GREG STUART

GEMS

Genetics and Environmental Mutagenesis Society

24th Annual Fall Meeting

"Oxidative Stress and Damage"

Thursday October 26, 2006

UNC Friday Center
Chapel Hill, NC

GEMS

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** Replacing Tasha Smith for remainder of term

GEMS

ANNUAL FALL MEETING

October 26, 2006

Friday Center of the University of North Carolina

"Oxidative Stress and Damage"

8:00 Registration and Continental Breakfast

8:30 Welcome: Dr. Les Recio, GEMS President
Speaker Introduction: Dr. Greg Stuart, GEMS President-Elect

8:45 **Dr. Susan P. LeDoux**, University of South Alabama College of Medicine
"Mitochondrial DNA Repair: A Critical Player in the Cellular Response to Oxidative Damage"

9:30 **Dr. James A. Swenberg**, University of North Carolina at Chapel Hill
"Do Chemicals That Form DNA Adducts and Induce Mutations Always Have Linear Low Dose Risks?"

10:15 Break / Refreshments / Visit Sponsor Booths

10:45 Poster Session

11:30 Talks by Students:
"Environmental Influences On The Fetal Epigenome," D.C. Dolinoy
"Mouse Lung Tumor Model Dose Response Analysis of the Conditional Expression of the Mutant Ki-Ras^{G12C} Allele," S. Dance

12:00 Lunch

1:00 Business Meeting

1:30 Talks by Students / 1st or 2nd-Year Post-Docs:
"Aroclor Derived Oxidative Damage In the Bivalve Corbicula Fluminea" D.W. Lehmann
"Biomonitoring of Reactive Oxygen Species In Biological Fluids" B. Heidenfelder
"Proinflammatory Cytokines Induce NO-mediated DNA Damage in Articular Chondrocytes" B. Fermor
"Immuno-Spin Trapping of Oxidatively Generated Damage To The Genome" D.C Ramirez

2:30 **Dr. James A. Imlay**, University of Illinois at Urbana-Champaign
"Molecular Explanations For the Toxicity of Sub-Micromolar H_2O_2"

3:30 Awards

3:45 Reception

Dear GEMS Members,

Welcome to our 24th Annual Fall Meeting. The key person responsible for organizing the meetings is our current President-Elect, Greg Stuart, who has done an excellent job in putting together an outstanding group of speakers on a very interesting and timely topic "Oxidative Stress and Damage". I can't think of more outstanding individual to take over as president in the coming year.

I would like to extend a special thank you and appreciation to our corporate sponsors. We could not hold these meetings without the very generous financial assistance of our corporate sponsors, many of whom have been long time supporters of our organization. Our sponsors will have tables set up to exhibit their most up-to-date products and laboratory equipment at the meeting. We have set aside a special time slot during the meeting for you to interact with our corporate sponsors and we encourage each of you to show your appreciation for their continued support of GEMS by speaking with company representatives. Please discuss with them your laboratory needs and products of interest, and thank them for their continuing support.

Organizing the Fall meeting requires the help of a dedicated cadre of volunteers. One of the exciting aspects of being a member of GEMS and serving on the Board is the great group of people with whom you interact. I encourage each of you to get involved and volunteer. Serving as President-Elect and President over the last two years, has been a pleasure especially since I had the privilege of working with a great group of interactive and engaged people. I want to thank Susan Ross, our Treasurer, and Gloria Jahnke, our Secretary, and other GEMS Board members who have done an outstanding job throughout the year. I and all the Board members of GEMS acknowledge the support of CIIT Centers for Health Research for hosting our bimonthly Board meetings and supplying the meeting badges and registration materials for a number of years. I also want to express my appreciation to our current Board members, Janice Allen, Jayne Boyer, Cindy Innes, Sharon Oxendine, Jeffrey Ross, Rose Anne McGee, and Errol Zeiger as well as our outgoing Board members, Karin Scarpinato, Mary Smith, and Alan Townsend. We especially appreciate the time and effort that Frank Stack, has devoted to GEMS maintaining and updating our web and blog site at http://www.ncneighbors.com/. We thank ILS for providing abstract and program booklets for the spring and Fall GEMS meeting.

There are a number of other people who deserve special recognition for their constant efforts on behalf of GEMS. Kristine Witt, who arranges for the award plaques and coffee mugs for both the Fall and Spring meetings, and Julie Ginsler, who has taken over as our Corporate Sponsor Representative. Julie has done an enormous amount to work encouraging our cooperate sponsors to exhibit at the meeting despite the many cutbacks that companies have adopted in recent years. This is another reason for you to visit their booths.

One of the most important activities of GEMS is to encourage the growth and development of our young scientists. The GEMS meetings offer them the opportunity to learn something new about a scientific area in which they may not be very familiar and permit their interaction with colleagues and other mentors, and I would encourage our junior colleagues to take full advantage of this opportunity. At this fall meeting we will have the Best Student Talk and the Best Post-Doctoral talk awards. The winner of the Best Student Talk serves on the Board as the Student Representative.

I have very much enjoyed my 2-year tenure as a member of the GEMS Board. It has been an honor and privilege to serve as your President.

