UNIVERSITY of ALASKA ANCHORAGE

UNIVERSITY OF ALASKA FAIRBANKS
Biochemistry and Molecular Biology Program

Second Annual University of Alaska Biomedical Research Conference

May 22-23, 2006

University of Alaska Anchorage

Second Annual
University of Alaska Biomedical Research Conference

Organizer: Jocelyn E. Krebs

Sponsored by:

UNIVERSITY of ALASKA ANCHORAGE

UNIVERSITY OF ALASKA FAIRBANKS
Biochemistry and Molecular Biology Program

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UABRC Schedule of Events

Monday May 22

8:00 am: Breakfast (Creekside Eatery)

9:00 am: Welcome and introductory remarks (Commons 107)

9:15 am-12:00 pm: Morning presentation session

Cindy Knall, University of Alaska Anchorage Biomedical Program
Characterization of Altered Gene Expression in the Lungs of Mice Exposed to Cigarette Smoke: Resistance Versus Sensitivity to Emphysema

Anshul Pandya, University of Alaska Fairbanks
Allosteric Potentiation of Neuronal Nicotinic Receptors

David Robinson, University of Alaska Anchorage
Posttranslational modification of histone H2A during repair of DNA double-strand breaks in *Saccharomyces cerevisiae*

Manuela Schmoll, University of Alaska Fairbanks
Translocation of Yeast Flavohemoglobin into the Mitochondrion

20 minute break

Sayali D. Kulkarni, University of Alaska Fairbanks
Effect of Oxidative Stress on Actin Cytoskeleton.

Njideka Chukwu, University of Alaska Fairbanks
Sublethal and Lethal Arsenic Exposure and Protein Oxidation in Neurons

Oya Yazgan, University of Alaska Anchorage
Autoregulation of the *CUP1* gene in *Saccharomyces cerevisiae*

12:00 pm-1:00 pm: Lunch (Commons 106)

1:00 pm-2:00 pm: Plenary Speaker Dr. Amy Bernard, Allen Institute for Brain Science, Seattle WA
"Refining Cellular Neuroanatomy Through a Genome-Wide Analysis of Gene Expression"

Afternoon presentation session (Commons 107)

Sreepurna Malakar, University of Alaska Anchorage and Fairbanks
Role of ISWI-Dependent Chromatin Remodeling Complexes in Development in *Xenopus laevis*

Brian Barth, University of Alaska Fairbanks
Ceramide Mediates Tumor Necrosis Factor Alpha-Induced NAD(P)H Oxidase Activation and Consequential Oxidative Damage to the Neuronal Actin Cytoskeleton

Carol Jones, University of Alaska Anchorage
Molecular Toxicology of Environmental Contaminants—Developing an Alaska Perspective

20 minute break

Abraham E Harms-Smyth, University of Alaska Fairbanks
Acetylcholine-Binding Protein (AChBP) as a Model for Predicting the Binding site structure and Function of Ligand-Gated Ion Channels.

Ileana Bembenek, University of Alaska Anchorage and Fairbanks
TCDD and HCB Effects on BRCA1 Gene Expression and the Role of BRG1 in Modulating These Effects in MCF-7 and MDA-MB-231 Cells

Robert A, Furilla, University of Alaska Anchorage Biomedical Program
Teaching in the Biomedical Sciences using Computer Simulations

4:00 pm-7:00 pm: Poster session and reception (Commons 106)

Tuesday May 23

8:00 am: Breakfast (Creekside Eatery)

9:00 am-11:30 am: Morning presentation session (Commons 107)

Lynne Lucher, Alaska State Public Health Laboratory
Clinical Diagnosis of Pertussis using Real-Time PCR to Discriminate between *Bordetella pertussis* and *Bordetella holmseii*.

Stephanie Massay, Alaska State Public Health Laboratory
Analytical and Clinical Comparisons Using Alaska Pertussis Test Data to Assess Test Validity

Lesa D. Hollen, University of Alaska Fairbanks
Heterogeneous Warming from Hibernation Monitored by Forward Looking Infrared Radiometer

Break

Jestina F. Kusina, University of Alaska Fairbanks
Analyzing Biomolecular Interactions using SPR Technology: Biacore 2000

David Freistroffer, University of Alaska Anchorage
Translational control of gene expression by eIF3

11:30 am-12:00 pm: Presentation of awards for best talk and best poster

12:00 pm: Lunch (Creekside Eatery)

Conference adjourns

SPEAKER ABSTRACTS

KEYNOTE SPEAKER
Dr. Amy Bernard

Refining Cellular Neuroanatomy Through a Genome-Wide Analysis of Gene Expression.

Amy Bernard
Allen Institute for Brain Science, Seattle, WA

The complete sequencing of the mouse genome has allowed a scaling of histological techniques in ways that have great importance for our understanding of the functional cellular anatomy of the brain. The Allen Brain Atlas project has taken the approach of using high-throughput RNA *in situ* hybridization to produce a genome-wide, cellular-resolution gene expression atlas of the adult mouse brain. This data is freely available through an open-access web portal, **http://www.brainatlas.org**. Analysis of this expansive dataset reveals tremendous cellular diversity and combinatorial complexity of transcriptional regulation that give rise to phenotypic differences between distinct neuronal populations. Certain genes show specificity for discrete cell populations, which lends great potential for targeted analysis and genetic manipulation of these cell types. Furthermore, novel divisions of cytoarchitectural fields revealed by cellular mRNA distribution suggest that there are functionally relevant subdivisions in a variety of brain regions that are not well defined. Finally, the cellular and subcellular localization of transcripts may facilitate predictions of gene function and neuronal connectivity on a molecular level. Thus, Allen Brain Atlas provides valuable resource for the scientific community through its scale and accessibility. The preliminary findings presented in this talk demonstrate that the Allen Brain Atlas is a powerful tool that should facilitate research into understanding the mammalian brain, in both normal and diseased states.

