optimized a facile and rapid method for obtaining direct shoot organogenesis from cotyledonary node (CN) explants excised from peanut seedlings germinated on cytokinin-supplemented Murashige and Skoog (MS) basal salt medium. A mass of multiple shoot-initials formed at the axillary bud region of CN explants derived from seedlings germinated in the presence of 4 mg l⁻¹ 6-benzylaminopurine (BAP) within three weeks of culture on the same cytokinin-containing medium. Adventitious shoots elongated rapidly over the next three weeks, and rooted efficiently on MS medium supplemented with 1 mg l⁻¹ α -naphthaleneacetic acid (NAA). Although adequate initiation of adventitious shoot buds was also observed from the CN explants in the presence of another commonly used cytokinin, thidiazuron (TDZ), the elongation of shoot buds was negatively impacted, thus compromising overall regeneration potential. Starting from mature seed, the described protocol yielded rooted plantlets within 12-15 weeks, in contrast to 15-18 months required for initiating and regenerating somatic embryogenic cultures. This procedure is highly reproducible and worked for several peanut genotypes tested.

48. Targeting nucleocytoplasmic shuttling mechanisms in esophageal cancers

Hu C, Wu L*

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

Esophageal squamous cell carcinoma (ESCC) is usually drug resistant tumor and has a dismal 5-year survival rate of <10%. Therefore, there is an urgent need to develop effective therapeutic approaches. In this study, we investigated whether CRM1 (XPO1), a major mediator of nuclear export, represents a therapeutic target in ESCC. CRM1 is responsible for the nuclear export of a variety of proteins including tumor suppressors and cell growth and survival regulators. CRM1 overexpression was correlated with poor prognosis in many cancers. Specific blockade of CRM1 caused selective cell growth suppression and apoptosis in several hematological malignancies and solid cancers, in part due to nuclear accumulation and activation of tumor suppressors. In this study, we evaluated CRM1 expression in ESCC cancer cells and determined whether CRM1 is required for maintaining cancer cell phenotypes of ESCC. We found that CRM1 is highly expressed in the majority of ESCC cancer cell lines. Blocking CRM1 expression and activity, through respective RNA interference and pharmacological inhibition with the CRM1 inhibitor LMB, reduced esophageal cancer cell growth and survival. The molecular action of CRM1 in ESCC cancer cells was further demonstrated by nuclear retention of the CREB coactivator CR2 and activation of CREB transcription when there was loss-of-function of CRM1. Therefore, CRM1 has an essential role in the maintenance of esophageal cancer cells, suggesting that CRM1 is a therapeutic target in ESCC. Our data thus warrant the preclinical investigations into the efficacies of newly developed small molecule CRM1 inhibitors with better specificity and less toxicity in blocking ESCC.

49. Development of an adeno-associated virus vector targeting the Keap1-Nrf2 pathway for dry age-related macular degeneration

Idefonso CJ¹, Jaime H², Lewin AS^{1,*}

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

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

Age-related macular degeneration (AMD) is a leading cause of blindness in developed countries. This ocular disease has two forms known as wet AMD (characterized by the growth of blood vessels in the retina) and dry AMD

(characterized by drusen accumulation). Currently there is no treatment available for dry AMD. This form of AMD has been associated with an increase in oxidative stress within the eye retinal pigmented epithelium. The Keap1-Nrf2 signaling pathway is involved in the modulation of antioxidant genes. In this pathway the repressor Keap1 binds and sequesters the transcription factor Nrf2 within the cytoplasm and targets it for degradation. The present study hypothesizes that the expression of antioxidant genes can be increased by knocking-down the levels of Keap1 and increasing the levels of Nrf2 therefore alleviating the oxidative stress. Several siRNA sequences that target the murine Keap1 were screened by co-transfection into HEK293T cells. One siRNA sequence which decreased the levels of murine Keap1 by 90% was selected. This sequence was embedded within the sequence of the pre-miRNA30 and cloned in an AAV plasmid between the Nrf2 cDNA and its poly-A signal. The co-transfection of this vector with the murine Keap1 in HEK293T cells resulted in a 50% knock down of Keap1. Future experiments will determine if this knock-down of Keap1 and overexpression of Nrf2 results in a significant increase of antioxidant genes and its correlation with cell viability under acute oxidative stress conditions. These *in vitro* studies will lead to future preclinical safety and efficacy testing in a model of dry AMD.

50. Exposure to the G protein-coupled estrogen receptor 1 (GPER) selective agonist G-1 results in elevated levels of vitellogenin in adult fathead minnows (*Pimephales promelas*)

Jayasinghe BS¹, Denslow ND^{1,2,*}, Sabo-Attwood T¹

¹Center for Environmental and Human Toxicology,
University of Florida, Gainesville, FL

²Department of Physiological Sciences, University of Florida, Gainesville, FL

Using a variety of human cell lines and animal models, several research groups have shown that G protein-coupled estrogen receptor-1 (GPER) (formerly known as GPR30) mediates 17 β -estradiol (E2) activation of signal transduction through non-genomic membrane initiated pathways. As the GPER is not well characterized in fish compared to mammalian organisms, we began studies to; (1) assess the tissue-specific expression of GPER in adult fathead minnows (FHM) (*Pimephales promelas*), (2) determine the effect of GPER activation on vitellogenin synthesis in adult FHM males and females using a selective GPER agonist (G-1) and (3) to study at molecular level whether the GPER antagonist, G-15, can block G-1 induced effects. Results from these studies show that GPER mRNA is detectable by qRT-PCR in many vital organs, and is most highly expressed in the brain followed by gall bladder, trunk kidney, intestine, liver, heart, ovary and muscle. Exposure to G-1 (5, 30 and 100 ng/L) for 48 hours

resulted in increased level of vitellogenin mRNA expression compared to vehicle control in both male and female FHM. These data suggest that GPER activation or inhibition result in activation of estrogen receptor (ER) down stream signaling or G-1 and G-15 directly activate ERs. To begin to understand alternate downstream targets of GPER, we are currently performing microarrays on various organs using G-1 exposed fish. Our findings to date suggest that control of vitellogenin synthesis likely involves both nuclear and membrane receptors that are sensitive to E2 activation.

51. Functional differentiation of AFL and VAL B3 transcription factors regulating the transition from embryo formation to germination

Jia H¹, Suzuki M^{1,2}, McCarty DR^{1,2,*}

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

²Horticultural Sciences Department, University of Florida, Gainesville, FL

The transition between seed and seedling is coordinately regulated by a family of plant specific B3 transcription factors (TFs). As master regulators of embryo development, AFL B3 TFs (ABI3, FUS3 and LEC2) and two HAP3 family TFs (LEC1 and L1L) positively regulate embryogenesis and embryo maturation, whereas VAL B3 factors (VAL1, VAL2 and VAL3) repress the AFL/LEC1 network during germination. AFL TFs recognize the Sph motif through their B3 DNA binding domain. The non-redundant roles of individual AFL and VAL B3 TFs in regulating the transition process have not been fully dissected. *val1 val2* double mutant seedlings exhibit embryonic callus. Seed specific genes in the AFL/LEC1 network are highly up-regulated in the *val1 val2* mutant. Therefore, a series of triple mutants (*val1 val2 abi3*, *val1 val2 fus3*, *val1 val2 lec2*, *val1 val2 lec1*, and *val1 val2 l1l*) were created in order to study the genetic interactions of AFL/LEC1 and VAL TFs. We found that *fus3* and *lec2* mutants can completely suppress the *val1 val2* embryonic seedling phenotype, while *abi3*, *lec1*, and *l1l* are partial suppressors. The seedling phenotype of the partially suppressed triple mutants is strongly dependent on the embryo developmental stage: seedlings rescued early in embryo development (7-9 DAF) uniformly show embryonic phenotypes; embryos rescued at mid stage (10-12 DAF) have intermediate phenotypes; while embryos rescued late (13-14 DAF) produce normal seedlings. Consistent with the genetic results, expression of FUS3 and LEC2 is elevated in seedlings of partially suppressed triple mutants that are rescued early compared to seedlings from late rescued embryos (12-14 DAF). Our results indicate that FUS3 and LEC2 expression is required for the embryonic seedling phenotype. These results highlight the distinctive

functional roles of AFL and VAL B3 TFs in regulating the progression from seed formation to germination.

52. Structural insights into *Bocavirus*

Kailasan S¹, Kantola K², McKenna R¹, Chipman P¹, Brown K³, Kapoor A⁴, Söderlund-Venermo M², Agbandje-McKenna M^{1,*}

¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

²Department of Virology, University of Helsinki, Helsinki, Finland

³Virus Reference Department, Health Protection Agency, London, United Kingdom

⁴Mailman School of Public Health, Columbia University, New York, NY

Human Bocavirus (HBoV1) was first isolated in 2005 from nasopharyngeal aspirates of children (< 2 years of age) with acute respiratory tract infections (ARTI). During 2008-10, several strains of HBoV, namely HBoV2-4, were identified in children suffering more frequently from gastrointestinal infections than ARTIs. HBoV1-4 occurrence has been reported in more than 17 countries worldwide with limited knowledge regarding its transmission and temporal dependence. HBoVs belong to the small, non-enveloped, ssDNA virus family of *Parvoviridae*. Other members of genus *Bocavirus* like Porcine Bocavirus (PBoV1), Bovine Parvovirus (BPV1), Gorilla Bocavirus (GBoV1), and Canine Minute Virus (CnMV or MVC) are known to cause similar respiratory and gastrointestinal infections in their respective hosts. These viruses encode two structural proteins called VP1 and VP2; the latter being the predominant protein of an icosahedral capsid (T=1). Our efforts to purify baculovirus/*Spodoptera frugiperda* expressed VP2 virus-like particles (VLPs) of HBoV1-4, BPV1, PBoV1, MVC and GBoV1, and determine high resolution three-dimensional structures of these viruses using X-ray crystallography and/or cryo-electron microscopy (cryo-EM) will be presented here.

53. Breeding genetically improved non-invasive elephantgrass hybrids for lignocellulosic biofuel production

Kannan B, Sollenberger L, Altpeter F*

Agronomy Department, University of Florida, Gainesville, FL

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

Elephantgrass (*Pennisetum purpureum*) is best adapted perennial grass and has the ability to produce large amounts of high quality forage biomass. The biofuels industry has identified elephantgrass as one of the

productive feedstocks for lignocellulosic biofuel production in the southern US. However, elephantgrass is listed as invasive in Florida by the Florida Exotic Pest Plant Council. Plant propagation and establishment of new elephantgrass plantings occurs through vegetative plant parts. Therefore, unlike most seeded crops, seed production is not necessary for elephantgrass biomass production and its suppression will significantly reduce its potential for invasiveness. To develop non-invasive genetically improved genotypes of elephantgrass we made interspecific hybridization between elephantgrass ($2n=4x=28$) and pearl millet ($2n=2x=14$) to result in drought tolerant, high biomass with male and/or female sterile triploid hybrids ($2n=3x=21$) which will eliminate production of wind dispersed seeds. Tall, stress tolerant elephantgrass parents were chosen to generate interspecific hybrids with good productivity and persistence. Pearl millet (AA genome) multiline population with A4 CMS was pollinated with allopolyploid napiergrass (A'A'BB) genotypes Merkeron and N51. We produced more than 3000 triploid, interspecific hybrids between elephantgrass and pearl millet. Phenotypic variability present in these hybrids allowed selecting lines which produced similar or higher biomass amounts as the seed producing elephantgrass cultivar Merkeron. We will present data describing the biomass yield and related traits of interspecific hybrids evaluated in replicated field trials.

54. RcrRPQ affects development of genetic competence in *Streptococcus mutans* through multiple pathways

Kaspar J, Ahn S-J, Burne RA

Department of Oral Biology, University of Florida, Gainesville, FL

Streptococcus mutans displays complex regulation of the development of natural genetic competence. The central regulator in the development of competence is ComX, an alternative sigma factor that activates late competence genes. Expression of comX is activated by a small hydrophobic peptide (ComS) that is secreted, reinternalized and bound by ComR, the proximal regulator of comX. Previously, the rcrRPQ operon, which encodes a MarR transcriptional repressor and a pair of co-transcribed ABC transporters, has been shown to link stress tolerance, (p)ppGpp production and genetic competence in *S. mutans*. Notably, an rcrR-polar ($\Delta 835P$) mutant is hyper-transformable, but the rcrR-non-polar ($\Delta 835NP$) mutant cannot be transformed; consistent with the inability of the latter strain to activate late competence genes. To explore these phenotypes further and dissect the mechanisms by which RcrRPQ affect competence development, we show that overexpression of comS in the rcrR mutant backgrounds impacts growth

inhibition by competence stimulating peptide (CSP) in complex medium, but does not affect the transformation phenotypes. In contrast, in a comX overexpressing background, the rcrR-non-polar mutant is able to be transformed and late competence genes are activated. Based on these results and our prior work, we conclude that RcrRPQ govern ComX-dependent activation of late competence genes, that hyper-expression of comX can overcome the effects of the non-polar rcrR mutation, and that levels of effectors that are inhibitory to the activity of ComX are modulated by the RcrPQ transporters.

55. Variability in *Wolbachia* prevalence among human louse (*Pediculus humanus*) populations

Kassu G, Ascunce MS, Reed DL*

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

Wolbachia is a genus of endosymbiotic α -proteobacteria infecting a wide range of arthropods and filarial nematodes. *Wolbachia* can manipulate host reproduction resulting in reproductive abnormalities such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing, thus affecting biology, ecology and evolution of its hosts. In addition, *Wolbachia* can affect host mitochondrial DNA evolution, because of the linkage between *Wolbachia* and associated mitochondrial haplotypes, and thus confound host phylogeny based on mtDNA. Previous research has shown that human lice have *Wolbachia* infections; therefore, we wanted to know whether *Wolbachia* could influence the divergence of mitochondrial haplotypes in human lice. We surveyed 59 human lice collected from 7 sites throughout the Americas. Nine *Wolbachia* primers targeting seven loci were evaluated in this study including fbpA, 16-S2 and coxA. None of the PCR primers tested in these populations of human lice yielded PCR products related to *Wolbachia*. Therefore, we conclude that *Wolbachia* are not present in the samples surveyed here, or that they are present at undetectable levels. This does not exclude the possibility that some other populations do harbor infections. It may be possible that *Wolbachia* in human lice follow a 'most-or-few' infection pattern within the species, such that populations have either a very high (>90%) or very low (<10%) prevalence of *Wolbachia*. We suggest that metapopulation dynamics and environmental fluctuations may partially explain the abrupt difference in prevalence reports of *Wolbachia* in human lice.

56. Expression of alpha and beta subunits of colony-stimulating factor 2 receptor during preimplantation embryonic development in the cow

Khan FA, Moss JI, Hansen PJ*

Department of Animal Sciences, University of Florida,
Gainesville, FL
DH Barron Reproductive and Perinatal Biology Research
Program, University of Florida, Gainesville, FL

Colony-stimulating factor 2 (CSF2) is an important cytokine regulating preimplantation embryonic development in several mammalian species. Studies on bovine *in vitro* produced embryos have shown that addition of CSF2 to culture medium improves development. In hematopoietic cells, CSF2 signaling involves binding of the ligand to the α -subunit of CSF2 receptor (CSF2RA) and downstream signaling by the β -subunit (CSF2RB). However, studies in mice and humans have shown that β -subunit is absent in preimplantation embryos. There is paucity of information on expression of *CSF2RA* and *CSF2RB* in bovine embryos. The present study was, therefore, conducted to evaluate expression of *CSF2RA* and *CSF2RB* during preimplantation stages of development. Embryos cultured *in vitro* were harvested at 2 cell [32-40 hours post insemination (hpi)], 3-4 cell (48 hpi), 5-8 cell (48 hpi), 9-16 cell (72 hpi), morula (120 hpi), and blastocyst stages (168 hpi). Matured oocytes were also collected. RNA was extracted, reverse transcribed, quantified by real-time PCR, and product was visualized using 1.5% (w/v) agarose gel electrophoresis. Five replicates, each involving 25-30 oocytes/embryos at each stage, were used. Leukocyte RNA was used as a positive control. *CSF2RA* was expressed at all stages of development examined while *CSF2RB* was not expressed at any stage. Expression of *CSF2RA* varied with development ($P < 0.0001$), with maximum expression at the 9-16 cell stage (i.e., coincident with embryonic genome activation). These findings suggest that CSF2 signaling in bovine embryos may occur independently of the β -subunit of CSF2R and through an alternate cytokine receptor coupled signaling pathway.

