FLORIDA GENETICS 2011

THE SEVENTH ANNUAL SYMPOSIUM OF THE UF GENETICS INSTITUTE

NOVEMBER **9-10**

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UFGI Strategic Plan

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

2011 Florida Genetics Symposium Schedule

Wednesday, November 9, 2011

Noon – 1:00 p.m.: Check-in and poster set-up at the Cancer/Genetics Research Complex, posters no. 1-60

1:00 p.m. – 1:15 p.m.:

Opening Remarks:
Connie Mulligan* and Kenneth Berns*

Session I

Extending Life and Limb
Chair: Marty Cohn*

1:15 p.m. – 2:00 p.m., Ergun Sahin:
"Telomere dysfunction induced mitochondrial compromise and ageing"

2:00 p.m. – 2:30 p.m., Malcolm Maden*:
"Will extending amphibian limbs lead to extending mammalian life?"

2:30 p.m. - 4:30 p.m.: Poster Session I:
Posters no. 1-60, award presentation for the Codified Art + Genetics competition, and reception (for registered attendees)

****5:00 p.m. – 6:00 p.m., Carol Greider:**
"Telomeres in cancer and stem cell failure"

***= UF Genetics Institute Faculty**

**All activities will be at the Cancer/Genetics Research Complex except for Dr. Greider's presentation, which will be at 5:00 p.m. Wednesday in the auditorium of the HPNP building on the Health Science Center campus.

Thursday, November 10, 2011

8:00 a.m. - 8:30 a.m.: Check-in, coffee, set up posters no. 61-119

Session II

Genetics of Personalized Medicine
Chair: Julie Johnson*

8:30 a.m. – 9:15 a.m., David Goldstein:
"Sequencing and the genetics of disease"

9:15 a.m. – 9:45 a.m., Mark Brantly*:
"The molecular basis of alpha-1-antitrypsin deficiency"

9:45 a.m. – 10:00 a.m.: Break

10:00 a.m. – 10:30 a.m., Reginald Frye:
"Pharmacogenomic biomarkers and personalized medicine: focus on cytochrome P450 enzymes"

10:30 a.m. – 11:15 a.m., Julie Johnson*:
"Translating genetics research to improve patient care"

11:15 a.m. – 1:30 p.m.: Poster Session II:
Posters no. 61-119
11:30 a.m.: Lunch (for registered attendees)

Session III

Host-Pathogen Co-Evolution
Chair: Glenn Morris*

1:30 p.m. – 2:15 p.m., Andrew Bent:
"The molecular back-and-forth of co-adapted plant/bacterial pathogen interactions"

2:15 p.m. – 2:45 p.m., Erica Goss*:
"Plant-pathogen coevolution gone awry: pathogen emergence in the age of globalization"

2:45 p.m. – 3:15 p.m., Jeff Jones*:
"A possible strategy for citrus canker control using a bacterial-derived transgene that triggers programmed cell death"

Presentation Abstracts

Telomere dysfunction induced mitochondrial compromise and ageing

Sahin E

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Telomere dysfunction induced functional decline and ageing has been mainly thought to be secondary to p53-mediated cellular growth arrest, senescence and apoptosis. Recent unbiased studies in telomerase deficient mice have uncovered a significant repression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha and beta (PGC-1α and PGC-1β), an impairment in mitochondrial biogenesis and function, gluconeogenesis, cardiac function, and increased reactive oxygen species in hematopoietic stem cell, liver and heart. Telomere dysfunction associated PGC repression and defects in oxidative phosphorylation, gluconeogenesis and cardiomyopathy can be reversed upon enforced Tert or PGC-1a expression or p53 germline deletion. Mechanistically, p53 directly binds to PGC-1α and PGC-1β promoters and represses their expression. These studies establish a direct molecular link between telomere and mitochondrial biology and we propose that this telomere–p53–PGC axis contributes to functional decline and metabolic failure across different tissues with critical short telomeres.

Biography for Ergun Sahin, M.D., Ph.D.

Ergun Sahin received his M.D. and Ph.D. from the Free University of Berlin, Germany. He completed his residency and fellowship in the Department of Gastroenterology and Hepatology at the Benjamin-Franklin Hospital in Berlin. His scientific interests focus on understanding fundamental aspects of telomere biology in the context of ageing and disease states. He completed his postdoc with Ronal A. DePinho at the Dana-Farber Cancer Institute, Harvard Medical School, Boston, with whom he studied and published on molecular mechanism of telomere driven ageing. He is currently an instructor at Harvard Medical School.

Telomeres in cancer and stem cell failure

Greider CW

Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD

Telomeres protect chromosome ends from recombination and from being recognized as DNA damage. Telomeres shorten with each cell division, but they can be elongated by telomerase. Telomerase is required for cells that undergo many rounds of divisions, including tumor cells and tissue specific stem cells. To understanding the consequences of telomere dysfunction we generated telomerase null mice that are viable and show progressive telomere shortening over six generations. Crosses of these telomerase null mice to other tumor prone mice show that tumor formation can be greatly reduced by short telomeres. These mice have also allowed us to generate an outstanding model of telomere shortening diseases such as dyskeratosis congenita and the associated bone marrow failure. We continue to focus on understanding how short telomeres cause these diseases as well as the mechanisms that regulate telomere length equilibrium.

Biography for Carol W. Greider, Ph.D.

Dr. Greider received a BA from the University of California at Santa Barbara in 1983 and a Ph.D. in 1987 from the University of California at Berkeley. In 1984, working together with Dr. Elizabeth Blackburn, she discovered telomerase, an enzyme that maintains telomeres, or chromosome ends. Dr. Greider first isolated and characterized telomerase from the ciliate *Tetrahymena*. In 1988, Dr. Greider went to Cold Spring Harbor Laboratory where, as an independent Cold Spring Harbor Fellow, she cloned and characterized the RNA component of telomerase. In 1990, Dr. Greider was appointed as an Assistant Investigator at Cold Spring Harbor Laboratory followed by appointment to Investigator in 1994. She expanded the focus of her telomere research to include the role of telomere length in cell senescence, cell death and in cancer. Together with Dr. Calvin Harley, she showed that human telomeres shorten progressively in primary human cells. This work, along with work of other researchers, led to the idea that telomere maintenance and telomerase may play important roles in cellular senescence and apoptosis. In 1997, Dr. Greider moved her laboratory to the Department of Molecular Biology and Genetics at The Johns Hopkins University School of Medicine. In 1999, she was appointed Professor, and in 2004 she was appointed as the Daniel Nathans Professor and Director of the Department of Molecular Biology and Genetics. At Johns Hopkins University Dr. Greider's group continued to study the biochemistry of telomerase and determined the secondary structure of the human telomerase RNA. She also expanded her work on a mouse model of dyskeratosis congenita and stem cell failure in response to short telomeres. Dr. Greider currently directs a group of 10 scientists studying both the biochemistry of telomeres and telomerase as well as the cellular organismal consequences of short telomeres. Dr. Greider has won a number of awards for the work on telomerase, and she shared the Nobel Prize in Physiology or Medicine with Drs. Elizabeth Blackburn and Jack Szostak in 2009.

Sequencing and the genetics of disease

Goldstein D

Duke Center for Human Genome Variation, Duke University School of Medicine, Durham, NC

One primary challenge in the interpretation of large-scale sequencing studies is the huge number of candidate variants that emerge. This occurs both because there are many functional variants in every sequenced genomes and also because sequencing is associated with considerable artifact. There are a number of possible directions for parsing amongst these variants including low to medium throughput functional evaluation, genotyping many candidate variants in large samples sizes, and resequencing targeted genes. Here I describe the application of these approaches in large scale sequencing studies.

Biography for David B. Goldstein, Ph.D.

Dr. Goldstein, Richard and Pat Johnson Distinguished University Professor, is Director of the Center for Human Genome Variation and Professor of Molecular Genetics & Microbiology and Biology at Duke University School of Medicine. Dr. Goldstein is the author of over 175 scholarly publications in the areas of population and medical genetics. His principal interests include human genetic diversity, the genetics of disease, and pharmacogenetics. In April 2007, he was appointed Honorary Professor, Institute of Neurology, University College London, UK. He is the recipient of one of the first seven nationally awarded Royal Society / Wolfson research merit awards in the UK for his work in human population genetics. Most recently, he was appointed co-chair and chair of the Gordon Research Conference meeting on human genetics and genomics for 2011 and 2013.

The molecular back-and-forth of co-adapted plant/bacterial pathogen interactions

Bent A

Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI

The last decade has seen the development of a paradigm for plant/pathogen interactions, describing successive levels of defense and counter-defense between the plant and a co-adapted pathogen. Like many paradigms, this one both provides a useful summary and also some misleading over-simplifications. Our work examining molecular mechanisms of host recognition of bacterial pathogens, and of subsequent plant responses to those pathogens, will be discussed, with a particular focus on FLS2 (the plant flagellin receptor) and other plant innate immune system receptors. Ways in which the prevailing paradigm is challenged by our work and the work of others will also be discussed.

Biography for Andrew Bent, Ph.D.

Dr. Bent received his Ph.D. from Massachusetts Institute of Technology in 1989 and conducted his postdoctoral research at the University of California – Berkeley. Dr. Bent is currently Professor of Plant Pathology, Department of Genetics, College of Agriculture and Life Sciences and the Department of Medical Genetics, School of Medicine and Public Health, University of Wisconsin. Dr. Bent's current research are focused on three projects, 1) Leucine-rich repeat (LRR) structure/function, and plant detection of bacterial flagellin; 2) Study and manipulation of disease resistance in soybean; and 3) Previously unidentified biochemical responses of plants to pathogen infection.

***= UF Genetics Institute Faculty**

Will extending amphibian limbs lead to extending mammalian life?

Maden M*

Department of Biology, University of Florida, Gainesville, FL

The reason for studying regenerative mechanisms animals in animals such as salamanders and newts is that, apart from its intrinsic fascination, we hope to gain insights into how to induce organ regeneration in mammals including humans. This will lead to life extension for those with organ that have become damaged or diseased, but may also give us insights into the ageing process itself. The evidence for this comes from our studies on one specific molecule widely involved in regeneration, namely retinoic acid and this work will be described. This signaling molecule is crucial for limb development, limb regeneration and when administered in excess induces the duplication of limbs. Its mechanism of action and targets in the nucleus are gradually being revealed and not surprisingly it interacts with other developmental signaling pathways. Retinoic acid has also been shown to be involved in the regenerative response in several organ systems including the heart and, most surprisingly, has been shown to induce a regenerative response in mammalian organs which cannot normally regenerate such as the lung or the spinal cord. We have therefore referred to this molecule, whose remarkable properties were originally discovered in the amphibian limb, as a regeneration-inducing molecule. Recent studies have also revealed the role of retinoic acid in neurodegenerative diseases such as Alzheimer's disease and thus this compound may really have life-extending properties.

The molecular basis of alpha-1-antitrypsin deficiency

Brantly ML*

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

Alpha-1-Antitrypsin (AAT) Deficiency is an inherited disorder associated with a substantial increase in risk for obstructive lung disease, cirrhosis and hepatocellular carcinoma. The gene that encodes alpha-1-antitrypsin is SerpinA1 located in the long arm of chromosome 14, nested in a cluster of related genes. While there are more than 120 variants of AAT including null and other disease associated variants, the primary allele associated with greater than 95% of all individuals with deficiency is PI*Z. The Z allele is the result of a single base substitution, G to A, in the terminal exon that creates a single amino acid substitution of Glu to Lys at residue 342. This amino acid change is located in the hinge region of the reactive site loop, is associated with polymerization, rough endoplasmic accumulation, rapid degradation and activation of the unfolded response genes. Expression of the Z allele is associated with a 5-fold decrease in plasma AAT and cellular injury within cells that express the mutant protein. The frequency of the Z AAT gene is one in 1500-4000 individuals. Among Caucasians, 1 per 100 individuals is heterozygous for the allele. Clinical disease is strongly modified by environmental factors. Homozygous individuals that smoke cigarettes have a life-span that is 20 years less than non-smokers. Importantly heterozygous individual have a 1.5-3 fold increased risk of obstruction lung disease and passive smoking, particularly in childhood, is a significant risk factor. Environmental factors are less clear for the development of liver disease but likely include fatty liver disease and heavy alcohol consumption. AAT deficiency is widely under-diagnosed by the medical community largely because a lack of knowledge of the deficiency. Current therapy includes augmentation with plasma purified AAT for lung disease. Novel therapies for both the lung and liver disease are in the pipeline and include gene therapy and drugs that modify folding/degradation. Early identification of AAT deficiency and avoidance of risk factors may substantially decrease the morbidity and mortality of this disorder.

Pharmacogenomic biomarkers and personalized medicine: focus on cytochrome P450 enzymes

Frye R

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

Variability in drug response can be attributed in part to variability in the activity of drug–metabolizing enzymes. One of the most important drug-metabolizing enzyme systems in humans is the cytochrome P450 (CYP) enzyme family, which is responsible for the oxidative metabolism of numerous endogenous compounds and xenobiotics. Several pharmacogenomic biomarkers have been identified that can help predict the outcome of treatment with drugs metabolized by the polymorphic CYP enzymes. In this presentation, the clinical relevance of recent discoveries in this area will be described and implications for personalized medicine will be discussed.

Translating genetics research to improve patient care

Johnson JA*

Department of Pharmacotherapy and Translational Research, University of Florida, Gainesville, FL
Division of Cardiovascular Medicine, Department of Medicine, University of Florida, Gainesville, FL

Since publication of the human genome project there have been substantial advances in genomics technologies such that the cost for generating a human genome sequence is expected to fall below $1000 in the next couple years. As such, it is anticipated that at some point in the future, whole genome sequence data will be available on individual patients as part of their electronic medical record. This presents tremendous opportunities and challenges for beginning to incorporate genetic information into patient care settings. The University of Florida & Shands Hospital Personalized Medicine Program will be among the first in the country to move toward this genetically-guided care approach of translating genetics research to patient care. The research data driving this program, the plans for the program, the genetics research opportunities created, and the long-term view of personalized medicine as an approach to health care will be discussed.

Plant-pathogen coevolution gone awry: pathogen emergence in the age of globalization

Goss EM*

Plant Pathology Department, University of Florida, Gainesville, FL
Emerging Pathogens Institute, University of Florida, Gainesville, FL

The interaction between a plant pathogen and its plant host has been described as an arms race or trench warfare, in which each organism responds in turn to coevolutionary changes in the other. Recent genome sequences of problematic crop pathogens have revealed genomic flexibility that may allow rapid evolutionary responses to changing host populations. Meanwhile, a growing challenge for both agricultural and natural ecosystems is the global movement of plant pathogens and their vectors. Each of these mechanisms of new pathogen or strain emergence is problematic on its own; together they create unprecedented opportunities for global gene flow followed by rapid adaptation to new hosts and environments with potentially devastating effects on global food security and native ecosystems. The Oomycete genus *Phytophthora* includes notoriously damaging pathogens of crops and forest trees. *P. infestans*, the pathogen responsible for the Irish potato famine as well as recent epidemics on potato and tomato, will be discussed as a compelling and ongoing cautionary tale of plant pathogen emergence and re-emergence.

A possible strategy for citrus canker control using a bacterial-derived transgene that triggers programmed cell death

Figueiredo JFL[1,2], Römer P[3], Moore G[1,4,*], Horvath D[5], Stall RE[1], Lahaye T[3], Jones JB[1,2,*]

[1]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
[2]Plant Pathology Department, University of Florida, Gainesville, FL
[3]Institute of Genetics, Ludwig-Maximillians University, Martinsried, Germany
[4]Horticultural Sciences Department, University of Florida, Gainesville, FL
[5]Two Blades Foundation, Evanston, IL

Asiatic citrus canker (ACC), caused by the plant pathogenic bacterium, *Xanthomonas citri*, has recently become established in Florida. ACC adversely affects citrus production worldwide. Conventional control practices rely heavily on copper-based bactericides. However, copper resistance (Cu^r) by way of horizontal gene transfer of Cu^r genes occurs and over time renders copper ineffective. We have designed a strategy for control of ACC based on recent findings predicting activation of the UPA (*UP*-regulated by *A*vrBs3) or UPT (*UP*-regulated by TAL effector) boxes in host cells by transcription activator-like (TAL) effectors (members of the AvrBs3/PthA TAL effector family) and the fact that most *X. citri* strains contain at least two TAL effectors. TAL effectors are injected via the type III secretion pathway into plant cells. Once in the plant cell, they enter the nucleus, bind to UPT boxes and turn on the downstream genes. A major TAL effector target is PthA4, which is present in *X. citri* and is critical for virulence. We have hypothesized that by engineering a promoter which contains several putative UPT boxes fused to a hypersensitive reaction (HR)-inducing gene, we could target PthA and other prevalent AvrBs3 homolog proteins in *X. citri* that when injected by the bacterium into the plant cell would activate transcription of the engineered gene, resulting in expression of an HR. We will discuss preliminary findings based on transient assays and stable transformants.

Poster sessions

***= UF Genetics Institute Faculty. Presenters are underlined.**

1. Genome-wide genetic diversity analysis of two *Pinus* species

Acosta JJ[1], Neves LG[2], Davis JM[1,2,*], Kirst M[1,2,*]

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL
[2]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL

Recent advances in DNA sequence-capture and sequencing make possible the genome-wide analysis and identification of genes under natural selection. We applied this strategy to the analysis of two conifer species, *Pinus taeda* and *Pinus eliottii*, that are widely distributed in the south-southeastern US. Haploid DNA from megagametophytes was extracted from seeds of 24 trees of each specie, collected from natural populations in 11 states (AL, AR, FL, GA, LA, MD, MS, NC, SC, TX and VA), representing the natural range of the species. For genotyping, libraries were prepared by sequence-capturing a portion of the *Pinus* genome composed of 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). Interspecific and intraspecific SNPs are currently being detected from the sequence data. We anticipate that these markers will allow us to identify genes under different selection regimes using statistical tests that fall into three broad categories: (1) frequency-spectrum of mutations within-species (Tajima's D); (2) differentiation among populations (FST outlier detection) and; (3) comparative approaches based on the level of synonymous versus non-synonymous substitutions between species (McDonald-Kreitman test) and on the relationship between within-species diversity and between-species divergence (Hudson-Kreitman-Aguade test).

2. Basal cell carcinoma shows distinct patterns of nucleosome distribution and chromosomal accessibility

Alam PM[1], Elgin M[2], Soni BP[2], Dennis JH[1]

[1]Department of Biological Science, Florida State University, Tallahassee, FL
[2]Dermatology Associates of Tallahassee, Tallahassee, FL

Basal cell carcinoma (BCC) is the most common non-melanoma skin cancer worldwide. Studies of cancerous cells have revealed that normal and malignant cells differ in their chromatin architecture. The degree to which chromatin structure plays a role in the etiology of BCC has not been investigated. We carried out genome-wide analysis of chromatin accessibility in BCC clinical specimens and their matched normal skin tissue samples from 21 patients. We also mapped nucleosome distribution around transcription start site (TSS) of 425 reported cancer associated genes in these samples. We compared genome-wide chromatin accessibility patterns of BCC specimens with normal skin tissue and observed differential accessibility patterns between them at specific loci with striking differences at chromosome 9. We also found that out of 425 genes we analyzed, approximately 80 genes have altered TSS nucleosome distribution when comparing normal and BCC samples. Importantly, gene ontology analysis reveals that these genes are significantly ($p=10^{-4}$) enriched for regulation of kinase activity. These data suggest that global chromatin remodeling and redistribution of nucleosomes around TSS of key cancer related genes contribute to BCC tumorigenesis. We anticipate that these findings might lead to identification of novel biomarkers in basal cell carcinoma with possible diagnostic and therapeutic potentials.