Leslie Recio
GEMS President

A special thanks to NIEHS, ILS, and CIIT Centers for Health Research for their support

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PLATFORM PRESENTATIONS

S1

MITOCHONDRIAL DNA REPAIR: A CRITICAL PLAYER IN THE CELLULAR RESPONSE TO OXIDATIVE DAMAGE

Dr. Susan P. LeDoux University of South Alabama College of Medicine

Cells of the body are constantly exposed to agents that damage DNA. Although much attention has been paid to the effects of this damage on nuclear DNA, the nucleus is not the only organelle containing DNA. Within each cell, there are hundreds to thousands of mitochondria. Within each mitochondrion are multiple copies of the mitochondrial genome. These genomes are extremely vulnerable to insult and mutations in mitochondrial DNA (mtDNA) have been linked to several diseases, as well as the normal process of aging. The principal mechanism utilized by cells to avoid DNA mutations is DNA repair. Multiple pathways of DNA repair have been elucidated for nuclear DNA. However, it appears that only base excision repair is functioning in mitochondria. This repair pathway is responsible for the removal of most endogenous damage including alkylation damage, depurination reactions and oxidative damage. Within the body, there are cell-specific differences mtDNA repair. In the CNS, astrocytes exhibit efficient repair. Whereas, other glial cell types and neuronal cells exhibit a reduced ability to remove lesions from mtDNA. Additionally, a correlation was observed between those cells with reduced mtDNA repair and an increase in the induction of apoptosis. To demonstrate a causative relationship, a strategy of targeting DNA repair proteins to mitochondria to enhance mtDNA repair capacity has been employed. Enhancement of mtDNA repair in cells provided protection from ROS- and cytokine- induced apoptosis. Conversely, disruption of mtDNA repair in breast cancer cells leads to an increase in cell killing following an oxidative insult. These experiments provide evidence that mtDNA repair plays a critical role in the cellular response to genotoxic agents and a novel therapeutic strategy for either protecting sensitive cells or enhancing the killing of tumor cells.

S2

DO CHEMICALS THAT FORM DNA ADDUCTS AND INDUCE MUTATIONS ALWAYS HAVE LINEAR LOW DOSE RISKS?

Dr. James A. Swenberg University of North Carolina at Chapel Hill

The 2005 EPA Guidelines for Carcinogen Risk Assessment states that agents that are DNA reactive and have mutagenic activity should use linear extrapolation. Genotoxicity tests are generally not quantitative and represent Hazard Identification. DNA adduct data is expected to be linear at low doses and go through zero unless identical endogenous adducts are also formed. In contrast, mutations in surrogate genes such as hprt have background rates and do not go through zero. The same response is likely for cancer genes. These four key events form the Mode of Action for carcinogenesis, but have different dose responses. This talk will show examples of the above issues and will raise questions related to how we might improve the scientific underpinning of risk assessments for genotoxic agents.

S3

MOLECULAR EXPLANATIONS FOR THE TOXICITY OF SUB-MICROMOLAR H_2O_2

Dr. James A. Imlay University of Illinois at Urbana-Champaign.

Our lab employs *E. coli* to address fundamental questions about oxidative stress: How rapidly are reactive oxygen species formed inside cells? What biomolecules do they most readily damage? How does the cell defend itself? We have constructed mutants that cannot scavenge H_2O_2. These strains have allowed us to directly measure the rate of intracellular H_2O_2 formation, and we have used this information to calculate that the steady-state concentration of H_2O_2 is very low inside wild-type cells. However, we have also found that 0.1 micromolar H_2O_2 is sufficient to activate H_2O_2 stress-response systems -- which implies that evolution has recognized that such low concentrations constitute a hazard for *E. coli*. By investigating the growth defects of these strains, we have identified cell processes that are disrupted by submicromolar H_2O_2, and in several cases we have been able to pinpoint the molecular mechanisms of damage. This information, in turn, has revealed the rationale for aspects of the stress response. Historically bacteria have been excellent model systems for studies of oxidative damage, since the biochemical bases of injury are common to all organisms. Further, many of the defensive strategies that are used by eukaryotes were inherited from their microbial ancestors.

ORAL PRESENTATIONS

T1

ENVIRONMENTAL INFLUENCES ON THE FETAL EPIGENOME Dana C. Dolinoy [1,2,3], Jennifer R. Weidman [1], and Randy L. Jirtle [1,2,3]. [1] Department of Radiation Oncology, Duke University Medical Center, Box 3433 Durham, North Carolina 27710, [2] University Program in Genetics and Genomics, Duke University, [3] Integrated Toxicology and Environmental Health Program, Duke University

Traditional studies on the combined effects of genetics and the environment on individual variation in disease susceptibility primarily focus on single nucleotide polymorphisms that influence toxicant uptake and metabolism. A growing body of evidence, however, suggests that epigenetic mechanisms of gene regulation, such as DNA methylation, are also influenced by the environment, and play an important role in the early origins of adult disease susceptibility. Using the viable yellow Agouti (A^{vy}) mouse, we have investigated environmental determinants of DNA methylation following maternal exposures. Methylation of cytosine-guanine (CpG) dinucleotides varies among isogenic A^{vy} mice, causing variation in coat color from yellow (unmethylated) to pseudoagouti (methylated). Unmethylated mice also experience higher rates of obesity, diabetes, and tumorigenesis due to constitutive ectopic *Agouti* expression.

Since the epigenome is particularly susceptible to dysregulation during embryogenesis, we investigated the effect of dietary genistein supplementation of mice during gestation, at levels comparable with humans consuming high-soy diets. Maternal genistein shifted the coat color distribution of A^{vy} offspring toward pseudoagouti and increased methylation of six CpG sites in the A^{vy} retrotransposon. We next investigated the effect of low-level dietary bisphenol a (BPA) exposure of mice during gestation. Maternal BPA shifted the coat color distribution of A^{vy} offspring toward yellow and decreased methylation of six CpG sites in the A^{vy} retrotransposon. These studies provide the first evidence that *in utero* dietary exposure to genistein or BPA affects gene expression and alters susceptibility to disease by permanently modifying the epigenome. Studies are underway to investigate whether concurrent maternal exposure to both genistein and BPA affects methylation and coat color phenotype at the A^{vy} IAP. Similar to rescuing the adverse phenotype of a genetic knockout, these studies seek to determine whether environmental co-exposures may cancel each other out and restore normal stochastic variability at the A^{vy} and other metastable epialleles.