57. Role of glutaredoxins in high temperature stress tolerance in *Oryza sativa*

Krishnamurthy A, Nichols G, Rathinasabapathi B*

Horticultural Sciences Department, University of Florida,
Gainesville, FL

In the present global warming scenario, development of crop varieties with improved tolerance to high temperature stress is an important goal for modern agriculture. High temperature stress is a major limitation for productivity in cereals. Rice being a major food crop in the world, serves as an excellent model crop to test biotechnologies to improve heat tolerance in cereals. The objective of our project is to evaluate the role of glutaredoxins in imparting oxidative and heat stress tolerance in rice. We found heat shock induced glutaredoxin specific activity in young rice seedlings. In a previous study, PvGrx5 cDNA encoding

a glutaredoxin was isolated from an arsenic hyper-accumulator fern, *Pteris vittata*. Transgenic expression of PvGrx5 in *Arabidopsis* resulted in improved whole plant tolerance to high temperature stress. To test whether a similar approach can be used to improve heat tolerance in rice, we generated stable transformants of *Oryza sativa* cv. Nipponbare expressing PvGrx5 via agrobacterium-mediated transformation. We also generated transgenic rice lines over-expressing *Arabidopsis* glutaredoxins for comparison. The expression of glutaredoxin transgenes were confirmed by RT-PCR and enzyme activity assays. PvGrx5 over-expressing lines and control lines were evaluated for high temperature stress tolerance by using a seedling bioassay. PVG19-2 line over-expressing PvGrx5 was significantly less inhibited to a heat stress treatment compared to the vector control line VC19-6.

58. Development of an open urethra: comparative external genitalia development in the mouse and turtle

Larkins C¹, Cohn MJ^{1-3,*}

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 congenital defect characterized by an ectopic opening of the urethra on the ventral side of the penis. It is one of the most common congenital defects and can be caused by both environmental and genetic factors, although, despite its prevalence, it is still unclear how hypospadias develops embryonically. Interestingly, mammals are the only group of amniotes that normally develop a closed urethra, while all other groups have an open urethral groove or sulcus. Therefore, hypospadias in mammals may be interpreted as a reversion to a more primitive condition. We are taking advantage of this evolutionary characteristic as a means to identify genes and processes that are important for the generation of a closed urethra by comparing external genitalia development in mice and turtles. We show that during normal embryonic development of the red-eared slider turtle, *Trachemys scripta*, the urethra is initially closed but later opens along the entire ventral side of the penis generating the urethral groove. Importantly, we show that the morphology, with the exception of the open urethra, and transcription of several genes within the turtle penis are highly similar to that in mammals. Interestingly, our comparative analysis has led to the identification of the Bmp and Fgf signaling pathways as potential regulators of urethral opening in both mice and turtles.

59. Effects of mode of transmission on virulence

Larsen JS, Bermudez AL, Culbreth EM, Abbas R, Wayne ML*

Department of Biology, University of Florida, Gainesville, FL

Is virulence of parasites evolvable? Multiple theories have been proposed to answer this question, such as the trade-off hypothesis, which proposes an optimal balance between virulence's positive effects on transmission and its negative effects on the host organism. This experiment seeks to study the effects of mode of vertical transmission on virulence, and hence its evolution. We use the Sigma virus/*Drosophila melanogaster* system. Three different vertical transmission modes were studied: uninfected male with infected female, infected male with uninfected female, and infected male with infected female. A control was also included (neither parent infected). Data were collected for egg to adult viability, development time, fecundity, and body size, all of which are components of fitness in *Drosophila melanogaster*, as well as for viral titer (load) using quantitative PCR. This data could serve to increase our understanding of the evolution of virulence by quantifying the effects of mode of transmission. The results could also be applied to other vertically transmitted viruses carried by dipteran vectors that infect human hosts.

60. Novel regulatory genes affect thaxtomin production and pathogenesis in *Streptomyces scabies*

Laskaris P, Francis IM, Loria RL

Plant Pathology Department, University of Florida, Gainesville, FL

Potato scab disease, caused by *Streptomyces scabies* and other streptomycetes, has a global distribution and causes significant economic losses. Production of thaxtomin A, a nitrated dipeptide phytotoxin that potentiates virulence, is regulated by the *txtR* gene. Additional regulatory genes likely play a role in the production of thaxtomin, or in the expression of other genes involved in pathogenicity; their discovery may suggest new methods of combating potato scab. A total of 21 previously unstudied regulatory genes, that are conserved among the plant pathogens *S. scabies*, *S. turgidiscabies* and *S. ipomoeae*, were deleted in *S. scabies* 87-22 and mutants were evaluated for production of thaxtomin in oat bran broth (OBB). One knockout was reduced in thaxtomin production, while two overproduced thaxtomin. RT QPCR revealed that the thaxtomin biosynthetic cluster was over expressed in all three knockouts, though other pathogenicity genes were unaffected. Two of the regulators belong to the GntR

family, a member of which is a global developmental regulator. Their effect may be due to the link between development and secondary metabolism. The third regulator belongs to the ribonuclease III family, a member which is a global regulator of secondary metabolism; the homolog in *S. scabies* may have a similar role. The overexpression of thaxtomin biosynthetic genes in the knockout with reduced production indicates that the regulation of thaxtomin production is a complex process.

61. Comparative analysis of the limb-specific enhancer of the Sonic hedgehog (Shh) gene and its relation to vertebrate limb evolution

Leal F¹, Cohn MJ^{1-3,*}

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

Digit and limb loss have occurred many times during vertebrate evolution, however the underlying molecular mechanisms are largely unknown. Pythons lack forelimbs and have only rudimentary pelvic girdles and femora. We showed previously that the arrest of python hindlimb bud development is associated with absence of an AER and lack of Shh expression. However, Shh can be induced in python limb bud cells grafted under a chick AER. Shh expression in the ZPA is regulated by a long-range, limb-specific enhancer known as the ZRS. Previous studies have been unable to identify a ZRS in limbless reptiles, linking loss of this enhancer to the evolution of limblessness. We isolated the ZRS-containing region from the basal snake *Python reticulatus* and identified a well-conserved ZRS domain. We therefore revisited whether Shh is expressed at very early stages of python hindlimb development, and found a transient, posterior domain of Shh in early limb buds. Interestingly, the Shh expression domain extends further anteriorly than the typical ZPA pattern; consistent with our finding that polarizing potential is widespread in python limb buds. Together, these results suggest that the genomic regulatory elements required for digit development have been retained during the 100+ million years of snake evolution.

62. BLIMP1 binds the ZRS enhancer and negatively regulates Shh expression in the limb bud

Lee C, Harfe BD*

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

The hedgehog signaling protein SHH is responsible for patterning many different types of tissues during organogenesis. In the limb buds, Shh is expressed in the zone of polarizing activity (ZPA) located in the posterior mesenchyme of the developing limb bud and is required for normal limb patterning. Expression of Shh in the limb bud is controlled by the limb-specific ZPA regulatory sequence (ZRS). A few positively acting factors have been identified that can activate expression from this enhancer, however it has been unclear how ZPA-specific expression is achieved since the positively acting factors are all expressed more broadly than Shh. We uncovered a negative regulator of Shh expression, BLIMP1. Blimp1 is expressed in cells proximal to the ZPA. BLIMP1 protein binds three sites within the ZRS enhancer and is required for repressing Shh expression in proximal posterior cells in the limb bud. Our data suggests that BLIMP1 is a negative regulator of Shh expression in cells that will later migrate into the ZPA and express Shh.

63. Measures of sequence diversity for sizing taxon sampling

Lee HW^{1,2}, Brocchieri L^{2,*}

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

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

With the increasing size of genome, gene and protein databases, evaluation of the size of the available samples for different taxa, of how densely each taxon is represented, and of how much diversity is represented in each sample, is becoming increasingly relevant for optimization of sampling strategies. Thorough taxon sampling is crucial in increasing accuracy of phylogenetic tree reconstructions and for estimating patterns of functional differentiation. Taking into consideration the evolutionary relationships among sampled sequences, we developed several metrics based on alignments and phylogenetic trees and estimated size and diversity of available samples of bacterial phyla. We expect our metrics are useful for constructing balanced samples of phylogenetic groups.

64. Combinatorial splicing regulation by muscleblind-like proteins in development and disease

Lee K-Y^{1,2}, Li M¹, Manchanda M¹, Chamberlain C³, Charizanis K¹, Mohan A¹, Hong H¹, Shiue L⁴, Ares M⁴, Swanson MS^{1,*}

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

²Department of Neurology, Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan

³Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN

⁴RNA Center, Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA

The muscleblind-like (MBNL) proteins regulate the alternative splicing of hundreds of genes during development and loss of MBNL splicing activity is a key pathogenic feature underlying the neuromuscular disease myotonic dystrophy (DM). Since the MBNL gene family consists of three paralogs, which vary in temporal and spatial tissue expression patterns, we have proposed that the multi-systemic disease manifestations of DM result from combinatorial loss of MBNL proteins. To test this hypothesis, we have generated conditional Mbnl1, Mbnl2 and Mbnl3 knockout (KO) mice and studied the effects of single and multiple Mbnl knockouts on embryonic and postnatal development. Splicing microarrays, RNA-seq and HITS-CLIP analyses demonstrate that the Mbnl1 and Mbnl2 proteins regulate hundreds of splicing events in skeletal muscle and the brain, respectively, via the recognition of a similar RNA core sequence motif, YGCY. While Mbnl1 KOs develop DM-associated muscle and eye pathologies, loss of Mbnl2 expression does not significantly affect muscle function but instead has a profound impact on the brain with spatial learning/memory deficits on a hippocampal-dependent task, a decrease in NMDAR synaptic transmission, impaired hippocampal synaptic plasticity and REM sleep abnormalities. Mbnl1^{-/-}; Mbnl2^{-/-} double KOs are embryonic lethal but Mbnl1^{-/-}; Mbnl2^{+/-} are viable and show enhanced pathological phenotypes in skeletal and cardiac muscle suggesting that Mbnl2 partially compensates for Mbnl1 loss in these tissues. The molecular basis of this compensatory function will be discussed.

65. Proteomic analysis of high NaCl-induced changes in abundance of nuclear proteins

Li J^{1,2}, Ferraris JD², Yu D², Singh T², Izumi Y², Wang G², Gucek M², Burg MB²

¹Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

²Systems Biology Center, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD

Mammalian cells are normally stressed by high interstitial NaCl in the renal medulla and by lesser elevation of NaCl in several other tissues. High NaCl damages proteins and DNA and can kill cells. Known protective responses include nuclear translocation of the transcription factor NFAT5 and other proteins. In order to better understand the extent

and significance of changes in nuclear protein abundance, we extracted nuclear and cytoplasmic proteins separately from HEK293 cells and measured by LC-MS/MS (iTRAQ) changes of abundance of proteins in the extracts in response to high NaCl at three time points: 1 hour, 8 hours, and adapted for two passages. We confidently identified a total of 3190 proteins. 163 proteins changed significantly at least at one time point in the nucleus. We discerned the biological significance of the changes by Gene Ontology and protein network analysis. Proteins that change in the nucleus include ones involved in protein folding and localization, microtubule-based process, regulation of cell death, cytoskeleton organization, DNA metabolic process, RNA processing, and cell cycle. Among striking changes in the nucleus, we found a decrease of all six 14-3-3 isoforms; dynamic changes of "cytoskeletal" proteins, suggestive of nucleoskeletal reorganization; rapid decrease of tubulins; and dynamic changes of heat shock proteins. Identification of these changes of nuclear protein abundance enhances our understanding of high NaCl-induced cellular stress, and provides leads to previously unknown damage and protective responses.

Poster Session II, Posters no. 66 – 130
Thursday, November 29, 11:45 a.m. – 1:30 p.m.

66. Unexpected role of Rad51 in *Babesia bovis* antigenic variation

Mack E, Xiao Y, Allred DR*

Department of Infectious Diseases and Pathology,
University of Florida, Gainesville, FL

Babesia bovis is an intraerythrocytic protozoan parasite of cattle that causes pathology sharing many parallels with falciparum malaria. *B. bovis* undergoes antigenic variation of at least one virulence factor, VESA1, using a segmental gene conversion-type mechanism. Rad51 is a canonical DNA repair protein that plays a major role in gene conversion-mediated repair in most organisms. To determine whether Rad51 similarly plays a key role in the segmental gene conversion process associated with antigenic variation in *B. bovis*, we created a Rad51 knockout clonal line, *rad51*^{ko1}, using a double crossover gene replacement strategy. The success of gene replacement was confirmed by PCR and Southern blotting, and the lack of *rad51* transcripts by RT-PCR. Preliminary experiments with *B. bovis* *rad51*^{ko1} revealed several unexpected results. First, no apparent defects in parasite growth rate or morphology were observed following recovery from transformation with the plasmid used for targeted gene disruption, despite the significance of Rad51 to DNA replication. Perhaps more significantly, there was no difference in the survival rate of *B. bovis* *rad51*^{ko1} compared with the CE11 parental line after exposure to DNA-damaging γ -irradiation. Finally, the absence of Rad51

appears to have a stabilizing effect on the locus of active ves transcription (LAT), which encodes the VESA1 protein. This result raises the possibility that in wild type parasites Rad51 may serve to target actively transcribed ves genes for dsDNA breaks, focusing antigenic variation-associated gene conversion mutational events on this family.

67. Genome-wide effect of the *mop1-1* mutation on chromatin structure in maize

Madzima TF¹, Fincher JA¹, Vera DL¹, Dorweiler JE², Bass HW¹, Dennis JH¹, McGinnis KM¹

¹Department of Biological Science, Florida State University, Tallahassee, FL

²Department of Biological Sciences, Marquette University, Milwaukee, WI

The *mop1* gene encodes a putative RNA-dependent RNA polymerase required for several examples of epigenetic regulation of endogenous genes and transgenes in maize. Its orthology to the *Arabidopsis* RDR2 and pleiotropic mutant phenotypes suggest that Mop1 influences chromatin structure via an RNA-dependent silencing pathway and plays an important role in maize development. Nucleosome occupancy and higher order chromatin structure have been characterized using micrococcal nuclease (MNase) protection and sensitivity assays. We developed two types of Nimblegen microarray-based assays to characterize chromatin structure responses in maize. One assay (nucleosome occupancy) produces data on promoter/TSS architecture. The other assay (nuclease sensitivity) produces genome-wide data on global chromatin accessibility. Here we describe new findings from our chromatin accessibility assay used to characterize the global changes in chromatin structure in response to the *mop1-1* mutation. Using the *mop1-1* mutation segregating in a B73 genetic background we observed two large trends in genomic response, an increase in chromatin accessibility in gene-rich areas, and an intriguing decrease in accessibility around centromeres, which are enriched in LTR retroelements. These observations are consistent with Mop1's proposed role in global regulation of chromatin structure. This work demonstrates the utility of genome-wide, microarray-based nuclease sensitivity assays to determine changes in chromatin structure.

