Biochemistry and Molecular Biology Graduate Student Association

4th Annual Graduate Research in the Biological Sciences Symposium

Twenty-seventh and twenty-eighth of September 2007

Sponsored by: DASNR, VET MED, GPSGA, BMBGSA and VP for Research & Technology Transfer
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*All Presenters Must Be At Your Poster For Judges Questions. The posters will be up the entire duration of the symposium, and the judges will evaluate them at any time during the symposium.*
4th Annual Graduate Research in the Biological Sciences Symposium

Oral Presentations Abstracts, September 27th, 2007
Alveolar type I cells mediate ATP release via P2X7R activation: new insights in surfactant regulation

Mishra Amarjit *, Narendranath Reddy Chitangari and Lin Liu

Alveolar epithelium is composed of alveolar epithelial type I (AEC I) and type II (AEC II) cells. Lung surfactant is secreted from AEC II. We have previously shown that P2X7 receptors (P2X7R) are specifically expressed in AEC I. We hypothesize that the activation of P2X7R in AEC I cells releases ATP and stimulates surfactant secretion from AEC II cells via a paracrine manner. To test this hypothesis we first developed a heterologous system of AEC I and AEC II culture. We isolated primary rat alveolar epithelial cells by releasing them from lung tissues using elastase digestion (8 U/ml), followed by macrophages and leukocyte removal by rat IgG ‘panning’ and immunomagnetic sorting. The yield per rat was ~60 x 10^6 with more than 97% viability. Immunophenotyping with polyclonal anti-P2X7R and monoclonal anti-LB-180 antibody showed 32% and 64% positive AEC I and AEC II, respectively with minor contaminating cells. Cytometric analysis of FITC-P2X7R labeled freshly isolated alveolar heterocellular cells also revealed 34% of P2X7R positive AEC I. To examine effects of P2X7R on surfactant secretion, we incubated overnight cultured AEC I and AEC II cells with 3'-O-(4-benzoyl) benzoyl adenosine 5'-triphosphate (BzATP), which is known to be a potent P2X7R agonist. Surfactant lipid secretion was measured using the cells metabolically labeled with [3H] choline. BzATP significantly increased surfactant lipid secretion in the mixed cells in a dose dependent manner. The increase secretion was significantly inhibited by P2X7R antagonist, Brilliant Blue G (BBG). However, BzATP had little effect on surfactant secretion in purified AEC II cells alone. To elucidate the mechanism of P2X7R-mediated surfactant secretion, we scavenged the ATP with apyrase and ADA, which significantly decreased the BzATP evoked surfactant secretion. BzATP-mediated secretion was also reduced in the presence of suramin and staurosporine, indicating the possible involvement of P2Y2R and downstream PKC-mediated signaling pathway. Our results suggest that AEC I communicate in a paracrine fashion with AEC II cells in modulating surfactant secretion. The in vitro cell system described here is close to in vivo conditions and would be a valuable tool for investigating AEC I and AEC II cell communications.
Expression, Purification, Crystallization and Structural Analysis of Aes from Escherichia coli
Mamiko Nishida and Stacy D. Benson

Aes is an Escherichia coli soluble protein consisting of 319 amino acids (36kDa). It acts as an acetyl esterase and belongs to the family of hormone sensitive lipases (HSL). The regulatory domain of the HSL family is the GXSXG motif with the active site Ser residue. Gly163, Asp164, Ser165, and Gly167 are the components of the GXSXG motif in Aes and Ser165, Asp262, and His292 function as the catalytic triad with Asp164 being a critical residue in enzymatic activity. In addition to the acetyl esterase activity, Aes is the down-regulator of MalT, which is the central transcriptional activator of the maltose regulon in E. coli. It has been shown that Aes down regulates MalT through protein-protein interaction directly. MalT consists of four domains (DT1-DT4) and is regulated by several signals, including being activated by ATP and maltotriose. Maltotriose binds to DT3 while ATP and Aes bind to DT1. It has been found that Aes competes with maltotriose in binding MalT. Besides Aes, there are two additional proteins that down regulate MalT: MalK and MalY whose structures have been solved. The crystal structures of three bacterial proteins that are members of the HSL family have also been determined by X-ray analysis. Recently, the interaction between Aes and alpha-galactosidase, which is involved in metabolism of raffinose, has been found. Therefore, Aes plays an important role in the regulation of carbohydrate metabolism. The aim of my research is to investigate the structures of Aes, alpha-galactosidase, MalT, and complexes between them. In this presentation, the expression, purification and crystallization of Aes with an N-terminal His-tag is discussed.