3. Next-generation sequencing technologies at UF's ICBR

Almira EC, Moraga-Amador D, Shanker S, Farmerie WG*

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

The use of next-generation (nextgen) sequencing is changing the landscape of genomic exploration as new and innovative technologies and applications evolve having great impact on how research is performed in the laboratories. Here at UF's ICBR, our Genomics Division has been offering nextgen sequencing services using various platforms such as the Roche 454 GS FLX-plus Titanium, the Illumina GAIIx analyzer, and the Applied Biosystems SOLiD v4 (being upgraded to the AB 5500xl). Recently, we acquired two Life Technologies Ion Torrent PGM machines and the third-generation, single-molecule sequencing technology in Pacific Biosciences (PacBio) SMRT system. The ever-broadening range of applications includes *de novo* sequencing of genomes, targeted resequencing, metagenomics, transcriptome sequencing (RNA-seq), and epigenetics (ChIP-seq, methylation). This poster presents a bird's eye view of the nextgen sequencing instrumentation available to UF investigators through the ICBR Genomics Division. An attempt is made to summarize the optimum areas of application, as well as the associated costs for each of the platforms. Due to the availability of different choices of technology, cost considerations, platform-specific applications, plate configurations and turnaround times, interested users are encouraged to discuss with Core personnel their sequencing project before commencing any service request.

4. Statistical models for RNA-Seq data

Bacher RL[1,2], Oberg AL[3], Young LJ[1], McIntyre LM[4*]

[1]Department of Statistics, University of Florida, Gainesville, FL
[2]Department of Mathematics, University of Florida, Gainesville, FL
[3]Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, MN
[4]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

RNA-Seq is a tool used for assessing gene expression based on read counts from high throughput sequencing. Many analysis methods to detect differential expression thus far have focused on discrete distributions. Looking at both the underlying data and the measurement on which we perform the analysis, we propose that RNA-Seq data are continuous. The underlying libraries are made from a solution of mRNA quantified by concentration. This solution is sampled and sequencing technology is used to estimate the number of molecules in the sample for a particular gene. Normalization techniques, which result in non-integer values, are often applied to RNA-Seq data in order to account for systematic effects on the total number of counts, for example the length of the exon/transcript and the total number of reads mapped to the reference per sample. Furthermore, the raw read counts themselves take on a large range of values (0, 1, to 6 and 7 digit numbers). Given this evidence, we will evaluate whether various continuous models for RNA-Seq data fit the data reasonably well.

5. Exploring the mutational landscape of *Caenorhabditis*

Denver DR[1], Wilhelm LJ[1], Dolan PC[2], Howe DK[1], Gafner K[1], Salomon MP[3], Baer CF[3,*]

[1]Department of Zoology, Oregon State University, Corvallis, OR
[2]Division of Science and Mathematics, University of Minnesota, Morris, Morris, MN
[3]Department of Biology, University of Florida, Gainesville, FL

Mutation is often referred to as "the fuel of evolution" because without mutation, evolution would grind to a halt. Different groups evolve at different rates, but the extent to which variation in mutation underlies variation in the rate of evolution is unknown. The causes of variation in genome-wide mutational properties are difficult to disentangle, because variation in mutational properties could result from the effects of genotype, environment or both. We report single-nucleotide mutation (SNM) rates and spectra for two genotypes of *C. briggsae* (HK104, PB800) and *C. elegans* (N2, PB306) in which mutations accumulated under relaxed selection for 250 generations. Five to seven mutation accumulation ("MA") lines from each genotype were sequenced with Illumina technology at ~6X average coverage. The average SNM rate is ~2 x 10^{-9}/gen. The overall SNM did not vary among the four genotypes, but the PB306 strain had a significantly elevated mutation rate specific to the X-chromosome. Of the six paired mutation types, two (A:T->T:A and A:T->G:C) varied between genotypes. G:C->T:A transversions were more common than in the standing within-species genetic variation, as found previously in N2. Also as found previously in N2, mutations from G and C to T and A are much more common than from A and T to G and C. These findings generalize the conclusion that mutational bias alone cannot explain the observed genome-wide base composition or the transition/transversion ratio.

6. Analysis of maize seed development mutants with maternal parent-of-origin effects

Bagadion A[1], Cooper K[2], Evans M[3], Settles AM[2,4,*]

[1]Howard Hughes Medical Institute-UF Science for Life Program, University of Florida, Gainesville, FL
[2]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL
[3]Department of Plant Biology, Carnegie Institution for Science, Stanford, CA
[4]Horticultural Sciences Department, University of Florida, Gainesville, FL

Maize is a central crop to world-wide agriculture with the seed being the primary product for food and industrial applications. Mature maize seeds contain an embryo and an endosperm. The endosperm accumulates seed reserves of starch and protein and accounts for more than 80% of seed weight. Epigenetic regulation, specifically imprinting within the endosperm, is thought to play an important role in determining endosperm and seed size. However, imprinted genes that regulate maize endosperm size have not been identified. We are screening for defective kernel mutants with maternal parent-of-origin effects. We present preliminary analysis of one locus, maternal rough endosperm isolate 594 (mre*-594). When the female gametophyte is mre*-594, seeds develop a rough, etched, or pitted endosperm surface regardless of pollen genotype. The mre*-594 mutant fully transmits through the pollen and does not cause seed phenotypes when fertilizing wild-type plants suggesting it confers a maternal parent-of-origin effect on seed development. Laser confocal microscopy of pre-pollinated female gametophytes suggests mre*-594 alters antipodal cell morphology, and 4 days after pollination mre*-594 seeds showed delayed endosperm development. These data suggest the Mre gene is required prior to pollination and help explain the reduced size of mre*-594 seeds. We are currently mapping mre*-594 using a backcross population to identify the Mre gene.

7. Analyzing the role of beta-globin gene locus associated *cis*-regulatory DNA elements using artificial DNA binding domains

Barrow J, Massannat J, Bungert J*

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

Introduction: The drive to understand the regulation of the globin gene locus has been warranted by the need to improve the treatment of hemoglobinopathies. Our work focuses on exploring the role of various *cis*-regulatory DNA elements within the beta-globin locus.
Research Goal: Employing a novel technology, we are generating a series of artificial zinc finger DNA binding domains (ZF-DBD) that can bind with high affinity and specificity to 18bp recognition elements, a sequence adequate in length to address a unique signature within the genome. By blocking *cis*-regulatory DNA elements, we aim to delineate key *cis*-DNA elements that are important for globin gene regulation and whose activity can ultimately be altered in order to restore the synthesis of the fetal gamma-globin genes to improve the treatment of hemoglobinopathies.
Findings: Currently, we have successfully designed a ZF-DBD that binds to the EKLF binding site in the murine beta major-globin gene promoter and are in the process of designing additional ZF-DBDs that will neutralize the function of putative regulatory DNA elements in the beta-globin gene locus, including the +60 Ebox in the beta major-globin gene promoter as well as E-box and MARE sequences in the locus control region (LCR). We will present data on the functional characterization of the ZFDBDs.

8. Mbnl3 expression during embryonic development

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Microsatellite CTG triplet repeat expansions in the 3' untranslated region (3'UTR) of the DMPK gene cause the neuromuscular disease myotonic dystrophy (dystrophia myotonica) type 1 (DM1). Longer CTG expansions (>1,000 repeats) result in a congenital form of DM1 (CDM) which is characterized by hypotonia, delays in muscle development and mental retardation. DM1 is caused by loss of muscleblind-like (MBNL) protein function due to nuclear sequestration by CUG expansion RNAs, which result from the transcription of mutant DMPK alleles. Although MBNL1 loss-of-function explains adult-onset DM1 symptoms, the molecular etiology of CDM remains elusive. To investigate the possible role of other MBNL proteins in normal muscle development and pathogenesis of CDM, we analyzed the spatial-temporal expression pattern of mouse Mbnl3. Mbnl3 is primarily expressed during embryonic myogenesis (E9.5-E15.5) and declines towards birth although this gene is re-expressed during muscle regeneration in adults. Surprisingly Mbnl3DE2/E2 isoform knockout mice develop normally but exhibit significant upregulation of another Mbnl3 isoform which suggests that some Mbnl3 expression is essential for embryonic myogenesis. We have used crosslinking/ immunoprecipitation combined with RNA-Seq (HITS-CLIP) and microarray analysis to identify the global RNA targets for Mbnl3 in mouse E15 forelimb and in the C2C12 myoblasts. Our results suggest that Mbnl3 may be essential for a distinct developmental pathway during embryogenesis.

9. Transdifferentiation by bacterial mediated MyoD protein delivery

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Forced exogenous gene expression has been well characterized as an effective method for directing both cellular differentiation and dedifferentiation. However, transgene expression is not amenable for therapeutic application due to the potential for insertional mutagenesis. Protein based techniques provide a safe alternative, but current protein delivery methods are quite limited by labor-intensive purification processes, low protein yield and inefficient intracellular targeting. Such limitations may be overcome by using a naturally occurring bacterial protein injection system. *Pseudomonas aeruginosa* utilizes a Type III Secretion System (T3SS) to inject bacterial proteins directly into the eukaryotic cell cytoplasm. Our previous studies describe the ability of this system to easily deliver a high quantity of protein to both differentiated and pluripotent cells using a genetically attenuated strain. Using Cre recombinase as a reporter, we have demonstrated high frequency loxP mediated recombination in the chromosome of the recipient cells, suggesting the protein is not only efficiently targeted to the nucleus, but also retains its biological function. MyoD is a key muscle regulatory factor, the over-expression of which is able to induce transdifferentiation of numerous cell types, such as fibroblasts, into functional myocytes. Here we demonstrate transient injection of MyoD protein by *P. aeruginosa* to be sufficient to induce myogenic conversion of mouse embryonic fibroblast.

10. ZmNlr1: a structure-function analysis

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We recovered a maize mutant that impacts the development of multiple tissues and possesses a distinct narrow leaf and rough endosperm (nlr1) phenotype. We identified a Robertson's Mutator (Mu) transposon insertion tightly linked to nlr1. The transposon disrupts the coding sequence of an Hsp40 protein. Hsp40 proteins activate Hsp70 ATPase activity and characteristically contain a J-domain. In addition, Nlr1 contains two nuclear localization signals (NLS) and an Arginine/Serine (RS)-rich domain. RS domains are found in pre-mRNA splicing factors and presence of this domain suggests NLR1 is associated with spliceosomal complexes. Transient expression of N and C terminal fusions with GFP shows subnuclear localization consistent with nuclear speckles. Pre-mRNA splicing factors are frequently localized to nuclear speckles. Domain deletion assays revealed that the N-terminal RS domain is required for the speckling pattern, while deletion of either the C-terminal NLS or J-Domain has no affect on the localization pattern. A yeast-two-hybrid (Y2H) screen using NLR1 as bait retrieved FK506-binding protein 12 (FKBP12). Members of the FKBP family are ubiquitous and serve in protein folding, cell stress response, signal transduction, transcription and cell cycle regulation. Interestingly, AtFKBP12 is known to interact with AtFIP37, a homolog to two metazoan proteins involved in splicing. These data support a model in which NLR1 is involved in transcriptional regulation.

11. Population genetic admixture and adaptation

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Genetic admixture between different populations has been proposed as a factor contributing to both the success as well as the failure of adaptation in a novel environment. On the one hand, genetic introgression from other populations can swamp locally adapted alleles, known as outbreeding depression. On the other hand, admixture of multiple populations can increase additive genetic diversity, decrease inbreeding depression, and create novel beneficial trait combinations. How any given population's fitness will be affected by genetic admixture is difficult to predict and depends on many parameters that are not possible to manipulate in nature. Using *Drosophila melanogaster*, we conducted an artificial evolution experiment to simulate an invasion to a novel environment both with and without genetic admixture. Our results indicate that admixture does indeed have both positive and negative affects which depend on the original and introduced environments and the degree of similarity between them.

12. QTL mapping and candidate gene analysis of telomere length control factors in maize (*Zea mays* L.)

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Telomere length is a quantitative trait important for many cellular functions. Failure to regulate telomere length contributes to genomic instability, cellular senescence, cancer, and apoptosis, but the functional significance of telomere regulation in plants is much less well understood. To gain a better understanding of telomere biology in plants, we used QTL mapping to identify genetic elements that control telomere length variation in maize. We measured telomere lengths from 178 recombinant inbred lines of the IBM mapping population and found multiple regions that collectively accounted for 33–38% of the variation in telomere length. Two-way ANOVA revealed interaction between two QTL (2.09 and 5.04). Candidate genes within these other significant QTL intervals along with other genes known *a priori* to regulate telomere length were tested for correlations between expression levels and telomere length by qRT-PCR. Many of the candidate genes showed a slight but significant direct correlation between expression levels and telomere length, but Ibp2, a homolog of genes known to reduce plant telomere length, showed a negative correlation. One candidate gene, encoding a RAD51-LIKE protein was strongly supported by several lines of evidence. Our results highlight the value of combined QTL mapping and candidate gene expression analysis in a genetically diverse model system, and establish maize as an ideal organism for plant telomere research.

13. Artificial selection on Sigma virus titer and correlated response of virulence

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One hypothesis to explain the evolution of parasites toward high levels of virulence (where virulence corresponds to a decrease in the host fitness) is that parasites maximize intra-host replication rate in order to increase transmission. Here, using the vertically transmitted Sigma virus (Rhabdoviruses) of *Drosophila melanogaster*, we propose to directly study the effect of within host growth rate on the virus evolution. Using QPCR, this experimental evolution work consisted of selection over eleven generations of host for both increased and decreased viral titer in seven replicates of a single effectively isogenic line of *D. melanogaster*. We observed an increase over time in the difference in virus titer between the two treatments, including significant difference in the last generation. This difference in virus titer had also effects on parasite virulence, whose three proxies showed significant differences between the two treatments. These results illustrate how parasite virulence can be positively linked with increase in intra-host replication rate, as stipulated by the trade-off hypothesis for the evolution of virulence. The sequencing of the full genome of the viruses from the two treatments suggested that most of the differences between the two treatments are due to fixation of new mutations in the treatment consisting of decreasing the virus titer. Evolution of the host genome during the experiment is also suspected.

14. Genetic ancestry as a predictor variable in an association study of blood pressure

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Hypertension is a leading risk factor for cardiovascular disease and stroke and its prevalence is characterized by significant racial disparity. Genetic ancestry (GA) has been included in recent disease association studies to represent a presumed racial contribution to health disparities. Here we investigate the association of African GA and blood pressure variation under two sets of conditions: 1) different racial composition of study sample and 2) continuous vs. categorical characterization of the GA variable. The relationship between African GA and systolic blood pressure (SBP) was measured in a series of multiple linear regression models designed to compare 1) pooled sample vs population subsets divided according to self-identified racial or ethnic designations and 2) GA as a continuous or categorical variable. The continuous GA variable was significantly associated with SBP in All Individuals, Non-Hispanic Whites and All Whites, but not in African Americans or Hispanic Whites. One out of two categorical GA variables was significantly associated with SBP. Our results suggest that the association between genetic estimates of ancestry and health outcomes may differ depending on the racial or ethnic composition of the sample and characterization of the ancestry variable. Therefore, caution is advised when interpreting results of disease association studies that incorporate GA as a predictor of disease, particularly when one is investigating racial disparities in disease.

15. Comparison of genetic variation in a non-migratory population of monarch butterflies (*Danaus plexippus*, Miami, FL)

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Genetic connectivity between populations is a key factor in developing appropriate conservation strategies, and is particularly important for maintaining spatially dispersed populations. Monarch butterflies (*Danaus plexippus*) lay their eggs exclusively on milkweed plants, so monarch distribution is closely tied to the patchy distribution of milkweeds. These butterflies are found throughout North America during the summer months, but most migrate in the fall to overwintering colonies in California and Mexico. Interestingly, there are also several non-migratory, or resident, populations in the southern United States. To varying degrees, these small resident populations lie along the major migratory flyways, but the degree to which migrating monarchs interact with resident monarchs is unknown. We are in the process of evaluating two complementary hypotheses: 1. Resident populations are genetically distinct from migratory populations. 2. Gene flow from migratory into resident populations is higher than from resident into migratory populations. Our preliminary results for the Miami, FL population show low overall genetic variation at both mitochondrial and nuclear loci, but they also indicate that these resident monarchs are distinctly different from those found along the migratory routes. These preliminary results are consistent with our hypothesis that resident populations are genetically distinct from migratory populations.

16. Multiplexing eight SSR molecular markers to fingerprint diverse *Fragaria* germplasm

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Strawberries (*Fragaria* species) are grown around the world for their delicious taste, aesthetic appeal, and nutritional benefits. The most commonly cultivated strawberries are interspecific hybrids (or descendants) of *F. chiloensis* and *F. virginiana* known as *F.* x *ananassa.* Developing molecular tools for strawberry will help unlock true molecular diversity in both wild and cultivated material and potentially aid in improving important horticultural characteristics in cultivated strawberry. Simple Sequence Repeats (SSRs) are DNA markers defined by repeating, tandem nucleotide patterns found throughout a genome, and are useful for determining diversity between related genotypes. We have combined eight SSR markers to create a universal fingerprinting platform of mostly high nucleotide (> 4) repeats for strawberry. The results provide greater insight into the evolutionary relationships between diverse genotypes specifically in the strawberry "supercore" used in our studies. Our analysis also helps clarify the classification of genotypes that were previously elusive. We are especially interested in the long term goal of using this data to guide parental selection decisions for incorporating more diversity into the UF strawberry breeding program. Our results will be useful to strawberry breeders and molecular researchers, as well as to those developing similar platforms in related systems.

17. Chromatin structural control of the anti-inflammatory response

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Inflammation is a highly complex phenomenon, which is regulated at numerous levels. The downregulation of the inflammatory response is as critical as its induction. IL-10 has a key regulatory function, with the ability to simultaneously block the expression of proinflammatory genes while enhancing the anti-inflammatory response. Little is known about the molecular mechanisms underlying this anti-inflammatory response. We study the effect of IL-10 on the chromatin of macrophages by means of combined measurements of nuclease sensitivity, nucleosome position, and gene expression. We have identified genes for which nucleosome positioning is modified in an inflammatory environment (media with LPS) compared with a non-inflammatory environment (media). We classified the effect of IL-10 in the LPS induced inflammatory response into three groups: 1) unchanged nucleosome distribution genes, 2) reversion to the non-inflammatory environment, and 3) new distinct organization different from the inflammatory and non-inflammatory distributions. We present a model in which IL-10 alters the transcriptional potential of a locus by altering nucleosome distribution. We have identified an overall "signature" of the anti-inflammatory response associated with IL-10 signaling. This work gives insight into the contribution of epigenetics to immunologically relevant gene regulation, which could permit the rational design of therapeutics to enhance control of the inflammatory response.

18. Proteomics and mass spectrometry applications in genetics and biomedical research

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Studying at the protein level allows researchers to understand how proteins, their dynamics and modifications affect cellular processes (e.g., gene expression) and how cellular processes (e.g., genetic perturbation) and the environment stimuli and drugs affect proteins. The mission of our facility is to provide excellent service and training in proteomics and mass spectrometry to UF scientists and students. Here we present our capabilities in proteomics and other analytical services. The tools include a gel-based 2D-DIGE (Two Dimensional Difference Gel Electrophoresis) and gel-free iTRAQ (Isobaric Tags for Relative and Absolute Quantitation). We have a suite of state-of-the-art mass spectrometers, including a tandem time-of-flight (4700 Analyzer, AB), quadrupole-time-of-flight (QSTAR XL and Elite), quadrupole-linear ion trap (4000 QTRAP), and linear ion trap-Obitrap (LTQ-Orbitrap). These instruments are mainly used for protein identification, posttranslational modification and protein expression analysis (e.g., SILAC and Mass Western). Our facility is also set up to provide Edman N-terminal protein sequencing and Biacore biomolecule interaction analysis. Proteomics and mass spectrometry are useful in large-scale survey of proteome for hypothesis generation as well as in detailed analysis of target proteins for hypothesis testing. Our services also include accurate molecular weight analysis, MRM-based protein screening and targeted metabolite profiling.