68. *Foxa1* and *Foxa2* are required for intervertebral disc formation

Maier JA, Lo YT, Harfe BD*

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

The intervertebral disk (IVD) is composed of the collagenous annulus fibrosus, which surrounds an inner, gel-like nucleus pulposus (NP). The NP is derived from the notochord. Disc degeneration results in back pain, for which effective treatments are limited. Uncovering the mechanisms of IVD development and degeneration could lead to improved treatments for back pain. The forkhead box (*Fox*) genes are expressed in the early embryo and function in development and post-natal life. Both *Foxa1* and *Foxa2* are expressed in the notochord, but due to embryonic lethality of the *Foxa2* null mouse, their role in IVD formation has not been characterized. We removed *Foxa2* from the notochord using a conditional *Foxa2* allele under control of the *ShhcreER¹²* allele, allowing us to inactivate *Foxa2* where Sonic hedgehog was expressed. This mouse was combined with the *Foxa1* null allele to make double knockouts. Histology and fate-mapping with the *Rosa26* reporter allele were also done. Mice null for *Foxa1* and lacking *Foxa2* in Shh-expressing cells have a severely deformed NP and a shortened tail. Fate-mapping in these mice suggests defects in the notochord to NP transition in *Foxa1*; *Foxa2* knockout mice, cell death studies indicate cells of the posterior midline and somites are dying. Hedgehog signaling is decreased in the double mutants, and the neural tube is abnormally patterned. Study of the role of *Foxa* family action in IVD development may provide insight into new treatments for disk degeneration.

69. Structure-function analysis of the RGH3 splicing factor in maize

Martin F, Fouquet R, Fajardo D, Gault C, Settles AM*

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

Alternative RNA splicing produces multiple mRNA species from individual genes increasing protein diversity and regulating gene expression. About 42% of intron containing genes in plants are alternatively spliced, yet little is known about the biological function of alternative splicing. The rough endosperm3 (*rgH3*) mutant causes lethal seed and seedling developmental defects and encodes a U2AF35 related protein (ZmURP). U2AF35 identifies splice acceptor sites during RNA processing by interacting with U2AF65 and other SR proteins. Rgh3 is alternatively spliced, producing at least 19 different isoforms. Only one variant is predicted to encode a full-length URP ortholog containing an N-terminal acidic domain followed by two zinc fingers flanking a UHM domain and a C-terminal RS-like domain. Several Rgh3 splice variants produce truncated proteins missing one to several domains. GFP fused to full-length RGH3 localized to the nucleolus and nuclear speckles. Functional analysis with endogenous truncated protein variants and artificial domain

deletions fused to GFP showed that the acidic domain contains a nuclear localization signal and the RS-like domain is important for its localization to speckles. These results suggest Rgh3 is regulated by splicing to produce truncated variants that render the protein unstable and excluded from spliceosomal speckles.

70. Differential regulation of *Salmonella typhimurium* genes involved in O-antigen capsule production and their role in persistence within tomatoes

Marvasi M, Noel JT, Teplitski M*

Soil and Water Science Department, University of Florida, Gainesville, FL

Enteric pathogens including non-typhoidal *Salmonella* and enterovirulent *E. coli* are capable of persisting within plants and multiplying within fruit tissues consumed by their animal hosts. Yet, little is still known about the mechanisms that *Salmonella* uses to colonize and persist within plants including crops important to humans. This study identified the *Salmonella yihT* gene (involved in synthesis of the O-antigen capsule) as involved in persistence in immature tomatoes. Deletion of *yihT* reduced competitive fitness of *S. enterica* sv Typhimurium by over 3 logs. The *yihT*TRIVET reporter was strongly resolved in immature tomatoes. Expression of *yihT* in mature tomatoes (regardless of their color) was low, and *yihT* did not affect competitive fitness within mature fruit. Fitness tests within tomato rin, Nr mutants (defective in synthesis and perception, respectively, of the plant hormone ethylene essential in ripening and defense responses) suggest a role for functional ethylene-mediated signaling in the persistence of *Salmonella* within tomato fruit.

71. Is mutation rate fitness-dependent? Fitness decay and mutation rate in mutation accumulation lines of the nematode worm *Caenorhabditis elegans*

Matsuba C¹, Salomon MP^{1,2}, Ostrow D^{1,3}, Sylvestre L¹, Ungvari-Martin J¹, Tabman B¹, Lewis S¹, Baer CF^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Section of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California, Los Angeles, CA

³Molecular Pathology and Genomics Laboratory, Children's Hospital Los Angeles, Los Angeles, CA

Disparate lines of evidence suggest that physiological condition may affect mutation rate, which in turn suggests that the mutation rate may depend on the underlying

fitness of the organism. To investigate that possibility we chose five high-fitness and five low-fitness “first-order mutation accumulation” (1° MA) lines that had previously evolved under extremely relaxed selection ($N_e \approx 1$) for 250 generations subsequent to divergence from a common ancestor. Forty-eight replicate “second order MA” (2° MA) lines were initiated from each 1° MA progenitor and allowed to evolve under relaxed selection ($N_e \approx 1$) for an additional 150 generations. On average, the rate of decay of fitness of the 2° MA lines did not differ between high-fitness and low-fitness lines, but several non-significant trends are consistent with low-fitness lines having greater mutation rate. Re-sequencing of the genomes of 2-4 2° MA lines and their 1° MA progenitors revealed that the base-substitution mutation rate does not differ between high and low fitness groups, but mutations in the mtDNA genome may partially explain the observed fitness patterns.

72. Assessment of atenolol induced HDL change in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study

McDonough CW^{1,2}, Gillis NK^{1,2}, Alsultan A^{1,2}, Buford TW³, Chang SW^{1,2}, Lang JE⁴, Shahin MHA^{1,2}, Suzuki-Kawaguchi M^{1,2}, Gong Y^{1,2,*}, Langa TY^{1,2,*}, Gums JG⁵, Chapman AB⁶, Turner ST⁷, Cooper-DeHoff RM^{1,2,8}, Johnson JA^{1,2,8,*}

¹Department of Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL

²Center for Pharmacogenomics, University of Florida, Gainesville, FL

³Department of Aging and Geriatric Research, University of Florida, Gainesville, FL

⁴Division of Pulmonary and Sleep Medicine, Nemours Children’s Hospital, Orlando, FL

⁵Department of Community Health and Family Medicine, University of Florida, Gainesville, FL

⁶Renal Medicine Division, Department of Medicine, Emory University, Atlanta, GA

⁷Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN

⁸Division of Cardiovascular Medicine, Department of Medicine, University of Florida, Gainesville, FL

We sought to identify novel pharmacogenomic markers for HDL response to atenolol in patients with mild to moderate hypertension. We genotyped 768 hypertensive patients from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study on the Illumina Human CVD Beadchip. During PEAR, patients were randomized to receive atenolol or hydrochlorothiazide first as monotherapy and then as combination therapy. Blood pressure and additional laboratory measures, such as HDL, were evaluated at baseline, after monotherapy, and after combination therapy. This analysis focused on patients treated with atenolol during monotherapy. Association

with atenolol induced HDL change was evaluated in 232 Caucasian patients and 152 African-American patients using linear regression. Top regions were selected as those with a P-value $< 10^{-3}$ in one race, and a nominal signal (P-value < 0.05) in the gene region in the other race. We identified 26 regions that replicated across races. Most interesting were seven gene regions with prior associations with HDL or other metabolic traits, or functional implications in the lipid pathway: *GALNT2*, *STARD3NL*, *ABCB1*, *LEPR*, *FTO*, *LRP5*, *LIPC*, and *ESR1*. In conclusion, we identified multiple gene regions associated with atenolol induced HDL change that replicated across race groups, several with functional implications or prior associations with HDL.

73. A dual transgene system as an approach to study DNA methylation and silencing mechanisms in maize

McGivern J¹, Irsigler A², Madzima TF¹, Stroud L¹, McGinnis KM¹

¹Department of Biological Science, Florida State University, Tallahassee, FL

²Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasília, Brasil

In maize and other organisms, transcriptional gene silencing acts to prevent gene expression. This epigenetic pathway can be mediated by siRNAs acting in association with directed DNA methylation. Transgenes have also been demonstrated to be regulated by these pathways. We are developing a two transgene system to study these pathways. The first transgene encodes a reporter gene whose expression results in a distinct purple phenotype. The second transgene contains a DNA methyltransferase fused to protein sequence which binds to the first transgene. When the two transgenes are present and expressed, the fusion protein binds to the reporter construct, allowing methylation and silencing of adjacent promoter regions. Independent transgenic lines were created for each of these constructs, and plants containing the transgenes were crossed to create segregating populations of plants with either one or both transgenes. In a segregating population, plants with both transgenes exhibit hypermethylation in the promoter of the reporter gene and reduced gene expression. These results are consistent with what we would expect given successful tethering. However, there is evidence of DNA methylation of the selectable marker cassette that each transgene encodes, silencing by small RNAs or chromosome pairing. Additional experiments are being carried out to demonstrate whether methylation is occurring by tethering-dependent or -independent mechanisms.

74. Y-chromosome library construction for next-generation sequencing

McNulty S¹, Miro-Herrans A^{2,3}, Mulligan CJ^{2,*}

¹Biotechnology Track, Biology Major, College of Agriculture

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

³Graduate Program in Genetics and Genomics, University of Florida, Gainesville, FL

The Y-chromosome is particularly useful for population history inferences through the investigation of haplogroup diversity in males. These haplogroups are defined by single nucleotide polymorphisms (SNPs). The cost of high-throughput sequencing of the entire Y-chromosome of multiple individuals for population analyses renders it prohibitive for SNP investigation. Selectively enriching for areas of the Y-chromosome that provide the most useful information, via library construction, can make next-generation sequencing cost-effective. We are creating a protocol in which we enrich for Y-chromosome fragments containing lineage-defining SNPs through hybridization with PCR products of selected haplogroups. Two thousand base pair fragments were generated from these PCRs to serve as probes to capture complementary sequences on the Y-chromosome. The resulting PCR products were sheared to increase sequence diversity and coverage during hybridization. Individual samples were tagged with identifying sequences, allowing multiple samples to be pooled and sequenced on a single lane of the Illumina GAIIx after capture on the custom probes. Sixty-three primer pairs were designed to produce probes containing over 150 SNPs. SNPs were chosen that define major haplogroups, as well as more divergent subgroups of E and J haplogroups, which are of particular interest in our samples. Ninety-five samples were uniquely tagged and pooled for library construction. Approximately 1.824 billion bases will map to the Y-chromosome, allowing for haplogroup determinations of all 95 samples. This method provides an unprecedented amount of sequence data in order to address questions that have been intractable.

75. Investigating the alternative splicing landscape in maize – analysis from B73/Mo17 hybrids and B73 x Mo17 recombinant inbred line (IBM RIL) population

Mei W¹, Liu S^{2,3}, Yeh C-Y^{2,3}, Li X^{2,3}, Springer NM^{4,5}, Schnable PS^{2,3}, Barbazuk WB^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Center for Plant Genomics, Iowa State University, Ames, IA

³Department of Agronomy, Iowa State University, Ames, IA

⁴Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN

⁵Department of Plant Biology, University of Minnesota, Saint Paul, MN

Alternative splicing (AS) produces multiple isoforms from a single pre-mRNA through selective use of splice sites. AS can influence protein diversity or affect protein levels by regulating message processing. AS is known to play roles during plant development, stress response, and flowering, but the extent of AS in plants is not well understood.

Maize, the major crop plant in the USA, lacks information about genome-wide alternative splicing. We are examining RNA-Seq data to investigate tissue specific AS within endosperm and embryo in B73 and Mo17 maize, identify parent of origin alternative splicing events or isoforms, *cis*- and *trans*- regulation in the hybrids and sQTL in the IBM RIL population. The results identified here will help us to further understand the cross talk between endosperm and embryo during seed development and embryogenesis, the regulation of alternative splicing in the hybrids and IBM RIL population. By comparing AS between B73 and Mo17 we can test for the existence of genotype-specific AS. Currently we are developing a new computational approach to detect parent of origin effects in B73/Mo17 hybrids, *cis*- and *trans*-regulation in the IBM RIL to identify genome-wide patterns of regulation of isoform abundance and alternative splicing. Our analysis centers on huge amount of RNA-Seq read alignment, allele specific splice site detection, alternate isoform detection and identification of allelic gene structures and isoforms. Our current results have identified differences in transcript isoform abundance between embryo and endosperm as well as some evidence for genotype-specific AS and parent of origin AS.

76. HD-ZIP III lost the HD: evolution of a transcription factor regulator

Miles NW¹, Soltis DE^{1,2,*}, Soltis PS^{2,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Laboratory of Molecular Systematics and Evolutionary Genetics, Florida Museum of Natural History, University of Florida, Gainesville, FL

LITTLE ZIPPER (ZPR) is a leucine zipper gene that regulates the activity and expression of Class 3 Homeodomain-Leucine Zipper (HD-ZIP III) genes. This interaction is well studied in *Arabidopsis* and is known to affect the polarity of lateral organs. However, little is known about its evolution in plants. To get a phylogenetic history of these genes, we sampled sequences from ferns and gymnosperms from the 1KP Transcriptome Project. The genome of the basal angiosperm, *Amborella*, was also sampled to understand the evolution in angiosperms and

intron/exon evolution. We have found that ZPR evolved from a paralog of HD-ZIP III in gymnosperms by truncation of the HD-ZIP III gene down to just the leucine zipper region. This means that ferns do not have ZPR genes which might have implications for interpreting the evolution of lateral organ development in vascular plants.

77. High throughput sequencing of pre-historic Taíno samples

Miro-Herrans AT^{1,2}, Wang A³, Mulligan CJ^{2,*}

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

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

³Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

DNA allows us to reconstruct the evolutionary history of populations. A challenge in inferring evolutionary histories is identifying, from the current genetic variation, the different processes that have shaped the genetic variation of a population. Ancient DNA (aDNA) allows the unique opportunity to reconstruct an event from the genetic variation present at the moment the event occurs. Until recently, it was unfeasible to accurately infer ancient events because of the cost and time required to generate a large aDNA dataset. Next-generation sequencing platforms now make it possible to generate large amounts of aDNA data. We test the use of an indexed whole mitochondrial DNA (mtDNA)-enriched library to generate sequence data for a pre-historic Native American population of Puerto Rico. Twenty-seven Taíno samples from the Punta Candelero archaeological site were simultaneously sequenced for whole mtDNA genomes, on one lane of the Illumina GAIIx, using an indexing and library enrichment approach. Sequence reads were sorted into samples according to the index identifier, trimmed to remove the index and adapters, and mapped to the NC012920.1 reference whole mitochondrial genome. Sequence coverage was highly variable among samples (725bp to 10,358bp) with mostly 1x coverage for each read. Sequences showed very low variability with only 21 variant sites among all samples, of which at least 4 sites are artifacts of C to T deamination conversions. These results show that indexed library approaches are useful for aDNA data generation, but will require further improvement to address the particular challenges of aDNA, such as DNA degradation and low quantities of DNA.