Vijay Muthukumar
Paper (Oral) Presentation
BMB Ag

Metagenomics for identification of novel plant viruses
Vijay Muthukumar, Ulrich Melcher, Marlee Pierce
The number of virus species identified by the ICTV is likely to be far less than the actual number of viruses existing globally. A metagenomic approach is being taken to discover novel viruses from plants. In this approach, nucleic acids from virus-like particle fractions of plant specimens from The Nature Conservancy’s Tallgrass Prairie Preserve in Osage Co., Oklahoma were amplified, cloned and sequenced. Aliquots of plant material were subjected to homogenization and differential centrifugation to isolate virus-like particles. The pellet obtained was treated successively with DNase-I to remove contaminating plant DNA and proteinase-K, sodium dodecyl sulfate to digest the DNase-I and capsid proteins. The released viral nucleic acid (DNA/RNA) was amplified by reverse transcription and PCR using degenerate primers. The sequences obtained from cloned PCR products were assembled and characterized by comparison with known viral sequences. To date, 269 plants were analyzed; of these 139 were PCR positive. Of the 21 sequence sets available to date, 40% suggested the presence of six viruses, all previously uncharacterized by sequence analysis. Two double infections were found. Polymorphisms in the sequence of a frequent tymovirus reveal aspects of its population structure. Plant specimens yielding virus sequences showed no obvious symptoms of infection. Evidence of bacteria and fungi was also found in several samples. The study thus is identifying viruses and microbes that may emerge as plant pathogens to crop plants. Supported by NSF-EPSCoR.

Samodha Fernando
Paper (Oral) Presentation
Animal Science  Oklahoma State University and University of Oklahoma

Characterization of Porcine Tissue Kallikreins: Towards Understanding the Biology of a complex family of genes

Samodha Fernando, Najar, F.Z.b, Guo, X.a, Zhou, L.b, Fu Y.b, Geisert, R.D.a, Roe, B.A.b, and DeSilva, U.a
a Department of Animal Science, Oklahoma State University, Stillwater, OK, 74078 USA b Advanced Center for Genome Technology, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73109, USA

Kallikreins belong to a family of serine proteases that are widespread throughout living organisms, expressed in diverse tissue specific pattern, and known to have highly diverse physiological functions. The 15 human and 24 mouse kallikreins have been implicated in patho-physiology of brain, kidney respiratory and reproductive systems, and often are used as cancer biomarkers. To better understand the structure and evolutionary origin of this important gene family in the pig, we have constructed a fully contiguous BAC clone-derived physical map of the porcine kallikrein gene region and have fully sequenced a BAC clone containing 13 kallikrein genes, 11 of which are novel. Radiation-hybrid mapping assigns this kallikrein gene-rich region to porcine chromosome 6. RT-PCR based expression analysis of porcine kallikreins showed a complex expression pattern across different tissues with the thymus being the only tissue expressing all 13 kallikreins
genes. We have also conducted quantitative real-time PCR based, expression analysis and insitu hybridization studies of porcine kallikreins to evaluate the expression and tissue localization of kallikreins in the porcine endometrium during the estrous cycle and pregnancy to better understand the role of kallikreins in placental development and embryonic survival in the pig.

Lisa Rigdon  
Paper (Oral) Presentation  
Microbiology and Molecular Genetics  Oklahoma State University, Stillwater

**A New RNAi Tool Using Conidiation Gene brlA in Aspergillus nidulans**  
Rigdon L., Prade R.

RNA Interference (RNAi) is a eukaryotic mechanism where small RNA molecules regulate gene expression, and researchers are using RNAi as a tool to down-regulate targeted genes. We have used Aspergillus nidulans, a multicellular fungus, to test a new alcohol-dependent inducible system for RNAi. This new system was first constructed in a plasmid, consisting of inverted repeats of an alcohol dehydrogenase promoter (alcAp) with the gene of interest located in a unique restriction enzyme site (BamHI) between the promoters. The plasmid also contains a nutritional selection marker for easy selection of fungal transformants. Our first gene of interest was bristle (brlA), a two-transcript, differentially transcribed gene, encoding a transcription factor that regulates conidiation. The RNAi plasmid, containing the alcA promoters flanking the brlA gene, was transformed into a wild-type strain, and Southern blot analysis indicated the construct was randomly integrated into the genome. The mutants show normal phenotypes on standard media, but a remarkable loss of conidiation on alcAp inducible media, similar to than seen of brlA null mutants. Expression and down-regulation of brlA was confirmed with Northern blotting and RT-PCR. These results are proof that our RNAi construct induces down-regulation of a targeted gene. Even though only one transcript of brlA was targeted for RNAi, both transcripts were silenced. This was a surprising result, and we may be able to contribute to the further understanding of the brlA differential transcription and regulatory mechanisms. We further plan to use Real Time RT-PCR and small RNA Northerns to address this non-differential transcript targeting mechanism.

Manoj Bhaskaran  
Paper (Oral) Presentation  
Physiological Sciences  Center for Veterinary Health Sciences