19. The removal of the irradiation responsive enhancer region (IRER) and the effects on regulatory functions in *Drosophila*

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As has been studied before, *Drosophila* embryos are known to have two pro-apoptotic genes when followed by irradiation. These two genes are known to be reaper and hid. Before differentiation of the embryos (pre-stage twelve), the two genes are highly sensitive to the gamma irradiation whereas the embryonic differentiation leads to a resistant state. An enhancer region called irradiation responsive enhancer region (IRER) explains the transition between this sensitive to resistant state. IRER is known to induce the pro-apoptotic gene hid during irradiation. The deficiency in IRER will suppress reaper and hid; thus, a suppression to apoptosis. In this study, the removal of the IRER reveals that there is a correlation between the role of IRER and the regulatory functions of development in the *Drosophila*. The difference in body weight and organ size portrays that the homeostatic cell development is interfered with the deletion of the enhancer region; therefore, indicating that without IRER, possible overgrowth occurs.

20. Non-specific activity of VMD2 promoter in various tissues

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Age related macular degeneration (AMD) is an incurable genetic disease that affects individuals 50 years of age and older. AMD is caused by oxidative stress in the macula of the retina and primarily deteriorates vision quality. The pathology behind fovial deterioration is unique in that one looses center field of view but maintains peripheral; this effect can either be gradual or sudden depending on the etiology of AMD one is afflicted with. Utilizing mouse models that exhibit this disease is necessary in understanding the disease's pathology. The mice models are engineered using a retinal specific promoter, villiform-macular-dystrophy-2, VMD2, which expresses cre-recombination to delete a gene, super oxide dismutase-2, SOD2 in the retina. SOD2 regulates radical products of cellular respiration. Without this gene the tissue expresses oxidative stress that mimics the symptoms of AMD. It is in these mice that unusual traits were noticed such as increased body mass and aggression. VMD2 is a retinal specific promoter that obligates cre-recombination in the retinal pigment epithelium (RPE). Cre-recombination is a genetic tool used to delete segments of DNA flanked by lox-p sites. However, due to the unusual phenotype expressed by these mutant mice, there must be recombination in other tissues. In order to quantify a difference in the mice a preliminary study was executed weighing the amount of food consumed by Cre+ mice compared to Cre- mice as well as weighing the mice intermittently throughout the study. Data supports the hypothesis of adverse expression, as there was a statistical difference in weight between the transgenic mice and the wild type control. Additional staining of tissues, using the reporter gene b-galactosidase, against X-Gal illustrated Cre-driven expression in areas of the hindbrain, kidney, and epithelium to name a few. Immunohistology of adult tissues expressing oxidative stress via 4-Hydroxynonenal will qualify, without a reasonable doubt, that there is adverse Cre-driven recombination in tissues other than the RPE, causing oxidative stress and the unusual noted phenotypes.

21. Possible membrane association of a novel regulator of actin depolymerizing factor (ADF)

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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. Because ADF plays a central role in severing actin filaments, understanding the regulation of ADF activity is essential for understanding actin dynamics. We recently identified a new regulator of ADF in plants named RPA for REGULATOR OF PLANT ADF. Our *in vitro* analysis of RPA1 function showed that it interacts with ADF and inhibits actin binding to ADF. RPA1 is a member of a moderately sized gene family. Interestingly, about half of the members possess a putative signal sequence (SS). Given that signal sequences direct proteins for secretion, we have to reconcile the fact that ADF is not secreted. We thus hypothesize that the putative signal sequences are novel N-terminal transmembrane (TM) anchors. To test this hypothesis, we have constructed GFP fusions to the RPA11 protein, which contains a putative SS. We are also conducting *in vitro* transcription/translation of the RPA11 protein in the presence of eukaryotic microsomes to test for co-translational membrane insertion. Understanding the function of membrane association of RPA proteins will provide key insight into the role of actin dynamics in membrane trafficking.

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

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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 other parasites than sigma and evaluating other selected lines, are needed to draw conclusions.

23. Histone deacetylase inhibitors prevent nucleosome redistribution at transcription start sites

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The relationship between histone acetylation and chromatin structure is uncharacterized and represents a gap in understanding the regulation of the human genome. This relationship is critical to understand the mechanism of an important new class of drugs, the histone deacetylase inhibitors (HDI). HDI prevent the removal of acetyl groups from histones, and are emerging as significant drugs in the treatment of a broad spectrum of disease. We have measured nucleosome distribution at 505 genes involved in the immune response. We treated human macrophages with the innate immune agonist lipopolysaccharide (LPS) and have measured the chromatin structural changes from LPS in both the presence and absence of HDI. We show that at a majority of the genes tested, HDI alone induce only modest changes in nucleosome distribution, while treatment with LPS induces drastic changes in nucleosome distribution at a majority of genes. However, pretreatment with HDI abrogates 70% of LPS induced changes in nucleosome distribution, demonstrating that that HDI restrict LPS-induced nucleosome redistribution at transcription start sites. These results indicate that HDI "lock-in" chromatin structural states, and suggest that the anti-inflammatory mechanisms of HDI involve regulation of chromatin structure. We anticipate that these results will provide a foundation to understand functional relationships that will give insight into HDI therapeutic roles in disease.

24. Quorum activation at a distance: spatiotemporal patterns of gene regulation from diffusion of an autoinducer signal

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Quorum sensing, a cell-cell communication process, may allow bacteria to probe the physical and chemical properties of their local environment and regulate behaviors such as biofilm formation, secretion of virulence factors, and symbiotic interactions. This is accomplished by the production and detection of diffusible chemical signals known as autoinducers (AI). The aim of this study is to measure spatiotemporal patterns in gene regulation that result from quorum communication in a spatially extended system. We study a quorum sensing *Escherichia coli* strain harboring a plasmid based on the luxI/R system of *Vibrio fischeri* which responds to an AI (N-3-oxo-hexanoyl-L-homoserine lactone) by expression of GFP. This bacteria, which lacks the AI synthase, is embedded in agar which is loaded into a long, quasi-one-dimensional lane and injected with AI at the terminus of the lane. As the AI diffuses, it initiates a wave of GFP expression that propagates down the lane. The resulting spatial and temporal patterns can be simulated by a simple mathematical model incorporating population growth, diffusion of AI, and transcriptional activation. Our experiments demonstrate that AI can regulate gene expression over distances greater than a centimeter and the resulting patterns of expression can be represented by a mathematical model.

25. Identification of chromatin-based patterns across lung cancer grades

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The progression of cancer is classified by histologic grade, identifying nuclear changes that cells undergo as they de-differentiate. Although there have been numerous studies on chromosomal aberrations in cancer, broad assessment of chromatin structure information in malignant transformation has been understudied. We have identified chromatin-based patterns across different lung adenocarcinoma cancer grades. We compared the chromatin-structure from lung adenocarcinomas of grades one, two and three to their normal adjacent tissue. We developed nucleosome distribution and chromatin accessibility microarray mapping platforms to analyze chromatin structure genome-wide. We measured chromatin structure at three levels of resolution: nucleosome distribution, chromatin accessibility and three-dimensional molecular cytology. Grade one lung adenocarcinomas have greatly altered nucleosome distributions compared to the adjacent normal tissue, but nearly identical chromatin accessibility. Conversely, the grade three samples show extensive rearrangements in chromosomal accessibility, but modest changes in nucleosome distribution. We have developed a model in which early grade lung adenocarcinomas are linked to changes in nucleosome distributions, while later grade cancers are linked to large-scale chromosomal changes. These results indicate that we will be able to use these chromatin structural changes to identify grade sub-type specific cancer biomarkers.

26. Studying the interactions between ITB3L04, actin depolymerization factor and other ADF regulators

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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 see the effect that this protein has on regulating ADF and interacting with other ADF regulators. In order 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, ADF and the other ADF regulators can not only confirm its effect as a regulator of ADF and quantify the extent to which it interacts with the other proteins, but also allow us to start identifying relevant biological pathways that contribute to actin dynamics.

27. Differential gene expression inside and out. QTL analysis using HiSeq

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Understanding the genetic and biochemical basis of sugar accumulation is an important step towards the improvement of sweet sorghum as a bioenergy crop. Sweet sorghums accumulate soluble sugars in their stems, which can be directly converted to fuels and chemicals by microorganisms. A major quantitative trait locus (QTL), for stem sugar concentration, was identified on chromosome 3 based on the analysis of a recombinant inbred line population derived from the sweet sorghum 'Rio' and the grain sorghum BTx623. To identify the gene(s) underlying this QTL, an F5 individual heterozygous for the QTL was self pollinated. Stem and leaf tissue was collected from progeny homozygous for both QTL alleles at two different developmental stages. Sixteen Illumina libraries were multiplexed and sequenced in 4 lanes of HiSeq®. A total of 481,083,373 reads were obtained, with 356,958,375 (74%) reads aligning to the sorghum transcriptome. Additional SNP variation was detected and used to improve mapping. Differential expression of *cis*- and *trans*- genes give insight into the underlying biochemical basis of sugar accumulation.

28. Phylogenetic structure in Amaryllidaceae tribe Hippeastreae (Asparagales) gains resolution with an expanded ITS tree

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Amaryllidaceae tribe Hippeastreae constitutes a horticulturally valuable group of petaloid monocots; however, its taxonomy at the generic level has been controversial, with several segregates proposed during the last 40 years. Previous phylogenetic analyses of the nrDNA ITS/5.8S region showed that certain genera are not monophyletic, but that study lacked good representation of Chilean-Argentinean groups. The hypothesis of possible early lineage reticulation in the tribe was suggested. We have expanded the taxon sampling to approximately 110 species by including members of the Chilean endemic genera and additional species of previously sampled groups. The tribe comprises two major clades: a) *Traubia, Placea*, *Phycella*, *Rhodolirium*, and *Famatina maulensis*, characterized by $x = 8$, lack of polyploidy, and a capitate stigma, and b) *Rhodophiala*, *Habranthus*, *Hippeastrum*, *Sprekelia*, *Zephyranthes*, and the remainder of *Famatina*, characterized by several basic chromosome numbers ranging between $x = 6 - 12$, and frequent polyploidy and aneuploidy. No clear morphological features diagnose the latter clade. We are currently working on obtaining a robust phylogenetic framework for this group based on low-copy nuclear genes and several plastid regions, which will serve as a basis for reclassification of the tribe and for study of its chromosomal evolution, and will facilitate several tests of the ancient inter-clade reticulation hypothesis in Hippeastreae.

29. Deep sequencing of maize endosperm culture transcriptomes to understand the impact of the ROUGH ENDOSPERM3 splicing factor

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The maize rough endosperm3 (*rgh3*) mutant encodes the U2AF35-related protein (ZmURP). In humans, URP is associated with the minor and major spliceosomes, but its biological role is unknown. Prior characterization of the rgh3 mutant indicates that the gene is required to repress cell proliferation and promote cell differentiation. The *rgh3* mutants are highly proliferative in endosperm cell culture when compared to wildtype endosperm culture that undergoes endoreduplication. Our data indicate that *rgh3* endosperm cells remain frozen in this early, undifferentiated stage throughout kernel development. We hypothesize that *rgh3* has an effect on RNA splicing and gene expression. To test this hypothesis, we compared *rgh3* and normal sibling transcriptomes in an RNA-seq experiment with the ABI SOLiD platform. About eight million reads from each genotype were aligned uniquely to the genome. These sequences mapped to 26% of predicted exons in the genome and detected >26,000 annotated genes. *rgh3* had subtle effects on transcript levels with only 87 genes showing a four-fold difference compared to wildtype. Additionally, there were 63 splice junctions that were differentially expressed in *rgh3* and WT samples, indicating that Rgh3 affects the splicing of genes involved in carbon metabolism and translation. Splice junction analyses also indicate that Rgh3 may influence the selection of 5′ or 3′ splice sites during intron cleavage.

30. The role of acid stress response genes in the interactions of *Salmonella typhimurium* with *Pectobacterium carotovorum* during tomato infections

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In tomatoes infected with a soft rot plant pathogen *Pectobacterium*, *Salmonella* grows to numbers 10 fold higher than in tomatoes without *Pectobacterium*. There are three hypotheses which attempt to explain this phenomenon. The first two hypotheses (which tested (1) potential metabolic interactions within soft rots, and (2) the role of the production conditions) did not adequately explain the aforementioned findings. The third hypothesis is currently being tested. We hypothesize that within soft rots, *Salmonella* does not experience acid stress, which promotes increased multiplication of the human pathogen. The experiments compare the fitness of *Salmonella typhimurium* 14028 with the mutants which lack genes which play roles in the acid tolerance response (ATR) mechanism. Regulatory genes (ompR, envZ) and structural genes (ompC, ompF) involved in ATR were excised using Datsenko-Wanner mutagenesis. For control experiments, complementation constructs were developed on low copy number plasmids. The hypothesis is being tested using the constructed mutants, which are co-infected with the wild type into tomatoes that are (or are not) seeded with *Pectobacterium*. Rotting by *Pectobacterium* tends to increase fruit pH, which should allow the mutants to be

more competitive due to a decrease of acid stress. Ongoing experiments suggest that the presence of a soft rot increased fitness of mutants, whereas the conditions of an intact fruit seem to inhibit the growth of mutants.

31. Daxx and USP7: novel regulators of mitosis and taxanes sensitivity

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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 taxanes 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 show 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. *In silico* analysis of Daxx and USP7 expression pattern negatively correlates with response to taxanes in cancer cell lines indicating that Daxx and USP7 can be used as predictive factors for taxanes response in cancer patients.

32. Hypertension susceptibility loci associated with blood pressure response to antihypertensives – results from the pharmacogenomic evaluation of antihypertensive responses (PEAR) study

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BACKGROUND: To date, 39 SNPs are associated with blood pressure (BP) in genome-wide association studies (GWAS) in whites. We assessed the association of these loci with BP response to atenolol (ATEN) and hydrochlorothiazide (HCTZ), with particular interest in loci with opposite associations for the two drugs, given their contrasting pharmacological mechanisms.
METHODS: PEAR evaluated BP response in 768 hypertensive patients randomized to either ATEN or HCTZ monotherapy then the combination. Genotypes of 37 loci were obtained from Illumina 50K cardiovascular or Omni1M GWAS chips. Associations with systolic (SBP) and diastolic BP (DBP) responses to ATEN or HCTZ mono- or add-on therapy was evaluated in 464 white individuals using linear regression adjusting for baseline BP, age, gender and principal components for ancestry.
RESULTS: Eight SNPs reached nominal significance ($p<0.05$) and 3 were associated with ATEN BP response at $p < 0.01$. Rs1458038 near FGF5 was associated with BP response to ATEN and HCTZ monotherapy, with genotype effects in the opposite direction. Rs871606 near CHIC2 (DBP p=0.0037 and SBP p=0.017) and rs2932539 near MOV10 (DBP p=0.0054 and SBP p=0.06) were associated with BP response to monotherapy with ATEN, but not HCTZ.
CONCLUSION: Eight hypertension/BP loci are also associated with BP response to ATEN and/or HCTZ. These data highlight that disease GWAS may provide strong candidates for the relevant drug response phenotype.

33. Allelic imbalance in *Drosophila* hybrid heads: exons, isoforms and evolution

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Unraveling how regulatory divergence contributes to species differences and adaptation requires identifying functional variants from among millions of genetic

differences. Analysis of allelic-imbalance (AI) reveals functional genetic differences in *cis* regulation and has demonstrated differences in *cis* regulation within and between species. Regulatory mechanisms are often highly conserved, yet differences between species in gene expression are extensive. What evolutionary forces explain widespread divergence in *cis* regulation? Allelic-imbalance was assessed in *D. melanogaster-D. simulans* hybrid female heads using RNA-Seq technology. Mapping bias was virtually eliminated by using genotype specific references. Allele representation in DNA sequencing was used as a prior in a novel Bayesian model for the estimation of AI in RNA. *Cis* regulatory divergence was common in the organs and tissues of the head with 41% of genes analyzed showing significant AI. Using existing population genomic data, the relationship between AI and patterns of sequence evolution was examined. Evidence of positive selection was found in 30% of *cis* regulatory divergent genes. Genes involved in defense, RNAi/RISC complex genes, and those that are sex regulated are enriched among adaptively evolving *cis* regulatory divergent genes. For genes in these groups, adaptive evolution may play a role in regulatory divergence between species.

34. Tissue-specific roles of *FgfR2* in development of the external genitalia

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Incomplete closure of the urethral tube, or hypospadias, is the second most common birth defect in the United States. The incidence of hypospadias has been increasing in recent years without explanation, although researchers have speculated that this rise may be due to an increase in environmental endocrine disrupting compounds. Research has elucidated some key developmental pathways necessary for urethral development, but the link between genetics and hormones remains poorly understood. FgfR2 is necessary for development of the urethral tube and is transcriptionally regulated by hormones in a dose-dependent manner. FgfR2 is expressed in two regions of the external genitalia: the endodermally derived urethra and the preputial (foreskin) ectoderm. Either or both of these regions may be responsive to circulating hormones during embryonic development. In order to identify the tissue-specific functions of FgfR2 in each of these compartments, we have conditionally deleted FgfR2 from the urethral endoderm and the preputial ectoderm. We have found that endodermal FgfR2 is required for maturation of the urethral epithelium and ectodermal FgfR2 is necessary for urethral tube closure. These results decouple the tissue-specific roles of FgfR2 in each of these compartments and identify the cellular mechanisms by which FgfR2 orchestrates urethral epithelial maturation and tubulogenesis.

35. Identification of multiple cellular targets of Kaposi's sarcoma-associated herpesvirus (KSHV) miRNAs by Ago-HITS-CLIP

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KSHV is the etiological agent of Kaposi's sarcoma, primary effusion lymphoma and some types of multicentric Castleman's disease. With the increasing evidence for the involvement of miRNAs in cancer, the fact that herpesviruses including KSHV and EBV express multiple miRNAs suggests a potential role for these miRNAs in viral tumorigenesis. However, to date only very few targets have been experimentally confirmed and little is known about the function of these miRNAs in the viral life cycle. To address this question, we performed Ago-HITS-CLIP using the 2A8 antibody in combination with Solexa sequencing to identify viral and cellular miRNAs and their targets in KSHV-infected lymphoma cells. Initial analysis of canonical seed sequences (nts 2-8) for the 25 KSHV miRNAs in the mRNA derived sequence tags revealed more than 1000 cellular targets. Importantly, we identified reads for THBS1, BACH-1 and BCLAF1, the three best characterized KSHV miRNA targets to date. Selected proteins involved in tumorigenesis (TP53INP1, TPD52), immunity (HLA-A, -C, and –E), and the high mobility group family are currently under validation by luciferase reporter and western blot analysis. Gene ontology analysis revealed that KSHV miRNAs directly regulate genes involved in cell cycle control, apoptosis, tumor suppression, immune evasion, and chromatin remodeling, suggesting a role in KSHV pathogenesis and potentially tumorigenesis. Target analysis for non-canonical seeds as well as for cellular miRNAs is currently ongoing.

36. Peptide-regulated innate immunity in plants

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Plant defense against invading organisms is initiated through perception of molecules associated with attacking microbes and herbivores. In addition to elicitors derived

from attacking organisms, plants recognize host-derived molecules. These endogenous elicitors induce and amplify the defense responses against invading organisms and their role regulating immunity to both herbivores and pathogens is becoming increasingly appreciated. Arabidopsis plant elicitor peptide 1 (AtPep1) functions together with its receptors to activate innate immune responses in Arabidopsis, and enhances resistance to both *Pythium irregulare* and *Pseudomonas syringae*. Recently the maize ortholog of the gene encoding AtPep1 was found to encode a bioactive peptide, ZmPep1. In maize, ZmPep1 activates *de novo* synthesis of the hormones jasmonic acid (JA) and ethylene (ET), induces expression of genes encoding defense proteins and biosynthetic enzymes for benzoxazinoid defenses, and promotes accumulation of the phytoalexin precursor HDMBOA-Glc in leaves. Maize plants pretreated with ZmPep1 prior to infection with fungal pathogens displayed enhanced resistance to both southern leaf blight and anthracnose stalk rot caused by *Cochliobolis heterostrophus* and *Colletotrichum graminicola* respectively. Peptides belonging to the plant elicitor peptide family have conserved function across plant species as endogenous regulators of innate immunity and may have potential for enhancing disease resistance in crops.