Ceramide Mediates Tumor Necrosis Factor Alpha-Induced NAD(P)H Oxidase Activation and Consequential Oxidative Damage to the Neuronal Actin Cytoskeleton

Barth, B.M.[1,2], LaVictorie, D.L.[1], and Kuhn, T.B.[1,2,3]
[1]Department of Chemistry and Biochemistry, University of Alaska Fairbanks
[2]Alaskan Basic Neuroscience Program
[3]Institute of Arctic Biology

Acute and chronic disorders of the central nervous system (CNS) are very debilitating. Recently, sphingolipids have been identified as important mediators of various signaling pathways linked to both degeneration and survival. Specifically, in many cell types, ceramide has been implicated as a negative regulator of cell function, and as a potent inducer of apoptosis and oxygen radical formation in response to various timuli. Additionally, the NAD(P)H oxidase is a key generator of reactive oxygen species (ROS), and may play a role in oxidative damage to critical components of neurons such as the cellular actin cytoskeleton.

Tumor necrosis factor alpha (TNFα) and ceramide were evaluated for their potential to impede cellular motility in a serum-stimulated assay in SH-SY5Y human neuroblastoma cells. The production of ROS was monitored utilizing a redox-sensitive fluorescent dye assay. Actin was immunoprecipitated and carbonylation was detected by western blotting against a 2,4-dinitrophenylhydrazine modification using dinitrophenyl-KLH specific primary antibodies. Western blotting and membrane labeling was also used to identify stimulus-dependent NAD(P)H oxidase subunit activity.

Together, these results implicate ceramide as a key intermediate of TNFα-induced NAD(P)H oxidase activation and subsequent oxidative damage of the neuronal actin cytoskeleton. Thus, inflammation associated with acute and chronic CNS insults could directly contribute to the failure of axon regeneration. A more detailed understanding of this molecular pathway in primary neurons would allow for the development of more specific treatments and approaches to improve axonal regeneration and neuronal connectivity.

[Supported by NIH grant number U54 NS41069 (SNRP: NINDS, NIMH, NCRR, NCMHD)]

TCDD and HCB Effects on BRCA1 Gene Expression and the Role of BRG1 in Modulating These Effects in MCF-7 and MDA-MB-231 Cells

Ileana Bembenek [1,2], Jocelyn E. Krebs [2], Carol Jones [2]

[1]Dept. of Chemistry and Biochemistry, University of Alaska Fairbanks
[2]Department of Biological Sciences, University of Alaska Anchorage

The purpose of this study is to determine if hexachlorobenzene (HCB) and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), both aryl hydrocarbon receptor (AhR) ligands, can contribute to breast cancer incidence/progression. We hypothesize that these POPs inhibit expression of the breast cancer gene BRCA1 (invoved in cell cycle and DNA repair), by altering the chromatin remodeler BRG1.

BRCA1 is up-regulated in response to 10nM estradiol (E2) in estrogen receptor positive (ER+) cell line MCF-7 at both transcriptional and protein level (determined by RT-PCR and immunofluorescence microscopy, respectively). Treatment of MCF-7 cells with TCDD (1nM and 10nM) results in a dose dependent downregulation of E2-induced BRCA1 mRNA. Immunofluorescence microscopy reveals that TCDD treatment decreases both basal and E2-induced BRCA1 protein levels. Increased BRG1 protein levels are observed with TCDD exposure, but not with HCB exposure. Neither BRCA1 nor BRG1 were altered in MDA-MB-231 (ER-) cells upon TCDD exposure

Our results suggest that activation of the AhR pathway by the strong ligand TCDD, inhibits estrogen-induced expression of BRCA1 transcription in ER(+) MCF-7 but not in the ER(-) MDA-MB-231 cells. While the weak AhR ligand, HCB, does not alter BRCA1 or BRG1 at the concentrations examined, its participation in synergistic AhR activation remains to be determined.

Acknowledgement:

This work was supported by grants from NIH through the Alaska INBRE program

Sublethal and Lethal Arsenic Exposure and Protein Oxidation in Neurons

Njideka Chukwu
University of Alaska Fairbanks

Environmental toxins are linked to neurodegeneration and abnormalities in CNS development. The research will focus on arsenic and its suspected effects on neuronal cells by exposing neuronal cells to sublethal (chronic) and acute toxic levels of the toxin. The aim is to find biomarkers that are indicative of metal stress in the developing CNS. The cells will be tested for protein oxidation and possible NOX activation that would indicate active toxicity of arsenic. The oxidized proteome will be compared to that of other metal toxins which are passively toxic. Arsenic has been shown to affect several pathways including that of cell survival, apoptosis, ROS generation, and protein oxidation. Another aspect of the research will be to test the beneficial effects of dietary uptake of polyphenols from blueberry extracts by analyzing how neuroprotective they are against metal-induced ROS production.