**Differential expression of microRNAs modulate fetal lung development**  
Manoj Bhaskaran, Yang Wang, Honghao Zhang, Deming Gou, Tingting Weng, Pradyumna Baviskar, and Lin Liu
MicroRNAs (miRNAs) are small endogenous RNAs and are widely regarded as one of the most important regulators of gene expression in both plants and animals. miRNAs are generally 21 to 24 nucleotides in length and they switch off the expression of specific protein by either interfering with the translational machinery via binding to complementary sequences on the mRNA (common in animals) or by directly degrading the mRNA (common in plants). Even though thousands of miRNAs have either been identified or predicted in almost 40 species of living organisms, only a handful have been verified at the functional level. We are interested in identifying miRNAs that regulate fetal lung development and their mechanism of action. Screening for miRNAs differentially expressed during different stages of rat lung development was done earlier in our lab using microRNA microarray. Three clusters of miRNAs showing three specific trends of expression were identified. The aim of the present study is to verify and functionally characterize selected miRNAs from the microarray data. Fetal rat lung collected at days 16, 19, 21 of gestation, from newborn, days 6 and 14 post partum and adult were used for these studies. The expression profiles of microRNAs representative of the three clusters namely miR-18, miR-20a, miR-29a and mir-127 were verified and confirmed using real-time PCR, thus validating the microarray data. In situ hybridization using DIG labeled LNA probes for the relatively abundant miR127, miR-20a and miR-351 were done to verify both their expression patterns and to visualize the cell types in which they were localized. Both miR-127 and miR-351 expression seemed to gradually shift from mesenchymal cells to epithelial cells as development progressed indicating a role for them in the cell differentiation process. miR-20a expression was seen only on Day 16 in mesenchymal cells. Preliminary experiments showed that miR-127 over expression in fetal whole lung cultures using adeno viral vectors caused defective tubular formation and decreased pulmonary surface area during development. Concurrently the expression of Hox B4 protein, a predicted target of miR-127, decreased when miR-127 was over expressed. Also Thyroid transcription Factor (TTF1), known to be both a key mediator of fetal lung development and to be regulated by Hox B4 protein also decreased in fetal lungs which over expressed miR-127. These results indicate that differential expression of miR-127 maintains the appropriate levels of Hox B4 and TTF1 which, in turn, is important for normal fetal lung development.

Veenita Grover
Paper (Oral) Presentation
Biochemistry & Molecular Biology OSU, Stillwater

**Development of microarray hybridization for detection of known & related novel plant viruses in natural settings.**

Veenita Grover, Dr. Ulrich Melcher, and Dr. Marlee Pierce

The International Committee on Taxonomy of Viruses recognizes only about 2000 viral species in the world, a number which is undoubtedly an underestimate. The possibility of future emergence of unknown viruses as crop pathogens requires an active search for
novel viruses. This study aims at developing microarray hybridization, a rapid method for
detection and characterization of known & related novel plant viruses, irrespective of
their pathological behaviors. In this approach, target nucleic acids extracted from plant
samples are amplified using random primers; the products, tagged with fluorescent dye
are hybridized to a microarray of more than a thousand oligodeoxynucleotide probes. The
probes were designed for each genus (sometimes subgenus) of plant or fungal viruses
from the NCBI reference genomes of those viruses. Preliminary experiments using full
length cDNA control targets (2000-5000bp) and short probes (20-30mer) failed to
produce sufficient intensity of hybridization. In this study, we examined the effects of
target and probe lengths on hybridization. Experiments using different target lengths
showed that smaller targets (125-150bp) hybridize much more strongly than longer
targets (300bp and above), possibly due to secondary structure formation in longer
targets. To achieve better fragmentation of the longer products into shorter targets, RNA
was synthesized from cDNA, which was further fragmented using fragmentation buffer
and used as targets. When probe length was varied (20, 30, 50 & 70mers) an increase in
hybridization intensities from 20mer to 70mer occurred. Initial control experiments in
which T tails (thymidylate tails, T20) were attached to shorter probes (20-30mer) at their
3’ends and using shorter targets (125-150bp) showed as strong hybridizations as with
longer probes (70mers). The T-tails are believed to facilitate the UV cross linking of the
probe to the chip making the rest of probe sequence accessible for the target. The use of T
tails was adopted to avoid the decrease in selection potential of detection due to longer
probes. Incorporation of poly A in fragmented RNA targets from plant samples in
hybridizations against tailed probes showed reduction in non specific hybridizations.
Thus, microarrays using tailed probes and shorter length targets show promise in
detection of a broad spectrum of viruses, assuring homeland security and predicting
outbreaks of agricultural diseases.

Preston Dilbeck
Paper (Oral) Presentation
Microbiology and Molecular Genetics  CAS

**Mutation of arginine 357 of the CP43 protein of photosystem II severely impairs the
catalytic S-state cycle of the H2O-oxidation complex.**

Hong Jin Hwang, Preston Dilbeck, Richard J. Debus, and Robert L. Burnap

Basic amino acid side chains situated in active sites may mediate critical proton transfers
during an enzymatic catalytic cycle. In the case of photosynthetic water oxidation, a
strong base is postulated to facilitate the de-protonation of the active site Mn4-Ca cluster
thereby allowing the otherwise thermodynamically constrained transfer of an electron
away from the Mn4-Ca cluster to the oxidized redox active tyrosine radical, Yz•,
generated by photosynthetic charge separation. Arginine 357 of the CP43 polypeptide
may be located in the second coordination shell of the O2-evolving Mn4-Ca cluster of
photosystem II (PSII) according to current structural models. An ostensibly conservative
substitution mutation, CP43-357K, was investigated using polarographic and
fluorescence techniques to evaluate its potential impact upon the S-state cycling. Cells containing the CP43-357K mutation lost their capacity for autotrophic growth and exhibited a drastic reduction in O2-evolving activity (~15% of the wild-type) despite the fact that mutant cells contained over 80% the concentration of charge-separating PSII reaction centers and more than half of these contained photooxidizable Mn. Fluorescence kinetics indicated that acceptor side electron transfer, dominated by the transfer of electrons from QA- to QB, was unaffected, but the fraction of centers containing Mn-clusters capable of forming the S2 state was reduced to about ~40% of the wild-type. Analysis of O2 yields using a bare platinum electrode indicated a severe defect in the S-state cycling properties of the mutant H2O oxidation complexes. Although O2 evolution was delayed to the third flash during a train of single turnover saturating flashes, pattern of O2 emission did not exhibit a discernible periodicity indicating a very high miss factor, which was estimated to be ~45% compared to ~10% for the wild-type. On the other hand, the multiflash fluorescence measurements indicate that yield of formation of the S2 state from S1 is diminished to ~20%, although this latter estimate is complicated by presence of damaged PSII centers. Taken together, the experiments indicate that the high miss factor observed during S-state cycling is likely due to a defect in the higher S-state transitions. These results are discussed in relation to the idea that CP43-R357 may serve as a ligand to bicarbonate or as the catalytic base proposed to mediate proton-coupled electron transfer (PCET) in the higher S-states of the catalytic cycle of H2O-oxidation.