37. An apportionment of human gene expression variation

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It is well documented that most of human genetic variation (>85%) is shared among human populations, and although small, genetic differences among human groups plays an essential role in human phenotypic variation, disease susceptibility, and response to medical treatment. What we have yet to fully realize is how phenotypic variation or specifically, expression variation is apportioned among human groups. Given the genotype - phenotype relationship and the genetic structure observed among human groups we may hypothesize that phenotypic variation would mirror genetic variation. As such, to evaluate the contribution of genetic ancestry to expression variation we have collected a unique sample of 40 human placentas derived from individuals with ancestry from four human populations - Europe, Africa, South Asia and East Asia – and sequenced the transcriptomes of all individuals on an Illumina GAIIx. Analyzing the human transcriptome in this population genetics framework allows us to identify not only genes but also pathways that are differentially regulated among human groups and to gage how the environment and evolutionary history of these groups has putatively influenced biological variation in the human placenta. Finally, we will just begin to evaluate if transcriptome variation, a phenotype, presents structure among human groups at levels observed for genomic variation.

38. Salivary PYY: a novel physiological domain

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Our objective is to identify and characterize functions of peptide YY (PYY) in saliva. PYY is a satiation hormone released postprandially into the bloodstream from L-endocrine cells in the gut epithelium. We have previously demonstrated that PYY is secreted into saliva from plasma, that the cognate receptor Y2R is abundantly expressed in cells of the tongue epithelium and that the acute augmentation of salivary PYY induced a decrease in food intake. The anorexigenic action of salivary PYY was corroborated by an increase in neuronal activity in the paraventricular and arcuate nuclei. In this report we show the effect of chronic augmentation of PYY in saliva using PYY transgene delivery in the submandibular salivary gland (recombinant adeno-associated virus - rAAV). We have documented an absolute increase of salivary PYY concentration in rAAV-PYY treated mice compared with controls. Interestingly, in the former group, PYY plasma concentration remains unchanged after the treatment. Over the course of 28 weeks, after a single treatment, rAAV-PYY treated mice gained 30% less weight than rAAV-GFP injected mice (controls). However, there is no difference in food intake in these two groups suggesting that salivary PPY may have a role in energy expenditure as well and not only in energy intake. Thus this study provides evidence for new functions of the previously characterized gut PYY suggesting a potential efficient alternative therapeutic approach for the treatment of obesity.

39. Endoplasmic reticulum visualization in plant cells deficient of a novel regulator of actin depolymerizing factor

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The cytoskeleton is responsible for the trafficking of materials in the cell and plays a key role in cell expansion and growth. Previous results from our lab have shown that loss of a novel regulator (RPA1) of actin depolymerizing factor (ADF) function in *Arabidopsis thaliana* causes dramatic changes in actin organization that lead to changes in the shape of epidermal hair cells (trichomes). In addition, we have shown that the altered trichome actin organization accompanies defects in Golgi body organization. The rpa1 trichome phenotype provides a clear connection between actin dynamics, Golgi organization, and cell expansion. Since the endoplasmic reticulum (ER) is the first step in trafficking of cell wall materials needed for proper expansion, we hypothesize that ER dynamics will also be altered in trichome cells of *rpa1* mutants. To examine ER dynamics in living cells, we used an ER-targeted green florescent protein (GFP) to transform wildtype and *rpa1* mutants. We are using confocal microscopy to visualize and quantify the differences in ER dynamics. These results will allow us to define a broader role for actin dynamics in membrane trafficking and cell expansion in plants.

40. Improvement of turf quality of apomictic bahiagrass following *in vitro* mutagenesis

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Bahiagrass (*Paspalum notatum* Flugge) cultivar "Argentine" is a prime low-input and drought tolerant turf species. However its apomictic reproduction mode hinders the genetic improvement by conventional breeding. Our objective was to explore the potential of chemical and tissue culture derived mutagenesis for generation of uniform mutagenized seed progeny with improved turf quality. Scarified and surface sterilized bahiagrass seeds were treated with the mutagen sodium azide. Callus was induced from these seeds and regenerated via somatic embryogenesis to obtain uniformly mutagenized plants. 2,000 of the 20,000 regenerated seedlings were selected based on their morphological characteristics and transferred to soil. 46 independently mutagenized lines with reduced stem length, higher tiller density or reduced or delayed seedhead formation were established under field conditions in 1.2m x 1.2m plots in a randomized block design with 4 replications for further evaluation of density, leaf texture, tiller length, color, growth pattern, biomass, seedhead and seed production, as well as seedling vigor. Several mutagenized lines with improved characteristics and viable seed production have been identified and were evaluated in larger plots. Superior turf-type apomictic bahiagrass breeding lines with higher density and darker green color were identified. Beside the improved turf quality several lines retained the seedling uniformity with superior drought tolerance of apomictic bahiagrass.

41. Blends of ascarosides regulate dispersal in nematodes

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Dispersal is an important behavior for many organisms. It can easily be observed when infectious juveniles of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) leave a consumed insect host. Dauer larvae of the phylogenetically related nematode, *C. elegans*, show a similar behavior when exposed to crowding and low food resources. Here we show that *C. elegans* and *S. feltiae* dispersal is regulated by ascaroside semiochemicals. Four known ascarosides were identified in a dauer forming growth media. When *C. elegans* dauer were exposed to a synthetic blend of these components (ascr#2, ascr#3, ascr#8 and IcasC5), twice as many nematodes moved away from the food compared to a control with just food. Furthermore, the same blend was also recognized by *S. feltiae* IJs and by J2s of plant parasitic nematodes (*Meloidogyne* spp). A major component of the *S. feltiae* blend found in insect cadavers after dispersal was ascr#9, a structural analog of asc#2, which is a comparable major component of the *C. elegans* dispersal pheromone. Similarly *C. elegans* dauer responded to a blend where asc#9 was substituted for asc#2. We propose that ascaroside blends represent common communication systems among nematodes. Thus, nematodes sharing the same habitat can monitor and respond to other nematodes signals, maybe to avoid already depleted food sources. We anticipate the use of dispersal blends in a broad strategy to control parasitic nematode species by deterring them from a host.

42. Phylogenetic supermatrix analysis of Obtectomeran Lepidoptera: total evidence from 7 genes and 1796 taxa

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A phylogenetic supermatrix analysis of the hyper-diverse butterfly and moth group, Obtectomera was conducted. In total, 1796 species were sampled for six protein-coding nuclear genes and one mitochondrial gene. Sequences generated from the LepTree Team were combined with available sequences from GenBank. A semi-automated pipeline was designed to construct the supermatrix. Multiple sequence alignments were conducted separately for each gene, and the seven datasets were concatenated into a single data matrix. Multiple sequences of the same species were fused using IUPAC/IUB ambiguity codes and made into a single consensus sequence representing that particular species. Maximum likelihood analyses were conducted using parallel grid computing and processors on the University of Maryland Altus 3400 server. Results were generally in concordance with a preliminary molecular analysis with lesser taxon and gene sampling. However, unlike prior analyses, results from the present study recovered a monophyletic Obtectomera, a result which has traditionally been supported by morphology.

43. *In planta* production of a hyperthermostable xylanase converts sugarcane hemicellulose to fermentable sugars for biofuel production

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Biofuel production from lignocellulosic biomass depends on technology that efficiently and economically releases fermentable sugars from multi-polymeric cell wall components. Xylan is after cellulose, the most abundant polysaccharide in grass and wood biomass and must be hydrolyzed to its component sugars (xylose or xylobiose) before fermentation to ethanol. *In planta* production of cell wall degrading enzymes will reduce costs of enzyme production. Sugarcane is the main source for production of table sugar and is the most efficient photosynthesizer in the plant kingdom. Adding value through *in planta* production of cell wall degrading enzymes offers an alternative use for this abundant biomass resource. Constitutive, apoplast or chloroplast targeted expression cassettes of the codon optimized, hyperthermostable GH10 xylanase from *T. maritima* (xyl10B) were generated for *in planta* expression. Transgene integration, expression and enzymatic activity were evaluated following biolistic co-transfer of the xyl10B and the selectable nptII expression cassettes by Southern blot, RT-PCR, ELISA, western blot, flourometric xylanase activity analysis and sugar release assay. Highest expression was detected in mature leaves. The *in planta* produced enzyme was purified and sugarcane xylan was used as a substrate. TLC analysis confirmed the superior catalytic activity and stability of the in planta produced enzyme with directly fermentable xylobiose as the main degradation product.

44. Bacterial genome finishing using optical mapping

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ICBR has several ongoing bacterial genome projects addressing a variety of challenges to genome assembly and closure. In the absence of a physical map, we adopted whole-genome optical mapping as a tool to validate bacterial genome assemblies. OpGen, Inc. (Gaithersburg, Maryland) prepared the optical maps used in these projects. Briefly, an optical map is a complete genome restriction map deduced from a number of partial restriction maps. Optical maps are generated by spreading carefully extracted genomic DNA onto a treated glass surface containing many narrow channels, followed by digestion *in situ* with restriction enzymes. About 50–100 overlapping partial optical contigs are combined by alignment software to produce a contiguous whole genome restriction map. The contiguous optical map can be aligned and compared with the *in silico* restriction map of contigs obtained from whole-genome assembly. We successfully used optical mapping for guiding the closure of four closely related bacterial genomes. The optical map not only orients scaffolds but also allowed us to identify assembly errors, which was not possible using shotgun DNA sequencing data alone. Thus, we conclude that, in order to ensure the accuracy of a finished bacterial genome and to accelerate overall finishing process, optical mapping is an important tool to *de novo* assemblies generated by next-generation DNA sequencing.

45. Evolutionary basis of birth defects: the turtle as a model for hypospadias

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Hypospadias is a congenital defect of the penis characterized by an ectopic ventral opening of the urethra, and can range from a small opening anywhere along the glans or shaft, to a large opening along the entire length of the penis. It is one of the most common congenital defects in humans, however little is known about the developmental events that lead to this defect. Importantly, a closed urethral tube is a unique feature of mammalian genitalia, while the phallus develops an open urethral groove, or sulcus, in reptiles and birds. Thus, hypospadias may be interpreted as a disruption of a developmental process that evolved after the divergence of mammals and reptiles, in which the primitive condition (an open groove) persists in the mammalian penis. Here we compare the mechanisms of external genital development in turtle and mouse embryos and show that during normal embryonic development of the red-eared slider turtle, *Trachemys scripta*, the urethra is initially closed but opens along the entire ventral side of the penis at later stages of development generating the urethral groove or sulcus that is found in the adult turtle. Importantly, we show that penile morphology, with the exception of the open urethra, and transcription of several genes import ant for phallus development are highly similar between turtles and mammals, making it an excellent model for the identification of signaling pathways that when disrupted can lead to hypospadias in humans.

46. Comparative analysis of the limb-specific enhancer of the Sonic hedgehog (*Shh*) gene and its relation to the evolution of limblessness in snakes

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Digit and limb loss has occurred many times during vertebrate evolution, but its molecular basis remains unknown. Sonic hedgehog (*Shh*) is a growth factor that regulates the morphogenesis of several organs, including the limbs. During limb development *Shh* is expressed in the posterior mesenchyme of the limb bud (or ZPA), where it controls development of the digits along the anteroposterior axis of the limb. *Shh* expression at the ZPA is regulated by a non-coding limb specific enhancer, called ZPA regulatory sequence (ZRS), localized at 1 Mb away from the *Shh* gene. The ZRS is a highly conserved sequence across vertebrates, but has never been identified in a limbless species, suggesting that the ZRS loss might be correlated to the evolution of limblessness. The goal of this study was to analyze if digit loss and limblessness is reflected by significant modifications in ZRS. We have isolated the ZRS from the basal snake *Python reticulatus* and also searched genomic databases to identify the ZRS in a variety of limbed vertebrates with and without digit reduction. Since, pythons conserve the ZRS but they only have a rudimentary femur in the hindlimb, we used *in situ* hybridizations to analyze if *Shh* is expressed in the hindlimbs of python embryos. Despite the distal truncation of hindlimb in pythons, *Shh* is expressed in a ZPA-like domain, suggesting that the genomic regulatory elements required for digit development have been retained during the 100+ million years of snake evolution.

47. Blimp1 as a negative regulator of Shh in mouse limb buds

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Shh functions in the hedgehog signaling pathway and is a key player in vertebrate organogenesis. In the limbs, Shh is expressed in the zone of polarizing activity (ZPA) and Shh protein functions as a morphogen to pattern limb development. Currently, it is not known whether all cells in the ZPA express Shh continuously or whether Shh is turned on and off in a subpopulation ZPA cells. Recent studies suggest that only a small population of ZPA cells actively express Shh at any given time and fate mapping of Shh-expressing cells using cre alleles showed that cells that have expressed Shh expand out of the ZPA while new cells within the ZPA turn on Shh. We investigated the molecular mechanisms responsible for regulating Shh-expression within the ZPA and have focused on the role Blimp1, a protein known to interact with histone modifiers. Based on the expression pattern of Blimp1, it has been suggested that this protein may interact with Shh in the vertebrate limb. RNA *in situ* hybridization indicates that Blimp1 and Shh are expressed in partially overlapping domains in the mouse limb bud. In addition, conditional knockout of Shh in Blimp1 expressing cells result in a Shh-null like phenotype. These data suggest that Blimp1 expressing cells may be progenitors of Shh expressing cells. We have also found that Blimp1 directly binds to the Shh ZPA enhancer (ZRS) and represses Shh expression. Therefore, Blimp1 may be a negative regulator of Shh and function in controlling how Shh is turned on and off in ZPA cells.

48. Functional differentiation in protein evolution: perspectives from switch positions

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Protein evolution can be modeled as a combination of neutral and functional differentiation. An analysis based on non-synonymous and synonymous substitution ratio on codon sites (Ka/Ks) suggested that substitutions between amino acid types of different physico-chemical properties correlate more frequently with events of functional differentiation, whereas substitutions between similar amino acid types more likely represent events of constrained neutral evolution. Here we identify patterns of functional differentiation in the evolution of bacterial proteins based on "switch positions", sites in a protein family where amino acid types are conserved within individual phylogenetic groups of bacteria, indicating strong lineage-specific constraining negative selection, but differ ("switch") between different groups. From the analysis of the alignment of 168 protein families conserved across 31 well-defined bacterial groups, we identified a small fraction of switch positions. Amino acid exchange matrices based on switch positions indicated that, in contrast to inferences from Ka/Ks ratios, amino acid switches ascribable to events of functional differentiation corresponded most frequently to replacements between similar amino acid types. Being a class of rare genomic changes, switch positions should be appropriate markers to reconstruct phylogenetic relations of bacterial groups. The resulting trees supported the model of biological big-bang in the evolution of bacterial phyla.

49. Muscleblind-like 2 regulates alternative splicing during brain development

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The muscleblind-like (MBNL) proteins are a family of zinc finger related factors which function in the developmental regulation of alternative splicing. Inhibition of MBNL activity has been linked to the neuromuscular disease myotonic dystrophy (DM). Previously, we have shown that Mbnl1 is essential for the switch from embryonic to adult splicing patterns in skeletal muscle and this switch is blocked in *Mbnl1* knockout mice which develop muscle pathology characteristic of DM. Here, we report that Mbnl2, which is most highly expressed in the hippocampus and cerebellum of the adult mouse brain, controls the brain-specific alternative splicing of genes implicated in neuronal plasticity, dendritic density regulation and spatial memory. *Mbnl2* conditional knockout ($Mbnl2^{\Delta E2/\Delta E2}$) mice have been generated and global splicing analysis revealed that these mice are an important new resource for investigations on the molecular events which underlie normal brain development and DM-relevant brain pathology. Moreover, $Mbnl2^{\Delta E2/\Delta E2}$ knockouts provide a useful model for ongoing drug development designed to reverse the clinical features of this disease in the central nervous system.

50. Optimization of a protein tag-based system for testing protein-protein interactions

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Almost all proteins in cells interact with other proteins, either for regulation or as part of their normal function. For this study of protein-protein interactions, we use ITB3 and ADF – two proteins that regulate actin dynamics in plant cells. To facilitate the study of the ITB3 and ADF interaction, tagged versions of these proteins have been constructed in bacterial expression vectors. Because inclusion bodies were present when ITB3 was expressed, optimization of the expression and purification of this fusion protein was necessary. Various modifications of growth conditions, including media, growth temperature, and concentration of inducing agent, were tested to optimize the expression of ITB3. Purification of ITB3 was also optimized and this included varying cell lysis methods and imidazole concentrations in the wash and elution buffers. Protein concentrations of purified ITB3 and ADF were quantified through the use of a modified Bradford assay to ensure that equal amounts of each protein were used for the protein-protein interaction assays. Currently preliminary tests of the interactions of these proteins have begun, and future experiments will include the optimization of long term storage conditions for both ITB3 and ADF.

51. Influence of viral vector-mediated delivery of superoxide dismutase and catalase to the hippocampus on spatial learning and memory during aging

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Studies employing transgenic mice indicate overexpression of superoxide dismutase 1 (SOD1) improves memory during aging. It is unclear whether the improvement is due to a lifetime of overexpression, decreasing the accumulation of oxidized molecules, or if increasing antioxidant enzymes in older animals could reduce oxidative damage and improve cognitive function. We used adeno-associated virus (AAV) to deliver antioxidant enzymes (SOD1, SOD2, CAT and SOD1+CAT) to the hippocampus of young (4-month) and aged (19-month) F344/BN F1 male rats and examined memory-related behavioral performance one month and four months post injection. Overexpression of antioxidant enzymes reduced oxidative damage; however, memory function was not related to the level of oxidative damage. Increased expression of SOD1, initiated in advanced age, impaired learning. Increased expression of SOD1+CAT provided protection from impairments associated with overexpression of SOD1 alone and appears to guard against cognitive impairments in advanced age. In conclusion, oxidative stress is a likely component of aging; however, it is unclear whether increased production of ROS or the accumulation of oxidative damage is the primary cause of functional decline. The results provide support for the idea that altered redox sensitive signaling rather than the accumulation of damage may be of greater significance in the emergence of age-related learning and memory deficits.

52. Homeostatic regulation of the WDR-23/SKN-1 stress response

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The cap 'n' collar transcription factor family (CnC) orchestrates the transcriptional responses to oxidants and xenobiotics. We recently showed that WDR-23, a highly conserved WD40 repeat-containing protein, directly interacts with the *C. elegans* CnC SKN-1 to regulate nuclear abundance and activity of the transcription factor. Elevated SKN-1 activity caused by *wdr-23* loss of function can promote stress resistance and longevity but also inhibits larval growth and reproduction. Interestingly, our microarray and real-time RT-PCR data indicate that *wdr-23* is strongly elevated by xenobiotics and by a deletion allele of *wdr-23(tm1817)*; these increases in *wdr-23* mRNA require *skn-1*. The *wdr-23* promoter contains five SKN-1 binding motifs and ChIP-seq data rates *wdr-23* as the most likely gene promoter to be bound by SKN-1. These observations support a negative auto-feedback loop in which activation of SKN-1 by stress enhances *wdr-23* expression to repress SKN-1 and limit detrimental effects on growth and reproduction. *wdr-23::GFP* reporters containing the putative SKN-1 binding sites revealed that *wdr-23* is broadly activated after stress by a mechanism that requires *skn-1*. We are conducting biochemical and transgenic approaches to define the bona fide SKN-1 binding sites that control *wdr-23* expression *in vivo*. We are also using translational *wdr-23* transgenes lacking SKN-1 regulatory sites to test, for the first time, the physiological function of CnC auto-regulation.