T2

MOUSE LUNG TUMOR MODEL DOSE RESPONSE ANALYSIS OF THE CONDITIONAL EXPRESSION OF THE MUTANT Ki-RAS^{G12C} ALLELE Stephanie T. Dance [1], Heather S. Floyd[2], Joseph E. Moore[1], Libyadda J. Mosely[1] Nancy D. Kock[3], and Mark Steven Miller[1] Departments of [1]Cancer Biology and [3]Comparative Medicine, Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157 [2]NC State College of Veterinary Medicine Molecular Biomedical Sciences Raleigh, NC 27606

Previous studies from our laboratory have described the development of a novel bitransgenic mouse lung tumor model that conditionally expresses the human Ki-*ras*^{G12C} allele in a

doxycycline (DOX)-inducible, lung-specific manner. This model appears to recapitulate the earliest stages of lung tumorigenesis, as mice treated with DOX (500μg/ml) for 12 months develop benign foci of hyperplasias and adenomas, showing little progression to malignant ACs. Most laboratories using inducible expression systems utilize the dose of ligand that results in maximal gene expression. In this study, we determined if different levels of mutant RAS expression, resulting from doses of 25 and 100μg/ml of DOX, would influence the phenotype seen in the lung. Treatment with different doses of DOX resulted in a dose-dependent increase in tumor multiplicity. At the higher 500μg/ml dose, all lesions were <1mm; however in the bitransgenic mice that received either the 25 or 100μg/ml dose, there was a significant number of lung lesions that were ≥1mm, with some reaching up to 4mm in size. Interestingly, there was a dose- dependent difference in the morphology of the proliferative lesions. The 25 and 500μg/ml treated bitransgenic mice exhibited hyperplasias and relatively benign adenomas whereas the 100μg/ml treated mice also exhibited more severe, high grade adenomas with atypic features of AC. Immunohistochemical analysis of signaling pathways suggested similarities and differences in the expression and/or phosphorylation of specific signaling molecules. Cyclin D1 expression was increased with all 3 doses of DOX whereas increased expression of $p19^{ARF}$, along with the concomitant activation of the *p53* pathway, was only seen at the 500μg/ml dose. Elevated levels of Ki-67 staining were found at all 3 levels of transgene expression. Interestingly, increased caspase-3 activity was noted at the 25 and 100, but not the 500μg/ml, dose. Increased expression of survivin was seen at the 100μg/ml and 500μg/ml doses but not at the 25μg/ml dose. Using phospho-specific antibodies, we found no effect on the levels of phosphorylated AKT at any dose, whereas an increase in phosphorylated JNK was seen at the 25μg/ml dose, but not at the two higher doses. Our results suggest that different levels of transgene expression may influence the levels of expression and/or phosphorylation of specific signaling molecules. It is possible that the molecular alterations driving tumorigenesis may differ at different levels of transgene expression, and this should be taken into consideration when inducible transgene systems are utilized to promote tumorigenesis in mouse models.

T3

AROCLOR DERIVED OXIDATIVE DAMAGE IN THE BIVALVE *CORBICULA FLUMINEA* **D. W. Lehmann**[1], J. M. Law [2], J. Levine [2]; [1]Dept of Environmental and Molecular Toxicology, NCSU, Raleigh, NC 27695. [2] Dept of Population Health and Pathobiology, NCSU College of Vet Medicine, Raleigh, NC 27606.

Oxidative damage is hypothesized to be an underlying cause of many chronic disease states. Acutely, oxidative damage can lead to irreversible cell injury and pathological consequences. Reactive oxygen species, while naturally occurring at low levels in biological systems, can also be brought about by severe environmental conditions or by chemical contaminants. We evaluated the oxidative effects of PCBs in *Corbicula* clams, as possible surrogates for endangered bivalve mollusks. The Ward Transformer site in Wake County, North Carolina was found to have released Aroclor 1260 in quantities sufficient to cause measured levels as high as 1.7 mg/kg in fillets from fish collected miles downstream from the source. Concern over the effects of PCB exposure to the native wildlife, especially at-risk populations of native bivalves, prompted the hypothesis that oxidative damage due to Aroclor exposure would cause changes in biomarkers and pathology associated with decreased health status. We hypothesized that exposure to Aroclor 1260 via the water column would cause alterations in oxidative biomarkers

and visual pathological indices. Specifically, we conducted a laboratory study to determine the effects of Aroclor 1260 in *Corbicula fluminea* clams in order to validate common biomarkers of oxidative damage without confounding environmental factors. Secondly, we performed field deployments of *Corbicula* into the polluted Brier Creek system to gauge the oxidative status of animals in the field relative to a downstream distribution of Aroclor 1260. Results from the laboratory study at 0, 1, 10, and 100 ppb indicated that exposures were detrimental to the clams. Antioxidant biomarkers responded significantly at 3 weeks exposure and morphologic changes included anasarca, Brown cell accumulation, severe gonad atrophy, inflammation, and necrosis in both the field and the laboratory. These studies indicate that oxidative damage, as a result of exposure to Aroclor 1260, is sufficient to cause negative effects in bivalves.