Narendranath Reddy Chintagari
Paper (Oral) Presentation
Veterinary Physiological Sciences Center for Veterinary Health Sciences

**Vacuolar ATPase regulates lung surfactant secretion by alveolar type II cells**

Vacuolar ATPases (v-ATPase) are multi-subunit enzymes which utilize energy of ATP hydrolysis to transport protons across cell membranes. Lung alveolar type II cells (AT2) synthesize, store and secrete a lipid rich surface-active substance called surfactant. Before being released, the surfactant lipids and proteins undergo extensive processing in the acidic environment of the lamellar bodies. Additionally, lamellar bodies enrich calcium in a pH dependent manner, acidic pH favoring accumulation. We hypothesize that v-ATPases mediate acidification of lamellar bodies and that inhibition of v-ATPases leads localized increase in calcium concentration owing to mobilization from lamellar bodies. The increased calcium levels might activate enzymes such as PKC and CaCMKII resulting in increased surfactant secretion. To this end, v-ATPase was inhibited by Bafilomycin A1 (Baf A1) which effectively dissipated the lamellar body pH as indicated by loss of quinacrine accumulation in the lamellar body. V-ATPase inhibition resulted in increased surfactant secretion which was effectively blocked by intracellular Ca2+-chelator, BAPTA-AM. The Baf A1-mediated increase was also blocked by staurosporine and KN-62, the inhibitors of PKC and CaCMKII, respectively. Moreover, thapsigargin, a
endoplasmic reticulum calcium store depletor inhibited the Baf A1-induced increase in surfactant secretion, indicating the role of ER calcium pool in Baf A1-mediated secretion. Further, quinacrine accumulation in the lamellar bodies was reduced following stimulation of AT2 cells indicating an increase in lamellar body pH and an inhibition of v-ATPase activity during the process of regulated secretion. In summary, we conclude that v-ATPase regulates surfactant secretion via increased calcium mobilization which activates PKC and CaCMKII. [NIH R01 HL-052146; R01 HL-071626; R01 HL-083188; Student Seed Grant, CVHS(to NRC)].

Christy Baker
Paper (Oral) Presentation
Entomology & Plant Pathology Oklahoma State University

**Evaluation of Repeat Markers for Forensic Strain Identification of Pseudomonas syringae**

Christina Baker, Carol Bender, Ulrich Melcher, and Jacqueline Fletcher

Agriculture is economically important, and US crops are vulnerable to accidental or deliberate introduction of pathogens. Pseudomonas syringae pv. tomato DC3000 (Pst DC3000), is a gram-negative plant pathogenic bacterium which causes bacterial speck of tomato. P. syringae shares survival and virulence mechanisms with pathogens that are currently on the USDA APHIS select agent list, but is a model pathogen that can easily be utilized for forensics without biocontainment facilities. Related studies demonstrated that repeat loci, which are found throughout bacterial genomes, were useful for rapid and reliable strain identification in other organisms. In the current study, the genome of Pst DC3000 was analyzed, and primers were designed to amplify by polymerase chain reaction (PCR) 34 repeat loci. The primers were used to screen representative strains, and five loci were identified as polymorphic. Primers for these loci were used to survey a collection of diverse pv. tomato isolates to distinguish among strains. These loci represent a promising molecular tool for tracing bacterial outbreaks and dissemination, as well as studying population structure and diversity. Research and development on technologies for improved strain detection and typing are very valuable to the new field of Microbial Forensics, and will benefit society through improved safety and security of our food supply.