53. Genome-wide identification of chromatin transitional regions reveals the diversity of chromatin barriers and the association with dynamic nucleosomes

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The juxtaposed distribution of heterochromatin and euchromatin along chromosomes is central to defining cellular identity and property. The boundary of heterochromatin is often set up by specific DNA elements called chromatin barriers. Comprehensive understanding of chromatin barriers will provide important insights into the study of the regulation of distinct chromatin states and gene expression in different cell types. In this study, we seek to perform genome-wide analysis of chromatin transitional region (CTR), which is a genomic region where chromatin state switches. A bioinformatics program CTRICS (Chromatin Transitional Regions Inference from ChIP-Seq) was developed and applied to identify CTRs for H3K27me3 in *Drosophila* S2 cells. By comparing the CTRs with the distribution of known insulator proteins in *Drosophila*, we found that chromatin barriers in general locate on euchromatic side of CTRs with about 2 nucleosomes in between. Hierarchical clustering revealed multiple groups of CTR marked by different combinations of associated proteins, while known insulator proteins only account for less than half of all chromatin barriers. Interestingly, we found that immediate to the open side of CTRs, there exist a dynamic-nucleosome region, characterized by low level of nucleosome density, as well as high levels of H3.3 incorporation and DNA accessibility. These findings suggest that H3.3 incorporation and

dynamic nucleosomes are essential to the formation of chromatin boundary.

54. *mtND2*^{a} protects human β-cells from type 1 diabetes-related effectors

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Oxidative stress is associated with β-cell failure that results in type 1 diabetes (T1D) development. We have linked a single nucleotide polymorphism within the mt-DNA gene for NADH dehydrogenase subunit 2 (*mtND2*) to reactive oxygen species (ROS) and T1D resistance in the mouse at the β-cell level. The human *mtND2*^{c} allele is also present at a higher frequency in T1D patients than in controls. Here, we test cybrid human β cell lines with equal nuclear genomes and differing in the mt-DNA haplotype, encoding either *mtND2*^{c} or *mtND2*^{a} allele, for susceptibility to cytokine and Fas-induced death. Cybrid cell lines were developed by first depleting the human β cell line, βlox5, of mt-DNA with low dose ethidium bromide, and then fusing the resulting cell line with human donor platelets with *mtND2*^{c} or *mtND2*^{a}. This process produced βlox5-*mtND2*^{c} (JC-2) and βlox5-*mtND2*^{a} (JC-3). JC-2 and JC-3 cells were incubated with the cytokine combination of TNFα and IFNγ, or the combination of IFNγ and α-Fas for 48h before assaying cell death, mitochondrial ROS production, and mitochondrial membrane potential (MMP). JC-3 cells showed increased resistance to cytokine and α-Fas killing compared to JC-2, which correlated with lower levels of ROS generation and increased resistance to MMP changes. Our results indicate that, like in the mouse, *mtND2*^{a} protects a human β cell line from insults associated with T1D progression by suppressing ROS produced by mitochondria in response to pro-death signals.

55. The polycomb protein Sfmbt1 regulates MyoD-mediated epigenetic silencing in skeletal myogenesis

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The biological functions and molecular basis of a newly identified polycomb protein SFMBT1 are poorly characterized. Here, we studied the molecular mechanisms and functions underlying SFMBT1 regulation of cell proliferation and differentiation. SFMBT1 is associated with components of multiple transcriptional repressive complexes including CtBP/LSD1/HDACs complexes, MBT family proteins and polycomb repressive complex 1 (PRC1). Sfmbt1 negatively regulates myogenic differentiation in both cultured and primary myogenic progenitor cells, as its over-expression represses myogenic differentiation while its depletion promotes differentiation. Mechanistically, Sfmbt1 mediates epigenetic silencing of muscle transcription factor MyoD via interacting with MyoD and recruiting its associated repressive proteins to MyoD target loci including myogenin and Mef2C that are essential for myogenic differentiation, and subsequently effects epigenetic changes. Our overall data reveals a novel mode of action for SFMBT1 in chromatin compaction, and suggests its essential roles in regulating MyoD-mediated transcriptional silencing and maintaining undifferentiated states of myogenic progenitor cells. Currently the *in vivo* roles of Sfmbt1 in muscle regenerative capacity are being evaluated.

56. Induction of reaper ortholog mx in mosquito midgut cells following baculovirus infection

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Many vertebrate and insect viruses possess antiapoptotic genes that are required for their infectivity. This led to the hypothesis that apoptosis is an innate immunoresponse important for limiting virus infections. The role of apoptosis may be especially important in insect antiviral defense because of the lack of adaptive immunity. However, the cellular mechanism that elicits apoptosis in response to viral infection in insects has not been determined. Using an *in vivo* infection system with the mosquito baculovirus CuniNPV (*Culex nigripalpus* nucleopolyhedrovirus), we demonstrated that michelob_x (mx), the mosquito ortholog of *Drosophila* proapoptotic gene reaper, is specifically induced in larval midgut cells following viral infection. Interestingly, the dynamics of mx induction corresponds with the outcome of the infection. In the permissive mosquito *C. quinquefasciatus*, a slow induction of mx failed to induce prompt apoptosis, and the infected cells eventually undergo necrosis with heavy loads of encapsulated viruses. In contrast, in the refractory mosquito *Aedes aegypti*, a rapid induction of mx within 30 min p.i. is followed by apoptosis within 2–6 h p.i., suggesting a possible role for apoptosis in limiting viral

infection. When the execution of apoptosis was delayed by caspase inhibitors, viral gene expression became detectable in the *A. aegypti* larvae.

57. The role of sensory perception during osmotic stress

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Cell structure and function are highly sensitive to osmolarity and volume. Accordingly, organisms have developed behavioral and physiological responses to cope with osmotic changes in the environment. How animal cells perceive osmolarity and the signals that regulate the physiological responses remain largely unknown. *C. elegans* is a powerful model for studying the osmotic stress response due to its genetic tractability and well-understood nervous system. Like other animals, *C. elegans* has both behavioral and cellular responses to high environmental osmolarity. Recent studies have shown that cellular responses to some environmental stressors are coordinated by the nervous system. Here, we wanted to determine if cellular responses to high osmolarity require sensory perception. The gene osm-9 encodes a TRPV channel homologue that is required for detecting high osmolarity in the sensory neurons of *C. elegans*. By comparing the induction levels of stress-responses between osm-9 defective and wild-type strains, we provide evidence that cellular responses to high osmolarity are largely independent from the sensory perception of osmolarity.

58. Methamphetamine alters nucleosome distribution at specific immune loci

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Methamphetamine is a potent suppressant of innate and adaptive immune function. Its effects are characterized by altered cytokine and chemokine expression programs and increased pathogenesis of viruses and bacteria. Consequently, it has been implicated as a cofactor in the increased progression of diseases of suppressed immunity. An overwhelming majority of research has examined the immunomodulatory characteristics of methamphetamine from the perspective of cell signaling and transduction. Nonetheless, a few studies show that methamphetamine alters gene expression patterns in target cells through epigenetic modifications. Here, we show that methamphetamine treatment induces chromatin alterations in cells of the innate immune system. Our research has identified nearly 200 immune-related genes whose transcription start sites exhibit time- and dose-dependent nucleosome redistributions following acute methamphetamine treatment. Further gene ontology assessment confirms that genes involved in T-cell co-stimulation and acute phase response are enriched in this population. Our research represents the first characterization of the effect of methamphetamine on the organization of chromatin in immune cells. Furthermore, it is the first step in the identification of chromatin structural signatures associated with methamphetamine-induced immune suppression. These studies are a new line of inquiry into the molecular pharmacology of methamphetamine and its role in immune suppression.

59. Investigating a replicative mechanism for the establishment and maintenance of recombinant adeno-associated viral vector genomes *in vitro* and *in vivo*

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Over the past few decades, adeno-associated virus (AAV) has been developed as a recombinant vector for gene transfer. Recombinant AAV (rAAV) vectors consist of a transgene cassette flanked by the endogenous viral inverted terminal repeats. rAAV vectors have been used successfully in multiple clinical trials, but there are still aspects of the biology of the vector that remain unknown. Upon infection with rAAV, the vector is trafficked through the cell and delivered to the nucleus, where it establishes a persistent infection. It is known that these vectors can persist *in vivo* and maintain stable transgene expression for at least 7 years in large animal models. It was previously thought that integration of the vector genome into the host cell chromosome was responsible for this long-term persistence. However, it has now been demonstrated in muscle and other tissues that rAAV vector genomes persist in the host cell nucleus as episomal circular monomers and concatemers, and these forms are believed to be responsible for the observed long-term expression of the transgene. While there is sufficient evidence for the presence of these episomal circular forms, it is unknown how they are established and maintained over time. To elucidate this aspect of the vector life cycle, we are investigating the role of DNA replication in the establishment and maintenance of episomal circular rAAV monomers and concatemers *in vitro* and *in vivo*.

60. Improving diploid strawberry Yellow Wonder genotype 5AF7 as a functional genomic resource

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Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is one of the major crops in United States and Florida with a substantial high contribution to the economy. Many studies focus on the cultivated strawberry which has an octoploid genome, making genetic and genomic analyses complicated. An alternative is to investigate strawberry biology using diploid strawberry, which shares a common ancestor with the cultivated strawberry. Unlike octoploid strawberry, diploid strawberry grows quickly from seed to seed and has a simple and remarkably small genome. Diploid strawberry has become an attractive system for studies in all rosaceous crops. We have developed protocols for the Yellow Wonder *F. vesca* genotype 5AF7 (YW5AF7) is a seven-generation inbred diploid strawberry that has been well phenotyped. The optimization of *in vitro* growth seedlings and leaf disks regeneration of YW5AF7 determined that MS media with B5 vitamins and 1% sucrose supported healthy *in vitro* plant growth after two months. Optimization on various combinations of plant growth regulators (PGRs) and media types was conducted to obtain robust, high regeneration efficiency. A combination of 1.5 µM IBA with 15 µM BA gave the highest percentage of shoots, (about 70% of explants) and 5 shoots per explant within the same period. We also have identified light conditions that best support adventitious shoot formation, increasing the efficiency of the system.

61. *Foxa1* and *Foxa2* in intervertebral disk formation

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

62. ZmURP influences alternative splicing in maize and may function by interacting with the U2AF spliceosome complex

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Alternative RNA splicing produces multiple mRNA species from individual genes increasing protein diversity and providing an added level of gene expression regulation. Genome sequencing projects have shown that about 35% of genes in plants are alternatively spliced, but little is known about how this process is controlled. The *rough endosperm3* (*rgh3*) mutant causes developmental defects that are either seed or seedling lethal. *Rgh3* encodes a $U2AF^{35}$ related protein (ZmURP), which is a predicted RNA splicing factor. $U2AF^{35}$ proteins identify splice acceptor sites during RNA processing and function through protein-protein interactions by creating complexes with $U2AF^{65}$ and other SR proteins. Semi-quantitative RT-PCR analyses of alternatively spliced genes showed that *rgh3* effects splicing in a subset of genes supporting a role for ZmURP in alternative splicing. ZmURP is alternatively spliced itself, producing at least 19 different spliced variants with only one predicted to encode a full-length URP ortholog. GFP fused to full-length ZmURP localized to the nucleolus and nuclear speckles. Co-expression of ZmURP-GFP with $U2AF^{65}$-RFP demonstrates the proteins co-localize supporting interactions between these proteins. Intriguingly, GFP fusions to truncated ZmURP variants were excluded from nuclear speckles identifying domains required for incorporation into functional splicesomes.

63. Fitness decay and mtDNA mutation in mutation accumulation lines of the nematode worm *Caenorhabditis elegans*

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Under the hypothesis that an organisms' own fitness may affects its mutation rate, mutation accumulation (MA) lines of the nematode *C. elegans* were evaluated for fitness and genomic mutation rate. Five high fitness lines and five low fitness lines were chosen from a previous MA experiment, expanded to 48 sub-lines, and allowed to accumulate mutations for an additional 150 generations. All but one of the sub-lines groups showed average fitness decay as compared to the parental lines. In addition, both fitness groups showed a similar decay as the parental lines. The observed pattern of fitness decay might be explained by an early mutational diversification in the first MA process (~a few hundreds generation prior). To examine this possibility, whole-genome re-sequencing was performed on a subset of high and low fitness MA lines. The re-sequencing data suggests that mutations in the mtDNA genome may partially explain the observed fitness patterns. Substitutions and small insertions/deletions were distributed throughout the genomes of both fitness groups, however three large deletions (>3kbp) were found in two of the low fitness lines. Of the three large deletions, two deletions were found to have occurred independently in two different lines in the initial MA process. Furthermore, the third mtDNA deletion appears to have originated subsequent to second order MA experiment in one of the low fitness lines.

64. Non-synonymous SNPs in *SELE*, *SELP*, and *SIGLEC12* associate with cardiovascular (CV) outcomes in the INternational VErapamil SR-Trandolapril STudy GENEtic Substudy (INVEST-GENES)

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We sought to identify novel pharmacogenetic markers for antihypertensive drug-associated CV outcomes in patients with hypertension and coronary artery disease (CAD). We genotyped 1345 patients with hypertension and CAD, comprising a 1:4 case:control from INVEST-GENES on the Illumina Human CVD Beadchip. Patients were randomized to an atenolol-based β-blocker strategy or a verapamil SR-based calcium channel blocker strategy with trandolapril and/or hydrochlorothiazide added if necessary. The primary outcome was defined as first occurrence of death, nonfatal myocardial infarction (MI) or nonfatal stroke. This analysis focused on non-synonymous SNPs in whites and Hispanics. Association with the primary outcome was assessed by race in each treatment strategy using logistic regression, adjusting for age, sex, principal components for ancestry, and history of MI, heart failure, and diabetes. SNP x treatment interaction analyses were also evaluated by race, adjusting for the same covariates. Two regions were noteworthy. The SEL region on chromosome 1 was among top hits: *SELE*, selectin-E in whites (Interaction P=0.048) and *SELP*, selectin-P in Hispanics (Interaction P=0.002). Also, *SIGLEC12*, sialic acid binding immunoglobulin-like, showed strong evidence of association in whites (Interaction P=0.033) and replicated in Hispanics (Interaction P=0.021). These results suggest CV outcomes may differ based on *SELE*, *SELP* and *SIGLEC12* genotypes and antihypertensive treatment strategy.

65. Y-genotyping of haplogroup E in Yemeni samples

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Yemen, located in the southwest corner of the Arabian Peninsula, occupies a key position to improve our understanding of human migration from Africa to Europe and Asia. Our goal is to assess the African genetic contribution in Yemen and understand the area's role in migration out of Africa and implications for the Southern Dispersal Route hypothesis for migration across the southern end of the Red Sea. To this end, we are genotyping Y-chromosome SNPs in 399 male subjects collected throughout Yemen. Approximately 13.0% of males were found to belong to the E haplogroup, which is found nearly exclusively in Africa. Haplogroup E is further divided into E1a and E1b subclades, where 10.6% haplogroup E individuals were found to belong to the E-M33 subclade and 67.3% were found to belong to the E-P2 subclade, with the remaining samples (22.1%) belonging to E-M96. When compared to the frequencies of E-M33 and E-P2 in other populations in Africa and Asia, our results will provide insight into the phylogeographic processes that shaped current genetic variation in Yemen.

66. Disentangling human demographic processes? What mtDNA simulations teach us

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Gene flow has played a defining role in the population structure of modern humans. The complexity of human demographic processes makes it difficult to disentangle the effects of geneflow from the effects of other demographic processes. To address this, we simulated mtDNA for 42 different parameter combinations describing modern humans migrating out of Africa, which include two times for the initial migration event, three sizes for the initial migrating population and seven levels of subsequent gene flow. We calculated genetic summary statistics on the simulated datasets and compared these values to identify which combinations generate distinguishable differences in genetic variation. Depending on the parameter combination, one to four summary statistics could capture differences in genetic variation. Our results show that different timings for the migration (2000 vs 4000 generations ago) generated indistinguishable patterns of genetic variation. Combinations with low initial migration size (1% of the source population) generated distinguishably different patterns of genetic variation from the higher initial sizes (10% and 30%). The genetic variation from the different levels of gene flow were only distinguishable between low ($4Nm<1$) and high ($4Nm>>1$) levels of gene flow. These results suggest that despite the complexity of human demographic processes, the genetic variation of mtDNA generated in human populations is not high enough to be captured by simple summary statistics.

67. Retinoic acid signaling during limb development, maintenance, and regeneration in the axolotl salamander

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Retinoic acid (RA) plays a necessary role in both limb development and regeneration, but the precise mechanism by which RA acts during these processes is unclear. Blocking RA synthesis before limb development or regeneration in mice, zebrafish, or axolotls inhibits limb formation, suggesting a conserved need for RA signaling. Here, we find that RA receptor-beta (RARB) signaling may be necessary for forelimb development, regeneration, and maintenance in axolotl salamanders. We first show that RA signaling takes place during limb development by monitoring a transgenic axolotl that carries a RA response element-GFP reporter construct. Inhibition of RARB signaling during development or regeneration allowed limb buds to form, but outgrowth was halted at the mid-bud stage suggesting a role of RA in limb differentiation. Inhibition of RARB signaling in mature limbs caused limb regression suggesting that RA signaling may also be involved in the maintenance of limb structures. Inhibition of all three processes was associated with a decrease in cartilage integrity suggesting that RA signaling is necessary in the differentiation and maintenance of skeletal structures. Overall, our data show that functional RAR signaling is necessary for the development, regeneration, and maintenance of forelimbs and these processes can be tracked using transgenic technologies in axolotls.

68. Function of Daxx at centromeres and pericentromeres

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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 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.

69. Biological research computing resources at the University of Florida

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The University of Florida Research Computing Center is a faculty-directed facility with the mission of providing high-performance computing, storage, and software resources as well as support to the UF research community. We will provide an overview of the computing infrastructure and support available to biological researchers including the Galaxy - web-based platform for accessible and reproducible biological computing, modules system for command line software environment loading, software for biological research, and support and consulting services.

70. Epigenetic alterations and stress among new mothers and infants in the Democratic Republic of Congo: a biocultural look at the intergenerational effects of war

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There is growing evidence that epigenetic modifications may serve as an intermediate adaptive mechanism that mediates between the rapidly changing environment and our slowly evolving genome. We test the idea that epigenetic modifications may create heritable changes in response to extreme environmental stressors that affect infant health in a multigenerational manner. Maternal blood and umbilical cord blood samples were collected from 25 mother-infant dyads in the eastern Democratic Republic of Congo. Detailed ethnographic interviews and peri-natal trauma surveys were administered to all mothers. A 321 bp promoter of *NR3C1* with 39 CpG sites was amplified, cloned and sequenced after bisulfite conversion. *NR3C1* is a glucocorticoid receptor that was previously implicated in methylation-mediated changes in gene expression associated with childhood trauma. Our preliminary results show increased methylation (20-34%) in high stress mothers (material deprivation, mundane stressor, war stressors) relative to low stress mothers. In contrast, infants of low stress mothers show increased methylation with respect to their mothers (45-51%) and with respect to infants of high stress mothers (16-26%). No differences in methylation were seen when mothers and infants were combined. Our results suggest that methylation patterns differ between mothers and infants and may correlate with maternal stress exposures.

71. Multi-factorial association studies for dissecting genetic architecture of complex diseases

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The study of the genetic basis of complex disorders is a great challenge in the field of translational genetics. Although genome-wide association studies (GWAS) have shown a lot of promise in investigating phenotype-genotype relationships on a large scale, they have had limited success in revealing the genetic mechanisms underlying complex traits, because the current "one SNP at a time" analytical approaches are unable to model the joint effects of multiple SNPs. To address such complexities, we have developed a hypothesis-based method which takes advantage of preexisting biological knowledge to reduce the dimensionality of genome-scale datasets and to generate hypotheses about the disease under investigation. Hypotheses are tested and ranked by a genetic algorithm (GA) which produces multi-SNP models relevant to the trait. The method was tested on the Wellcome Trust Case-Control Consortium (WTCCC) dataset to analyze the genetic factors influencing the pathogenesis of rheumatoid arthritis (RA). Our method was able to generate several multi-SNP models exhibiting significant association with RA. None of the SNPs in these models was previously known to have significant association with RA individually. The identified models were replicated in an independent case-control dataset, suggesting that our method can be used to investigate the genetic architecture of complex disorders. A software package implementing our method is available at: http://genome.ufl.edu/rivalab/kbas.