T4

BIOMONITORING OF REACTIVE OXYGEN SPECIES IN BIOLOGICAL FLUIDS
Brooke L. Heidenfelder, U.S. EPA, Human Studies Division, MD 58C, RTP, NC 27711

Elevated levels of reactive oxygen species (ROS) are associated with several disease processes in humans, including cancer, asthma, diabetes, and cardiac disease. We have explored whether ROS can be measured directly in human fluids, and their value as a biomarker of exposure and/or effect. Using a simple chemiluminescent technique, we have measured ROS levels in a variety of human biological fluids including plasma, whole blood, milk, urine, and exhaled breath condensate. These measurements were taken from several populations, including healthy adults, new mothers, pediatric and adult asthmatics, and elderly adults exposed to arsenic in water. We evaluated: 1) the stability of ROS over time, 2) intra- versus inter-individual variation of ROS levels, 3) the effect of known modulators of ROS, 4) ROS levels in different biological fluids taken from the same individual (breast milk vs. plasma), and 5) relationships between ROS and disease endpoints (lung function measures and cancer). Our data suggest that ROS are detectable in a variety of human fluids, but the levels are dependent on age and several other endogenous factors. In order to understand the role of ROS in disease pathogenesis, or their value as an integrated measure of environmental exposures, we need to view ROS in the context of other biomarkers of exposure and effect. We believe that, with further validation, ROS measurement may be a biomarker of general health and early effects. (These findings do not necessarily represent EPA policy).

T5

PROINFLAMMATORY CYTOKINES INDUCE NO-MEDIATED DNA DAMAGE IN ARTICULAR CHONDROCYTES
C.M. Davies [1], J. Son [1], A. Chen [1], F. Guilak [2], J.B. Weinberg, [2], and **B. Fermor** [1] Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA. [2] Department of Medicine, VA and Duke Medical Centers, NC 27705, USA.

Articular cartilage is the connective tissue covering the ends of long bones, which provides frictionless joint movement and load distribution. Loss and degeneration of articular cartilage is characteristic of osteoarthritis (OA). The pro-inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF) are involved in the pathogenesis of OA. IL-1 or TNF contribute to up-regulation of inducible nitric oxide synthase (NOS2) and increased nitric oxide (NO) production. Patients with osteoarthritis have elevated levels of NO. To date, no one has

evaluated the effects of these cytokines on DNA integrity in articular chondrocytes. We evaluated the susceptibility of porcine articular chondrocytes to oxidative DNA damage. The aims were to determine whether 0.1-100 ng/ml IL-1α or TNFα could induce DNA damage, via up-regulation of NO or superoxide ($O_2^{\cdot-}$), and to determine the type of DNA damage that occurs. DNA damage was quantified using the comet assay, and oxidative DNA damage evaluated by the FLARE assay, using either 1μl/ml formamidopyrimidine-DNA glycosylase or endonuclease III. NO levels were determined using the Griess reaction. Cell viability was determined using the live/dead assay. A concentration dependent increase in DNA damage in response to IL-1 or TNF was observed ($P<0.01$, ≥1ng/ml), which was associated with a concentration dependent increase in NO production ($P<0.01$, ≥1ng/ml). Cell viability was greater than 95%. Both oxidative DNA strand breaks and base modifications occurred. Cytokine induced DNA damage was inhibited by a NO synthase inhibitor, or by superoxide dismutase (SOD) (the superoxide anion and peroxynitrite scavenger) ($P<0.01$), thus supporting roles of NO and superoxide in the mechanism of DNA damage. Furthermore, an NO donor (NOC-18, 250μM) or peroxynitrite generator (SIN-1, 250μM) caused DNA damage ($P<0.001$). Our data show that pro-inflammatory cytokines induce DNA damage via reactive oxygen and nitrogen species, suggesting that DNA damage may be involved in the pathogenesis of OA.

T6

IMMUNO-SPIN TRAPPING OF OXIDATIVELY GENERATED DAMAGE TO THE GENOME **Dario C. Ramirez**, Sandra E. Gomez-Mejiba, and Ronald P. Mason, Laboratory of Pharmacology and Chemistry, NIEHS, NIH, DHHS, 111 T. W. Alexander Dr., MD F0-02, Research Triangle Park, NC 27709. e-Mail: ramirez1@niehs.nih.gov

Oxidatively generated damage to DNA must be repaired to ensure the continuity of the cell's normal life. Most of the techniques used to study oxidatively generated damage to the DNA are based on the detection of final oxidation products such as 8-oxo-7,8-dihydro-2`-deoxyguanosine (8-oxo-dG). However, 8-oxo-dG can be repaired by cell glycosylases and/or excreted; and its usefulness as a biomarker has been questioned due its artifactual generation during sample work-up and analysis. We thought that by detecting DNA radicals it must be possible to achieve an early and more sensitive and specific characterization of the oxidatively generated damage to the genome than detecting final products of oxidation. To increase DNA radical lifetime the nitrone spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) can be used because it possesses low toxicity and a convenient pharmacokinetics in biological systems. DMPO accesses the cell and traps, *in situ* and in real time, DNA radicals in the nucleus and/or mitochondria of cells exposed to an oxidizing insult forming DMPO-DNA radical adducts (radical adducts). However, the analysis of DNA radicals and radical adducts is difficult in biological systems due to the time required to isolate the DNA from the biological matrix relative to the decay of parent radical(s) and/or radical adduct(s). Radical adducts decay to DMPO-DNA nitrone adducts (nitrone adducts), in which DMPO is stably bound to the DNA facilitating their extraction and analysis. Accordingly, we have developed a new technology to detect oxidatively generated damage to the DNA through the detection of DNA radicals called immuno-spin trapping (IST). IST involves three main steps: i) trapping, *in situ* and in real time, DNA radicals with DMPO, thus forming DMPO-DNA nitrone adducts (nitrone adducts); ii) purification of nitrone adducts; and iii) analysis of nitrone adducts by heterogeneous immunoassays using antibodies against DMPO. The sensitivity of the original IST analysis of DNA radicals (Nature Meth. 2006, 3:123-127) has been enhanced and applied to detect DNA radicals in known models of genotoxicity.