Teaching in the Biomedical Sciences using Computer Simulations.

Robert A. Furilla
WWAMI Biomedical Program, University of Alaska Anchorage

Teaching is traditionally a didactic process, putting students in a passive mode in which they listen and take notes. Over the years, I have been developing computer simulations of physiological processes. I expected students to use these models, extracting the wealth of information included within. They did not. I set up a computer lab with a set of objectives for students to follow. Again, little educational value was achieved. To use simulations effectively, students must develop and test hypotheses, but most students are not trained to think in this way, so I brought the simulations into the lecture and set up hypotheses for discussion. To do this without increasing contact hours, half of the Power Point presentations were discarded, and replaced with discussions based on the models. After using this approach, I compared the results of a national standardized exam (Shelf Test). There was a significant improvement in test scores from the year in which the models were used compared with the previous year, and compared with the national average. Those sub-disciplines in which models were not used showed no significant improvement. Models can be used effectively to promote active learning, but require a complete restructure of the lecture format.

Acetylcholine-Binding Protein (AChBP) as a Model for Predicting the Binding site structure and Function of Ligand-Gated Ion Channels.

Presenting Author: Abraham E Harms-Smyth

Coauthors: Jestina F Kusina, Brian Guritz, Chelsea D Paskvan, Anshul Pandya, Marvin K Schulte

Abstract:

The recently discovered acetylcholine binding protein (AChBP) shares substantial amino acid-sequence homology with the pentameric cys-loop family of Ligand-Gated Ion Channels (LGIC`s). AChBP was recognized as a potential model for predicting the binding site structure and elucidating the mechanism for ligand binding to LGIC`s. Its crystal structure has been used as a model to predict the binding site for serotonin type 3 Receptors (5-HT3R) by our lab. Several ligands that bind to LGIC`s were also found to bind to AChBP. We expressed an intact pentameric AChBP, and we have seen that the nicotinic acetylcholine receptor (nAChR) ligand epibatidine binds to it using Scintillation Proximity Assay. Preliminary data using Surface Plasmon Resonance show that the nAChR antagonist D-tubocurarine and nAChR allosteric potentiator physostigmine also bind to our AChBP. We hypothesize that AChBP can be used as a sensor molecule for developing biosensors and assays that can rapidly identify drugs that interact with LGIC receptors. This project is the first step in a long term project to engineer binding proteins that resemble other LGIC receptors, which could be used for modeling LGIC's, screening new drugs, and form the basis for biosensor chips to detect LGIC regulators in the body or in the environment.

Authors' Affiliation: Department of Chemistry & Biochemistry, University of Alaska, Fairbanks.

Acknowledgments:

This work is supported by Alaska Idea Network for Biomedical Research Excellence (INBRE).

Heterogeneous Warming from Hibernation Monitored by Forward Looking Infrared Radiometer

L.D. Hollen*[1], J. Dehn[2] , M. Aoki[3], K.L. Drew[1].

[1]*Alaska Basic Neuroscience Prog., Inst. of Arctic Biology,* [2]*Alaska Volcano Observatory, Geophysical Inst.,*

[3]*Arctic Region Supercomputing Center, Univ. of Alaska Fairbanks, Fairbanks, AK 99775.*

Arctic Ground Squirrels (AGS) experience large fluctuations in temperature during the course of hibernation (0°-37°C). The purpose of the present study was to test the hypothesis that anterior regions of the body re-warm prior to posterior regions. To visualize thermal gradients during arousal from hibernation we used infrared thermography, a *novel* technique that allows direct measurement of the concentration of thermal energy by epidermal emissivity. The forward looking infrared radiometer (FLIR Systems Inc Portland, OR), an infrared digital imager was used to measure temperature changes (±0.2°C). In one animal, monitoring of core body temperature and ECG (TA11CTA-F40 transmitter), Data Quest IV (Data Sciences, St Paul, MN), and respiratory rate (videography) was used to create a multi-media representation. Data was acquired every 30 sec (~3hr), during the four stages of arousal. A statistically significant steep thermal gradient occurred between the thoracic and abdominal regions during re-warming ($p<0.001$, $n=5$). Since the head region has no known mechanism of thermogenesis, these results suggest warmed blood is preferentially shunted to the head, where the brain rapidly re-warms by convection of thermal energy from arterial blood to tissues.

Acknowledgments: Oivind Toien, PhD. Institute of Arctic Biology, Bill Brody, PhD. Arctic Region Supercomputing Center, Funded by NIH NINDS-Grant #331424-65878

Characterization of Altered Gene Expression in the Lungs of Mice Exposed to Cigarette Smoke: Resistance Versus Sensitivity to Emphysema.

Cindy Knall[1,2], Lois Herrera[2], James Aden[2], Thomas March[2], Susan Boggs[2].
[1]WWAMI Biomedical Program and Department of Biology, University of Alaska Anchorage, Anchorage Alaska & [2]Division of Pathophysiology, Lovelace Respiratory Research Institute, Albuquerque, NM.

Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the US. Although 90% of COPD cases are directly linked to cigarette smoking, the underlying mechanism by which cigarette smoking causes COPD is unknown. Utilizing a mouse model of smoke induced emphysema, we have defined a signature gene set which distinguishes a progressive from a resistant emphysema phenotype. Female strain C3H/HeJ, C57Bl6/J and B6C3F1 mice were exposed for 6h/day, 5days/wk to 250 mg TPM/m^3 of mainstream cigarette smoke or filtered air for 16 weeks. Lung tissue was isolated for both pathology and molecular analysis. Emphysema was established by mean linear intercept and surface area to lung volume ratio analyses. Glass microarrays were generated by the EBL-UNM using 70mer-oligonucleotides from Qiagen Operon to cover the entire mouse genome. Total RNA was isolated from filtered air controls and smoke exposed lung tissue. Competitive hybridization using Cy3 or Cy5 labeled cDNA was performed using a pair-wise multi-ringed strategy for maximal statistical efficiency. Genomic data sets were analyzed using both math/stat analysis and functional biological mapping. Altered gene expression patterns in the susceptible mouse strain are indicative of generalized epithelial damage/repair processes contributing to the molecular mechanism of emphysema induction.

The presenter acknowledges funding by LRRI, the State of NM, the NIH and HHMI.

Effect of Oxidative Stress on Actin cytoskeleton.

Sayali D. Kulkarni, Jestina F. Kusina, Marvin K. Schulte, Thomas B. Kuhn
Alaska Basic Neuroscience Program, Institute of Arctic Biology, University of Alaska Fairbanks, Ak 99775.

Reactive oxygen species (ROS) strongly contribute to the pathologies associated with many disorders (diabetes, inflammatory bowl syndrome, Alzheimer, Parkinson, HIV) as well as environmental toxicity. ROS inflict extensive damage to membrane lipids, proteins, nucleic acids and carbohydrates through oxidative modifications. The integrity and dynamics of the actin cytoskeleton is critical for the morphology, contractility and motility of all cell types. We hypothesize that oxidation of actin monomers decreases the rate of assembly into filaments.

Protein carbonylation is a hallmark of oxidative stress. Protein carbonyls in actin were revealed by immunocytochemistry after exposure of the actin monomer and polymer pools to hydrogen peroxide. To study kinetic parameters of actin dynamics in detail, we have employed surface plasmon resonance, which uses monomeric actin molecules covalently bound to a sensor chip. Immobilized actin monomers permit rapid evaluation of the interaction of antibodies, binding proteins and actin-actin interactions. Individual actin monomers are covalently bound to the chip surface by various methods.

Moreover, we will determine the effect of actin oxidation on the kinetic parameters of actin assembly. Simultaneously the specific amino acid residues oxidized will be identified by mass spectrometric analysis.

Title: Analyzing Biomolecular Interactions using SPR Technology: Biacore 2000

Presenting author: Jestina F. Kusina[1]

Co-author: Marvin K. Schulte[2]

Abstract

Life is based on molecular interactions as multitudes of compounds bind to and modify each other, to form stable complexes. When interactions occur, all the principles of thermodynamics, dynamics and molecular structure and recognition come into play. As scientists continue discovering new proteins and input DNA sequences into databases, rapid methods to accurately characterize these interactions are needed. Commercial instruments for such purposes using surface plasmon resonance (SPR) technology, are available from BAICORE AB. SPR technology allows protein interaction analysis in real time. The technology defines the characteristics of proteins in terms of specificity, rates and affinity of interaction with other molecules and also provides sensitive, accurate concentration measurements. This is based on the ability of the biomolecule of interest to interact with a specific binding partner, and may therefore be more informative than generic measurement techniques. The reliability and success of this technology is built on three technologies: Sensor chip, microfluidics and SPR detection. Biomolecular interactions are fundamental to research, not only within life sciences, but also for drug discovery and development, and food analysis. The following article presents the basics of SPR technology, its components, applications and an insight into the advantage and shortcomings associated with the technology.

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Acknowledgements

Work is supported by INBRE.

Clinical Diagnosis of Pertussis using Real-Time PCR to Discriminate between *Bordetella pertussis* and *Bordetella holmseii*.

Gloria Kragness* and Lynne A. Lucher, Ph.D.**
*University of Alaska, Anchorage AK; **Alaska State Public Health Laboratory, Anchorage AK

A real-time polymerase chain reaction (PCR) assay to detect *Bordetella pertussis* in nasopharyngeal swab specimens is currently in use at the Alaska State Public Health Laboratory. This PCR test amplifies the most commonly used *B. pertussis* target, IS481, a high copy-number (~80 copies) insertion sequence. A highly homologous, IS481-like sequence is also present in *Bordetella holmseii*, and is amplified by the IS481 PCR. Because *B. holmesii* is rarely associated with upper respiratory infections in immunocompetent patients, the cross-reactivity of IS481-targeted PCR for pertussis testing has not been of much concern. However, a report from Massachussetts describing isolation of *B. holmesii* from patients with pertussis-like symptoms prompted us to develop a PCR assay specific for *B. holmesii*, to rule out cross-reactivity in IS481-positive PCR results. The *B. holmesii*-specific BhIS1001 PCR was used to analyze specimens that were previously positive by the IS481 PCR assay. From February 14, 2005 to February 6, 2006, a total of 120 specimens were IS481-positive; five of these specimens were also BhIS1001 positive. An additional PCR test is under development to confirm that the five IS481/BhIS1001-positive specimens did not result from *B. pertussis* and *B. holmesii* co-infection.