Xinyi Peng
Paper (Oral) Presentation
Chemistry Oklahoma State University

**Structural Studies of the Human Retinoic Acid Receptor Gamma Ligand-Binding Domain Complexed with Anti-Cancer Heteroarotinoids Drugs**

Xinyi Peng, K. Darrell Berlin, Stacy D. Benson
Retinoids play an important role in the therapy and prevention of cancer by interacting with the retinoic acid receptors (RARs) and the retinoid receptors (RXRs), which are ligand-dependent transcription regulators. Human RAR gamma is involved in skin photoaging and carcinogenesis. The ligand binding domain (LBD; residues 178-423), which is the portion of hRAR gamma that specifically binds natural reinoids (retinol and retinoic acid) or synthetic retinoids (arotinoids and heteroarotinoids), has been cloned with an N-terminal tag containing six histidines and over-expressed in Escherichia coli. The tag allows for purification of the protein using immobilized nickel absorption followed by gel filtration chromatography. Currently, expression and purification are being optimized and preliminary crystallization trials are underway with the LBD in complex with all-trans retinoic acid (ATRA). The LBD/ATRA complex structure is known but the crystallization trials are being used to check the integrity of the construct. Small crystals have been obtained and await characterization by X-ray diffraction. The ultimate goal is to obtain X-ray crystal structures of the LBD in complex with novel heteroarotinoids, which have been shown to be effective anti-cancer agents against certain human cancer cell lines. Atomic resolution of the drugs interactions with the protein will aid in optimization of the drugs.

Alexandre Mello  
Paper (Oral) Presentation  
Entomology and Plant Pathology  
Agriculture Science and Natural Resources

**Genetic Diversity of Spiroplasma citri Strains from Different Regions, Hosts, and Isolation Dates**

Alexandre F.S. Mello, Raymond K. Yokomi, Ulrich Melcher, Jianchi C. Chen, Astri C. Wayadande and Jacqueline Fletcher

Spiroplasma citri, a phloem-resident, leafhopper-transmitted pathogen, causes citrus stubborn disease (CS). Concern over losses due to CS in California orchards has grown over the past decade. To investigate the possibility of introduction or emergence of a new spiroplasma strain, a study of genetic diversity among S. citri strains from various locations was conducted using RAPD-PCR of 35 strains cultured from 1980-1993, and another 35 strains cultured from 2005-2006. Although considerable diversity was observed among strains, no unique genetic signatures were associated with recent strains compared with those cultured 15-28 years ago. Further, no geographically associated pattern was distinguishable. Some DNA fragments were unique to carrot and daikon radish strains, suggesting some host plant influence. Multiple strains from a single tree also showed genetic diversity. Sequencing of five RAPD bands that differed among S. citri strains showed that diversity-related gene sequences include virus fragments, a membrane lipoprotein, a DNA modification enzyme, and a mobilization element. No differences in colony morphology were observed among the strains. The lack of correlation between PCR patterns and isolation date or collection site is inconsistent with the hypothesis that recent infections are due to the introduction or emergence of novel pathogen strains.
4th Annual Graduate Research in the Biological Sciences Symposium

Poster Presentation Abstracts, September 28th, 2007
Physiological and biochemical characterization of mannitol accumulating transgenic wheat under water deficit stress.

Shraddha Vadvalkar, Sathya Elavarthi, Dr. Bjorn Martin.

The spring wheat cultivar Bobwhite was transformed with the mtlD gene from E.coli by the biolistic method. The gene is responsible for mannitol accumulation and has shown to result in drought and salt tolerance. Four transgenic lines (pTA2-115, pTA2-118, pTA5-104, pTA5-108), a positive control (pAHC20, containing the transgenic bar gene only) and wild type Bobwhite were grown in a green house under well watered conditions as well as under water deficit stress. Volumetric soil water content in the pots of well-watered plants was maintained at 40% throughout the experiment while that of stressed plants was maintained at <14%. Physiological characterization was done by determining the net photosynthesis rate and conducting CO₂- and light-response curves on one transgenic line (pTA2-118) and one control line (pAHC20). All lines showed significantly higher net photosynthesis rate under well-watered conditions, and there were no differences in the net photosynthesis rate among the lines. Bobwhite had the greatest above-ground biomass under well-watered conditions, but pTA2-118 and pTA 5-108 had slightly higher biomass than the wild type under stress. Biochemical characterization included activity determination of antioxidant enzymes, glutathione reductase, catalase, and ascorbate peroxidase. The pTA2-118 line showed significant differences in the activities of all antioxidant enzymes after 30 days of water stress. There were no substantial differences in enzymatic activities between the transgenic lines and the wild type, however, which suggests that the plants were able to maintain a balance between the activities of all antioxidant enzymes even under water deficit stress. Mannitol is known to scavenge hydroxyl radicals. The possible role of mannitol in preventing lipid peroxidation when exposed to drought will be evaluated by testing for malon dialdehyde and by using a direct hydroxyl radical scavenging assay.
assay identifying strong and weak adherence to abiotic surfaces in strains of L. monocytogenes isolated from raw and ready-to-eat (RTE) meats and environmental samples in RTE meat processing facilities. Since cellular adherence is the first stage of infection with L. monocytogenes, we were interested to see whether the strong adherence observed with abiotic surfaces would also facilitate cellular adherence and aid virulence. Our objectives were to examine the virulence characteristics of these strains using tissue culture adherence and invasion assays. Several strong (cw50, 99-38, cw62 and cw77) and weak (cw34, cw35, cw52 and sm3) adherent strains of L. monocytogenes were selected for adherence and invasion assays with the Caco-2 cell line. Cell monolayers were inoculated with bacterial suspension adjusted to obtain an MOI (multiplicity of infection) of ~100 bacteria per cell. Duplicate monolayers were infected with bacterial culture and incubated for 2 hrs. Nonadherent bacteria were then removed by washing 3 times with EMEM. Total associated bacteria were determined by lysing monolayer cells with 1 ml of 1% (v/v) Triton X-100 for 5 min and enumeration on TSA plates. For invasion assays, gentamicin-containing (1000 mg/ml) medium was used to kill extracellular bacteria and surviving intracellular bacteria were enumerated. Negative controls included L. innocua. Results indicate that there may be a correlation between adherence to abiotic surfaces and invasion for strong biofilm forming strains. The highest invasive indices were observed with several strains showing strong adherence to abiotic surfaces (cw50 with an invasive index of 0.857) whereas strain cw52 (weak adherence) had an invasive value of 0.530. With low MOI of 1:10 and less incubation time showed remarkable difference between strong and weak adherent strains. The data suggest the strong adherence characteristic not only promotes retention of such strains in food processing facilities, but may also demonstrate enhanced virulence characteristics.