78. Dualistic function of Daxx at centromeric and pericentromeric heterochromatin in normal and stress conditions

Morozov VM¹, Gavrilova EV¹⁻³, Ishov AM^{1,*}

¹Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

²Faculty of Biology and Soil Science, St. Petersburg State University, St. Petersburg, Russia

³Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia

Heterochromatin architecture is essential for the proper orchestration of nuclear processes, while transcription from this part of the genome is required for its own maintenance. Here we present the first evidence that depletion of protein Daxx affects transcription of human heterochromatin, reducing accumulation of centromeric (CEN) RNA in normal conditions and pericentromeric (periCEN) RNA after heat shock (HS) application. Searching for the mechanism of Daxx-dependent regulation of heterochromatin transcription, we found that depletion of Daxx decreases incorporation of transcription-associated histone H3 variant, H3.3, into both CEN and periCEN. In normal conditions, Daxx is mostly accumulated at ND10/PML nuclear bodies, with minor association with CEN/periCEN in subpopulation of cells. HS changes this balance forcing very robust accumulation of Daxx on CEN/periCEN. Surprisingly, this transient redistribution of Daxx does not further elevate the incorporation of H3.3 that remained steady during HS and recovery. Instead, depletion of Daxx leads to HS-induced changes in the balance of epigenetic modifications at heterochromatin, most dramatically elevating levels of H3K4Me2 at periCEN. We propose dualistic function of Daxx-containing complexes at CEN/periCEN: 1) regulation of H3.3 loading in normal conditions, and 2) protection of epigenetic status upon stress application, thus collectively guarding epigenetic identity of heterochromatin and genome integrity.

79. Biocomputing resources at UF Research Computing

Moskalenko O¹, Gitzendanner MA^{2,*}

¹Research Computing, University of Florida, Gainesville, FL

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

The University of Florida High Performance Computing Center, part of UF Research Computing, provides comprehensive computing infrastructure and support including an instance of Galaxy to UF biological researchers. Galaxy is a web-based platform for accessible and reproducible biological computing. We will provide an overview of Galaxy; the modules system for easy command line software environment set up; software for biological research; and support, consulting and training services.

80. Carbohydrate availability modifies gene expression and virulence traits in *Streptococcus mutans*

Moye Z, Zeng L, Burne RA

Department of Oral Biology, University of Florida,
Gainesville, FL

Human oral biofilms are usually limited for carbohydrates during fasting periods of the host, but large quantities of sugars can be intermittently introduced via the diet. Here, we investigated the phenotypic and transcriptional responses of *Streptococcus mutans*, the primary pathogen in dental caries, to carbohydrate availability. Steady-state planktonic populations of *S. mutans* UA159 were generated using continuous chemostat culture in conditions of excess (100 mM) or limiting (10 mM) glucose; with pH, temperature and growth rate held constant. Cells growing under glucose-limitation were found to be able to acidify the environment more rapidly and to a greater extent than cells growing with excess glucose. Further, transport of glucose, fructose or mannose via the phosphoenolpyruvate:sugar phosphotransferase system (PTS) was dramatically more active in glucose-limited cells. Microarrays revealed that 123 genes were differentially expressed in cells grown in the presence of limiting versus excess carbohydrate ($p < 0.001$). Glucose-limited cells upregulated genes involved in energy metabolism and carbohydrate transport, as well as those encoding two-component systems. Cells grown in excess glucose showed elevated levels of transcripts associated with carbohydrate storage and biosynthesis of certain amino acids. Thus, carbohydrate availability dramatically influences physiological and biochemical pathways that contribute directly to the virulence of *S. mutans*.

81. The re-discovery of non-additive effects with genomic relationship matrices and its implication in breeding

Munoz P¹, Resende M², Gezan S³, Resende M⁴, Kirst M^{1,3,*}, Dudley H³, Peter GF^{1,3,*}

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

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

³School of Forest Resources and Conservation, University of Florida, Gainesville, FL

⁴Department of Forest Engineering, Federal University of Vicosa, Vicosa, Brazil

Non-additive effects have been highly overlooked in breeding, mainly because traditional methods for variance

estimation yield usually small – if ever significant parameters – compared to the additive. This has caused that many breeding programs make progress using only breeding values. We compare estimation, partition and prediction of different linear mixed models under additive and full (additive plus non-additive) assumptions with the use of either the pedigree- (BLUP) or marker-derived relationship matrices (GBLUP) in a clonal population of the tree species *Pinus taeda*. Our results show that additive REML/GBLUP increased the precision of the heritability estimate compared to additive REML/BLUP. We studied the partition of the components and found that the marker-based models were able to partition the estimations more efficiently. Finally, we evaluated all models in a cross validation and found a 36 and 30% higher predictive ability of the marker-based models that include additive, dominance and two way epistasis, when compared to the traditional GBLUP and to the additive BLUP models. We conclude that the use of relationship matrices derived from markers in a model including additive and non-additive effects had the best performance not only to partition the genetic variances but to improve considerably the breeding value prediction ability in trend, magnitude and top individual selection.

82. Regulation by KaeR mediated through interaction with flavonoids

Murdoch C, Chen A, Pagliai F, Lorca GL*

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

The focus of this study will be LVIS1989 (KaeR), a LysR family transcriptional regulator identified in *Lactobacillus brevis*. Through previous work, it was discovered that interaction with flavonoids (kaempferol and myricetin) stabilizes the regulator's binding to its cognate DNA inducing transcription. Two binding sites were identified through DNase I footprinting, one at the intergenic region between LVIS 1989 and LVIS 1988 and the other within LVIS1988. The aim of this work will be to determine critical residues responsible for KaeR's ability to respond to the presence of flavonoids. Using structure-guided, site-directed mutagenesis the following residues will be altered: glutamic acid at position 127, proline at position 201, and serine at position 203. These residues will be changed to aspartic acid, alanine, and alanine respectively. It is hypothesized that these mutations will result in a lack of response of KaeR to the ligand. *In vivo* and *in vitro* approaches will be utilized to compare the wild type protein and its mutant versions on ligand binding. It is also possible that mutations on KaeR may result in lower affinity of the protein to DNA or changes in the oligomeric state of the transcriptional regulator. To test these effects, electrophoretic mobility shift assay (EMSA) and size exclusion chromatography experiments will be performed

with the purified mutant proteins. Ligand binding will be tested in EMSAs by varying concentrations of kaempferol. *In vivo*, *lacZ* fusions to the KaeR regulated promoter will be used to test the effect of kaempferol and myricetin on activation of KaeR.

83. Genetic diversity of world collection of sugarcane and its related grasses

Nayak SN¹, Villa A¹, Pathak B¹, Todd J², Ayala-Silva T³, Glaz B², Wang J^{1,*}

¹Agronomy Department, University of Florida, Gainesville, FL

²Sugarcane Field Station, Agricultural Research Service, U.S. Department of Agriculture, Canal Point, FL

³Subtropical Horticulture Research Station, Agricultural Research Service, U.S. Department of Agriculture, Miami, FL

Sugarcane (*Saccharum* spp.) cultivar improvement programs have not yet systematically utilized most of the genetic sources of yield potential and resistance to biotic and abiotic stresses that may exist in the *Saccharum* germplasm. Two collections of genetic material potentially useful to sugarcane breeding programs, which are maintained in India and USA, are known collectively as the 'World Collection of Sugarcane and Related Grasses'. The objectives of this study are 1) to genotypically evaluate the World Collection of Sugarcane and Related Grasses maintained at the National Plant Germplasm System in Miami, FL, and 2) to select representative accessions in the collection that sugarcane breeders can utilize as a core breeding collection. In total, 1,002 accessions in the world germplasm collection, comprising 16 species, were sampled for genotypic evaluation using microsatellite markers. Initial screening of 192 microsatellite markers on eight species of sugarcane accessions yielded 76 polymorphic markers with polymorphic information content ranging from 0.22 to 0.89. Of these, 26 polymorphic markers were used to genotype the 1,002 accessions yielding around 200 alleles. Genotyping data was used for diversity analysis of the 1,002 accessions. Meanwhile, these accessions will be phenotypically evaluated by taking the observations on several plant growth parameters. Based on diversity parameters of the cluster and phylogenetic analysis, the sugarcane core collection will be formed, which will be useful in sugarcane breeding programs after thorough evaluation and later for genetic studies such as association mapping and genomic selection.

84. KBAS: a software tool for knowledge-based association studies

Nazarian A¹, Riva A^{2,*}

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

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

Genome-wide association studies (GWAS) have revolutionized the genotype-phenotype analysis of complex traits in which multiple genetic and non-genetic factors act jointly to form a phenotype. However, despite the successes of GWAS in detecting a number of previously unknown susceptibility loci, a remarkable amount of missing heritability still exists for most of the studied traits, in part because GWAS are unable to reliably detect small contributions of individual genetic risk factors, and to capture the interactions existing among genetic factors. We have developed a framework for analyzing the complexity underlying the genetic architecture of complex diseases, based on a hypothesis-based method which integrates hypotheses about the genetic basis of a complex phenotype, generated from pre-existing biological knowledge, with a genetic algorithm-based search engine (GA) which generates and optimizes multi-SNP models associated with the trait under investigation. We implemented the method in the KBAS software package, a command-line 64 bit executable. The program provides users with an interface to generate sets of SNPs related to the hypotheses of interest, and to test their association with the phenotype through the GA engine. Finally, the best multi-SNP model generated by the GA engine is written to a fully annotated output file that also contains the GA parameters and the results of the model validation. KBAS is freely available at:

<http://genome.ufl.edu/rivalab/kbas>.

85. Characterization of an n-type ATP pyrophosphatase required for thiolation of tRNA in the haloarchaeon, *Haloflex volcanii*

Nembhard NE¹, Englert M², Elbanna D¹, Holman M¹, Söll D², Maupin-Furlow JA^{1,*}

¹Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

Two small archaeal modifier proteins (SAMP1 and SAMP2) and their activating enzyme, UbaA, were recently described in *Haloflex volcanii*. SAMP 1 participates in molybdenum cofactor biosynthesis and SAMP2 is important for thiolation of tRNAs. It is apparent from our previous work that SAMPs function in the mobilization of sulfur for the biosynthesis of sulfur-containing molecules. However, the enzymes mediating the thiolation of tRNA remain unclear. In this study, we generate an in-frame markerless deletion in *H. volcanii* of an Urm1-associated tRNA thiolation protein Ncs6p homolog belonging to the n-type

ATP pyrophosphatase family. The n-type ATP pyrophosphatase family is characterized in adenylation of tRNA leading to acceptance of activated sulfur in biomolecule synthesis. Mutant strain analysis revealed the requirement of the n-type ATP pyrophosphatase (HVO_0580) in *H. volcanii* for 2-thiouridine synthesis and the deletion strain exhibited high-temperature sensitivity similar to other n-type ATP pyrophosphatases which are required for thiolation of tRNA at high temperatures.

86. Whole-genome genotyping of gene copy number variation in forest trees

Neves LG¹, Davis JM^{1,2,*}, Barbazuk WB^{1,3,*}, Kirst M^{1,2,*}

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

²School of Forest Resources and Conservation, University of Florida, Gainesville, FL

³Department of Biology, University of Florida, Gainesville, FL

Trees present a remarkable adaptation to a variety of environments. The diversity in their genome might explain their broad plasticity. For instance, most flowering plants have undergone one or more events of whole-genome duplication. As a consequence, in a population of plants from the same species, genes may be present in different copies in the genome, referred to as copy number variation (CNV). What is not known, however, is the extent to which CNV is responsible for changing the phenotype of the plant, particularly for complex traits. We are currently addressing this topic by developing a high-throughput, genome-wide, genotyping pipeline for CNV based on targeted-resequencing and, subsequently, genotyping a large population of 579 unrelated trees of *Populus deltoides*. For the genotyping, targeted genes are captured from the genome using complementary probes and sequenced using next-generation sequencing. The resequencing data is being analyzed in a mixed-model to identify genes for which some individuals are consistently sequenced with higher or lower depth, characterized by both CNV and presence/absence variation. Data from several species are being analyzed, namely, *Pinus taeda*, *Pinus elliottii*, *Populus trichocarpa* and *Populus deltoides*, with 24, 24, 48 and 10 individuals, respectively. The optimized model will be used to genotype the *P. deltoides* population containing 579 individuals, with the objective of associating population level CNV with phenotypic variation.

87. Comparison of SIV *in vitro* recombination rates using traditional clonal sequencing and single genome sequencing

Nolan DJ^{1,2}, Watson AR^{1,2}, Norstrom MM^{3,4}, Strickland SL^{1,2}, Lamers SL⁵, Salemi MM^{1,2,*}

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

²Emerging Pathogens Institute, University of Florida, Gainesville, FL

³Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

⁴Center for HIV Research, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

⁵Bio Info Experts, Thibodaux, LA

Simian immunodeficiency virus (SIV), like human immunodeficiency virus (HIV), can interchange portions of genetic sequence between viral genomes to generate recombinant progeny *in vivo*. Recombination rates are commonly calculated to assist in the interpretation of viral evolution, but sequences obtained by traditional cloning could be skewed by PCR-mediated recombination, where Taq polymerase can switch templates during cycles creating *in vitro* recombinants. The potential for *in vitro* recombination can be reduced with single genome sequencing (SGS) using limiting-dilution nested PCR. By diluting cDNA until there is just a single copy to amplify during SGS, the *in vitro* recombination effect is minimized due to sequence similarity of every amplified product. Using cDNA generated from SIV isolated from 4 plasma samples, we performed triplicate cloning and SGS experiments to produce six alignments of ~22 env gp120 sequences for each of the cDNA samples. The resulting 24 alignments were analyzed with SplitsTree, an application that uses a split decomposition network and a powerful statistical test to identify likely recombinants within the population. After doing chi-square analysis, no statistically significant difference was found between the numbers of recombinants in the cloning and SGS experiments. Given our experimental results, we concluded that PCR-mediated recombination occurs at a rate not high enough to be significant against the background rate of *in vivo* recombination.

88. Bioinformatics, data management, and general information needs of clinical and translational science researchers

Norton HF, Garcia-Milian R, Tennant MR*, Lyon JA, Botero CE

Health Science Center Library, University of Florida, Gainesville, FL

The UF Health Science Center Library (HSCL) strives to serve the diverse information needs of UF's biomedical researchers, including those affiliated with the Clinical and Translational Science Institute (CTSI). In the fall of 2011 we began a comprehensive assessment of CTSI researchers' information needs. This exploration included online assessments, focused discussions and one-on-one

interviews, and concentrated on bioinformatics, data management, and general information needs. Preliminary analysis of the bioinformatics results suggests that researchers are interested in training on both the free bioinformatics tools available through the National Center for Biotechnology Information and commercial tools. Data suggest researchers tend to use the resources with which they are familiar, and tend not to explore alternatives to those resources. From the data management assessment, we learned that the resources most needed for effective management and analysis of research data include computing expertise, storage capacity, organizational systems, and training on best practices in data management. The general information needs assessment addresses areas of interest including assessing research impact, facilitating research collaboration, meeting the NIH Public Access requirements, and various activities related to community engagement. We will present results from these assessments and indicate future directions for library services to support CTSI researchers' needs.

89. Hormonal regulation of chondrichthyan fin development: implications for the evolution of copulatory organs

O'Shaughnessy KL^{1,2}, Dahn RD³, Cohn MJ^{2,4,5,*}

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

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

³Countrywood Lane, Madison, WI

⁴Howard Hughes Medical Institute, University of Florida, Gainesville, FL

⁵Department of Biology, University of Florida, Gainesville, FL

The most primitive vertebrate copulatory organs are penis-like extensions of the posterior pelvic fins, known as claspers. Claspers are found in the fossil record extending back to the arthrodires, jawed fishes that predate the origin of sharks by 25 million years. Today only male members of the class Chondrichthyes develop claspers, and this represents an interesting sexual dimorphism of the fins that suggests a potential role for sex steroids during fin development. We reported previously that Sonic Hedgehog (Shh), Hoxd12, and Hoxd13 expression persists in the developing clasper of male fins after downregulation in the rest of the fin. This study seeks to determine the potential role of sex hormones in clasper development by examining the distribution of androgen receptor, as well as sexually dimorphic patterns of developmental genes in chondrichthyan embryos. Immunohistochemistry and *in situ* hybridization results reveal the presence of androgen receptors within the developing pelvic fin buds, suggesting their competence to respond to androgens. We find that the apical ectodermal ridge (AER) persists in the posterior

region of male pelvic fins, suggesting that clasper formation is achieved by prolongation of Shh-Fgf8 feedback loop. These results suggest that primitive copulatory organs evolved by hormonal regulation of the fin development program in early jawed vertebrates.