Title: Role of ISWI-Dependent Chromatin Remodeling Complexes in Development in *Xenopus laevis*

Presenting Author: Sreepurna Malakar

Co-author: Jocelyn E. Krebs

DNA in eukaryotic cells is in the form of chromatin, a highly compacted state that is necessary to fit into the nucleus. Chromatin consists of DNA wrapped around histone proteins to form nucleosomes. Imitation switch (ISWI) is an ATP-dependent chromatin remodeler, one of the group of enzymes with the ability to disrupt or alter the association between the DNA and histones or slide nucleosomes along DNA. These processes can facilitate the activation of genes by allowing the necessary transcription machinery access to the DNA or deactivate the genes by hiding necessary binding sites in the chromatin. There are at least four complexes that contain ISWI present in *Xenopus* oocytes: xWICH, xCHRAC, xACF, and one complex that has yet to be fully identified. Interfering with ISWI function can result in gastrulation defects, delayed development, eye malformations and the formation of cataracts. However, since ISWI is present in several complexes, we have begun to address which complexes are responsible for the observed phenotypes. We are determining the individual effects of ISWI-containing complexes through microinjection of *Xenopus* oocytes with either antisense mRNA, or Morpholino oligonucleotides to prevent expression of the subunits unique to different complexes.

Author affiliations: University of Alaska Anchorage and University of Alaska Fairbanks

Acknowledgements: This work is supported by NIH/NEI grant #EY016027-01

Analytical and Clinical Comparisons Using Alaska Pertussis Test Data to Assess Test Validity

Stephanie Massay, Alaska State Public Health Laboratory, Anchorage, AK

A retrospective descriptive study of pertussis laboratory data was conducted to assess test analytical and clinical validity of real-time polymerase chain reaction (PCR) and culture. Data (N=283) was obtained from routine clinical diagnostic specimens submitted for pertussis testing at the Alaska State Public Health Laboratory (ASPHL) from February to August 2005. Culture, the recognized laboratory "gold standard" was used as reference for the presence of *Bordetella pertussis* in analytical comparisons. Clinical symptoms provided on laboratory requisition forms were used to define case-patients using case definition criteria defined by the Centers for Disease Control and Prevention. Analytical sensitivity and specificity of PCR were 100% and 85.6%, respectively; the positive predictive value (PPV) and negative predictive value (NPV) were 13.0% and 100%, respectively. Clinical sensitivity, specificity, PPV, and NPV of PCR were 100.0%, 97.1%, 84.1%, and 100%, respectively, and 16.2%, 100%, 100%, 88.2%, respectively for culture. Results indicate that the new real-time PCR method available at ASPHL, offers improved diagnostic sensitivity over culture. PCR results when in correlation with patient history, can be used to confirm a case of pertussis infection.

Title: Allosteric Potentiation of neuronal nicotinic receptors

Presenting author: Anshul Pandya [1]

Co-authors: Chelsea Paskavan[1] Zsolt Bikádi, [2] and Marvin K. Schulte[1]

Abstract:

Neuronal Nicotinic acetylcholine receptors (nAChRs) are members of the ligand gated ion channel (LGIC) super family. Allosteric modulation is a common phenomenon in this type of receptors. In the nicotinic acetylcholine receptor allosteric potentiation offers a promising approach for receptor modulation which would be useful in the therapeutics of diseases such as Alzheimer's, Myasthenia Gravis, dementia and other cognitive disorders. The mechanism of allosteric potentiation is not known for nicotinic acetylcholine receptors. Understanding the mechanism of allosteric potentiation and the modeling of drug interactions with allosteric binding sites will facilitate development of a new class of nAChR drugs. Here we propose to identify the interaction of allosteric potentiators with the homomeric α7 heteromeric $\alpha 4\beta 2$ neuronal nicotinic receptors. Using site-directed mutagenesis and molecular modeling, we will develop models of the binding site on these receptors that will be used to synthesize new potentiating agents with enhanced specificity and potency. Finally with the aid of single channel patch clamp recording, we will monitor the effect of these agents on the conformational stability of the ion channel which will elucidate the mechanism by which these potentiating compounds influence the receptors.

Author affiliations

[1] Department of Chemistry and Biochemistry, University of Alaska Fairbanks.

[2] Department of Molecular Pharmacology, Institute of Chemistry, Chemical Research Center, Budapest, Hungary.

Acknowledgements:

This work was supported by Alaska INBRE.

Posttranslational modification of histone H2A during repair of DNA double-strand breaks in *Saccharomyces cerevisiae*

Presented by: David Robinson
Co-author: Jocelyn E. Krebs

The DNA in all cells is constantly being bombarded by chemicals, radiations, metabolic products, and physical stresses that can induce damage, the most serious of which is the double-strand break (DSB). To repair this damage, the cell must make changes to chromatin structure. Chromatin is the condensed form of DNA, which allows it to be packaged inside the nucleus. In order to study the changes in chromatin structure necessary for repair of DSBs, I will be designing a strain in which I can create DSBs in vivo that will be repaired solely by the non-homologous end-joining (NHEJ) pathway of repair, rather than by homologous recombination (HR). By using chromatin immunoprecipitation I will identify modifications made to chromatin during the repair of a double-strand break. Using a genomic library, I will perform a genetic screen to try to elucidate the specific pathways that lead to the phosphorylation of serine 122 of histone H2A, a modification known to be important in a number of DNA repair pathways. This screen will identify proteins which, when overexpressed, rescue the sensitivity to DNA damage seen in the H2A S122A mutant. I will confirm any direct roles in repair for proteins identified in this screen by performing chromatin immunoprecipitation analysis, to corroborate the presence of these enzymes at the site of DNA damage.