72. Exploring the megagenome of pine by targeted resequencing

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Loblolly pine is an ecological and economically important conifer species native to the southeastern US. The loblolly pine 22,000 Mbp genome is one of the largest and most

complex among plant species, hindering a full characterization of its sequence. We carried out an initial analysis of this complex genome by resequencing genic regions of 72 individuals of a segregating family using Agilent's SureSelect sequence capture, followed by sequencing with Illumina GAIIx. Custom probes were designed to capture 6.6 Mbp (0.03% of the genome) of 14,729 genes. Sequence capture was successful and resulted in an enrichment of ~1000 times on the target region. We developed a bioinformatics pipeline that combines alignment of the reads to the target region followed by a *de novo* assembly step to reconstitute and expand the coding portion of the genome. This analysis increased the reference unigene for 10,576 genes and identified intronic regions in 21% (2,207) of them. A draft gene model was built for the genes captured that will be used in the annotation of the pine genome. We also identified 4,563 high-confidence SNPs for 2,210 genes that segregate in the mapping population. Finally, we are developing methods to identify gene copy number variation based on targeted resequencing data.

73. Genome-wide linkage scan for familial IgA nephropathy among Southeast Asian Chinese: identification of a novel susceptibility locus on chromosome 8p22-23

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IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. Over the past decade, three independent genome-wide scans for linkage to familial IgAN among Caucasians have identified two susceptibility loci on chromosomes 6q22-23 IGAN1 and 2q36, as well as a locus suggestive for linkage on 4q26-31. We now report the results of a genome-wide scan based on a large 4-generation Singaporean Chinese family (F66) as well as 23 smaller Chinese IgAN kindreds from Hong Kong (HK23). Linkage analysis of F66 was first performed at 10 cM resolution using microsatellite markers. By parametric analysis (assuming autosomal dominant inheritance, allele frequency of 0.001, phenocopy rate of 0.01 and penetrance of 75%), a region of suggestive linkage with a maximum multipoint LOD score of 2.23 was identified on chromosome 8p23. By non-parametric (NPL) analysis, a significant linkage to 8p23 (maximum multipoint LOD score 3.89, p-value 0.004) was confirmed. We are genotyping 11 additional markers to conduct fine resolution mapping of the 8p22-23 locus, expected to increase the HLOD score and narrow the critical region. Maximum multipoint LOD score of 5.13 was identified on chromosome 8p23.1. Our results indicate that a novel locus on chromosome 8p23.1 contributes a large genetic effect in 52% of IgAN kindreds in our study, representative of large populations of Southeast Asian Chinese among whom familial IgAN is not uncommon.

74. Assessing the research data ecology of the University of Florida

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Research data represent a valuable resource to academic and research communities. While they often require large investments of time and money to be created, many datasets have significant value beyond their original research purposes. Access to data can facilitate scrutiny of research outcomes, lead to new collaborations, and increase impact and visibility of research. Adequate management of data is necessary in order to make the best use of these valuable resources. Among the benefits of proper data management practices are: efficiency savings, risk management, access, and reuse. Proper data management provides an idea of data storage and processing requirements as well as potential improvement of workflows throughout the data lifecycle. Given the breadth of research occurring here, the University of Florida faces many of the challenges inherent to preserving, storing, managing, and making accessible large quantities of research data. The Health Science Center Libraries, George A. Smathers Libraries, and UF High Performance Computing Center (HPCC) are committed to developing local solutions to these challenges based on a comprehensive understanding of the UF data environment. To that end, we are in the process of performing a data services needs assessment, using surveys and interviews of UF researchers. We will present the preliminary results of this assessment, with an indication of future directions for library and HPCC services supporting campus data needs.

75. The coding potential of the human genome: insights from sequence compositional contrasts

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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 on the base content at the three codon positions as a function of the overall base content can be identified and exploited to score sequence regions for their coding potential. More generally, the period-three structure of coding regions imposes compositional periodicity to the sequence that, irrespective of the specific type of contrasts that we may expect to see, result in a significantly non-random distribution of bases. Applying these principles we have devised two algorithms to detect potential coding regions in sequences of any composition, one based on overall compositional expectations and one based on overall contrasts. We have applied our procedure to the human genome detecting a plethora of regions, not overlapping with any of the currently annotated gene sequences, that display with high statistical significance a periodic structure often conforming to expectations for coding regions in terms of base-type composition. The frequency of these regions is far higher than the random frequency observed in corresponding scrambled sequences and show levels of complexity that distinguish them from repetitive elements. Although a fraction of these regions has been characterized as pseudogenes or transposases, most are previously uncharacterized potential coding regions.

76. Hormonal regulation of shark fin development: implications for the evolution of copulatory organs

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The most primitive vertebrate copulatory organs are 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 Chondricythes develop claspers, and this represents an interesting sexual dimorphism of the fins that suggests a potential role for sex steroids in fin development. Previous studies have shown that *Sonic Hedgehog*, *Hoxd12*, and *Hoxd13* expression persists in the developing clasper of male fins after downregulation in the rest of the fin. How these genes are maintained only in male fin buds is unknown. This study seeks to determine the potential role of sex hormones in clasper development by examining the distribution of androgen and estrogen receptors in catshark (*Squalus acanthias*) embryos. Immunohistochemistry results demonstrate the presence of both steroid receptors within the developing pelvic fin buds, revealing their competence to respond to androgen and estrogen. Male copulatory organs may have evolved by hormonally-regulated modification of the fin development program in early jawed vertebrates.

77. Evolutionary tug of war: examining proteome regulation in tomato defense against *Pseudomonas*

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The study of pathogen response and defense in crop species is of essential importance as its applications are directly related to agricultural production. *Pseudomonas syringae* pv *tomato* (*Pst* DC3000) causes speck disease in tomato (*Solanum lycopersicum*), a crop having both nutritional and economical value. *Pst* utilizes effector proteins to develop disease within the plant. Resistant line of tomato Rio Grande-Pto has the R gene *Pto* that interacts with *Pst* effector proteins and creates a defense response in tomato. Susceptible RG-prf3, has a 1kb deletion in *Prf3* (*prf3*), a gene required for resistance against *Pst*. Our central hypothesis is that the underlying proteins and regulatory mechanisms will vary and play important roles in developing resistant and susceptible responses. The goal of this project is to observe in greater detail, mechanisms that tomato utilizes in response to pathogen infection. Changes in protein levels between resistant and susceptible genotypes is one such change that tomatoes can modify. Two time points were chosen based on preliminary ROS and bacterial growth data for proteome analysis. Isobaric labeling for relative and absolute quantification (iTRAQ) was performed in order to examine changes in protein levels between the resistant and susceptible lines. LC-MS/MS data was analyzed using ProteinPilot. Proteins with significant expression changes were grouped into functional categories and will be discussed.

78. Identification of markers linked to Avr1 in *Cronartium quercuum f.sp. fusiforme* using a novel next-generation sequencing approach

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Fusiform rust disease, caused by infection of the fungal pathogen *Cronartium quercuum f.sp. fusiforme*, produces galls on stems and branches of southern pines. Stands of susceptible genotypes are often poorly stocked because stem galls weaken stems and make trees susceptible to lodging. Gene-for-gene interaction between the *Pinus taeda* resistance gene Fr1, and the corresponding pathogen avirulence gene Avr1 has been documented in previous work (Wilcox et al., 1996, *Proc Natl Acad Sci USA* 93(9):3859-64; Kubisiak et al., 2010, *Fungal Genet Biol* 48(3):266-74). Obtaining markers for avirulence loci would allow for surveying of pathogen populations to enable growers to plant trees with corresponding resistance genes that ensure stands are resistant to rust. Markers can be identified because its gene-for-gene relationship acts as a "sieve" for avirulence alleles when a heterozygous fungal culture is inoculated on a resistant host. After inoculation of a susceptible tree (fr1/fr1), spores harboring virulent and avirulent alleles (Avr1 and avr1) persist and produce haploid pycniospores. In contrast, pycniospores from resistant trees (Fr1/fr1) only contain spores with avr1, selecting against the avirulence allele. Here we evaluate an approach to identify markers linked to Avr1, using bulk segregant analysis to compare pycniospore DNA sequences obtained through next generation sequencing. Reads present from the susceptible host, but absent in spores from the resistant host, are likely linked to Avr1.

79. KSHV-encoded miRNAs regulate RTA promoter activation by targeting cellular transcription factors MYB, Ets-1, and C/EBP alpha

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MicroRNAs are short, non-coding RNAs that post-transcriptionally regulate gene expression by targeting 3' UTRs of mRNAs. KSHV miRNAs are encoded in the latency associated region of the viral genome which plays an important role in the maintenance of viral latency. Several cellular targets of KSHV miRNAs have been discovered implicating roles for KSHV miRNAs in promoting angiogenesis, regulation of transcription factors (TF), and inhibition of apoptosis. Here we report a mechanism in which viral miRNAs play a role in the maintenance of latency by targeting cellular TFs known to activate the promoter of RTA. BC-3G, a PEL-derived indicator cell line, was used to observe the effects of viral miRNA knockdown on lytic reactivation. Our data demonstrate that inhibition of miR-K12-3 and miR-K12-11, leads to increased sensitivity to reactivation from latency. Moreover, both miRNAs are predicted to target cellular TFs Myb, Ets-1, and C/EBP alpha, which are known to induce lytic reactivation by directly activating the RTA promoter. Upon knockdown of miR-K12-3 and miR-K12-11 in BC-3 and BCBL-1 cells, we see a de-repression of all three cellular genes along with increased viral gene expression. Lastly, specific miRNA deleted recombinant virus was generated to further study these viral miRNAs. In summary, our data show that KSHV-encoded miRNAs miR-K12-3 and miR-K12-11 target cellular TFs Myb, Ets-1, and C/EBP alpha which in turn are involved in the regulation of key steps in the viral life cycle.

80. A possible explanation for the population size discrepancy in tuna (genus *Thunnus*) estimated from mitochondrial DNA and microsatellite data

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A recent study using both mitochondrial DNA (mtDNA) and microsatellite data reported on a population size discrepancy in the eastern tiger salamander where the effective population size (N_e) estimate of the former exceeded that of the latter. That study suggested, among other hypotheses, that homoplasy of microsatellite alleles is responsible for the discrepancy. In this investigation, we report ten new cases of a similar discrepancy in five species of tuna. These cases derive from our Bayesian inferences using data from Pacific bluefin tuna (*Thunnus orientalis*) and yellowfin tuna (*T. albacares*), as well as from published estimates of genetic diversity for additional regional populations of yellowfin tuna and three other tuna species. Phylogenetic character analyses of inferred genealogies of Pacific bluefin and yellowfin tuna reveal similar low levels of mtDNA and microsatellite homoplasy. Thus, the discrepancy between inferred population sizes from mtDNA and microsatellite data in tuna is most likely not an artifact of the chosen mutation models used in the microsatellite analyses, but may reflect behavioral differences between the sexes such as female-biased philopatry and male-biased dispersal. This explanation now warrants critical testing with more local populations of tuna and with other animal and plant groups that have

different life histories (e.g., male-biased philopatry and female-biased dispersal).

81. Dietary restriction effect on epigenetic regulation in *Drosophila*

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Epigenetic regulations, by controlling DNA accessibility and gene expression, play important roles in many biological processes such as stem cell differentnation and animal development. Our laboratory demonstrated that the about 33kb irradiation responsive enhancer region (IRER) is required for radiation-induced expression of pro-apoptotic genes in early stage embryos (before stage 11). The accessibility of IRER is subject to epigenetic regulation. Chromatin in IRER forms a facultative heterochromatin structure post embryonic stage 12. Consequently, cells with closed IRER lose their sensitivity to radiation-induced pro-apoptotic gene expression and cell death (Anderson et al., 2009, *Toxicol Pathol* 37(1):47-51). A transgenic *Drosophila* strain (X3) has a fluorescent marker (ubi-DsRed) inserted into IRER and allowed us to monitor the epigenetic status of IRER by directly measuring the DsRed fluorescent signal from homogenized flies. As a result, when the chromatin of IRER is more condensed, the DsRed fluorescence reading would be lower compared to its open form. Dietary restriction (DR) has been linked to life prolongation in many species such as rhesus monkeys, rodents, flies, and yeast (Zhang et al., 2008, *Dev Cell* 14(4):481-93). There is also evidence that DR is closely related to chromatin function even though the mechanism is not clear. We will submit this strain to DR in both early developmental stages (e.g. larvae) and adulthood and measure the DsRed fluorescent signal to test for the effect DR effect on epigenetic regulation of IRER.

82. RAPID-Seq: A method for genome complexity reduction and high throughput genotyping

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Genotypic information from human, plant and animal populations have been used to uncover genetic relationships and identify genes that regulate clinical and agricultural traits, among many other uses. Some applications, such as genome wide association studies (GWAS) or whole genome prediction (WGP) require the analysis of very large numbers of polymorphisms, evenly distributed in the genome, in very large numbers of individuals. The most popular methods of genotyping use single base extension, which relies on invariant primer binding sites flanking the genetic variant. Limitations for these methods are the challenges of identifying consistently invariant regions flanking polymorphisms, the high cost per genotype and an elevated rate of failure in species where the genome has not yet been well characterized. To address these limitations we have developed a method to produce reduced genome representation libraries for next generation sequencing and genotyping. The approach relies on PCR amplification using degenerate oligonucleotide primers. Such primers contain a specific DNA sequence in the 3' end, followed by degenerate nucleotides. DNA polymorphisms are assayed based on the presence of variants in sequencing reads derived from PCR amplification. The method has shown to be efficient, high throughput, and approximately one order of magnitude cheaper than the most popular genotyping methods.

83. Functional characterization of a candidate gene for carbon partitioning in *Populus*

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The development of a plant feedstock more suitable for bioenergy production requires the understanding of the genetic regulation of wood chemical properties. Our group identified a major gene that regulates carbon partitioning and growth on chromosome 13 (cpg13) of a poplar hybrid population, using genetical genomics approach. A major QTL that explains 56% of the variation in cellulose to lignin ratio, as well as 20-25% of the heritable variation of several biomass productivity traits was detected. The expression of cpg13 is highly correlated with lignin ($r = 0.41$) and cellulose ($r = -0.41$), and moderately correlated with shoot biomass ($r = -0.18$) in the poplar hybrid mapping population. A transcriptome analysis shows that cpg13 is mostly expressed in tissues undergoing secondary cell wall formation. High expression correlation and sharing of motifs with other lignin biosynthesis genes provide further evidence of cpg13 interaction with this pathway. Putative homologues of cpg13 in *Arabidopsis* are annotated as proteins with unknown function; therefore, the functional characterization of cpg13 is essential. Currently, evidence of the functional role of cpg13 is being obtained by the analysis of poplar genetically modified that down-regulate

(RNAi) and up-regulate cpg13 expression levels. Transcriptomics, wood chemical and biomass traits are going to be analyzed in plants grown under controlled environment. Furthermore, enzymatic assay with cpg13 protein will be performed to uncover its function.

84. Identification of a 21 kDa connective tissue growth factor fragment from human corneal fibroblasts

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Stromal scarring due to corneal trauma, infection, or refractive surgery is the result of a complex cascade of multiple growth factors, cytokines, chemokines, and proteases. Connective tissue growth factor (CTGF) is a 38kDa fibrogenic cytokine that is a downstream mediator of many of the fibrotic actions of TGF-β. Low molecular weight bands have been detected in cell homogenates, biological fluids and tissue extracts by western blots. We used tandem mass spectroscopy and western blots to positively identify a 21 kDa CTGF fragment in extracts of human corneal fibroblasts (HCF). Cultures of HCF were serum starved for 48 hours then stimulated with 10 ng/mL of TGF-β1. After 24 hours, the cell extracts were removed. Colloidal Coomassie stained bands that migrated and corresponded to the immunoreactive CTGF bands were excised and analyzed by LC/MS/MS. Extracts of HCF stimulated by TGF-β1 contained two bands that were detected on western blots at apparent molecular weights of 38 kDa and 21 kDa. The 21 kDa CTGF fragment and 38 kDa full length protein where both identified by the following sequence, LEDTFGPDPTMIR, that spans amino acids 184-196 of CTGF. These results clearly show that full length (38 kDa) and a proteolytically processed fragment (21 kDa) of CTGF are present in the HCF cell extracts. The identification and purification of this fragment may allow for determination of the cleavage site of the full length CTGF. Proteolytic processing may be the mechanism that CTGF uses to elicit two completely independent cellular responses.

85. Members of the RPA protein family play an important role in actin organization in the plant *Arabidopsis thaliana*

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The dynamic actin cytoskeleton plays an important role in many cellular processes including intracellular motility, membrane trafficking, and cell shape control. The assembly and disassembly of the actin cytoskeleton depends on the interactions of actin monomers with various other proteins. Members of the actin-depolymerizing factor (ADF)/cofilin protein family regulate actin dynamics by severing actin filaments and increasing disassembly. ADF/cofilin activity is known to be regulated by phosphorylation and lipid binding. We have uncovered a novel protein in the plant *Arabidopsis* that binds to and inhibits ADF thus extending the known mechanisms of ADF regulation. We named this protein RPA1 for Regulator of Plant ADF 1. Analysis of the *rpa1-27* mutant using fluorescence confocal microscopy reveals grossly aberrant F-actin organization in the epidermal hair cells (trichomes). Two other mutant alleles of the *rpa1* gene, *rpa1-1* and *rpa1-3*, also show trichome shape defects, which suggests a similar disruption of cellular actin organization. Here we analyze trichome actin organization in these alleles, and compare it to the defects observed in the original *rpa1-27* allele. Our findings will extend our knowledge of ADF regulation in plants and its relationship to cell shape.

86. Gene therapy with self-complementary recombinant adeno-associated virus in a T17M autosomal dominant retinitis pigmentosa mouse model

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Self-complementary AAV (scAAV) holds great potential for increased transduction efficiency, gene onset and number of genome copies per cell. These attributes make it ideal for treatment of rapidly degenerating forms of autosomal dominant retinitis pigmentosa (ADRP). Here we report the use of a scAAV serotype 5 to express a hardened mouse rhodopsin and siRNA301 under the control of the mouse opsin promoter. The purpose of this construct is to globally knockdown endogenous rhodopsin, mutant and wild-type, while replacing it with a hardened wild-type rhodopsin. T17M mice will be used to serve as a model of rapidly progressing ADRP. Viral injections will be given in the right eye in two groups at p5 or p15 with the left eye serving as control. Photoreceptor preservation will be assessed monthly through electroretinography (ERG) and optical coherence tomography (OCT). One month post injection, mice will be sacrificed and western blot as well as immunohistochemical changes in rhodopsin expression.

87. Immune modulation in hemophilia A mice using anti-CD20 and liver gene transfer

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We used an anti-murine CD20 IgG2a antibody (provided by Biogen Idec) to deplete B cells in two strains of hemophilia A mice and investigated the potential of this treatment to induce tolerance to FVIII when combined with gene therapy. Mice were given either anti-CD20 before and after 1e11vg/ms of AAV8 carrying FVIII transgene under a liver specific promoter or vector alone. BL/6-129/sv had modest and transient correction of clotting times representing <1.25% FVIII activity. Following i.v. challenge with FVIII, mice in the AAV8-only group had a mean anti-FVIII IgG1 titer of 7001ng/ml (±867) and 336 BU (±88). AAV8+anti-CD20 mice had lower anti-FVIII IgG1 titers of 1609ng/mL (±868) and Bethesda titers of 22 BU (±11). Hemophilic BALB/c mice showed better efficacy of gene therapy (1-2% FVIII activity) in both treatment groups. Only 1 in AAV8-only mouse developed an antibody response after challenge. Seven weeks later, mice were challenged again and tested for the ability to have their clotting times corrected with FVIII protein. All tolerant mice showed correction of clotting similar to that in naïve mice receiving FVIII for the first time. The antibody response of mice receiving only anti-CD20 did not differ in any respect from untreated HA mice, indicating immune competence. Cytokine RNA analysis of splenocytes stimulated with FVIII showed a lack of typical immune response and no indication of Treg markers. Thus, B cell depletion may prove beneficial in inducing tolerance in gene therapy for hemophilia A.