90. Gene identification in *Pseudomonas aeruginosa*: from bioinformatics to experimental analysis

Oden S¹, Zhang Y¹, Jin S^{1,*}, Tornaletti S², Brocchieri L^{1,*}

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

²Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Gene coding regions generally exhibit contrasting global compositional properties in the three codon positions, depending on the overall base composition of the sequence. General rules of the base content at the three codon positions as a function of the overall base content of the sequence can be identified and exploited to score sequence regions for their coding potential. We developed procedures to predict and quantify coding regions in prokaryotic sequences. These procedures augment the visual approach of frame analysis for detecting significant contrasts in nucleotide usage among codon positions. The procedures are implemented in the web-based tool N-PACT (N-Profile Analysis Computational Tool) available at <http://genome.ufl.edu/npact/>. N-PACT produces gene predictions and characterizations, and a graphical output of predictions and sequence compositional features. We applied N-PACT to the genome of *Pseudomonas aeruginosa* PAO1 and other *P. aeruginosa* strains, identifying several potential new genes. Many of these new predictions consist of short sequence length and are conserved in different bacterial species. We compared our computational predictions with experimental evidence of transcription obtained by RNA-seq analysis. RNA-seq results revealed that many of the predicted genes were undergoing transcription, furthering the validity of their prediction as protein coding.

91. A dual role of TstR transcriptional regulator provides insights into cyanide detoxification in *Lactobacillus brevis*

Pagliai FA, Murdoch CC, Nath R, and Lorca GL*

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

In this study, a dual activity for a member of the MarR family of transcriptional regulators was discovered in *Lactobacillus brevis* ATCC 367. TstR modulates the expression and activity of the downstream encoded thiosulfate:cyanide sulfurtransferase (TstT). DNase I footprint identified the TstR binding site in a region of 34

nucleotides (-1 to +33, from *tstR* transcriptional start site). EMSA assays revealed that while sulfite, an end product of the reaction catalyzed by TstT, improved the interaction between TstR:*PtstR*, iron impaired this interaction. A structural model of TstR was used to identify putative ligand binding sites. Site-directed mutagenesis identified M64 as a key residue in the sulfite recognition, while a pocket comprising the residues H136-H139-C167-M171 was the site coordinating iron. Besides its role as a transcriptional repressor, TstR played a role in the thiosulfate:cyanide sulfurtransferase activity of TstT. Using a two-hybrid system, we demonstrated that TstT and TstR undergo a physical interaction promoted by iron. Enzymatic assays proved that this novel interaction increased the activity of TstT by 3-fold, comparable to other "active" single-domain sulfurtransferases. The protein-protein interaction between TstR and TstT described herein represents a novel mechanism of regulation of enzymatic activity performed by a transcriptional regulator.

92. Bulk segregant analysis generates markers linked to avirulence gene in fungal pathogen

Pendleton AL¹, Smith KE², Nelson CD², Davis JM^{1,3,*}

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

²Southern Institute of Forest Genetics, Southern Research Station, U.S. Forest Service, Saucier, MS

³School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Cronartium quercuum f.sp. *fusiforme* (Cqf), the fungus that incites fusiform rust disease of oak and southern pine, produces galls on stems and branches of southern pines. Gene-for-gene interactions between the *Pinus taeda* resistance gene Fr1, and the corresponding pathogen avirulence gene Avr1 have been documented. Obtaining markers for avirulence loci would allow pathogen populations to be surveyed where plantations were to be established, enabling growers to plant trees with corresponding resistance genes that ensure the stand is resistant to rust. We have coupled the power of bulk segregant analysis (BSA) with next generation sequencing to identify markers that segregate with avirulence to Fr1. Spores collected from resistant (Fr1/-) and susceptible (fr1/fr1) hosts were bulked and sequenced using the Illumina GAIIX platform. Following alignment to the reference Cqf genome, single nucleotide polymorphisms (SNPs) were identified in the bulked sequencing data. Significant deviation from a 1:1 ratio of all SNPs was calculated to determine markers tightly linked to Avr1, which generated an approximately 70kb interval with candidate Avr1 genes. Syntenic analyses with a closely related rust pathogen (*Melampsora larici-populina*) validated the interval generated through BSA. Subsequent

bulk sequencing and comparative analyses will be completed to refine the interval.

93. Comparing PCR conditions that detect rAAV harboring human erythropoietin cDNA: applications for gene doping

Perez IC¹, Ni W¹, Le Guiner C², Moser D³, Simon P³, Moullier P^{1,2}, Snyder RO^{1,2,4,*}

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

²Laboratoire de Thérapie Génique, Nantes, France

³Department of Sports Medicine Johannes Gutenberg-University, Mainz, Germany

⁴Center of Excellence for Regenerative Health Biotechnology, University of Florida, Alachua, FL

The development of molecular assays that help detect gene doping in sports can serve as a deterrent and can identify athletes who may be illicitly using gene transfer for performance enhancement. Promising technologies to detect foreign DNA with high reliability, sensitivity, and specificity include TaqMan real time, nested PCR, and internal threshold control PCR. Previous studies conducted in cynomolgus macaques (cm) have shown that optimized conditions for TaqMan real time PCR detected as low as three copies of foreign cm erythropoietin (EPO) in white blood cells with a false-positive rate of 0% and false-negative rate of 13%. In this present study, the reliability, sensitivity and specificity of three different PCR assays are being investigated for the detection of a recombinant adeno-associated virus (rAAV) vector harboring a promoterless human EPO (hEPO) complementary DNA (cDNA) administered to cynomolgus macaques, and on samples of known copy numbers of rAAV promoterless hEPO plasmid spiked into naïve human genomic DNA. The first two assays involve a one-step real time PCR, each having different primer sets. The third assay involves a nested PCR approach. Preliminary data shows that the nested PCR has the most sensitivity, detecting as low as two copies of the hEPO cDNA in human gDNA spiked with plasmid, and the presence of human naïve genomic DNA did not interfere. Future work involves injecting macaques intramuscularly with a rAAV promoterless hEPO vector in order to investigate the longevity and biodistribution of hEPO, as well as the sensitivity and specificity of the three different assays.

94. A MarR transcriptional regulator from "Candidatus Liberibacter asiaticus" affects cell shape and biofilm formation

Potts AH¹, Melanson N^{1,2}, Lorca GL^{1,*}

¹Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

²Undergraduate Honors Program, College of Agricultural and Life Sciences, University of Florida, Gainesville, FL

"*Candidatus Liberibacter asiaticus*" is an obligate intracellular pathogen of citrus and the causative agent of citrus greening disease. Although this pathogen has devastated Florida's citrus industry, little is known about this bacterium, and it has yet to be cultured. Our approach aims to use the genome sequence of "*Ca. L. asiaticus*" to identify protein targets for the development of small molecule therapeutics. In order to demonstrate the biological role of these small molecules, we will use the closely related *Sinorhizobium meliloti* 1021 as a surrogate. As with many intracellular pathogens, "*Ca. L. asiaticus*" has undergone reductive evolution and thus has much less complex regulatory networks than related species, presenting a target for the development of treatments. One of these few regulators is CLIBASIA_01180, a member of the MarR family of transcriptional regulators. Analysis of the genomic context in "*Ca. L. asiaticus*" and mobility shift assays suggest that it may regulate the gene directly downstream, CLIBASIA_01175. This gene encodes a hypothetical protein that contains a YkuD domain that is associated with L,D-transpeptidase activity as well as a PG_binding_1 peptidoglycan binding domain. This domain structure suggests CLIBASIA_01175 is likely a transpeptidase involved in cross linking peptidoglycan. In a *S. meliloti* strain with a mutation in this regulator, more biofilm formation is seen, potentially because the cells are aggregating as a response to stress. Thus, we hypothesize that disrupting the regulation of this gene may result in destabilization of the cell wall of "*Ca. L. asiaticus*".

95. Transcriptional regulation of wdr-23

Prasse S, Choe KP*

Department of Biology, University of Florida, Gainesville, FL

Tight regulation of inducible gene transcription responses is essential for basic animal function and for survival of environmental stress. In the nematode *Caenorhabditis elegans*, the gene wdr-23 tightly represses a stress-inducible transcription factor named SKN-1. Loss of wdr-23 activates SKN-1 and enhances stress resistance, but also impairs growth and reproduction. Interestingly, we have found that expression of the wdr-23 gene is regulated by SKN-1 in a feedback mechanism and by other unknown factors. Chromatin immunoprecipitation assays indicate that PHA-4, a transcription factor previously known to mediate development and longevity, associates with the promoter of wdr-23. Interestingly, PHA-4 and SKN-1 both associate with the same region of the wdr-23 promoter suggesting that the two transcription factors might act as co-regulators. This research is currently ongoing, and is

focused on being able to define the regulatory pathway for wdr-23.

96. The molecular evolutionary rate of scombroid fishes is predicted by the body size (but not metabolic rate) hypothesis

Qiu F¹, Kitchen A², Burleigh JG^{1,*}, Miyamoto MM^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Anthropology, University of Iowa, Iowa City, IA

The study of variable molecular evolutionary rates is confounded by the fact that the life history traits of groups typically co-vary with each other. Interestingly, in contrast to other groups (i.e., mammals), the mass-specific metabolic rate of scombroid fishes trends positively (not negatively) with body size. This study exploits this divergent pattern and its corresponding opposing predictions for scombroid rate variation to distinguish between the metabolic rate and body size hypotheses. This investigation uses a supermatrix of molecular and morphological characters to infer robust phylogenies, which are subsequently incorporated into two recent comparative methods that directly account for the phylogenetic non-independence of species in their tests of trait and substitution rate. As predicted, a negative correlation is supported between body size and substitution rate. Conversely, a negative (not positive) association is also unexpectedly found between metabolic and substitution rates. These relationships are consistent across all molecular data, genomes, and genes with large sample sizes. On the basis of these consistent relationships, we conclude: (i) that scombroid rate variation may be explained by the body size hypothesis; and (ii) that metabolic rate is tracking body size and not evolutionary rate. This study highlights how groups with divergent traits hold special promise for the isolation and testing of typically co-varying factors.

97. Genomic selection and association mapping identify candidate genes linked to fusiform rust resistance (Fr) loci in loblolly pine

Quesada T¹, Resende M^{1,2}, Muñoz P^{1,3}, Gezan S¹, Kirst M^{1,*}, Peter GF^{1,*}, Davis JM^{1,*}

¹School of Forest Resources and Conservation, University of Florida, Gainesville, FL

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

³Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Fusiform rust is a pine disease caused by the fungus

Cronartium quercuum f.sp. *fusiforme* (Cqf), producing galls in stems and branches. It affects survival in young trees and seedlings, generating significant economic losses to the timber industry. Studies in loblolly pine showed that resistance to fusiform rust involves major genes based on Mendelian cosegregation of resistance with RAPD markers in seedlings inoculated with single-spore lines of Cqf; however, no fine-mapping has been achieved. We used a genomic selection approach, using Bayes $C\pi$ to pre-select SNPs for association analyses based on their effects on breeding values for gall score in two loblolly pine tests. One test was inoculated with (Cqf) spores from a single gall and the other was inoculated with spores from a mixture of ten galls. Significant associations were detected for gall score using BAMD software at 99% confidence. We observed nine and 13 significant SNPs for the single gall and 10-gall test, respectively, of which two appear to be linked to Fr1. They are significant at high confidence, showed high LOD scores from single marker regression analyses for parent 17, which is known to be heterozygous for the Fr1 gene, and both mapped to the same linkage group that harbors Fr1. Additionally, other significant SNPs mapped to different linkage groups, suggesting the presence of new fusiform rust resistance loci.

98. Functional characterization of a candidate gene for hydraulic conductivity in *Populus*

Ribeiro CL¹, Dervinis C², Kirst M^{1,2,*}

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

²School of Forest Resources and Conservation, University of Florida, Gainesville, FL

In a previous study, moderate heritability and significant QTL for vessels per sapwood area, hydraulic vessel diameter, and specific hydraulic conductivity showed that these physiological traits are under genetic control in a *Populus* hybrid population. Co-localization between QTLs for hydraulic conductivity traits and growth increment further suggested a functional linkage between these traits, supporting the hypothesis that they are correlated in forest trees. To uncover potential regulators for these traits we used a genetical genomics approach that integrated hydraulic conductivity and growth trait QTLs with gene expression measured in the poplar hybrid population. This analysis identified a previously uncharacterized gene, referred hereafter as HC1 (hydraulic conductivity 1) as the most probable gene regulator. Currently, evidence of the functional role of this gene is being obtained by the analysis of poplar genetically modified plants, up-regulated in HC1 expression levels with the use of 35S promoter. Down-regulation (RNAi) of this gene was lethal in transgenic lines. Up-regulated plants grown under controlled environment have significant higher height and vessel diameter, based on

analysis on a micro CT scan, in comparison to wild-type plants. Further characterization will be obtained from analysis of T-DNA lines in *Arabidopsis* and vessel analysis under light microscope.

99. siRNA preservation in rapidly progressing autosomal dominant retinitis

Rossmiller B, Mao H, Lewin AS*

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

Purpose: We are interested in characterizing and treating the rapidly degenerating autosomal dominant retinitis pigmentosa (ADRP) mouse model, T17M. Work by our lab has shown this strain to be highly susceptible to light damage (White et al., 2007, *Invest Ophthalmol Vis Sci* 48(5):1942-51). We hypothesize that the knockdown of transducin-alpha in rods will reduce rod degeneration and preserve cone function. It is therefore the purpose of this study to determine if siRNA mediated knockdown of transducin-alpha can inhibit the phototransduction cascade in rod cells preventing apoptosis resulting from exposure to bright light.

Methods: A cell culture assay was conducted to determine the siRNA with greatest knockdown of the transducin-alpha gene (GNAT1). Two small hairpin RNAs (shRNAs) were designed to target homologous regions between mouse and dog GNAT1. Transfections were done in HEK293 cells, six replicates, using CMV-GNAT1 and H1-shRNA GNAT1 given at 1:0, 1:2, 1:4, and 1:6 ratios. Using a CBA-GFP-control miRNA expression plasmid as carrier, the total amount of DNA transfected was constant. The amount of transducin produced was observed at 24 and 72 hours post transfection using western blot with a mouse transducin specific antibody. The percent knockdown was then calculated using tubulin as the loading control and normalized against the control plasmid.

Results: siRNA GNAT1(a) showed the greatest knockdown with 24.0%, 28.6% and 30.9% at the 1:2, 1:4 and 1:6 ratios, respectively, after 72 hours.

Conclusions: We demonstrated siRNA GNAT1(a) to be a viable siRNA for testing in T17M RHO transgenic mice.