POSTER PRESENTATIONS

P1

DISTRIBUTION OF PROTEIN OXIDATIVE MODIFICATIONS BECOMES POLYMODAL UPON OXIDATION-INDUCED UNFOLDING Sanjay Venkatesh [1], Joshua S. Sharp [2] and Kenneth B. Tomer [2]; [1] Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 and [2] Laboratory of Structural Biology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709

Experimental strategies aimed at analyzing proteins through protein chemistry must ensure that modification events do not grossly alter the structure of the protein. The aim of this project was to develop a technique to quickly and easily identify any conformational changes to the protein by monitoring the rate of protein chemical modification. The accuracy of this technique was verified by examination of the corresponding circular dichroism spectra. Reactive sites of proteins are modified by chemical reagents at a rate based on a combination of their chemical reactivity and their accessibility to the modifying reagent. Modifications that cause the protein to unfold will result in a greater accessibility of other reactive sites, and therefore a higher rate of reaction. We hypothesize that changes in protein structure due to covalent modification of the protein will be reflected in a change in the rate of modification of the protein, which will be readily apparent in the distribution of modification states. In testing our hypothesis, we oxidized several small model proteins, lysozyme, β-lactoglobulin A, and ubiquitin, using hydroxyl radicals derived from ©-irradiation of water. The proteins were irradiated at different time points and then analyzed using electrospray ionization mass spectrometry and circular dichroism spectroscopy. The mass spectra of the proteins showed a shift from a uni-modal distribution of oxidation states to a poly-modal distribution as the irradiation time increased, reflecting a change in the rate of protein oxidation. Examination of the corresponding CD spectra showed that the appearance of poly-modal modification distributions corresponded with significant conformational changes in the proteins. These data indicate that there is a link between the distribution of oxidation states of a protein (and therefore, the apparent rate of oxidation) and the conformational changes of that protein, suggesting that monitoring of the rate of modification of proteins would be of use in determining if covalent modification strategies are causing conformational changes.

P2

RECOMBINANT EXPRESSION OF GLUTATHIONE REDUCTASE FROM *COWELLIA PSYCHREYTHREAEA IN ESCHERICHIA COLI* Mikyoung Lee Ji [1], Callie Barnwell [2], Alice Lee [1], Wendy Boss [3], Amy Grunden [1]; [1] Department of Microbiology; [2] Department of Zoology; [3] Department of Botany, North Carolina State University, Raleigh, NC 27695

Under certain environmental stresses such as exposure to extreme temperatures, radiation, and dehydration, plants face the challenges of protecting protein structure and preventing cellular damage. One method microbial extremophiles utilize under such conditions is the formation of adducts between thiol groups of the proteins and the small peptide, glutathione. Glutathione reductase reduces glutathione dimers to monomers. The reduced glutathione is then free to react

with sulfhydryl groups on proteins, forming adducts and stabilizing disulfide bonds. *Colwellia psychrerythraea* is a psychrophilic marine bacterium, which has been isolated from Arctic sea ice and grows at temperatures ranging from -6 to 19°C. Glutathione reductase (GOR) from *C. psychrerythraea* is stable at low temperatures and could be engineered into plants to aid in combating rapid changes in temperature and to protect against freezing and oxidative stress. *C. psychrerythraea* strain 34H was cultured in marine broth at 4°C for genomic DNA isolation. Two different constructs were designed to express the recombinant GOR protein. In the first construct, GOR was cloned into the pBAD/HisA expression vector, generating a recombinant *C. psychrerythraea* GOR protein with an N-terminal tag consisting of 6 histidine residues (His_6-GOR). Protein expression was optimized for transformed *E. coli* cells. The His_6-GOR protein was purified and enzyme assays were performed to verify the activity. For the second GOR expression construct, the IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) system was used. The IMPACT system vector (pTYB2) features an inducible self-cleavage activity of a protein-splicing element (termed intein) to separate the target protein from the affinity tag, resulting in the production of an untagged recombinant protein in a single chromatographic step. The *C. psychrerythraea* GOR was cloned into pTYB2 vector and over-expressed in *E. coli* strain ER2566. The *C. psychrerythraea* GOR protein was purified and enzyme assays were performed to measure activity.

P3

EFFECTS OF MECHANICAL STRETCH ON FETAL LUNG TYPE II EPITHELIAL CELLS **Kenny Esho** [1], Hyeon Soo Lee [2], Juan Sanchez-Esteban [2]; [1] North Carolina A&T State University, Greensboro, North Carolina, [2] Brown University Medical School, Providence, RI