Keywords: Listeria monocytogenes, cell culture, virulence assay.

Shawn Daley
Poster Presentation
Biochemistry & Molecular Biology  DASNR

Paralogous Evolution of a Family of LysR-Type Transcriptional Regulator’s (LTTR) Found In Synechocystis sp. PCC6803.
Shawn Daley; Marla Carrick; Robert Burnap

Paralogous regulators arise from gene duplication events allowing for divergence of function in one member while maintaining the original function of the other. There are 6 LysR-Type Transcriptional Regulators within Synechocystis sp. PCC6803; which appear to be the result of 5 separate gene duplication events. Two of three closely related members (ccmR & cmpR) from one duplication series control the high-affinity/low-flux inorganic carbon acquisition system, while the other (ycf30) controls an unknown but essential system, perhaps also involving carbon. Bioinformatic analysis has yielded a putative binding site in all three regulators and has subsequently been mapped onto a 3-D model constructed from the crystal structure (1IXC) of a homologous regulator. This
mapping/analysis has allowed for the identification of residues located both in the DNA binding domain and regulatory domains, each of which shows conservation among the two known inorganic carbon acquisition regulators (ccmR & cmpR) and the as yet undefined ycf30. Putative binding site has been identified with unexpected residue makeup and dimension. Evolutionary trace analysis has yielded additional buried residues which appear conserved between members of this protein family. Patterns of conservation and divergence may indicate regulatory/functional differences among the different family members and have implications for the complex regulatory networks involved in inorganic/organic carbon metabolism.

Shekher Mohan
Poster Presentation
Pharmacology and Physiology Oklahoma State University College of Osteopathic Medicine, Tulsa, Oklahoma

Cloning and bioinformatics of chick mu opioid receptor expressed in brain tissue
SHEKHER MOHAN and CRAIG W. STEVENS

Opioid drugs have various physiological effects, the actions of which are mediated by membrane associate G protein-coupled receptors: mu (MOR), delta (DOR), kappa (KOR) and nociceptin (ORL) opioid receptors; however the role of ORL in analgesia is less clear. Typically, GPCRs consist of 7 transmembrane (TM) domains, in mammals, members of the opioid receptor family have conserved intron/exon boundaries. Therefore, through opioid receptor cloning, gene duplication may prove to be the source of the opioid receptor gene. The present study reports on the sequence, expression, and bioinformatics of the mu opioid receptor (MOR) cDNA cloned from Gallus gallus brain tissue; ggMOR. Following RT-PCR used to represent brain tissue expression of opioid receptors and cloning, a comparative bioinformatic approach was used to compare ggMOR to other opioid receptor. From use of the basic online bioinformatic tool, BLAST was used to identify cDNA sequences obtained from Gallus gallus cloning. Our evolutionary bioinformatics’ approach is novel and we have used it compare vertebrate MOR, DOR and KOR protein sequences within vertebrate species and their groups. Concluding that in non-mammals and mammals a 70-80% protein similarity for MOR, DOR and KOR exists. Therefore, we hypothesis from the cloning and functional characterization of the opioid receptors from the Northern Leopard frog, Rana pipiens in our lab, that chick opioid receptors, as earlier-evolved vertebrates shows less opioid type-selectivity than those found in human and other mammals. The rapid rate of adaptive evolution of opioid proteins between species expressing opioid receptors could explain the increased type selectivity to opioid agonist recorded in human (hMOR) compared to Rana pipien (rpMOR) as shown using radioligand-binding assays. Using further studies, if confirmed, these hypotheses provide a novel understanding of the pharmacology of opioid receptors, providing an additional tool in the development of opioid analgesics.

Keywords: Analgesia – Bioinformatics – Chick - Evolution - Opioid receptors
Pleiotrophin Regulates Alveolar Epithelial Cell Differentiation During Fetal Lung Development

Tingting Weng, Li Gao, Kexiong Zhang, Manoj Bhaskran, Deming Gou, Lin Liu

Pleiotrophin (PTN) is a cytokine highly expressed at the late stage of embryogenesis. It plays important roles in angiogenesis, mesenchymal-epithelial interaction, cell differentiation and migration. Our previous studies have shown that PTN was highly expressed in fetal lungs and located in mesenchymal cells adjacent to the developing epithelium. We further investigated the roles of PTN in fetal lung development and alveolar epithelial cell differentiation using fetal lung organ culture and isolated fetal lung epithelial cells. PTN expression in fetal lung organ culture was knocked down by an adenoviral vector carrying siRNA targeted to PTN. The reduction of PTN decreased the branching morphogenesis of fetal lungs. Additionally, when isolated fetal alveolar epithelial type II cells were cultured on plastic dishes, PTN significantly promoted the cell migration and proliferation, and simultaneously arrested the trans-differentiation of type II cells to type I cells. Our study suggested that PTN may have important roles in the regulation of fetal lung development and alveolar epithelial cell trans-differentiation. (Supported by AHA 0610143Z, NIH R01 HL-52146, R01 HL-071628 and R01 HL-083188).