100. Detection of primary cilia in human glioblastoma

Sarkisian MR^{1,*}, Siebzehnrubl D¹, Deleyrolle L^{2,3}, Silver DJ^{2,3}, Siebzehnrubl FA^{2,3}, Guadiana SM¹, Steindler DA^{2,3}, Reynolds BA^{2,3}

¹Department of Neuroscience, University of Florida, Gainesville, FL

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

³Evelyn F. and William L. McKnight Brain Institute,
University of Florida, Gainesville, FL

Glioblastoma (GB) is the most common adult brain tumor and carries a poor prognosis due to primary and acquired resistance. While many cellular features of GB have been documented, it remains unknown if cells within these tumors possess primary cilia, a cellular organelle reported to promote cell proliferation and support survival of neural precursor cells. We used immunohistochemical and western blot analyses to screen both human GB biopsies and primary cell lines for presence of cilia and cilia-associated proteins. In two primary cell lines, we found that ~10-20% of cells contained acetylated alpha tubulin positive cilia. By combining immunostaining for cilia with a FUCCI cell cycle indicator, we found that cilia extended from cells in G0/1. Other key cilia genesis/function proteins, such as IFT88 and KIF3a, were detectable within the primary cilia and within protein lysates generated from >20 GB biopsies. Notably, cilia were also observed in several tumor biopsies (two primary and one recurrent). The cilia were especially identifiable within and surrounding the vasculature but also in necrotic areas. Collectively, our data indicate that ciliated cells and proteins required for ciliogenesis are detectable in human GB. The contribution of these organelles to GB requires further investigation.

101. The influence of genetic background and natal environment on testes development

Sasson D¹, Miller CW^{2,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Entomology and Nematology, University of Florida, Gainesville, FL

An organism's genetic make-up and natal environment can play a large role in its morphological, physiological, and behavioral development. These genetic and environmental effects on development are often studied separately. However, we know that the expression of genes changes across environments and thus any developmental outcome is likely due to the interaction between an organism's genetic make-up and its environment. In this study, we have explored the interaction of genes and the environment on testes development in the cactus bug, *Narnia femorata*. We are interested in three questions: 1) does a bug's genetic background influence testes development?; 2) do males from high and low quality environments differentially allocate resources to testes development?; 3) are there interactions between the genetic background and natal environment such that some male lines do better in certain environments than other male lines? Preliminary results suggest that genetics affect testes development. Additionally, males from the low

quality environment are smaller but allocate proportionally more resources to testes development than do males from high quality environments. This finding suggests that small males may shift resources to testes development in an effort to compete for reproductive success with other males through sperm competition rather than through direct fights. We are currently analyzing the degree to which genes and the environment interact to influence testes development.

102. Tissue regeneration and blastema formation in a mammal, the African spiny mouse (*Acomys*)

Seifert AW^{1,2}, Kiama SG², Seifert MG¹, Goheen JR^{3,4}, Palmer TM¹, Maden M^{1,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Veterinary Anatomy and Physiology, University of Nairobi, Nairobi, Kenya

³Department of Zoology and Physiology, University of Wyoming, Laramie, WY

⁴Department of Botany, University of Wyoming, Laramie, WY

Evolutionary modification has produced a spectrum of animal defense traits to escape predation, including the ability to autotomize body parts to elude capture. Following autotomy, the missing part is either replaced through regeneration (e.g. urodeles, lizards, arthropods, crustaceans) or is permanently lost (mammals). While most autotomy involves the loss of appendages (e.g. leg, cheliped, antennae, tail), skin autotomy can occur in certain taxa of scincid and gekkonid lizards. Here we report the first demonstration of skin autotomy in Mammalia (African spiny mice, *Acomys*). Mechanical testing revealed a propensity for skin to tear under very low tension and the absence of a fracture plane. Following skin loss, rapid wound contraction was followed by hair follicle regeneration in dorsal skin wounds. Surprisingly, we found regenerative capacity in *Acomys* extended to ear holes where they exhibited complete regeneration of hair follicles, sebaceous glands, dermis, and cartilage. Salamanders capable of limb regeneration form a blastema (a mass of lineage-restricted progenitor cells) following limb loss, and our findings suggest that ear tissue regeneration in *Acomys* may proceed through assembly of a similar structure. This study underscores the importance of investigating regenerative phenomena outside of traditional model organisms and suggests that mammals may retain a higher capacity for regeneration than previously believed. As re-emergent interest in regenerative medicine seeks to isolate molecular pathways controlling tissue regeneration in mammals, *Acomys* may prove useful in identifying mechanisms to promote regeneration in lieu of fibrosis and scarring.

103. DNA sequence directs nucleosome redistribution in the immune response to KSHV

Sexton BS, Avey D, Druliner B, Fincher J, Zhu F, Dennis JH

Department of Biological Science, Florida State University, Tallahassee, FL

In eukaryotic cells, DNA is organized into chromatin, and the fundamental subunit is the nucleosome: 150 base pairs of DNA wrapped 1.6 times around a histone octamer. The distribution of nucleosomes can regulate access to DNA and influence transcription. Although various chromatin regulatory complexes are known to regulate nucleosome occupancy, the role of primary DNA sequence in this regulation remains unclear, particularly in mammals. To address this problem, we measured nucleosome distribution at high temporal resolution in human cells at hundreds of genes during the reactivation of Kaposi's sarcoma-associated herpesvirus (KSHV). We show that nucleosome redistributions are widespread and transient. To clarify the role of DNA sequence in this regulation, we compared the genes with altered nucleosome distribution to a sequence-based computer model and *in vitro* assembled nucleosomes. We demonstrate that both the computational model and the *in vitro* assembly accurately predict the nucleosome distributions at a majority of genes studied. The surprising findings that nucleosome redistributions are transient and DNA-directed shifts the current perspective regarding regulation of nucleosome distribution in humans, and suggest that DNA sequence plays a more considerable role in the regulation of nucleosome positions than was previously appreciated.

104. *VKORC1* Asp36Tyr geographic distribution and its impact on warfarin dose requirements in Egyptians

Shahin MHA^{1,2}, Cavallari LH³, Perera MA⁴, Khalifa SI⁵, Dvorak A⁶, Langae TY^{1,2,*}, Patel S³, Perry K⁴, McLeod HL⁶, Johnson JA^{1,2,*}

¹Department of Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL

²Center for Pharmacogenomics, University of Florida, Gainesville, FL

³Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, IL

⁴Department of Medicine, University of Chicago, Chicago, IL

⁵Pharmaceutical Sciences Section, College of Pharmacy, Qatar University, Doha, Qatar

⁶Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, NC

Identifying predictors of higher warfarin dose has been challenging. *VKORC1* Asp36Tyr single nucleotide polymorphism (SNP) is one of the most promising predictors of high warfarin dose, but data on its population prevalence is incomplete. We determined the frequency of this SNP in participants from seven countries on four continents and investigated its effect on warfarin dose requirement. A total of 992 samples were analyzed to define the population prevalence of this SNP. Those samples included individuals from Egypt, Ghana, Sudan, Kenya, Saudi Arabia, Peru and African Americans from the United States. 206 Egyptian samples were then used to investigate the effect of this SNP on warfarin dose requirements. This SNP was highest among Kenyans and Sudanese, with a minor allele frequency (MAF) of 6% followed by Saudi Arabians and Egyptians with a MAF of 3.0% and 2.5%, respectively. It was absent in West Africans, based on our data from Ghana, and a large cohort of African Americans. Egyptian carriers of the *VKORC1* Tyr36 showed higher warfarin dose requirement (57.1 ± 29.4 mg/week) than those with the Asp36Asp genotype (35.8 ± 16.6 mg/week; $P < 0.0001$). In linear regression analysis, this SNP had the greatest effect size among the genetic factors (16.6 mg/week increase in dose per allele), and improved the warfarin dose variability explained in Egyptians (model R² from 31% to 36.5%). The warfarin resistant *VKORC1* Asp36Tyr appears to be confined to north-eastern Africa and nearby Middle-Eastern populations, but in those populations where it is present, it has a significant influence on warfarin dose requirement and the percent of warfarin dose variability that can be explained.

105. Comparative proteomics of maize basal endosperm in miniature1 seed mutant and its wild-type Mn1

Silva-Sanchez C¹, Chen S^{1,2,*}, Zhu N², Li Q-B³, Chourey PS^{3,4,*}

¹Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

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

³Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL

⁴Plant Pathology Department, University of Florida, Gainesville, FL

Developing endosperm in maize seed is a major site for biosynthesis and storage of starch and proteins, and of immense economic importance. The basal part of endosperm is however unique as it performs a major role in solute, water and nutrition acquisition from mother plant. The miniature1 (mn1) mutation is a loss-of-function

mutation of the Mn1-encoded cell wall invertase, which is entirely expressed in the basal endosperm and is essential to many of the above functions. Here we report a comparative proteomic study between Mn1 and mn1 basal endosperm to better understand the possible basis of pleiotropic effects on many diverse traits in the mn1 seed. Specifically, we used iTRAQ based quantitative proteomics combined with Gene Ontology classification and the KEGG bioinformatics to conduct functional analysis of the proteomic information. A total of 2,518 proteins were identified from soluble and cell wall associated protein fractions, and 131 proteins were differentially expressed in the two genotypes. Based on the gene ontology classification, the main functional groups of proteins that were significantly enriched were those involved in the carbohydrate metabolic and catabolic process, and cell homeostasis.

106. Genetic mapping of *transgene reactivated mutant 1 (tgr1)*, a novel allele of the largest subunit of RNA polymerase IV in maize

Sloan A, Madzima TF, Mills ES, McGinnis KM

Department of Biological Science, Florida State University, Tallahassee, FL

Stably silent transgenic lines containing the *b1* genomic transgene (referred to as BTG-s) were used in a forward genetic screen to identify transgene reactivated (Tgr) mutants. In the M2 generation recessive homozygous mutants were identified by purple pigmentation, characteristic of BTG expression. In addition to the loss of transcriptional silencing of BTG-s, *tgr1-1* individuals exhibit hypomethylation of the promoter region of BTG-s and a reduction in 24 nucleotide siRNAs. These molecular phenotypes are consistent with *Tgr1* encoding a component of the RNA-directed DNA methylation gene silencing pathway in maize. Genetic mapping of *Tgr1* indicates that the mutation lies within a 12Mbp interval on chromosome 1, which also includes the *Rmr6* locus. Complementation assays were used to demonstrate that *tgr1-1* is an allele of *Rmr6*. We confirmed that *tgr1-1* plants do not contain any of the cloned *Rmr6* mutant alleles. Together, these results demonstrate that *tgr1-1* is a novel allele of *Rmr6*, a gene that has previously been shown to encode the largest subunit of RNA polymerase IV.

107. Intra-host phylodynamics in SIVmac251 infected CD8-depleted rhesus macaques with different neuropathology

Strickland SL^{1,2}, Veras NM^{1,2}, Prosperi MC^{1,2}, Suchard MA³⁻⁵, Williams K⁶, Salemi MM^{1,2,*}

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

²Emerging Pathogens Institute, University of Florida, Gainesville, FL

³Department of Biomathematics, University of California Los Angeles, Los Angeles, CA

⁴Department of Biostatistics, University of California Los Angeles, Los Angeles, CA

⁵Department of Human Genetics, University of California Los Angeles, Los Angeles, CA

⁶Biology Department, Boston College, Chestnut Hill, MA

Determining the intra-host evolutionary factors leading to the emergence of neurovirulent strains continues to be of importance for understanding development of neuroAIDS. We applied high resolution Bayesian phylodynamics analysis to longitudinal samples collected from SIV infected CD8-depleted rhesus macaques, a rapid disease model for AIDS-related neuropathology. The mode of transmission, the tempo of the appearance of neurovirulent strains and the spatial distribution of the virus were determined. Molecular clock analysis showed rate of evolution along the branches differed slightly among primates. The average evolutionary rate of the virus was $3.0e^{-4}$ substitutions per site per day, one log higher than what is usually observed in non-CD8-depleted primates. Bayesian skyline plots were inferred to quantify the dynamics of the effective population size (N_e) over time. Two primates (D03 and D06) showed a peak in the N_e at about 50 dpi. Whereas one primate (D04) showed two peaks at 50 and 85dpi and the last primate (D05) had three peaks occurring around 20, 50, and 85 dpi. Phylogeography and gene flow analysis showed multiple brain seeding events occurred throughout the infection, with the initial brain infection occurring as early as the first week post infection, and the median of the of the transitions taking place between 52-68 dpi. The data indicate that the depletion of CD8 immune response in the infected macaques, all of which developed neural damage, results in an accelerated evolution rate of the virus leading to an increase of genetic heterogeneity and the emergence of effective viral variants where the majority migrate to the brain several weeks post infection.

108. The inducible antioxidant transcription response has context-dependent effects on stress resistance

Tang L, Choe KP*

Department of Biology, University of Florida, Gainesville, FL

Pre-exposure to one type of stress often confers resistance to a distinct type of stress suggesting that inducible stress responses may be broadly beneficial. Cap "n" collar (CNC) transcription factors are principle regulators of antioxidant

and detoxification defense genes in animals ranging from nematodes to mammals. *Caenorhabditis elegans* has a single functional CNC named SKN-1 that is directly suppressed by the WD40 repeat protein WDR-23. The role of SKN-1 in mediating oxidative and xenobiotic stress resistance has been widely reported, but it is unclear what role SKN-1 has during other types of stress. Juglone (175 μ M), sodium chloride (425 mM), and heat exposure (34°C, 12 hrs) were used as models for oxidative, osmotic, and heat stress, respectively. Worms with increased SKN-1 activity due to *wdr-23* deletion were resistant to juglone and had slightly increased longevity. Alternatively, loss-of-*wdr-23* sensitized worms to osmotic and heat stress. We also found that loss of *wdr-23* dramatically reduced survival of diapause, a starvation-induced developmental state. All of the stress-resistance phenotypes of *wdr-23* were suppressed by *skn-1(RNAi)*. Taken together, our studies demonstrate that SKN-1 has complex effects on stress resistance that vary by the nature of the stressor. Surprisingly, these results also suggest that individual stress responses may be beneficial only under a narrowly defined set of conditions.

109. SFMBT1 is required in Snail1-mediated transcriptional repression during epithelial-mesenchymal transition

Tang M^{1,2}, Shen H³, Lin T^{1,2}, Jin Y¹, Cai QS¹, Quyen T⁴, Lin S⁵, Wu L^{5,*}, Lu J^{1,*}

¹Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

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

³Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, China

⁴Department of Biology, Agnes Scott College, Atlanta, GA

⁵Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Epithelial-mesenchymal transition (EMT) is a reprogramming event reflecting the plasticity of epithelial cells. EMT is a critical step during embryonic development and tumor progression. During this transition, epithelial markers such as E-cadherin, a gatekeeper of the epithelial phenotype and a suppressor of tumor invasion, are down-regulated. We previously demonstrated that the Snail1 zinc finger transcription factor represses epithelial gene expression by recruiting the LSD1 demethylase complex to remove dimethyl histone H3 lysine 4 (H3K4me2), a mark associated with active transcription. Here we show that the Snail1 protein complex also contains the malignant brain tumor (MBT) domain protein SFMBT1. We have confirmed that SFMBT1 associates with Snail1 through the LSD1 complex. Depletion of SFMBT1 impairs Snail-mediated E-cadherin repression and TGF β -induced EMT. Importantly, knockdown of SFMBT1

blocks demethylation of the H3K4me2 mark at the E-cadherin promoter by Snail1-LSD1. Because the MBT domains recognize dimethyl lysines, we propose that SFMBT1 functions as an essential histone reader of the LSD1 demethylase complex required for Snail1-mediated transcriptional repression.

110. Computational analysis of alternative splicing regulation using RNA-Seq data from a mouse model

Tang S^{1,2}, Riva A^{2,*}

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

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

Characterization of the RNA population plays an important role in studying the spatial and temporal expression patterns of gene isoforms and elucidating protein functions. Differential inclusion of exons and alternative usage of splice sites causes genes in metazoan organisms to produce multiple isoforms with distinct biological functions and compositions. In this study, we report on the application of the software package PASTA (Patterned Alignments for Splicing and Transcriptome Analysis) to study alternative splicing and differential expression of isoforms using RNA-Sequencing (RNA-Seq) data from an Mbnl2 knockout mouse. Results from PCR validation show that PASTA is very sensitive in discovering alternative splicing events, and has the ability to detect non-canonical splicing. The use of PASTA can lead to a more accurate characterization of alternative splicing and its regulation, and can be used to analyze changes in RNA isoform expression levels in a variety of applications including cancer research.