Many premature infants require mechanical ventilation for survival. However, overdistension of the lung by mechanical ventilation can produce an inflammatory lung disease called Bronchopulmonary Dysplasia (BPD). The mechanisms by which mechanical ventilation leads to BPD are not fully understood. We hypothesized that mechanical stretch induces lung inflammation by increasing apoptosis, cytotoxicity and proliferation of fetal type II epithelial cells. In addition, we speculated that this inflammatory response is mediated by imbalance between pro- and anti-inflammatory cytokines. Fetal type II epithelial cells were isolated on day 19 of gestation (term=22) and plated on Bioflex plates coated with fibronectin. Monolayers were then exposed to cyclic mechanical strain to simulate mechanical ventilation, using the Flexercell 4000 Strain Apparatus. Non-strained cells were otherwise treated in an identical manner and served as controls. Cell proliferation was analyzed by DNA incorporation of the thymidine analog 5-bromo-2'-deoxyuridine (BrdU). Type II cell cytotoxicity was assessed by lactic acid dehydrogenase (LDH) release. Detection and quantification of apoptotic cells were performed using the terminal deoxynucleotidyl transferase-mediated dUTP-FITC nick-end labeling method and by fluorescence microscopy. Our results show that 20% mechanical stretch increases type II cell proliferation by 3-fold when compared to unstretched control samples (10% versus 3%, respectively). Similarly, mechanical stretch increases LDH release (14%) compared to unstretched samples (10%). In contrast, 20% mechanical strain does not affect apoptosis index. These findings suggest that mechanical distention of this magnitude induces necrosis of fetal type II cells, which in turn stimulates cell proliferation to restore normal cell population and alveolar structure caused by lung injury (overstretching).

P4

A STUDY OF VARIATIONS IN SOIL AND WATER CHEMISTRY OF SELECTED PONDS AT BROOKHAVEN NATIONAL LABORATORY Jamie S. Brungard [1], Nina S. Kean [1], **Georgia L. Sawyer** [1], Roy Coomans [1] and Timothy Green [2]; [1] North Carolina A&T State University, Greensboro, NC 27411, [2] Brookhaven National Laboratory, Upton, NY 11973.

Brookhaven National Laboratory (BNL), a 5,265-acre site, contains a variety of wetlands; included are coastal plain ponds, vernal ponds, recharge basins, and streams. Wetland habitats in Pine Barrens communities serve important ecosystem functions including providing critical habitat for the state endangered tiger salamander (*Ambystoma tigrinum*) and a number of other rare species. Survey techniques were used to gather information on soil and water chemistry of seven coastal plain ponds at BNL: four natural ponds, one man-modified pond, and two man-made ponds. Each pond was tracked using Global Positioning System (GPS) technology and mapped using ArcGIS. Five water samples were collected at each pond; nine soil samples were collected at five of the seven ponds. Water samples were analyzed for iron, sulfate, total chlorine, copper, aluminum, nitrate, phosphorus, tannin-lignin, suspended solids, hardness, total chromium, and molybdenum using HACH DREL/2000 and HACH CEL/890 water test kits. Soil samples were analyzed for pH, nitrate nitrogen, phosphorus, potassium, aluminum, ferric iron, magnesium, sulfate, calcium, and chloride using LaMotte soil test kits. Soil temperature, color, texture, structure, and consistency were also determined. A YSI 650 MDS meter with multi-probe was used to field-test water temperature, pH, dissolved oxygen, turbidity, and conductivity at each sample point. The pH and temperature of the soil around the natural ponds was significantly lower than that of the anthropogenic ponds. The pH of the water from the natural ponds was significantly more acidic and the tannin-lignin content significantly higher than that of the anthropogenic ponds. We propose that these differences in the soil and water chemistry of the ponds can be explained by the nature of the surrounding vegetation. The results of this study provide baseline data for monitoring pond health in the future and for assessing the suitability of ponds as breeding sites for tiger salamanders.

P5

A COMPARISON OF WATER CHEMISTRY BETWEEN NATURAL, MODIFIED, AND MANMADE PONDS WITHIN BROOKHAVEN NATIONAL LABORATORY **Priscilla D. Randolph**, Roy Coomans (North Carolina A &T State University, Greensboro, NC 27411), Timothy Green (Brookhaven National Laboratory, Upton, NY 11973)

Brookhaven National Laboratory (BNL) is located in the center of the Long Island Pine Barrens. Within BNL's 5,265-acre site there are numerous coastal plain ponds. These ponds provide valuable breeding sites for the State endanger tiger salamander (*Ambystoma tigrinum*). Water samples were collected from seven coastal plain ponds on BNL was tested: four natural, one man-modified, and two manmade. Five water samples were collected from each pond. An eTrex Vista Cx Global Positioning System was used to mark each water sample point. A Yellow Springs Instrument 659 MDS meter fitted with a multiprobe was utilized to determine temperature, pH, dissolved oxygen, conductivity, and turbidity at each sample point. Water samples were analyzed for sulfate, nitrate, iron, phosphorus, chlorine, calcium, magnesium, copper, tannin-lignin, chromium, molybdenum, aluminum, and suspended solids using Hach

DREL/2000 and CEL/890 water test kits. The pH in the anthropogenic ponds was found to be more basic than that of natural ponds. Phosphorous, tannin-lignin, and hardness (magnesium and calcium) were elevated in the natural ponds when compared to manmade and modified ponds, but only the difference in tannin-lignin content proved statistically significant. The natural ponds were shaded by the canopy of the surrounding forest while the manmade and modified ponds where located directly in the sun. This had an affect on water temperature. The results from the other 12 tests did not show a significant difference between ponds when compared as groups (Natural VS Manmade or modified). The results of this research will give environmental scientists an insight into water chemistry and interrelationships between abiotic and biotic factors and will enable BNL to optimize the management of amphibian and reptile habitats.