Cadmium-Induced Bone Loss in Ovariectomized Rats Was Exacerbated by Potassium Phosphate and Moderated at Some Levels by Dried Plum.


ABSTRACT Cadmium (Cd) is a toxic heavy metal that has detrimental effects on bone mineral density (BMD). Chronic phosphorus (P) supplementation decreases bone mass through a decline in serum calcium concentration and resultant hyperparathyroidism. The purpose of our study was to examine the effects of Cd and P on bone and to test the hypothesis that dried plum would ameliorate the detrimental effects of Cd and P on bone. Fifty, 90 day-old Sprague-Dawley rats were ovariectomized (Ovx) and assigned to the following five treatments (n=10): 1) control, 2) 50 mg Cd/kg diet, 3) 50 mg Cd /kg diet with 1.2 % potassium phosphate (KPhos), 4) 200 mg Cd/kg diet, and 5) 200 mg cadmium/kg diet with 1.2 % KPhos. After 45 days of treatment, half the rats in each
group had 15% dried plum added to their diets. This second phase of the experiment continued for an additional 3 months. At necropsy, the distal femur was scanned using microcomputed tomography (uCT) to assess microarchitecture of the trabecular bone and cortical thickness at the midshaft. In the distal femur a volume of interest beginning 25 slices below the growth plate and consisting of 100 slices at 16.5 micron intervals was contoured and evaluated. Bone volume fraction was significantly lowered by Cd and by KPhos (p< 0.0001 and p < 0.0004, respectively). Trabecular number was decreased by KPhos (p< 0.04) while trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) were significantly increased by KPhos. Significant interactions affected connectivity density (ConnD). In rats fed Cd, feeding KPhos reduced ConnD. Dried plum was beneficial in rats fed 50 ppm Cd but detrimental in those fed 200 ppm Cd with KPhos. Cortical thickness was decreased by cadmium and by KPhos (p < 0.0001) but increased by dried plum (p = 0.002). Our results indicate that Cd causes loss of both trabecular and cortical bone. KPhos increased those losses. Dried plum increased cortical thickness; effects of dried plum on trabecular microarchitecture varied depending on the levels of Cd and the presence or absence of KPhos.

Michael Puckette
Poster Presentation
Biochemistry and Molecular Biology Oklahoma State University

Responses of Medicago truncatula accessions to chronic ozone, drought, and combined chronic ozone and drought stress
Puckette M and Mahalingam R

Reactive oxygen species (ROS) are key components of several different biotic and abiotic stresses in plants. Consequently they provide a potential convergence point where multiple stress induced signals may intersect. Previously we screened 38 accessions of Medicago truncatula to acute ozone stress and identified a resistant accession, JE154, and a sensitive cultivar, Jemalong. In this study we exposed these accessions to chronic ozone and drought stress and found that the resistant JE154 was tolerant to these two stresses while Jemalong was sensitive. In nature plants are often exposed to multiple stresses simultaneously. Hence these two accessions were exposed to combined drought and chronic ozone stress to identify if exposure to a combination of stressors altered plant responses. We found that the acute ozone resistant cultivar JE154 was more resistant to both chronic ozone and drought stress than sensitive cultivar Jemalong. In addition we found that chronic ozone stress delayed the onset of visual cell death lesions due to drought stress in both accessions. These results suggest that multiple stresses may interact in complex manner to impact plant responses. Analysis of biochemical and molecular changes to combined stresses in lines showing contrasting responses will provide rational targets for improving plant resistance to multiple stresses.
INVESTIGATION ON QH2 OXIDATION AT QO SITE DURING BC1 CATALYSIS
Shaoqing Yang, Hewen Ma, Linda Yu, Chang-An YU

Cytochrome bc1 complex catalyzes electron transfer from ubiquinol to cytochrome c in the electron transfer chain. However, the mechanism of bifurcated QH2 oxidation at Qo site in bc1 complex remains unclear. There are two kinds of hypotheses for QH2 oxidation at Qo site: sequential mechanism and concerted mechanism. To clarify the mechanism of this QH2 oxidation, mutants H198N, lacking of heme bL, and H111N, lacking of heme bH, were constructed. The loss of either heme bL or bH will break the low potential chain of Q-cycle. Fast-kinetics study showed that the rates of cytochrome c1 reduction by ubiquinol in mutants H198N and H111N were only around 10% of that of wild type bc1 complexes, suggesting that the electron transfer between heme bL and bH is responsible for the release of reduced ISP head domain from b-position to c1 position. Meanwhile, these two mutants can produce much more superoxide than wild type bc1 complexes. Qi inhibitor antimycin had little effects on their superoxide production. Experimental data showed that in Q-cycle, ISP reduction was independent on heme bL reduction by QH2 at Qo site. On the contrary, ISP reduction by QH2 at Qo site was required for heme bL reduction, indicating QH2 oxidation at Qo site follows the sequential mechanism.