Affiliations: University of Alaska Anchorage
Acknowledgements: NSF MCB-0315816, Alaska EPSCoR

Translocation of Yeast Flavohemoglobin into the Mitochondrion

Manuela Schmoll, Lisa Smith, and Kristin O'Brien
University of Alaska Fairbanks

Although required for many cellular processes, nitric oxide (NO) is also the source of both oxidative and nitrosative stress. Hemeproteins, including flavohemoglobins play a role in consuming NO and thus serve to protect cells against high levels of nitrosative and oxidative stress. In *Saccharomyces cerevisiae*, yeast flavohemoglobin (YHb) is a single gene product yet it is found in both the cytosol and the mitochondrial matrix. The mechanism of YHb import into the mitochondrion is not known and it lacks an obvious mitochondrial targeting sequence. YHb localization to the mitochondrion is of special interest since the electron transport chain in the inner mitochondrial membrane is the main source of reactive oxygen species (ROS) in the cell. We are using co-immunoprecipitation (Co-IP) with anti-YHb antibody on cytosolic and mitochondrial fractions to identify proteins that interact with YHb and are required for YHb import into the mitochondrion. Preliminary results indicate that there are about ten cytosolic and four to five mitochondrial proteins that co-purify with YHb using Co-IP. These proteins will be characterized using mass spectrometry and their effect on YHb translocation will be determined using knock-out mutants of these proteins.

This work is supported by AHA Beginning Grant-in-Aid to Kristin O'Brien (0560006Z)

WINNER: BEST ORAL PRESENTATION

Autoregulation of *CUP1* gene in *Saccharomyces cerevisiae*

Oya Yazgan and Jocelyn E. Krebs
Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK 99508

Abstract

The *CUP1* gene encodes a copper metallothionein (Cup1p), a protein involved in copper homeostasis. The transcription of *CUP1* is activated rapidly upon exposure of cells to copper. Cup1p removes excess free copper from the cellular environment, minimizing the deleterious effects of this toxic metal ion. However, if the expression of Cup1p is not turned off rapidly, it continues to bind all the copper ions in the cell. Complete removal of copper by the excess metallothionein leads to copper starvation and cessation of growth since a trace amount of copper is essential for normal functions of numerous proteins. Unlike the activation process, the mechanism of the shutdown is still not understood; therefore, we are working on shutdown of the *CUP1* gene. We are investigating Ruf5, an antisense RNA generated by the *CUP1* locus, as a candidate RNA involved in down-regulation of the *CUP1* gene. Real time RT-PCR assays suggest that Ruf5 transcript levels increase as the *CUP1* gene is being turned off, consistent with the idea that transcription from the *RUF5* gene might lead to shut down of *CUP1* expression through the transcriptional interference mechanism. We are currently identifying the key components that are involved in this process, and determining the role of chromatin remodeling/modification in regulation of *RUF5*.

Acknowledgements
Alaska EPSCoR

POSTER ABSTRACTS

POSTER: **Role of ISWI-Dependent Chromatin Remodeling Complexes in Development in *Xenopus laevis***

M. Anderson[1], S. Malakar[1,2] and J. E. Krebs[1]

[1]University of Alaska Anchorage
[2]University of Alaska Fairbanks

DNA in eukaryotic cells is in the form of chromatin, a highly compacted state that is necessary to fit into the nucleus. Chromatin consists of DNA wrapped around histone proteins to form nucleosomes. Imitation switch (ISWI) is an ATP-dependent chromatin remodeler, one of the group of enzymes with the ability to disrupt or alter the association between the DNA and histones or slide nucleosomes along DNA. These processes can facilitate the activation of genes by allowing the necessary transcription machinery access to the DNA or deactivate the genes by hiding necessary binding sites in the chromatin. There are at least four complexes that contain ISWI present in *Xenopus* oocytes: xWICH, xCHRAC, xACF, and one complex that has yet to be fully identified. Interfering with ISWI function can result in gastrulation defects, delayed development, eye malformations and the formation of cataracts. However, since ISWI is present in several complexes, we have begun to address which complexes are responsible for the observed phenotypes. We are determining the individual effects of ISWI-containing complexes through microinjection of *Xenopus* oocytes with either antisense mRNA, or Morpholino oligonucleotides to prevent expression of the subunits unique to different complexes.