88. KSHV reactivation alters both nucleosome distribution and large scale chromosomal accessibility

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Kaposi sarcoma-associated herpesvirus (KSHV), a gammaherpesvirus, is one of seven known oncogenic viruses and is implicated as the ethologic agent in Kaposi sarcoma, primary effusion lymphoma, and Castleman's disease. Recent work has focused on the role of chromatin structure in regulating the viral genome. The relationship between viral-cancer signals and chromatin regulatory architectures of the host genome represents a gap in our understanding of the mechanisms driving KSHV-associated cancer. Here we show the chromatin structural changes induced in the host genome upon activation. We measured changes in chromatin structure and gene expression in a human cell line model of KSHV, iSLK.219 and measured nucleosome distribution at the transcription start sites (TSS) of 909 immunity- and cancer-related genes using custom-designed microarrays at 12 bp resolution. Reactivation of KSHV changed nucleosome distribution at the TSSs of many human genes surveyed. We also measured chromosomal accessibility using whole genome microarrays with 12.5 kb resolution. KSHV causes domain-sized alterations in chromosomal accessibility throughout the human genome. Using these measures, we identified a chromatin structural "fingerprint" for KSHV reactivation in iSLK.219. These descriptions of chromatin structure will provide a new framework for understanding the etiology of KSHV-induced cancer and a platform for future studies involving the regulation of chromatin in viral-associated cancers.

89. Characterization of a new *Perkinsus* species in *Tridacna crocea*

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A new pathogen recently discovered after importation into the USA in photosynthetic ornamental clams, *Tridacna crocea*, was investigated. The organism readily enlarged in alternative Ray's thioglycollate media (ARFTM), a diagnostic media specific for *Perkinsus* species, but did not propagate under standard culture conditions for known members of the *Perkinsoa* genus. Furthermore, a small percentage of *P. olseni* also found in the host tissues as a dual infection rapidly overgrew the unpurified cultures. Light microscopy and ultrastructural (TEM) examinations revealed tissue stages and morphology consistent with *Perkinsus* species trophozoites. Extensive phylogenetic and population genetic analyses using nuclear DNA sequences for the 5.8S ribosomal RNA (rRNA) gene and its surrounding internal transcribed spacers were performed. These analyses, which are based on 357 rRNA sequences, confirm that our new form is strongly diverged from other known members of the genus and belongs to a separate distinct evolutionary lineage. In turn, our population genetic (coalescent) analysis documents that limited gene flow is occurring between our new form and its closest known relative (*P. olseni*). On the basis of our behavior-in-culture experiments, morphology, and evolutionary inferences, we conclude that our new *Perkinsus* constitutes a previously unknown species of the genus. As such, we are in the process of providing a formal description and name for our new species.

90. Interaction of FevR with the MglA/SspA complex and identification of FevR DNA binding region in *Francisella tularensis*

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Francisella tularensis is an intercellular pathogen capable of proliferating in a wide range of cells. MglA and SspA are proteins that interact with the RNA polymerase to regulate the expression of the *Francisella* Pathogenicity Island (FPI) genes, which are required for intramacrophage survival. In *F. tularensis* FevR, a hypothetical DNA binding protein, also interacts with MglA and SspA to regulate the FPI genes. FevR contains cysteine residues that may play a role in sensing oxidative stress within the host environment thus, affecting the interaction with the MglA-SspA complex. To test the importance of the cysteine residues in FevR, site-directed mutagenesis was used to change all four cysteines to alanine (C19, C28, C83 and C97). The effects of these mutations were assayed using a bacterial two-hybrid and bridge-hybrid systems in different genetic backgrounds. In these systems, the proteins of interest are fused to either a DNA binding protein (Zif), the omega subunit of the *E. coli* RNAP, or present in an inducible plasmid. Transcription will only occur if the proteins interact, which can be measured by beta-galactosidase assays. In the two-hybrid system MglA is fused to omega and FevR is fused to Zif while in the bridge-hybrid system an additional plasmid containing SspA is included. The results obtained show that the mutations in FevR did not affect its interaction with MglA in a wild-type reporter background. However, the interaction between mutated FevR and MglA were affected in a reporter strain deficient in the production of polyphosphate. Interestingly, in the bridge two-hybrid, the presence of SspA decreased the interaction between MglA and FevR. FevR shares homology with SoxR, a member of the MerR family, which senses oxidative stress in the environment. We are currently characterizing the binding of FevR or its mutated derivatives to the *pdpD* promoter region within the FPI under oxidative stress conditions.

91. Exploring stochasticity in competence circuit of *Streptococcus mutans* using microfluidics

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Streptococcus mutans has the ability to take up exogenous DNA, a phenomenon called 'genetic competence'. Competence in *S. mutans* is regulated by complex biological circuits that receive inputs from multiple quorum-sensing signals, including secreted peptides designated as CSP and XIP. These complex circuits effect differential expression of the competence genes among cells in identical environments. Microfluidics has proven an ideal tool for studying such stochasticity at a single cell level. We developed a microfluidic mixer to monitor individual *S. mutans* in a defined chemical environment. A strain harboring a fusion of the gene for green fluorescent protein (GFP) to the promoter of comX, encoding an alternative sigma factor that activates other competence genes, was used to study transcriptional activation of comX. In the microfluidic device, expression of comX could be induced by XIP alone, but not by CSP alone. At low XIP concentrations, we observed highly stochastic expression of comX, suggesting a possible bistable mechanism in the XIP circuit. Our microfluidic device is also capable of rapidly switching between different growth media, allowing us to study the kinetics of induction and repression of the competence state in response to changes in the concentration of signal peptides and environmental signals. We explore possible bistability in the competence regulon of *S. mutans* as a source of stochasticity in single cell behavior.

92. A screen designed to target MKS-like genes yields a novel allele of MKS-5, possibly the core MKS protein

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Cilia are developmentally essential organelles projecting from most mammalian cells. Altered cilia function is the underlying cause of autosomal recessive disorders including nephronophthisis (NPHP) and Meckel-Gruber syndrome (MKS). The primary NPHP symptoms include renal interstitial infiltration, basement membrane disruption, and tubular atrophy. MKS additionally features CNS malformations, occipital encephalocele, post-axial polydactyly, heart malformations, and hepatic developmental defects. Several genetic loci have been linked to these diseases; however, the causative mutations have not yet been identified in the majority of patients. Conservation of these genes in *C. elegans* allows its utilization for the study of these disorders. Single mutations in nphp or mks genes in *C. elegans* minimally affect ciliogenesis; however, any combination of nphp with mks gene mutations alter cilia formation. From a forward EMS mutagenesis screen at least nine novel loci were identified and a new mks-5 mutation (yhw91) was uncovered. Hierarchy analysis was subsequently performed to determine that all other known MKS complex proteins were dependent on MKS-5. Interestingly, MKS-5

localization remained unaffected by nphp mutations. This insinuates that MKS-5 may be the core anchoring protein in the MKS portion of the basal body complex. Ultimately, MKS families will be screened for mutations in the human homologs of genes identified from this screen.

93. Zinc finger protein CTCF in regulation of VEGF expression and angiogenesis

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Angiogenesis is meticulously controlled by a fine balance between positive and negative regulatory activities. VEGF is a predominant angiogenic factor and its dosage is precisely regulated during normal vascular formation. In cancer, VEGF is commonly overproduced, resulting in abnormal neovascularization. VEGF is induced in response to various stimuli including hypoxia, however, very little is known about the mechanisms that confine its induction to ensure proper angiogenesis. Chromatin insulation is a key transcription mechanism that prevents promiscuous gene activation by interfering with the action of enhancers. Here we show that the chromatin insulator-binding factor CTCF binds to the proximal promoter of VEGF. Consistent with the enhancer-blocking mode of chromatin insulators, CTCF has little effect on basal expression of VEGF but specifically affects its activation by enhancers. CTCF-knockdown cells are sensitized for induction of VEGF and exhibit elevated pro-angiogenic potential. Cancer-derived CTCF missense mutants are mostly defective in blocking enhancers at the VEGF locus. Moreover, during mouse retinal development, depletion of CTCF causes excess angiogenesis. Therefore, CTCF-mediated chromatin insulation acts as a crucial safeguard against hyperactivation of angiogenesis.

94. Spliced transcripts analysis using RNA-Seq data

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Next generation transcriptome sequencing (RNA-Seq) is able to produce tremendous short read sequences. These sequence reads enable us to identify novel transcripts and alternative splice variants. The identification of splicing events has been a crucial step in RNA-Seq data analysis. However, previous work primarily focuses on sequence alignments based on "seed-and-extend" methods. Furthermore, the canonical signal "GT-AG" is used in many splicing junction prediction algorithms. These restrictions will cause failures in identifying splice events with non-canonical signals and splicing exons enriched with sequence polymorphisms. In this work, we have developed a splice junction prediction algorithm PASTA (patterned alignments for spliced transcripts analysis), using reference-sequence guided alignments modeled by a logistic regression. PASTA allows us to detect splice junctions without a requirement for canonical splice signal. In addition, several biological *cis*-regulatory elements are included in PASTA to make the predictions more biology oriented. A probability score is also provided for each splicing event prediction to allow a description of the prediction precision. The application of PASTA will enable more in-depth investigations of genome-wide alternative splicing events through the deep sequencing method.

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

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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. Collagen2alpha1 is the major structural protein in the cartilage ECM, and the Collagen2alpha1 gene is regulated

by members of SoxE and SoxD families of transcription factors. Signaling proteins such as Sonic hedgehog act upstream of Sox genes, and each of these components is required for cartilage formation. Although vertebrate chondrogenesis has been studied extensively, little is known about how this gene network was assembled during evolution. 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*. We cloned the invertebrate orthologs of Collagen2alpha1, SoxE, SoxD and Hedgehog and examined their expression during embryonic development. Clade A collagen and Sox genes are expressed in prechondrogenic cells of both species, and the expression of Hedgehog in adjacent tissues suggests a possible regulatory role. The findings suggest that the independent evolution of cartilage in these taxa was facilitated by a deeply conserved genetic program for chondrogenesis, which evolved in the common ancestor of Bilateria.

96. Research Computing Center overview

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The University of Florida Research Computing Center is a faculty-directed facility with the single 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 Research Computing Center.

97. Utilizing bibliographic databases for social network analysis: co-authorship networks

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Social network analysis has emerged as an important method for understanding research efforts and can play a role in helping to shape an institution's strategic direction and evaluating research programs. This project aims to utilize traditional bibliographic resources in developing value-added services such as social network analysis for an institution, department, or specialized research center. Forty faculty from the University of Florida's Genetics Institute were selected for social network analysis, based on their academic standing (20 assistant and associate professors and 20 full professors). Bibliographic data from the literature search (Scopus and Web of Science) was downloaded and used to create a network to compare collaborations, evidenced by co-authorship on papers. Analysis was carried out using the Network Workbench Tool (NWB) from the Cyberinfrastructure for Network Science Center, a freely available social network analysis platform which is downloadable from the web. Co-authorship networks can be an insightful approach to better understand collaboration efforts by academic authors. NWB software provides a visualization of the co-authorship patterns as well as quantitative metrics to describe the network. Because the data used in such analyses is widely available through library subscriptions and because library staff have expertise in navigating these bibliographic databases, this type of analysis is an ideal service to offer through the library.

98. The modification N6-threonylcarbamoyladenosine is a positive determinant for the PrrC ribotoxin

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The ribotoxin PrrC is a nuclease used by bacteria to prevent phage infections. PrrC cleaves the anticodon of $tRNA^{Lys}$, 5' of the wobble uridine at position 34, leading to translation inhibition and cell death. As PrrC cleaves native $tRNA^{Lys}$ *in vitro* but does not cleave transcript, it was proposed that a post-transcriptional modification of $tRNA^{Lys}$ was a required for PrrC activity. Two complex post-transcriptional modifications are found in the anticodon stem loop (ASL) of $tRNA^{Lys}$ in both *Escherichia coli* and *Saccharomyces cerevisiae*. First, the U at position 34 is modified to 5-methylaminomethyl-2-thiouridine (mnm^5s^2U) in *E. coli* and to 5-methoxycarbonylmethyluridine (mcm^5U) in *S. cerevisiae*. Second, the A at position 37 is modified to N6-threonylcarbamoyladenosine (t^6A_{37}) in both organisms. It was recently shown that PrrC expression in *S. cerevisiae tot3*Δ(*elp3*Δ) and *trm9*Δ (eliminating the mcm^5U_{34} modification) was still toxic. We investigated if the other modification of the $tRNA^{Lys}$ ASL, t^6A_{37}, was required for PrrC activity in yeast. Our laboratory recently discovered several genes involved in the synthesis of t^6A_{37}, and we have shown that *kae1*Δ yeast mutants are viable and devoid of t^6A_{37}. We found that PrrC was not toxic in a *S. cerevisiae kae1*Δ strain suggesting that the highly conserved t^6A_{37} modification is the determinant of the PrrC ribotoxin. We are currently testing other mutants in the t^6A_{37} biosynthetic pathway, to establish a relationship.

99. Association of *FTO* with hydrochlorothiazide (HCTZ)-induced elevation in uric acid (UA) in African American (AA) hypertensives in the Pharmacogenomic Evaluation of Antihypertensive Response (PEAR) study

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BACKGROUND: HCTZ and atenolol cause adverse metabolic effects, including increased UA, but the exact mechanisms are unknown. We undertook pharmacogenomic studies to shed light on potential genetic influences on UA levels.
METHODS: PEAR studied 768 hypertensive patients initially treated with atenolol or HCTZ for 9 weeks, then with the combination for 9 weeks. UA was measured on a Hitachi 911 Chemistry Analyzer (Roche). Genotyping was conducted with 50K HumanCVD BeadChips (Illumina). Data were analyzed using linear regression in PLINK (Broad Institute), controlling for age, sex, baseline UA, treatment group and waist circumference.
RESULTS Rs4784333, an intronic SNP in *FTO*, was associated with changes in UA in AAs taking HCTZ monotherapy ($p = 0.00016$) and replicated in the HCTZ add-on group ($p = 0.028$), for a combined $p = 7.29 \times 10^{-6}$. The G allele was associated with a greater change in UA. GG patients had ~1 mg/dL greater increase than CC patients, a clinically important difference. The SNP was not associated with baseline UA.
CONCLUSIONS These data suggest the *FTO* gene may play an important role in regulating UA rise in response to HCTZ therapy in AAs. *FTO* polymorphisms have previously been linked to increased risk of obesity and type 2 diabetes, both of which are associated with UA. Additional replication and functional studies are needed to further understand the role of *FTO* in regulating the rise in UA following HCTZ exposure, including potential clinical application.

100. Maize kauralexins: inducible diterpenoid phytoalexins protect against fungal pathogens

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Phytoalexins constitute a broad category of pathogen and insect-inducible biochemicals that locally protect plant tissues. In rice, a complex array of inducible diterpenoid phytoalexins constitute an important component of the plants anti-pathogen defenses. In contrast, despite the demonstration of fungal-induced ent-kaur-15-ene production in maize over 30 years ago, the identity of functionally analogous maize diterpenoid phytoalexins has remained elusive. In response to stalk damage by the European corn borer (*Ostrinia nubilalis*) and fungi, we observed the induced accumulation of six ent-kaurane-related diterpenoids, collectively termed kauralexins. Isolation and identification of the predominant induced metabolites revealed ent-kaur-19-al-17-oic acid and the novel analog ent-kaur-15-en-19-al-17-oic acid, assigned as kauralexin A3 and B3, respectively. Fungal-induced An2 transcript accumulation precedes highly localized kauralexin production which can eventually exceed 100 g g-1 FW. In maize, An2 gene encodes an ent-copalyl diphosphate synthase and thus exists as a logical candidate protein involved in the production of diterpenoid phytoalexin precursors. Consistent with other defenses, the combined application of jasmonic acid and ethylene synergize the induced accumulation of these phytoalexins. Kauralexins appear ubiquitous in maize and occur at high levels in the scutella of all inbred lines examined following seedling germination. At concentrations as low as 10 g ml-1, kauralexin B3 significantly inhibits the growth of the opportunistic necrotroph *R. microsporus* and the causal agent of anthracnose stalk rot, *Colletotrichum graminicola*. Kauralexins also exhibit significant *O. nubilalis* antifeedant activity. Our work establishes the presence of highly-inducible diterpenoid phytoalexin defense responses in maize and enables a more detailed analysis of the associated biosynthetic pathways, regulation and defense functions.

101. Chromatin accessibility is largely unaltered by the transition to mitotic chromosomes

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Eukaryotic chromosomes undergo extensive condensation into mitotic chromosomes that facilitates the segregation of sister chromatids during cell division. The dynamics of the genome-wide histone modifications and chromatin

structure during mitosis is poorly understood in the context of genomic space. To understand how large-scale chromatin structure is affected by the formation of mitotic chromosomes, we have developed a microarray-based nuclease sensitivity assay to characterize the genome-wide chromatin accessibility of asynchronously-growing and mitotically-arrested cells. Surprisingly, we find that the large-scale chromatin accessibility of mitotically-arrested cells differs little from asynchronously-growing cells, suggesting that the condensation of chromatin into mitotic chromosomes does not accompany a dramatic alteration of accessibility to small soluble molecules. These results contrast with the finding that global levels of histone acetylation is reduced during mitosis. Thus, a loss of histone acetylation may facilitate the packaging of chromatin into mitotic chromosomes, but this does not affect the large-scale accessibility of mitotic chromosomes to small soluble molecules. This work highlights the utility of genome-wide, microarray-based nuclease sensitivity assays while revealing that large-scale chromatin accessibility appears unchanged by the packaging of chromatin into mitotic chromosomes.

102. Mutation in MYOF: a new cardiomyopathy gene?

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Mutations in a growing list of genes cause autosomal dominant cardiomyopathy. A family with dilated cardiomyopathy (DCM) was investigated for mutations in known and novel candidate genes. The myoferlin gene (MYOF) was included although no mutations had been previously reported. Myoferlin is a large type II membrane protein associated with plasma and nuclear membranes. It binds calcium and phospholipids, and is highly expressed in heart and skeletal muscle. DNA sequencing of the exons of the proband from this family identified a single-base deletion in the open reading frame, on one allele. This is predicted to cause a frameshift at codon 405 (normally Gly), resulting in a premature translation stop 7 codons into the aberrant frame. This mutation was also found in the proband's mother, who is currently asymptomatic for signs of cardiomyopathy. The mutation was also found in a brother with DCM but not an unaffected sib. While there are several isoforms of MYOF, including some as short as 437 amino acids, this specific DNA change has not previously been reported. Its identification in a family with DCM implicates it in the etiology of this condition, although the mother would be considered currently non-penetrant. This is the first report of a MYOF mutation in cardiomyopathy, but further characterization is needed.

103. The *Amborella* Genome Project: generating a reference sequence for angiosperm evolutionary analysis

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Amborella trichopoda, as the sister to all other extant angiosperms, occupies a crucial evolutionary position, and its genome sequence is an important reference for comparative genomic studies across the angiosperms. A complete genome sequence will help in understanding the evolution of key angiosperm traits and provide a baseline to examine genome organization throughout angiosperms. We are using a whole-genome shotgun strategy to sequence the ~790-980 Mbp *Amborella* genome using Roche's Genome Sequencer FLX system. To date, 28.5 plates of unpaired GS FLX, five plates of paired GS FLX, and 11 plates of unpaired GS XLR sequencing have been analyzed, providing ~18x coverage. We performed additional scaffolding using Bambus to incorporate BAC-end sequences, resulting in 10,967 scaffolds with average scaffold size of 66Kbp and N50 size of 3.56Mbp covering 78% of the genome. We are currently incorporating four lanes of short-insert Illumina HiSeq to close gaps in the assembly. Annotation of the assembled contigs is underway using DAWGPAWS and TWINSCAN. We are developing a GBrowse-based website to share these results with the community.