P6

EXPRESSION OF CDC13 AND RAP1 IN YEAST *KLUYVEROMYCES LACTIS*

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Telomeres are composed of repetitive, non-coding nucleotide sequences at the ends of chromosomes. Telomeres and the associated complex of telomeric DNA binding proteins, including CDC13 and RAP1, form a protective cap at chromosome ends that help maintain genome stability, and allow cells to distinguish natural chromosome ends from DNA double-stranded breaks (DSBs). The regulation of the size and maintenance of telomeres has implications in the development of human cancer cell lines, and human aging. The CDC13 and RAP1 proteins in the model yeast organism Kluyveromyces lactis are essential proteins that are involved in positive and negative regulation of telomeric length. We attempted to produce a plasmid that would over express the genes encoding CDC13 and RAP1 proteins. IPTG induction of the E.coli bacterial host strains yielded total protein fractions of expressed cloned genes. The proteins were harvested, and their expression levels determined by SDS-PAGE. CDC13 expression was abandoned due to time constraints and experiment delays. However, RAP1 protein expression was achieved although expression levels were low. Future experiments will include native protein purification procedures carried out on a nickel agarose gel, ultimately resulting in purified proteins that can be used for future binding assays with differing telomeric probes.

P7

METABOLIC ACTIVATION OF 5-METHYLCHRYSENE AND ITS DIOL IN V79MZ CELL STABLY TRANSFECTED WITH HUMAN CYP1B1 OR CYP1A1 WITH OR WITHOUT HUMAN GSTpi

Sarfaraz Ahmad, Sandra Leone-Kabler, Johannes Doehmer[1] and Alan J. Townsend Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC, USA and [1]GenPharm Tox., Munich, Germany.

5-Methylchrysene (5-MC), a methylated polycyclic aromatic hydrocarbon (PAH), is an environmental pollutant that is activated by phase I cytochrome P-450s (CYPs) to reactive electrophilic metabolites that are toxic, mutagenic, and carcinogenic. Glutathione-S-transferases (GSTs) are phase II enzymes that conjugate reactive electrophiles with glutathione to reduce their toxic effects.

The present study investigates the effects of competition between metabolic activation versus detoxification on the cytotoxicity and mutagenicity of 5-MC and its dihydrodiol intermediate (5-MC-1,2-diol) in V79MZ cells stably transfected with human CYP1B1 or hCYP1A1, either alone (V79MZh1B1 or V79MZh1A1, respectively) or together with transfected human GSTP1 (V79MZhCYP1B1 + hGSTP1-26 or V79MZhCYP1A1 + GSTp1-23, respectively). 5-MC cytotoxicity was modestly but significantly enhanced about 2-fold in V79MZhCYP1B1 cells as compared to the V79MZ control line. Co-expression of hGSTP1 in these cells did not significantly protect against 5-MC toxicity. The toxicity of 5-MC was similar to the parent V79MZ line in cells expressing hCYP1A1, without or with hGSTP1 expression. In contrast, 5-MC-1,2-diol (a dihydrodiol metabolite of 5-MC) was > 20-fold more cytotoxic in cells expressing either hCYP1B1 or hCYP1A1 as compared to control V79 MZ cells. Cells expressing both hCYP and hGSTP1 were approximately 3- to 4-fold less sensitive to 5-MC-1,2-diol toxicity (IC_{50} in hCYP1B1+GSTpi-26 = 0.164±0.018 or hCYP1A1+GSTpi-23 = 0.136±0.034 μM) as compared to cells expressing only hCYP1B1 or hCYP1A1, respectively (IC_{50} = 0.058 ± 0.007 μM, or 0.035 ± 0.008 μM, respectively). While neither 5-MC nor 5-MC-1,2-diol were mutagenic in V79MZ control cells (0-5 mutants), both 5-MC and 5-MC-1,2-diol were mutagenic in cells expressing hCYP1B1 or hCYP1A1 (336 ± 48 vs. 500 ± 45 mutant colonies per million cells, respectively). In contrast, 5-MC-1,2-diol was more mutagenic in hCYP1B1 cells (650±18 mutants) as compared to hCYP1A1 cells (272±23 mutants). Either 5-MC or 5-MC-1,2-diol were relatively 2-4 fold less mutagenic in cells co-expressing either hCYP together with hGSTP1. These results demonstrate that the 5-MC-1,2-diol metabolite is considerably more cytotoxic and mutagenic than the parent compound (5-MC) in cells expressing hCYP1B1 or hCYP1A1, and that that hGSTP1 expression can play a significant role in detoxification of 5-MC-1,2-diol.

P8

OVEREXPRESSION OF *PYROCOCCUS FURIOSUS* SUPEROXIDE REDUCTASE IN ARABIDOPSIS ENHANCES HEAT TOLERANCE Yang Ju Im [1], Mikyoung Ji [2], Alice Lee [2], Amy Grunden [2] and Wendy Boss [1], [1] Department of Plant Biology and [2] Department of Microbiology, North Carolina State University, Raleigh NC

Reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals, are produced as a part of basal metabolism and in response to environmental cues. ROS can serve as signaling molecules; however, the accumulation of ROS causes damage to living organisms. *Pyrococcus furiosus* superoxide reductase (SOR) is a thermostable archaeal enzyme that reduces superoxide without producing molecular oxygen unlike plant superoxide dismutases (SOD). Our hypothesis was that SOR which is not found in plants would provide a more effective ROS reducing system. We overexpressed *P. furiosus* SOR in Arabidopsis and produced a functional protein. Importantly, producing *P. furiosus* SOR increased heat tolerance. Specifically, SOR transgenic seeds had a 4-fold higher germination rate after exposure to 45 °C for 5 hours. In addition, dark grown seedlings tolerated a heat treatment of 48 °C for 30 min. Our results indicate that plants producing the functional *P. furiosus* SOR have enhanced heat tolerance without affecting plant growth and development.