POSTER**: Histone Modifications in UV Repair**

Yeganeh Ataian and Jocelyn E. Krebs
University of Alaska Anchorage

All organisms are constantly under threat from the cytotoxic and mutagenic effects of DNA damaging agents. These agents range from free radicals to a variety of chemicals and heavy metals encountered in food or as air and water-borne agents, ionizing radiations, and ultraviolet (UV) radiation. Failure to repair damaged DNA can lead to mutation, genomic instability, tumorigenesis, and cell death. Cells can use different methods of repairing DNA such as homologous recombination, non-homologous end joining, or nucleotide excision repair. All DNA transactions in the cell occur in the context of chromatin, which regulates transcription, DNA replication, and repair. A major means of regulating the structure of chromatin is through the modification of histone proteins. Studies of histone modifications, such as acetylation and phosphorylation, show that they play an early and important role in the cellular detection and response to DNA breaks and their repair. Here we report our study of the role(s) of histone modifications in UV-induced DNA repair pathway using genetic assays in the yeast *S. cerevisiae*. Our results show that histone code for UV-induced DNA damage is different from that which signals DNA double-strand breaks.

Acknowledgements: NSF MCB-0315816, Alaska EPSCoR

POSTER: **Regulation of Stress Response Genes in Brewer's Yeast**

R. Brewer, O. Yazgan and J. E. Krebs
University of Alaska Anchorage

All cells have mechanisms by which they respond to stressors, such as toxic metals, in their environment. This stress response includes transcriptional activation and regulation of specific stress response genes. The DNA has certain sequence elements and proteins that regulate this expression; however, DNA, which is normally compacted into nucleosomes, must modify its compaction to allow access to these genes. The *CUP1* gene encodes a copper metallothionein (Cup1p), a protein involved in copper level regulation. When cells are exposed to excess copper, *CUP1* transcription is quickly activated. Cup1p binds to the copper ions in the cell, thus limiting the exposure of the cell to the free copper ions. However, if *CUP1* transcription is not then turned off, Cup1p will bind all copper ions in the cell. Because trace amounts of copper are necessary for cellular activities, this can lead to cell death. Therefore, cells have developed a mechanism to rapidly shutdown transcription of the *CUP1* gene. We are investigating *RUF5* as a possible factor involved in down-regulating *CUP1* transcription. *RUF5* is a non-coding RNA transcribed at the same DNA location as Cup1p, though on the opposite strand. Therefore, *RUF5* transcription is likely to prevent *CUP1* transcription. These studies address how cells access specific stress response genes and which key components are involved in the process.

POSTER: **MK-801 Induces Arousal in Hibernating Arctic Ground Squirrels**

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Arctic ground squirrels (AGS; *Spermophilus parryii*),undergo hibernation. *N*-methyl-D-aspartate (NMDA) type glutamate receptors are thought to play a role in hibernation and respiratory control and glutamate may play a regulatory role in maintenance of torpor (Harris and Milsom, 2000). The purpose of the present study was to test the hypothesis that NMDA receptors play a role in maintenance of torpor in AGS. Saline or MK-801 (5 mg/kg, ip),), a non-competitive NMDA antagonist was administered on the 4^{th} day of a torpor bout (1mL/kg). Arousal was quantified from respiratory rate (breaths per minute, bpm) and from an arousal index based on a nominal scale of 0 to 6 where 0 was deep torpor indicated by a respiratory rate of less than 5 bpm and 6 was a fully active animal. Results showed that MK-801 induced arousal in all AGS tested ($p<0.05$, n=3 AGS) while saline injections did not evoke a statistically significant increase in respiratory rate or arousal index (n=3 AGS). Follow-up studies are planned to administer MK-801 into the lateral ventricle to test the hypothesis that arousal is induced via activation of central NMDA receptors (NMDAR) and to determine the dose-response relationship of MK-801-induced arousal following intracerebroventricular administration.

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Harris MB and Milsom WK, 2000, Is hibernation facilitated by an inhibition of arousal? In Life in the Cold (G. Heldmaier and M Klingenspor; Eds.) Springer-Verlag Berlin, pp241-250.

POSTER: **The Alaska State Public Health Laboratory: A Clinical Microbiology, Immunology, and Chemistry Resource for Bio-medical Research**

Dr. Lynne Lucher, Bonnie Bond, MS MT, Stephanie Massay, MS MT (ASCP), Alaska State Public Health Laboratory

The Alaska State Public Health Laboratory (ASPHL) collaborates with a wide variety of private, state and federal partners. An overview of the mission and capacities of ASPHL will be presented. The ASPHL maintains two facilities: the Anchorage laboratory, which specializes in clinical chemistry, bacteriology, parasitology, mycology, and is the only Select Agent licensed facility in the state; and the Fairbanks laboratory, which specializes in virology and immunology. Testing performed at ASPHL provides clinicians with diagnostic tools for patient care, and provides public health officials with disease surveillance support. Testing is also available to augment infectious disease and other health-related research projects conducted by other laboratories and agencies. Establishing and developing collaborative projects among Alaskan laboratories will strengthen the mission of all partners, and be beneficial to Alaskans.