104. Green light attenuates red-light-induced hypocotyl growth inhibition during early photomorphogenesis of *Arabidopsis* seedlings

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The transition from dark to light growth is a critical time for a developing seedling. The young plant adapts to the new light environment by balancing rapid hypocotyl elongation used to reach the solar irradiation with hypocotyl growth inhibition, cotyledon opening and greening for photosynthesis. Red, far-red, and blue light have a strong inhibition effect on the hypocotyl elongation. However, green light attenuates their effects by stimulating hypocotyl elongation. Here we report that the red-light-induced hypocotyl growth inhibition is attenuated by addition of low fluence-rate green light. The attenuation effect is obvious within the time frame of 2-3 hours after the co-irradiation of red and green light in our high resolution hypocotyl growth kinetic analysis. Genetic examination of this attenuation effect by testing photoreceptor mutants of *phyA*, *phyB*, *cry1cry2*, *phot1*, and *phot2* suggests that this effect requires the presence of phot1, but not other photoreceptors tested. The current results detail an important environmental signal input contributed by green light in the adjustment of early photomorphogenic events, as well as the unanticipated role of phot1 in the green light attenuation effect to red light.

105. Characterization of anoctamin Ca^{2+}-activated Cl^- channels in *C. elegans*

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Ca^{2+}-activated Cl^- channels (CaCCs) contribute to transmitter release from photoreceptors, excitability in neuron and myocyte membranes, sensory perception, and epithelial transport. Despite their importance, the molecular identity of CaCCs remained unknown until the anoctamin family was recently identified as CaCCs. There are 10 anoctamin family members in mammals with overlapping expression and potentially redundant functions that can hinder genetic and physiological analysis; there are only two anoctamin family members in *C. elegans*, ANOH-1 and ANOH-2. Using GFP reporters, we found that ANOH-1 is expressed in the cilia of sensory neurons that detect odors and noxious chemicals in the environment. Silencing of ANOH-1 causes an osmotic avoidance defect and a small deletion mutation in ANOH-1 causes a decrease in olfactory chemotaxis suggesting that the putative channel plays a role in sensory perception or signal transduction. ANOH-2 is expressed in the spermatheca, mechanosensory neurons, and ventral nerve cord. We have not observed any reproducible phenotypes caused by ANOH-2 loss-of-function suggesting that its functions are subtle or that other proteins are functionally redundant. We are currently using genetic approaches to identify proteins that function with ANOH-1 and testing if expression of human anoctamins can rescue ANOH-1 phenotypes to determine if functions of the proteins are conserved.

106. Differential role of Nkx2-5 in activation of the ANF gene in developing vs. failing heart

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Atrial natriuretic factor (ANF) is abundantly expressed in atrial cardiomyocytes throughout ontogeny and in ventricular cardiomyocytes in the developing heart. However, during cardiac failure and hypertrophy, ANF expression can reappear in adult ventricular cardiomyocytes. Transcription factor Nkx2-5 is one of the major transactivators of the ANF gene in the developing heart. We identified Nkx2-5 binding at three 5' regulatory elements (-34, -31 and -21 kb) and the proximal ANF promoter by ChIP assay using neonatal mouse cardiomyocytes. 3C analysis revealed close proximity between the distal elements and the promoter region. A 5.8-kb fragment consisting of these elements transactivated a reporter gene *in vivo* recapitulating endogenous ANF expression, which was markedly reduced in Nkx2-5-ablated mice. However, expression of a reporter gene was increased and expanded toward the outer compact-layer in the absence of transcription repressor Hey2 similar to endogenous ANF expression. Functional Nkx2-5 and Hey2 binding sites separated by 59 bp were identified in the -34 kb element in neonatal cardiomyocytes. In adult hearts, this fragment did not respond to pressure-overload, and ANF was induced in the absence of Nkx2-5. These results demonstrate that Nkx2-5 and its responsive *cis*-regulatory DNA elements are essential for ANF expression selectively in the developing heart.

107. Investigation into the parasitic infection of Pacific spiny lumpsuckers (*Eumicrotremus orbis*)

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The Pacific spiny lumpsucker (*Eumicrotremus orbis*) is a common aquarium exhibit fish. Routine necropsy revealed necrosis and severe histiocytic inflammation of multiple organs including heart (epicardium and myocardium), kidney, liver, ovary, and gills associated with large numbers of intralesional organisms within the coelomic cavity and affected tissues. Although the morphology at the light microscopic level and upon initial ultrastructural examination was not specific, it was somewhat suggestive of a group of fish pathogens called Mesomycetozoa which include *Dermocystidium* spp., *Ichthyophonus* spp. and the "rosette agent" of salmonid fish. Based on published phylogenetic analyses, Mesomycetozoa-specific polymerase chain reaction (PCR) primers were designed for conserved regions within the aligned 18S small subunit rRNA gene sequences of several species in the class Mesomycetozoa including species from the orders Dermocystida and Ichthyophonida. PCR was performed on formalin-fixed, paraffin embedded samples from affected fish. *Rhinosporidium* sp. archived case material was used as a positive control. Sequence analysis of the PCR product representing a 497bp partial sequence of the 18S SSU rRNA gene has revealed that the closest matches are Myxosporea including Myxidium rather than Mesomycetozoa suggesting that additional investigation is necessary.

108. Oxidative stress responses are induced when *Caenorhabditis elegans* are exposed to hyperosmotic stress

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A cell's ability to detect and respond to environmental stressors is essential. Our current understanding of how animal cells perceive osmotic stress, as well as the molecular mechanisms used to respond to that stress, is limited. It is well known that animal cells absorb inorganic ions as an acute response to cell volume decreases due to water loss and chronically accumulate compatible organic osmolytes; however how cells initially detect osmotic stress and the molecular signaling pathways utilized are poorly defined. The nematode, *Caenorhabditis elegans*, which has been shown to be capable of distinguishing between hyperosmotic conditions and other stressors such as oxidants, high temperature, and xenobiotics, offers several genetic and molecular advantages. Additionally, free-living nematodes like *C. elegans* experience osmotic stress in their natural environment. We show here that skn-1, a Cap-n-Collar (CnC) transcription factor associated with activation of cytoprotective genes during oxidative stress, is also activated when worms are exposed to hyperosmotic stressors. These results provide the foundation for 1) discovering how animal cells perceive high osmolarity, 2) elucidating how different stress responses are coordinated within the cell, and 3) defining the role of CnCs in a context outside of oxidative stress.

109. Acceleration of *Agrobacterium*-mediated genetic transformation of sugarcane

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Sugarcane (*Saccharum* sp. hybrids) is a highly productive C4 grass which is used as the main source of sucrose and more recently for biofuel production. Transgenic sugarcane with improved agronomic and value-added traits has been reported mostly via biolistic gene transfer. Compared with biolistic gene delivery, *Agrobacterium*-mediated transformation (AMT) results in simple transgene integration patterns. In this study, we inoculated alternative explants including immature leaf whorl explants after one week preculture, callus derived from 4-6 weeks or 3 months preculture of immature leaf whorl explants with Agrobacterium strain AGL1 harboring a binary vector with the nptII expression cassette. Geneticin resistant callus was identified following 3 biweekly subcultures on geneticin containing medium and followed by plant regeneration. The NPTII expression was confirmed using NPTII ImmunoStrip®Test. So far 27 independent transgenic lines were confirmed by Southern blot analysis and showed simple transgene integration patterns of 1 to 5 copies. No escape was found following the 6 weeks selection period, suggesting that the selection protocol can potentially be shortened. A similar number of geneticin resistant calli or transgenic plants were obtained from callus inoculated after 4-6 weeks or 3 months preculture of immature leaf whorl explants. These results suggest that the time in tissue culture can be reduced by 6-8 weeks compared to the standard 3 months preculture protocol.

110. Overexpression of human alpha-1 antitrypsin (AAT) in PiZZ liver reduced the polymerization; facilitate secretion *in vitro* cell model and *in vivo* PiZ mice model

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The alpha-1 antitrypsin deficiency disease is the most prevalent inherited lung disease next to cystic fibrosis and is also the most common metabolic-genetic indication for pediatric liver transplantation. However, in heterozygote PiMZ patients, the liver damage is mild. To evaluate the effect of human wild (M) type AAT (MAAT) as a possible chaperone on the trafficking of secretion-incompetent PiZZ AAT protein (ZAAT), we established a PiZZ cell model from pediatric patient. Over-expression of human MAAT in this cell model prevents process of polymerization of ZAAT in the cytoplasm. The result was confirmed by AAT western blot, immunostaining and electromicroscope (EM). We further test this promising treatment method on PiZ transgenic mouse in which E+11 vg AAV8 expression MAAT was injected through portal vein. The accumulation of ZAAT as polymer in hepatocyte which is the characteristic finding of PiZ mice has decrease sharply by polymerization specific Ab staining and liver tissue lysate ZAAT ELISA. Furthermore, the secretion of ZAAT in the serum increases about 5 folds after 12 weeks of treatment. The secretion of ZAAT is dosage dependent on the MAAT secretion level. Secretion of one molecule of MAAT, bring out approximately one molecule of ZAAT. The purified MAAT can also successfully block the formation of polymer of ZAAT *in vitro* which explains the mechanism of this treatment. The average serum SGOT level which reflects liver function decreased about 21% after MAAT treatment.

111. Identification of tissue-specific regulatory effect of KSHV miR-K12-11

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Short, non-coding microRNAs (miRNA) are regulators of gene expression. Altered miRNA expression may be associated with cancer, but the mechanisms by which miRNAs contribute to pathogenesis are not well understood. Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent for KS and two lymphoproliferative diseases. KSHV encodes 17 microRNAs, including miR-K12-11, which is a homolog of a human oncogenic miRNA, miR-155. To decipher the mechanism by which miR-K12-11 contributes to the regulation of gene expression in KSHV infected tumor cells, we combined microarray experiments with information stored in public databases. MiR-155 and miR-K12-11 were over-expressed in lymphatic and endothelial cells. Analysis of the transcriptome changes revealed a subset of genes affected by both miR-155 and miR-K12-11 and distinct targets for each miRNA. Common targets in both types of cells and tissue-specific targets were also identified. Computationally predicted target genes were obtained by integrating records from multiple databases. Direct targets were separated from indirect targets. The regulatory pathways of the indirect targets were modeled using DNA-protein and protein-protein interactions. Validation of the direct targets is in progress. This systems biology approach provides a more complete understanding of the common and distinct regulatory pathways that are modulated by these two homologous miRNAs. Furthermore, identification of miR-K12-11 targets in lymphatic and endothelial cells points to its role in KSHV lymphomagenesis.

112. DEB: A web interface for RNA-Seq digital gene expression analysis

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High-throughput RNA sequencing (RNA-Seq) is rapidly emerging as an efficient way to measure transcriptome quantatively. It is expected to overtake microarray technologies in the near future. Digital expression (DE) is an important application of RNA-Seq technology to quantify the transcriptome. Processing RNA-Seq sequencing data results in mapping of the reads to each individual gene or transcripts. However, the number of mapped reads to each gene or transcript varies under different conditions and replicates. So far, no software can process the data automatically to identify significantly expressed genes or transcripts. Here, we present a web application (DEB) which uses three different statistical algorithms (edgeR, DESeq and bayseq) to process the count data. The results of the three approaches are also

compared. DEB is freely accessible at http://www.ijbcb.org/DEB/php/onlinetool.php.

113. ICBR bioinformatics services: recent advances in the analysis of NGS data

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The emergence of high-throughput next-generation sequencing (NGS) technologies, e.g. SOLiD, illumina, 454, PacBio and Ion Torrent, have substantial impact throughout a broad range of biological applications. While the production of raw DNA sequence data is becoming routine, the most challenging task for researchers is finding ways to manage and analyze the considerable data output from NGS platforms. ICBR offers bioinformatics and biostatistics consulting and data analysis services to help campus researchers toward an in-depth understanding of their NGS data. ICBR staff scientists collaborate with researchers in designing experiments and analyzing complex data sets by applying various data-analytical and theoretical methods, mathematical modeling and computational simulation techniques. We developed multiple comprehensive pipelines that enable researchers to process large-scale data. Here, we present representative examples of ICBR assisted analysis including: metagenomics (characterization of microbial communities found in harsh Arctic climates, termite guts and premature infants); transcriptome and genome annotation (project based EST/genomic sequence assembly, annotation, gene prediction, and genome finishing of prokaryotes/eukaryotes); microarray-based data analyses (statistical and functional data analysis from major microarray platforms of various species); and other NGS data analysis that appeared in peer-reviewed journals.

114. Monitoring DNA accessibility and epigenetic status in cells and tissues with a fluorescent reporter

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Epigenetic regulations, by limiting DNA accessibility and gene expression, play an important role in biological processes such as stem cell maintenance and cellular differentiation. Deregulation of epigenetic status has been implicated in many diseases such as cancer and cardiovascular diseases. In contrast to static genetic changes, epigenetic regulations are dynamic, responsive to environmental and dietary factors, and under many circumstances, reversible. The dynamic nature of epigenetic regulation demands innovative techniques that allow continuous monitoring of epigenetic status in live animals. However, most biochemical methodologies for measuring epigenetic modification and DNA accessibility rely on homogenizing large amount of cells, which is inapplicable for monitoring dynamic epigenetic changes in live animals. In this study, we explored the application of using a fluorescent reporter in monitoring and quantitative assessment of epigenetic status *in vivo.* Using the "Ends-out" homologous recombination, we knocked an ubiquitin-DsRed reporter into IRER, a previously identified, epigenetically regulated enhancer region in *Drosophila.* DNase I sensitivity assay and ChIP analysis indicated that the expression of the ubi-DsRed reporter accurately reflected the DNA accessibility and histone modification of IRER. This reporter allowed us to monitor epigenetic changes of this locus in specific tissues/cells during development.

115. Sonic hedgehog expression in the limb bud mesoderm and its potential role in the ectoderm

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The early expression of sonic hedgehog (SHH) in the posterior mesenchyme of the developing limb bud functions in determining digit identity. Thus SHH production in each limb bud must be tightly regulated. The underlying molecular mechanisms responsible for SHH expression regulation remain unclear. A model suggests that hedgehog signaling in the limb bud ectoderm plays a role in specifying the initial SHH level through a "fast" epithelial-mesenchymal feedback loop involving SHH and fibroblast growth factor (FGF) (Harfe, 2011, *Dev Dyn* 240(5):915-9). To test this model, we quantified SHH expression in individual mouse limb buds in consecutive embryonic stages using western blot. The results indicate that SHH levels vary significantly between left and right in both fore limbs and hind limbs when expression first occurs and reach equality as development proceeds. Furthermore, constitutive activation of the hedgehog signaling pathway in the apical ectodermal ridge (AER) rescued the limb defects caused by inactivating of Fgf8 in early limb ectoderm. Our data suggest the hedgehog signaling pathway in the AER is able to compensate for variations by early SHH expression.

116. Gene expression profiling services at ICBR

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The ICBR Gene Expression Core (GE) is a full service laboratory employing microarrays for high throughput gene expression profiling starting with total RNA. Services include:

- gene expression analysis for prokaryotic and eukaryotic species using catalog and custom designed Agilent arrays;
- Affymetrix 3' expression arrays, GeneST array, exon arrays, GeneChip tiling arrays and miRNA array.

Our recently acquired Tecan HS 400™ Pro hybridization station and GenePix 4400A, four-laser microarray scanner, support hybridization and scanning of protein, tissue, quantum dots, carbohydrate and chemical compound arrays, along with other types of DNA and RNA arrays. GE also offers services for the construction of RNA-Seq libraries (nextgen sequencing platforms), DNA capture and Agilent SureSelect RNA capture services for targeted sequencing. Potential applications of sequence capture include:

- gene re-sequencing of specific genes of interest;
- discovering novel genomic sequence variants or somatic mutations in tumors;
- precise measurement of expression levels of specific genes of interest and rare or low abundance transcripts;
- detection of allele-specific expression, differential splicing events and gene fusions.

The Genetic Analysis and Genotyping core (GA/GT) provides high throughput gene expression analyses from catalog or custom arrays using the Illumina BeadArray Reader. For additional information, contact Yanping Zhang (yanp@ufl.edu) (GE) or Ginger Clark (gclark@ufl.edu).

117. Prenatal inactivation of androgen receptor activity feminizes external genitalia but masculinizes brain development

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Sexual differentiation of the external genitalia and brain has been proposed to occur around the same stage of embryonic development in mice, leading to dimorphic development of the penis and clitoris, and of specific brain nuclei, neural circuits and behaviors. Testosterone masculinizes the genitalia by activating androgen receptors (AR). In the brain, testosterone is aromatized to estrogen, which activates estrogen receptors (ER) to direct masculinization. Little is known about the role of AR in sexual differentiation of the brain. We inactivated AR during a narrow window of mouse embryonic development to determine its function during sexual differentiation of the external genitalia and brain. Inactivation of AR prior to the onset of sexual differentiation results in feminization of the external genitalia, causing micropenis and hypospadias, however the preoptic area (POA) of the brain was masculinized. To determine the long-term consequences of transient disruption of AR activity at embryonic stages, treated mice were raised to adulthood and their sexual behavior was investigated. Although treated males had feminized external genitalia, they exhibited more masculinized sexual behavior compared to controls. We find that AR acts on a common set of genetic targets in these two organ systems, but regulates them in different manners. The results suggest a mechanism by which the sex of the genitalia and the sex of the brain can differ in the same individual.

118. Functional characterization of a serine/threonine protein kinase in *Brassica napus* guard cells

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Protein kinases and phosphorylation events have been recognized as the central and pivotal regulatory mechanism in the 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 from a proteomic analysis of *Brassica napus* guard cells. The full-length cDNA encoding the protein (BnSnRK2b) was cloned and heterologously expressed in *E. coli*. The purified BnSnRK2b exhibited kinase activity in a manganese-dependent manner and preferentially phosphorylates myelin basic protein and beta-casein *in vitro*. The *in vitro* activity was sensitive to treatment of oxidants, indicating its responsiveness to oxidative stress conditions. Quantitative RT-PCR results showed the expression of BnSnRK2b in roots and guard cells, suggesting a role of this kinase in external signal transduction. Investigations are under way to characterize the function of BnSnRK2b in stomatal regulation, including the upstream kinases and downstream targets in guard cells.

119. Involvement of specific 14-3-3 isoforms in response to drought in *Arabidopsis thaliana*

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This study focuses on the possible involvement of isoforms of the 14-3-3 family of proteins in the drought response of *Arabidopsis thaliana*. The plant drought response is typically directed by signaling pathways that are either dependent or independent of the hormone abscisic acid (ABA). Both the ABA-dependent and the ABA-independent pathways show potential for 14-3-3 participation in drought signaling: 14-3-3s are known to bind drought related transcription factors, the kinases involved in drought and ABA responses are known to phosphorylate 14-3-3s, and the overexpression of a 14-3-3 isoform can enhance drought tolerance. By observing differences in drought-gene expression in 14-3-3 isoform knockout mutants, we can deduce whether specific 14-3-3 isoforms play a role in the regulation of these drought induced genes. We examined the induction profiles of five drought-induced genes: RAB18, RD20, ERD1, Cor15b, and DREB2A. The induction of RAB18 and RD20 are characterized as ABA-dependent, while ERD1, Cor15b, and DREB2A, can be induced independent of ABA signaling. Our data show that the plant lines carrying different 14-3-3 isoform knockouts differ in their ability to tolerate drought. Further, transcription analyses indicate that the loss of specific isoforms can influence differential expression of target genes from both the ABA-dependent and ABA-independent drought response pathways, suggesting 14-3-3s may play multiple roles in the drought response.

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