Expression Patterns of Conserved MicroRNAs in Switchgrass
Jessica Matts1, Yanqi Wu2, and Ramanjulu Sunkar1

Since microRNAs (miRNAs) were first discovered in C. elegans, they have been identified in numerous plants and animals. These small RNAs (21 to 23 nucleotides in length) help to regulate numerous aspects of the cell by binding to mRNA and either inhibiting translation or by facilitating cleavage of the mRNA and in turn down regulating protein production. In plants these miRNA have shown to play important roles in both development and the response to abiotic and biotic stresses. Within the plant kingdom, most of the work done with microRNAs and their targets has been done in Arabidopsis thaliana and rice. Here we are looking at another member of the Poaceae family—switchgrass. Switchgrass has become of more interest in recent years due to the possibility of using it for biofuel. This is a large, C4 plant that is native to the prairies and
plains of central North America. It is a contender for the production of biofuels because it grows tall, can withstand drought and high heat conditions, and does well in poor soil. Here we show the expression pattern of some of the conserved microRNAs in switchgrass, along with tentative gene targets for some of these conserved microRNAs.

Lila Peal
Poster Presentation
Biochemistry and Molecular Biology Agricultural Sciences and Natural Resources

A RNA Binding Protein Involved in Oxidative Stress in Arabidopsis thaliana
L. Peal, N. Jambunathan, R. Mahalingam

RNA binding proteins (RBPs) play a role in post-transcriptional regulation via mRNA stability, splicing, polyadenylation, transport and translation. RBPs containing RNA recognition motifs (RRM) are the most common among eukaryotes. In Arabidopsis thaliana, there are 196 RBPs containing different number of RRMs ranging from one to four. We examined the RBP gene sequences containing three RRMs in several other plant species too investigate the evolutionary conservation of RBPs containing three RRMs. Seven RBPs in Arabidopsis that contain three RRM are closely related to the RBP45 and RBP47 of tobacco. The expression profiles of those RBPs were examined under ozone stress. Of these seven, At1g11650 showed increased expression 2, 6, 10, and 18 hours after ozone fumigation. Interestingly, At1g11650 has 59 % similarity to yeast oxidative stress regulator protein, CSX1. This leads us to the hypothesis that the RNA targets of At1g11650 are important for oxidative stress regulation in Arabidopsis. Another interesting aspect gene At1g11650 is that it is alternatively spliced. The splice variant form of this protein has two complete RRMs and one incomplete RRM. The expression profiles of the full-length and splice variant form of this gene will be analyzed during ozone stress. We will also seek to identify the RNA targets of these RBPs to further understand their roles in oxidative stress regulation pathways.

GROWTH DIFFERENTIATION FACTOR-9 STIMULATES PROLIFERATION AND INHIBITS STEROIDGENESIS OF BOVINE THECA CELLS FROM SMALL BUT NOT LARGE FOLLICLES

Pauline Y. Aad¹, Leon J. Spicer¹, Dustin Allen¹, Sabine Mazerbourg², and Aaron J. Hsueh²

¹Department of Animal Science, Oklahoma State University, Stillwater, OK 74078, and ²Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA 94305-5317

Oocyte-derived growth differentiation factor-9 (GDF-9) is one of the many paracrine and
endocrine regulators of follicle development. Although GDF-9 null mice showed defective theca development, GDF-9 actions in theca cells has not been further investigated. Our objective was to study the effect of GDF-9 on theca cell functions. Pure theca cells from small (4-6 mm) and large (> 7.9 mm) follicles were isolated from bovine ovaries and cultured for 48 h in medium containing 10% FCS before treatment with various hormones in serum-free medium for an additional 48 h. In theca cells from small follicles, GDF-9 treatment (150 to 600 ng/ml) caused a dose-dependent increase \( (P < 0.05) \) in cell proliferation (maximum increase 2.5-3.2-fold of controls) in the presence of LH or LH plus IGF-I. In addition, both progesterone and androstenedione production were decreased by GDF-9 treatments in cells co-treated with LH and IGF-I, completely suppression steroidogenesis at 600 ng/ml of GDF-9. In contrast to its effect in theca cells from small follicles, GDF-9 had negligible effects on steroid production or cell proliferation of theca cells from large follicles co-treated with LH and IGF-I. Small follicle theca cells contained a greater abundance of ALK5 (type I GDF-9 receptor) mRNA than large follicle theca cells. Transfection studies demonstrated that GDF-9 activated the Smad2/3-induced CAGA promoter in theca cells from small follicles. Granulosa cells but not theca cells contained GDF-9 mRNA. We conclude that oocytes and granulosa cells (but not theca cells) produce GDF-9 which acts directly on theca cells during early stages of development to promote their proliferation and to prevent their premature differentiation and excess androgen production.

**Keywords:** Growth differentiation factor-9 (GDF-9); insulin-like growth factor-I (IGF-I); theca cell; progesterone production; androstenedione production; steroidogenesis.