POSTER**: Neuronal regeneration in the spinal cord after lesion during the permissive and late-permissive phase**

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Even though mature neurons retain their intrinsic ability to outgrow, lesions to the mature mammalian CNS normally result in an anatomical and functional failure of recovery. This is caused by inhibitory signals, i.e. the myelin sheet or myelin associated proteins from the surrounding environment and the formation of a glial scar as a secondary process following the primary lesion. In contrast, a distinct phase during neuronal development could previously be identified allowing anatomical and functional regeneration after neuronal lesion. This permissive phase is terminated with the onset of myelination. The aim of this project was to determine the neuronal ability of recovery after spinal cord lesion in chicken embryos during the permissive (E7) and the late-permissive (E10) phase before myelination occurred (E13). Therefore, fertilized eggs at a single cell state were incubated until the developmental stage E7 and E10 respectively. To injure the spinal cord, the chicken embryos were located in the eggs over an introduced window and injured at wing level with small spring-loaded scissors under sterile conditions. The injured embryos were further incubated for an additional six or 78 hours to allow the establishment of secondary processes and the potentially neuronal regeneration respectively. Chicken embryos at developmental stage E7, E10 and E13 were used as negative controls. The injured and non-injured chicken embryos were decapitated and dissected. The spinal cords were fixed in paraformaldehyde, frozen, sliced into 50 µm thick cryosections and stained with red fluorescent Nissl dye. No lesion could be found in the spinal cords of any developmental stages after further incubation of six and 78 hours. However, increased neuronal and non-neuronal cell death could be seen in the spinal cords and the surrounding tissue after incubation for an additional 78 hours compare to the non-injured and the additional six hour-incubated spinal cords.

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WINNER: BEST POSTER PRESENTATION

POSTER: **Identification of proteins involved in mitochondrial morphology**

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Antarctic icefishes (Channichthydae) are the only known vertebrates that lack hemoglobin (Hb) as adults. Moreover, some members of this family also lack myoglobin (Mb) in cardiac muscle. Previous studies have shown that the presence or absence of Hb and Mb in cardiac muscle is correlated with differences in the density and size of mitochondria, as well as with the density of the inner-mitochondrial membrane (cristae). Currently it is not known what regulates the distinct differences in mitochondrial morphology. Changes in mitochondrial protein expression among three species were analyzed to address this question. Mitochondrial proteins from *Notothenia coriiceps* (+Hb/+Mb), *Chaenocephalus aceratus* (-Hb/-Mb) and *Chaenocephalus rastrospinosus* (-Hb/+Mb) were separated using 2D polyacrylamide gel –electrophoresis. Gels were scanned and analyzed with Image Master Platinum 2D software. An average of 500 proteins were detected on each gel and 180 of these proteins showed interspecific differences in their expression levels. Further work will use MALDI-TOF and MALDI-TOF-TOF to identify these proteins.

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POSTER: **The Potential Role of Reactive Oxygen Species in Regulating Mitochondrial Biogenesis in Response to Cold Temperature**

G. Petersen and K. O'Brien, University of Alaska Fairbanks, Institute of Arctic Biology

Mitochondrial biogenesis occurs in response to cold temperature and exercise and is essential to the health of all organisms. We are using the three-spine stickleback, *Gasterosteus aculeatus* as a model organism to understand the molecular mechanisms that regulate mitochondrial biogenesis in response to cold temperature. *G. aculeatus* were acclimated from 20°C to 5°C and maintained at 5°C for 6 weeks. Mitochondrial densities are approximately 10% higher in the oxidative pectoral adductor muscle of *G. aculeatus* maintained at 5°C compared to those at 20°C. Moreover, within the first day of cold-temperature acclimation, the level of oxidized proteins in the pectoral adductor increases and then decreases by day three of cold temperature exposure. This indicates that reactive oxygen species (ROS) are produced in response to cold temperature and may stimulate mitochondrial biogenesis. We are currently measuring the expression level of genes known to regulate mitochondrial biogenesis and comparing the timecourse of their induction to that of ROS production. We are also examining the potential role of nitric oxide in this process by sequencing the isoforms of nitric oxide synthase and quantifying their expression level.

This work is supported by an EPSCoR YIFA (K.OB.) and an INBRE Undergraduate Research Fellowship to G.P.

POSTER: **Regulation of the Expression of Yeast Flavohemoglobin in *Saccharomyces cerevisiae***

L.Smith, R. Tawney, and K. O'Brien. University of Alaska Fairbanks

Yeast flavohemoglobin (YHb) is a nitric-oxide oxidoreductase expressed in *Saccharomyces cerevisiae*. Previous studies have shown that YHb protects cells against nitrosative stress, but its role in protecting cells against oxidative stress is controversial. Mutants lacking superoxide dismutase (*SOD1* and *SOD2*) have approximately seven-fold higher levels of YHb compared to wild-type cells. Levels of YHb in *sod1/ sod2* mutants are reduced in *sod1/sod2* mutants are treated with the antioxidant glutathione. Surprisingly however, mutants lacking *YHb1*, *SOD1* and *SOD2* show enhanced growth rates, and reduced levels of protein oxidation compared to mutants lacking only *SOD1* and *SOD2*. High levels of YHb in *sod1/sod2* mutants may be important for protecting these mutants from enhanced nitrosative stress, as these cells are more sensitive to the NO-donor DETA-NO compared to wild-type cells. Similarly, respiring wild-type cells that are producing reactive oxygen species (ROS) from the respiratory chain, are more sensitive to DETA-NO compared to fermenting cells. Together these data suggest that elevated levels of ROS induce the expression of YHb required to protect cells against an increased sensitivity to nitrosative stress when ROS levels are increased.

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POSTSER: Fluorescent Analyses of Asthma Associated Mucin Protein Expression After Exposure to Environmental Contaminants

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