111. The genetic program for cartilage development evolved in the common ancestor of Bilateria

Tarazona OA^{1,2}, Slota L^{1,2}, Cohn MJ^{1-3,*}

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

Cartilage has been proposed to be a defining character of vertebrates, however this tissue type has evolved independently in a number of invertebrate lineages. Studies of vertebrate chondrogenesis have revealed critical proteins for the structure of the cartilage extracellular matrix (ECM) and the regulation of chondrogenesis. Collagen2A1 is the major structural protein in the cartilage

ECM, for instance, and the Collagen2A1 gene is directly regulated by Sox9, Sox5 and Sox6 transcription factors. Signaling proteins such as Sonic hedgehog regulate chondrogenesis by acting upstream of Sox genes. To test the hypothesis that invertebrates and vertebrates use a common genetic program to build cartilage, we studied chondrogenesis in the cephalopod *Sepia pharaonis* and the horseshoe crab *Limulus polyphemus*, two distantly related Protostome invertebrates. We cloned the invertebrate orthologs of the ancestral (pre-duplication) Collagen2A1, Sox9, Sox5/6 and Hedgehog genes and examined their expression during embryonic development by *in situ* hybridization. We found that Clade A collagen and Sox genes are expressed in pre-chondrogenic cells of both species, and the expression of Hedgehog in adjacent tissues suggests a possible regulatory role. The expression data presented here demonstrate that invertebrate cartilage is collagen-based and chondrogenesis is probably regulated by SoxE and SoxD genes. Therefore, invertebrate cartilages share homologous molecular building blocks, which supports the hypothesis of invertebrate cartilage as "true cartilage". The findings suggest the independent evolution of cartilage by a deeply conserved genetic program for chondrogenesis for Bilateria.

112. Genome engineering using ZFN and TALEN technology

Tremblay DC, Culver-Cochran A, Horakova A, Chadwick BP

Department of Biological Science, Florida State University, Tallahassee, FL

Significant progress in the field of genome engineering has recently been fueled by advances in the design of novel zinc finger nucleases (ZFNs) and TAL-effector nucleases (TALENs). Targeted gene disruption using ZFNs and TALENs can be useful for gene correction, mutation, insertion or deletion via homology-mediated repair (HMR) and/or non-homologous end-joining (NHEJ). While these powerful technologies have been extensively used to induce NHEJ-based mutations in various model organisms, there has been limited use of ZFNs and TALENs in HMR of human cells. As proof of principle that ZFNs and TALENs can be used to induce HMR in human cells, we have constructed a ZFN and a TALEN that target the human XIST and SETDB1 genes, respectively. The ZFN targets the minimal promoter of the XIST gene, and was introduced into RPE1 cells in combination with a homologous template that replaces the endogenous promoter with an SV-40 driven neomycin gene. We isolated one heterozygous clone in which the promoter has been successfully deleted from one allele. Alternatively, the TALEN targets the ATG-containing exon 2 of SETDB1, and was introduced into RPE1 cells with a promoter-trap repair

template that fully replaces the exon. Two independent clones from 74 total (2.3%) were isolated that successfully deleted exon 2 on both alleles. Our findings support the continued use and further development of both nuclease platforms for homology-mediated genome editing in human cells.

113. Bacterial expression of a plant regulator of actin dynamics

Turcotte M^{1,2}, Grey PH¹, Emmanuel M¹, Cuddy KK¹, Oppenheimer DG^{1,3,*}

¹Department of Biology, University of Florida, Gainesville, FL

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

³Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

The ability of the actin cytoskeleton to rapidly polymerize and depolymerize in response to various cellular signals underlies many important cellular processes including membrane trafficking and intracellular transport. Members of the actin depolymerizing factor/cofilin (ADF) family of proteins play an important role in regulating the depolymerization of actin filaments. We recently discovered a family of ADF regulators in plants that we named RPA for Regulator of Plant ADF. We previously demonstrated that the founding member of the family, RPA1, could regulate ADF *in vitro* by using bacterially expressed RPA1. An analysis of the protein sequences of other RPA family members revealed that about half of the members possess a putative membrane anchor sequence at their N-terminus. To determine if these RPA family members also regulate ADF, we attempted to express RPA11, which possesses a putative membrane anchor, in bacteria and purify the protein to use in our *in vitro* ADF activity assays. Unfortunately, the N-terminal membrane anchor sequence appears to inhibit growth of the bacteria and expression of RPA11. To overcome this problem, we created a bacterial protein expression vector that contains a yellow fluorescent protein tag in addition to the affinity purification tags. Using this vector will allow the expression levels of the RPA11 protein to be monitored under different bacterial growth conditions prior to its purification. This will allow us to determine appropriate bacterial media and growth temperature to optimize RPA11 expression levels. Subsequent purification and *in vitro* analysis of RPA11 function will shed light on this new family of ADF regulators.

114. Bayesian divergence dating of Yemeni mitochondrial haplogroups

Vyas DN¹, Kitchen A², Černý V³, Al-Meerī A⁴, Mulligan CJ^{1,*}

¹Department of Anthropology, University of Florida, Gainesville, FL

²Department of Anthropology, University of Iowa, Iowa City, IA

³Archaeogenetics Laboratory, Institute of Archaeology of the Academy of Sciences of the Czech Republic, Prague, Czech Republic

⁴Department of Clinical Biochemistry, Faculty of Medicine and Health Sciences, University of Sana'a, Sana'a, Yemen

The southern dispersal route (SDR) out of Africa posits that anatomically modern humans (AMHs) first left Africa by crossing the southern extent of the Red Sea. Yemen would be the first stop outside Africa. If AMHs did follow the SDR and left modern descendants, one would expect to see deep divergences in the Yemeni mitochondrial gene tree. On the other hand, if AMHs followed the SDR but no modern descendants remain or if AMHs did not pass through Yemen on their route, one would expect more recent divergence dates instead. We used data from a wide-array of haplogroups that were previously sequenced using traditional Sanger sequencing. We used multiple methodologies within the BEAST software package to generate divergence dates. One method, which we took from published literature, used a relaxed clock model and a prior on the tree root, but restricted analyses to the coding region. We also used a novel method that attempts to integrate both the coding and control regions by using multiple substitution models. Dates were assessed to see how they related to various theories, hypotheses, and models of human migration out of Africa.

115. Phototropin 1 and cryptochrome action in response to green light in combination with other wavelengths

Wang Y¹, Maruhnich SA¹, Mageroy MH¹, Justice JR², Folta KM^{1,2,*}

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

²Horticultural Sciences Department, University of Florida, Gainesville, FL

Light signals from different portions of the spectrum converge to shape seedling development. Studies in *Arabidopsis thaliana* have delineated discrete roles for far-red, red, blue and green light in the control of hypocotyl growth inhibition. In this study the effect of green light (525-550 nm) was examined in concert with other light qualities. During photomorphogenic growth, narrow-bandwidth far-red, red or blue light inhibit hypocotyl (stem) elongation. Conversely, green light induces elongation. Blue-green reversibility has been shown to occur via the cryptochrome receptors. While early hypocotyl growth inhibition during de-etiolation is triggered by blue light acting through cryptochromes, it is

not green light reversible. Instead, blue and green light act additively to enhance inhibition. On the other hand, green light reverses the effects of red and far-red phytochrome action on hypocotyl growth inhibition in both early (min-h) and late (h-d) responses. The effects of green light requires the presence of phototropin 1, suggesting that this was not a green light response, but instead a response to extremely low-fluence rate blue light. Tests with dim blue light (<0.1 $\mu\text{mol}/\text{m}^2\text{s}$) confirm a phot1-dependent promotion of stem growth, opposing the effects of red/far-red light. The results show that green light works additively with blue light via cryptochromes during initial acclimation to light, and those red and far-red responses are actually opposed by dim blue light, in a manner that requires phot1. These findings demonstrate how enriched green environments may adjust red and blue light photomorphogenic responses through both the crys and phot1 receptors, and define a new role for phot1 in stem growth promotion.

116. scAAV-mediated gene transfer of interleukin 1-receptor antagonist to synovium and articular cartilage in large mammalian joints

Watson RS¹, Broome TA², Levings PP¹, Colahan PT², Ghivizzani SC¹

¹Department of Orthopaedics and Rehabilitation, University of Florida, Gainesville, FL

²Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL

With the long-term goal of developing a gene-based treatment for osteoarthritis (OA), we performed studies to evaluate the equine joint as a model for AAV-mediated gene transfer to large, weight-bearing human joints. A self-complementary AAV2 vector containing the coding regions for human interleukin-1 receptor antagonist (hIL-1Ra) or green fluorescent protein (GFP) was packaged in AAV capsid serotypes 1, 2, 5, 8 and 9. Following infection of human and equine synovial fibroblasts in culture, we found that both were only receptive to transduction with AAV1, 2 and 5. For these serotypes, however, transgene expression from the equine cells was consistently at least 10-fold higher. Analyses of AAV surface receptor molecules and intracellular trafficking of vector genomes implicate enhanced viral uptake by the equine cells. Following delivery of 1×10^{11} vector genomes of serotypes 2, 5 and 8 into the forelimb joints of the horse, all three enabled hIL-1Ra expression at biologically relevant levels and effectively transduced the same cell types, primarily synovial fibroblasts and, to a lesser degree, chondrocytes in articular cartilage. These results provide optimism that AAV vectors can be effectively adapted for gene delivery to large human joints affected by OA.

117. Selection for cold tolerance alters the maintenance of metabolic homeostasis during cold exposure in *Drosophila melanogaster*

Williams CM¹, Watanabe M², Morgan T³, Edison AS⁴, Boroujerdi A², Hahn DA¹

¹Department of Entomology and Nematology, University of Florida, Gainesville, FL

²Department of Chemistry, Claflin University, Orangeburg, SC

³Division of Biology, Kansas State University, Manhattan, KS

⁴Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Low temperatures induce in insects a state of paralysis (chill coma), which is reversible following the return of favorable conditions, although the time taken to recover varies widely both inter- and intra-specifically. This variation may result from differences in the degree to which insects can maintain metabolic homeostasis during cold exposure. We selected replicate lines of *Drosophila melanogaster* for either fast or slow recovery from chill coma (cold-tolerant or -susceptible lines), then profiled and compared the polar metabolome before, during and after cold exposure using nuclear magnetic resonance spectroscopy. We found that the cold tolerant lines were smaller, and maintained a higher degree of metabolic homeostasis during cold exposure. Pathways that responded differently to the cold exposure between cold-tolerant and -susceptible lines included amino-acyl tRNA biosynthesis (indicating differential levels of translation during cold stress), proline and alanine metabolism, starch and sucrose metabolism, and the TCA cycle. Our results suggest that adaptation to cold environments results in evolution towards energetic pathways that function better in the cold. These lines are fully genotyped, allowing us to look for single nucleotide polymorphisms among the selection lines in genes belonging to these pathways.

118. Evaluation of agrobacterium-mediated and biolistic gene transfer to sugarcane

Wu H^{1,2}, Awan FS¹⁻³, Zeng Q^{1,2,4}, Vilarinho A^{1,2}, Phipps T^{1,2}, McCuiston J⁵, Wang W⁵, Caffall K⁵, Altpeter F^{1,2,*}

¹Agronomy Department, University of Florida, Gainesville, FL

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

³Current address: Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan

⁴Current address: Biochemistry and Biotechnology Department, Yunnan Agricultural University, Kunming, China

⁵Syngenta Biotechnology Inc., Research Triangle Park, NC

Sugarcane (*Saccharum* spp. hybrids) is one of the most productive crops and is extensively utilized for table sugar or biofuel production. Both agrobacterium-mediated transformation or biolistic gene delivery have been successfully used for efficient gene transfer to sugarcane. For biolistic gene transfer method we have previously employed various optimizations including reduced amounts of DNA and/or removal of the vector backbone prior to gene transfer. These modifications resulted in reduced copy number and backbone insertion of the transgenic loci as well as reduced soma-clonal variation. Data from a biolistic and agrobacterium-mediated gene transfer of minimal nptII expression cassettes to sugarcane will be presented. Callus derived from six weeks culture of immature leaf whorl cross sections of commercial cultivar CP88-1762 was used as target for either biolistic gene transfer of the minimal nptII expression cassette or agrobacterium-mediated gene transfer of the nptII expression cassette in five independent experiments. Selection of transgenic events followed the same concentrations and types of selective agents for both transformation procedures. PCR amplification by nptII specific primers confirmed a total of 383 transgenic plants. The number of transgenic plants generated per tissue unit, variability from experiment to experiment, the time required from explant to transgenic plants in soil, the number of non-transgenic escapes, the frequency of complex or simple transgenic loci and the transgene expression level of simple and complex loci events for both agrobacterium-mediated and biolistic gene transfer were compared.

119. Anti- α 1-antitrypsin single chain variable fragment prevents Z-AAT toxicity and aggregation

Xiao K^{1,2}, Levites Y³, Wang RL¹, Oshins RA¹, Rouhani FN¹, Song S^{4,*}, Brantly ML^{1,*}

¹Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, University of Florida, Gainesville, FL

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

³Center for Translational Research in Neurodegenerative Disease, University of Florida, Gainesville, FL

⁴Department of Pharmaceutics, University of Florida, Gainesville, FL

Alpha-1 antitrypsin (AAT) is a neutrophil elastase inhibitor. The Z mutation (E342K) of AAT causes retention of the mutant protein as an inclusion body within the ER of hepatocytes, which may cause severe liver disease such as fibrosis and cirrhosis. Current methods of blocking the loop- β -sheet polymerization of Z-AAT with ligands or small peptides have been proven to be feasible *in vitro*, but have

limitations in the delivery method to the cell. We designed a new strategy using a single chain variable fragment (scFv) derived from a monoclonal antibody to inhibit AAT polymerization; we can further improve the scFv-ZAAT complex degradation by adding a tag recognized by proteasome machinery. Anti-AAT scFv was constructed and validated based on a parent hybridoma. The mRFP-Z-AAT stable-expression CHO cells were transfected with scFv expression constructs or a GFP expression vector as a control and Z-AAT accumulation levels and cell stress were evaluated. The ability of the scFv to bind AAT was validated *in vitro* and *ex vivo*. To facilitate degradation of the complex, Hsp70 binding motif, FKBP12, or KDEL sequence was attached to the C-terminus of the scFv; of these, only scFv-FKBP12 treated cells showed significant reduction of polymers within the ER. In addition, the scFv-FKBP12-Z-AAT complexes were degraded by the proteasome pathway and reduction of Z-AAT polymerization lead to decreased cell stress levels. Furthermore, although the scFv can recognize both M and Z AAT, the expression of scFv-FKBP12 does not interfere with the secretion or activity of normal M-AAT in the cells.