P9

USING *IN VIVO* DNASE I FOOTPRINTING TO MEASURE QUANTITATIVE CHANGES IN TRANSCRIPTION FACTOR BINDING FOLLOWING IN UTERO PHTHALATE TREATMENT **A.J. Kuhl**, S.M. Ross, and K.W. Gaido, CIIT Centers for Health Research, 6 Davis Dr. RTP, NC 27709

Often exposure to environmental chemicals can induce changes in gene expression leading to a variety of developmental and physiological problems. Previous work in this laboratory and others has demonstrated that in utero exposure to phthalate esters reduced transcription of enzymes involved in testosterone biosynthesis. Reduced testosterone levels during a critical period of male reproductive development likely leads to an observed feminized phenotype characterized by malformed epididymides, hyposipadias, cryptorchism, and retained thoracic nipples among others. Understanding the underlying mechanism associated with phthalate esters will aid in assessing the human risk to these chemicals. Traditional methods for analyzing protein-DNA interactions include *in vivo* footprinting and chromatin immunoprecipitation (ChIP). However, ChIP does not provide binding location, and conventional footprinting is too insensitive for comparing protein binding in toxicological studies. In this study, *in vivo* DNase footprinting was adapted for use with the automated DNA sequencer to provide a semi-quantitative map of changes in DNA-protein interactions in the proximal promoter region of the steroidogenic acute regulatory (StAR) protein. StAR is the rate-limiting step in the testosterone biosynthesis pathway and is down-regulated following in utero di-butyl phthalate (DBP) treatment in rats through an unknown mechanism. *In vivo* footprinting identified four regions of altered DNase digestibility following DBP treatment at four locations. ChIP assays confirmed changes in protein binding levels of SF-1 and c/ebp β and suggest a disruption of cooperation between these factors is involved in DBP induced transcriptional changes. When combined with ChIP, the novel footprinting assay outlined here provides a map of *in vivo* protein-DNA interactions and changes in the structure of transcriptional machinery following an exogenous chemical treatment.

P10

THE TIME COURSE FOR EXPRESSION OF NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELL GENES INVOLVED IN SPECIFIC BIOLOGICAL PATHWAYS FOLLOWING EXPOSURE TO CIGARETTE SMOKE **R. Parsanejad** [1], W.R. Fields [1, 2], W. T. Morgan [2], B.R. Bombick [2], D.J. Doolittle [1, 2], [1] Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC, [2] Research and Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC 27105

Specific gene expression changes have been identified as indicators of inflammation, oxidative stress and DNA damage. Previously, our laboratory reported induction of GADD45, p21, COX-2, IL-8, HO-1 and HSP70-1 in NHBE cells exposed to smoke. Currently, our objective is to evaluate the temporal expression of genes associated with specific injury and repair pathways following cigarette smoke exposure. NHBE cells were exposed to whole smoke (0.03/0.12mgWTPM/L:1h) generated from Kentucky Reference 2R4F cigarettes under Federal Trade Commission conditions, followed by gene expression analysis 1, 4 and 24 hours after exposure. The expression of 84 genes, representing 18 signal transduction pathways was

examined by real-time quantitative PCR and PCR arrays. A confidence score (CS), calculated based on statistical analysis of the degree and reproducibility of changes in expression, was used to assess the significance of observed changes. Overall, 28 genes exceeded the critical CS, indicating that a broad range of genes was affected by the smoke. One hour after exposure, the CS was exceeded for 4 and 12 genes at the low and high levels of exposure, respectively; at 4 hours after exposure, the CS was exceeded for 4 and 11 genes, respectively. Twenty-four hours after exposure, the CS was exceeded for 0 and 5 genes, respectively. Stimulation of NIAP, an apoptosis inhibitor, suppression of NFKB1 and MYC, representing pro-apoptotic activity, and down-regulation of TCF7 and up-regulation of KLK2, representing anti-proinflammatory responses, respectively, were altered 1 hour after exposure. Stimulation of genes, including TNF, LTA, SELPLG and IL-8, representing inflammatory response, MMP-10, representing repair and wound healing activity, and GREB1 and EGR1, representing growth activity, exceeded the CS 4 hours after exposure, suggesting tissue repair in this period. While most genes returned to baseline expression 24 hours after exposure, IL-8 and SELPLG remained elevated suggesting activity of inflammatory signaling in this period.

P11

A UNIQUE ERROR SIGNATURE FOR HUMAN DNA POLYMERASE ν

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Human DNA polymerase ν is one of three A family polymerases conserved in vertebrates. Although its biological functions are unknown, pol ν has been implicated in DNA repair and in translesion DNA synthesis (TLS). Pol ν lacks intrinsic exonucleolytic proofreading activity and discriminates poorly against misinsertion of dNTP opposite template thymine or guanine, implying that it should copy DNA with low base substitution fidelity. To test this prediction and to comprehensively examine pol ν DNA synthesis fidelity as a clue to its function, here we describe human pol ν error rates for all 12 single base-base mismatches and for insertion and deletion errors during synthesis to copy the *lacZ* α-complementation sequence in M13mp2 DNA. Pol ν copies this DNA with average single-base insertion and deletion error rates of 7×10^{-5} and 16×10^{-5}, respectively. This accuracy is comparable to that of replicative polymerases in the B family, lower than that of its A family homolog, human pol ν, and much higher than that of Y family TLS polymerases. In contrast, the average single base substitution error rate of human pol ν is 3.5×10^{-3}, which is inaccurate compared to the replicative polymerases and comparable to Y family polymerases. Interestingly, the vast majority of errors made by pol ν reflect stable misincorporation of dTMP opposite template G, at average rates that are much higher than for homologous A family members. This pol ν error is especially prevalent in sequence contexts wherein the template G is preceded by a C-G or G-C base pair, where error rates can exceed 10%. Amino acid sequence alignments based on the structures of more accurate A family polymerases suggest substantial differences in the O-helix of pol ν that could contribute to this unique error signature.

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