120. Analysis of the maternally expressed gene1 family provides novel insight into the evolution and expression of tandem gene duplicates in maize

Xiong Y¹, Mei W², Kim D-H³, Mukherjee K¹, Hassanein H¹, Barbazuk WB^{2,*}, Kolaczowski B^{1,*}, Sung S³, Kang B-H^{1,4}

¹Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

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

³Cell and Molecular Biology Graduate Program, University of Texas, Austin, TX

⁴Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

Maternally expressed gene1 (Meg1) encodes a small cysteine-rich peptide that is required for the normal development of the basal endosperm transfer cells (BETCs) in maize. Twelve copies of *Meg1*-like genes were identified to be a gene cluster near the *Meg1* gene. Here we analyzed the genomic organization, gene structure, expression, and chromatin status of the family. Except *Meg1* and *Meg13*, one of the *Meg1*-like gene, all the other members are flanked by homologous sequences varying in lengths from a few hundred bp to >5 kb, indicating that the gene family expanded through unequal crossing-over. Eight genes with intact gene structures are transcriptionally active and exhibit the BETC-specific expression pattern. Chromatin immunoprecipitation revealed activating and repressive marks of these genes in BETC and non-BETC samples, respectively, indicating that chromatin modifications contribute to the cell type-specific expression of the *Meg* gene family. Phylogenetic analysis

and synteny mapping suggest that the major duplication events took place after the maize allotetraploidy at 4.8 million years ago (mya) but prior to the emergence of teosinte. The oldest member displays a tissue expression pattern different from rest of the members suggesting that *Meg* genes acquire new functions as they expand and evolve. A strong signature of positive selection in the youngest members suggests further functional diversification in the family.

121. JAZ2 and JAZ7 function in jasmonate signaling through different mechanisms

Yan H^{1,2}, Yoo M-J¹, Chen Y¹, Koh J³, Acikgoz D¹, Wang Q², Chen S^{1,3,4,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Department of Horticulture, Zhejiang University, Hangzhou, China

³Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

⁴Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Jasmonates (JAs) are recognized as one of the important plant hormones, which play a vital role in plant growth, development and stress response. In this study, we focused on characterizing JAZ2 (JASMONATE ZIM-DOMAIN 2) and JAZ7, which are two of the 12 JAZ family members presumably functioning as repressors in JA signaling in *Arabidopsis thaliana*. We have isolated homozygous T-DNA insertion mutants of *JAZ2* and *JAZ7*, and observed different growth phenotypes between the mutants and wild type (WT) in the presence or absence of methyl jasmonate (MeJA). We have also tested the effects of other phytohormones and conditions. To understand the phenotypes and elucidate the molecular mechanisms underlying the functions of *JAZ2* and *JAZ7*, we have employed functional genomics approaches to analyze different types of molecules and their dynamic changes in the above plant materials. Here we will report our recent findings from RNA-Seq, proteomics and metabolomics experiments. These data can be used to construct a molecular network of JA signaling.

122. Genetic suppressors of plastid translation mutants in maize

Yang J^{1,*}, Suzuki M^{1,2}, McCarty DR^{1,2,*}

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

²Horticultural Sciences Department, University of Florida, Gainesville, FL

Genetic studies indicate that plastids have an essential role in plant embryo development. Our recent findings indicate that in maize, the embryo lethal phenotypes typical of a large class of mutants that have defects in plastid ribosome assembly and protein translation can be suppressed in some inbred backgrounds. At least one genetic modifier of a non-lethal class plastid mutant is already known. *Inhibitor of Striate 2 (Isr1)* gene is a suppressor of the white leaf striping mutant, *striate 2 (sr2)*. *Isr1*, encodes a putative plastid localized hydrolase, has been proposed to modify striping by suppressing proliferation of a subset of cells in leaves of the *sr2* mutant that lack protein translation competent plastids. Hence, *Isr1* may serve as a model for suppressors of the embryo lethal plastid mutants. To understand the molecular mechanism responsible for the formation and suppression of the white striping, we have characterized natural allelic variation of *Isr1* in diverse maize inbreds as well as molecular cloning of *sr2* gene. A candidate gene for *sr2* identified by bioinformatic analysis of linked genes in the maize genome is shown to encode a putative plastid localized nudix hydrolase homolog 26 (NUDX26). To test whether *NUDX26* is *sr2*, we have sequenced alleles from wild type and *sr2* mutant plant. In addition, genetic analyses are underway to map modifiers of the embryo lethal plastid translation mutants. Together these studies will illuminate the relationship if any between *Isr1* and background suppressors of embryo lethal plastid mutants in maize. Our long term goal is to understand the role of plastids and plastid derived signals in regulation of cell proliferation and embryo formation.

123. Objective measures of variability and reliability in ChIP-seq

Yang Y^{1,2}, Fear J^{1,2}, Hu J^{2,3}, Haecker I^{2,3}, Jacobs D^{2,4}, Bloom DC^{2,*}, Renne R^{2,3,*}, McIntyre LM^{2,5,*}

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

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

³University of Florida Shands Cancer Center, Gainesville, FL

⁴Interdisciplinary Program in Biomedical Sciences, University of Florida, Gainesville, FL

⁵Department of Statistics, University of Florida, Gainesville, FL

ChIP-seq is a new technology which enables rapid identification of protein-DNA binding. The technique is noisy, requiring biological replicates to confirm peak identification and to quantify binding. Biological replicates have been found to be critical and are required for deposit of data in the ENCODE and MODENCODE projects. Yet, to date no consensus has emerged about how to determine peak presence/absence among multiple biological

replicates. In addition, there is no consensus about how to quantify peaks among replicates. We use objective measures of variability and reliability to determine the best approach to consensus peak identification among biological replicates. We used several different ChIP-seq datasets including PolII, transcription factors and histones from the ENCODE and MODENCODE projects to illustrate the utility of objective measures in the determination of consensus peaks.

124. De novo transcriptome assembly and proteome profiling of the recently formed allopolyploid *Tragopogon mirus* (Asteraceae) and its diploid parents

Yoo M-J¹, Koh J^{1,2}, Soltis DE^{1,3,*}, Soltis PS^{3,*}, Barbazuk WB^{1,*}, McIntyre LM^{4,5,*}, Chen S^{1,2,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

³Laboratory of Molecular Systematics and Evolutionary Genetics, Florida Museum of Natural History, University of Florida, Gainesville, FL

⁴Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

⁵Department of Statistics, University of Florida, Gainesville, FL

Polyploidy (whole-genome duplication) is recognized as an important evolutionary process in speciation and genome evolution of diverse organisms, particularly plants. However, much of our current understanding of polyploidy is based on analyses of crop species. Here, we examined the transcriptomes of naturally occurring allopolyploid *T. mirus* and its diploid parents (*T. dubius* and *T. porrifolius*) using Illumina HiSeq 2000 technology. In parallel, we employed iTRAQ LC MS/MS to investigate the global proteomes of the three species. A total of 480 million 100-bp paired-end reads was generated from leaf transcriptomes of the three species, which corresponds to 34 Gb of sequence. These reads were assembled *de novo* by the Trinity short-read assembler, and the assembled contigs were annotated by Blast2GO software. This assembly was utilized as a reference for RNA-Seq and proteomic data analyses. Differential gene expression between the allopolyploid and its diploid parents was analyzed and compared at transcriptomic and proteomic levels. Together with ongoing analysis of transcriptome profiles of another young allopolyploid, *T. miscellus*, and its diploid parents (*T. dubius* and *T. pratensis*), this study will provide valuable insights into transcriptomic as well as proteomic changes in recently formed allopolyploids. In addition, the transcriptome data set generated here provides the most comprehensive sequence resource for

the *Tragopogon* polyploid system, which enables further study of gene and protein expression patterns in different tissues and under different conditions.

125. Systematic characterization of multiple sucrose-metabolizing pathways in *Streptococcus mutans*

Zeng L, Burne RA

Department of Oral Biology, University of Florida, Gainesville, FL

Sucrose is perhaps the most efficient carbohydrate for the promotion of dental caries in humans, and the primary caries pathogen *Streptococcus mutans* encodes multiple enzymes involved in the metabolism of this disaccharide. Here, we engineered a series of mutants lacking individual or combinations of sucrolytic pathways to understand control of sucrose catabolism and to identify novel or alternative sucrases. Growth phenotypes indicated that *gtfBCD* (glucan exopolysaccharide synthases), *ftf* (fructan exopolysaccharide synthase) and *scrA*, *scrB* (sucrose-PTS permease and sucrose-6-P hydrolase, respectively) constitute the majority of the sucrose-catabolizing activity of *S. mutans*; however mutations of any of these genes alone did not negatively impact planktonic growth on sucrose. Conversely, the multiple sugar metabolism pathway (*msm*) was shown to play only a minor role in growth on sucrose. Notably, loss of *scrA* led to growth stimulation in fructooligosaccharide-based medium, due in large part to increased expression of the *fruAB* (fructanase) operon (shown as a *PfruA-cat* fusion). Using the LevQRST four-component signal transduction system as a model for carbohydrate-dependent gene expression in strains lacking combinations of sucrases, a *PlevD-cat* reporter was shown to be activated by pulsing with sucrose. Interestingly, *ScrA* was required for activation of *levD* expression by sucrose through components of the LevQRST complex, but not for activation by the cognate LevQRST sugars, fructose or mannose. Collectively, these results revealed novel regulatory circuitry for control of sucrose catabolism, with a central role of the *ScrA* PTS permease as an effector.

126. Map-based cloning of the maize defective kernel5 (*dek5*) mutant

Zhang J¹, Settles AM^{1,2,*}

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

²Horticultural Sciences Department, University of Florida, Gainesville, FL

Defective kernel 5 (*dek5*) is one of the classic *dek* mutants in maize, but little is known about the biochemical and

molecular function of the *dek5* gene. The *dek5* mutant has a shrunken and collapsed-seed phenotype with an opaque, chalky endosperm and a normal embryo. Mutant seeds germinate poorly, and seedling leaves are pale green with white stripes. The seed phenotype is similar to starch biosynthesis mutants such as *brittle1* and *brittle2*, and we hypothesize that *dek5* affects endosperm starch biosynthesis. Starch accumulates in the amyloplast, which is a non-green form of the chloroplast. The pale green seedling phenotype of *dek5*, suggests the gene is also required for chloroplast development. We are cloning this gene to understand the function of *dek5* in seed development. We developed F2 mapping populations from crosses of the *dek5-25* allele with the Mo17 inbred. Fine mapping identified a 460 kb interval on chromosome 3 as containing the *dek5* locus. Thirteen gene models are within this map interval and are currently being evaluated as candidates for the *dek5* gene.

127. Biochemical characterization of *Brassica napus* MAP kinase 4

Zhang T¹, Zhu M¹, Song WY^{2,3,*}, Harmon AC^{1,3,*}, Chen S^{1,3,4,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Plant Pathology Department, University of Florida, Gainesville, FL

³Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

⁴Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

Mitogen-activated protein (MAP) kinase pathway includes MAP triple kinases (MAPKKKs), MAP double kinases (MAPKKs) and MAP kinases. This kinase cascade plays an important role in plant growth, development and defense. We show here that a MAP kinase from *Brassica napus*, BnMPK4, has autophosphorylation activity in the presence of manganese. The phosphorylation sites were mapped to the tyrosine residue in the activation loop using mass spectrometry. In addition, BnMPK4 can phosphorylate myelin basic protein (MBP) and the *B. napus* MAP kinase substrate (MKS1) *in vitro*. Compare to MBP, the *in vivo* substrate MKS1 was a better substrate for MPK4. While substrate phosphorylation activity of BnMPK4 is much stronger than the autophosphorylation activity, the presence of MKS1 can enhance the autophosphorylation activity of BnMPK4. BnMPK4 exists in both oxidized and reduced forms *in vitro*, and both showed kinase activity. BnMPK4 aggregated under oxidized conditions, such as in the presence of hydrogen peroxide and cupric chloride, but it still retained the kinase activity. These results indicate that BnMPK4 is an active kinase and its activity may not be affected by redox *in vitro*. The functional

significance of substrate enhancement and aggregation of BnMPK4 will be discussed.

128. pHA4C plasmids: a new modular series of vectors for constitutive and GAL4-mediated expression in *Drosophila*

Zhang Y¹, Arcia S¹⁻³, Perez B^{1,3}, Fernandez-Funez P^{1,4,*}, Rincon-Limas DE^{1,*}

¹Department of Neurology, University of Florida, Gainesville, FL

²Howard Hughes Medical Institute-UF Science for Life Program, University of Florida, Gainesville, FL

³Department of Microbiology and Cell Science, University of Florida, Gainesville, FL

⁴Department of Neuroscience, University of Florida, Gainesville, FL

Control of gene expression relies on a variety of strategies, including gene overexpression, gene rescue, and gene silencing. The UAS/GAL4 system has become a popular choice for genetic manipulation. However, there are cases in which ubiquitous expression is desirable independently of GAL4. Traditionally, constitutive expression has been achieved by cloning cDNAs under control of ubiquitous promoters such as the Actin5C or Hsp70 genes. Unfortunately, the Actin5C promoter displays heterogeneous expression and the Hsp70 promoter requires heat induction, which may affect certain experiments. In a search for more homogeneous expression, the promoters of α 1-Tubulin, Armadillo or EF-1 α F1 were isolated. The first introns of these genes containing essential regulatory information were used together with the first exon containing the ATG and a fragment of the second exon for ligation of the cDNA, further complicating cloning. To overcome these limitations we created p Δ TubHA4C, which was designed for expression of cDNAs under control of a minimal Tubulin promoter carrying key regulatory regions and the intron 1 of α 1-Tubulin. We then incorporated an optimized polylinker to offer flexible cloning options. Finally, we added the option of double labeling the expressed proteins with two C-terminal tags, the viral epitope hemagglutinin (HA) and a synthetic tetracysteine (4C) tag that binds small fluorescent compounds. To expand the application of this optimized polylinker, we also subcloned it into the typical pUAST vector to allow GAL4-dependent expression as well as att-mediated integration of the transgene. The properties and advantages of the new series of vectors will be presented.

129. High Performance Computing Center overview

Taylor CA, Zhang Y

Research Computing, University of Florida, Gainesville, FL

The High Performance Computing Center, part of the University of Florida Research Computing, is a faculty-directed facility with the focused mission of providing high-performance computing and storage resources, including support, to faculty whose research depends on large-scale computing. We will provide an overview of the facilities, services, and resources available within the High Performance Computing Center.

130. Thiol-based redox regulation of serine/threonine protein kinase in *Brassica napus*

Zhu M¹, Zhang T¹, Silva-Sanchez C², Song WY^{3,4,*}, Harmon AC^{1,4,*}, Chen S^{1,2,4,*}

¹Department of Biology, University of Florida, Gainesville, FL

²Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

³Plant Pathology Department, University of Florida, Gainesville, FL

⁴Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Protein kinase mediated phosphorylation events have been recognized as pivotal regulatory processes in stomatal function under abiotic and biotic stress conditions. A serine/threonine protein kinase belonging to the SnRK2b subfamily was identified to be ABA- and MeJA-responsive in a proteomic analysis of *B. napus* guard cells. Heterologously expressed BnSnRK2b exhibited *in vitro* kinase activity in a manganese-dependent manner and preferentially phosphorylated myelin basic protein and β -casein. Phosphorylation of multiple residues during the kinase autophosphorylation was detected by mass spectrometry analysis, including the serine and threonine in the activation loop. Interestingly, the *in vitro* autophosphorylation activity was inhibited by treatment of oxidants, including hydrogen peroxide, oxidized glutathione (GSSG) and nitric oxide donor S-nitrosoglutathione (GSNO). Isotope tagging analysis revealed that specific cysteines are responsive to certain treatment and contributes to the redox response. Additionally, the recovery of the inhibited kinase activity by reductant such as dithiothreitol (DTT) or thioredoxin isoforms indicated the *in vitro* autophosphorylation activity is reversibly regulated by redox status of the microenvironment. Furthermore, the ability to phosphorylate a *B. napus* slow anion channel protein (BnSLAC1 NT) is also reversibly redox regulated. All these data suggested redox regulation of (auto)phosphorylation process in guard cell signal transduction.



FLORIDA GENETICS 2012

