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100th Annual Meeting

April 24, 2008

Grand Forks, North Dakota

EDITOR'S NOTES

HISTORY

The *Proceedings of the North Dakota Academy of Science* (NDAS) was first published in 1948, with Volume I reporting the business and scientific papers presented for the 40th annual meeting, May 2-3, 1947. Through Volume XXI**,** the single yearly issue of the *Proceedings* included both abstracts and full papers. Commencing with Volume XXII, the *Proceedings* was published in two parts: A, published prior to the annual meeting, contained an abstract of each paper to be presented at the meeting, and B, published later, contained full papers by some of the presenters.

In 1979 (Vol. 33) the *Proceedings* changed to an 8½ x 11-inch format. Produced from camera-ready copy submitted by authors, it was distributed at the annual meeting. Commencing with Vol. 51 submissions were on computer disk; the *Proceedings* was then assembled with desktop publishing software. This approach allows the Editor to format papers so as to assure the *Proceedings* a consistent look. This method also produces an electronic copy of the *Proceedings;* the Secretary-Treasurer has the capability to generate electronic copies of past issues.

VOLUME 62 ORGANIZATION

In 2003 the NDAS council voted to accept all abstracts scheduled for presentation at the Annual Meeting. Thus, communications in volumes 58 to present haven't undergone a "typical" peer review. Rather, they provide an accurate reflection of the material presented before the NDAS membership at the Annual Meeting. The presentations in this year's *Proceedings* are presented in three major sections. The first contains the undergraduate communications presented as part of the A. Rodger Denison Student Research Competition. The second section comprises the graduate Denison Competition papers, and the final section comprises professional communications presented by faculty members of the Academy. Readers may locate communications by looking within the major sections of these *Proceedings (see table of contents)* or by referring to the author index on page 91
.

SYMPOSIA COMMUNICATIONS

Commencing with the 88th Annual Meeting [Vol. 50], Symposia presenters at annual meetings have had opportunity to contribute full-length articles or multiple-page contributions, thus providing much greater depth and coverage than that ordinarily possible. Speakers have presented educationally-oriented lectures and workshop discussions, and have still provided rigorous, more technical professional papers to the *Proceedings.*

COLLEGIATE AND PROFESSIONAL COMMUNICATIONS

Each Collegiate and Professional presentation at the annual meeting is represented by a Communication. Designed as more than a typical abstract but less than a full paper. Communications report results and conclusions, and permit sharing of important data and conclusions. Crucially, they provide for timeliness and ease of production.

CONSTITUTION AND BYLAWS

This issue of the Proceedings also contains the Constitution and Bylaws of the Academy, a list of officers and committee members. We're working on maintaining a list of dues-paying members of the Academy (we'd appreciate your help in building and adding to this list with names of new and prospective members), a listing of past presidents of the Academy, and an index of presenters and paper authors. Copies of the financial statement and the unapproved minutes from last year's annual business meeting will be available at the business meeting.

IN APPRECIATION

The Academy wishes to acknowledge current and emeritus members of the Academy who continue to support the mission of the North Dakota Academy of Science Research Foundation through their special gifts. A listing of these supporters will accompany the Financial Report. The Academy also wishes to express its thanks to the presenters of papers at the Annual meeting, the session chairs, as well as all who have helped in organizing spaces and places, soliciting manuscripts, and compiling of this year's communications. The President of the Academy also wishes to sincerely thank Dr. Dwight E. Bergles who served as honored guest speaker at this year's meeting.

Van Doze
President

Siegfried Detke
Secretary-Treasurer, *Proceedings* Editor

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UNDERGRADUATE COMMUNICATIONS

IN THE

A ROGER DENISON COMPETITION

SCHEDULE OF PRESENTATIONS - UNDERGRADUATE SESSION #1

Undergraduate session #1 talks will be in the Hilton Garden Inn Dahl room – session will be chaired by A. Rodger Denison Competition judges

MORNING SESSION

7:30 Registration desk open

8:00 Greetings from President Van Doze in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center

8:20 ANALYSIS OF PROTEIN BINDING IN THE MYELOID-LYMPHOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT CLUSTER REGION, Aileen M. Aldrich*, Alysa L. Anderson and Heidi J. Super [p. 9]

8:40 ASSESSING DNA TOPOISOMERASE BINDING IN THE MYELOID-LYMPOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT REGION, Alysa Anderson, Cheryl A. Lepp, Heidi J. Super [p. 10]

9:00 DETERMINING GENE EXPRESSION IN THE *Drosophila melanogaster* AFTER MULTIGENERATIONAL EXPOSURE TO CHLOROPHENOXY HERBICIDES, Marcie A. Bata*, Heidi M. Gienger, Aaron D. Dobmeier, Bridget M. Blunck and Hilde E. van Gijssel [p. 11]

9:20 GENETIC DIVERSITY AND SELECTION OF COMMON BEAN GENOTYPES FOR MAPPING GENES CONDITIONING MINERAL CONTENTS., Erika Anderson, Kayla Schmidt, Zahirul Talukder, Phillip Miklas, and Khwaja Hossain [p.13]

9:40 EFFECT OF FIBROBLAST GROWTH FACTORS AND CHLOROPHENOXY HERBICIDES ON CELL PROLIFERTION OF HUMAN LUNG FIBROBLASTS, Wylie E. Wisnewski*, Bridget M. Blunck and Hilde E. van Gijssel [p.14]

10:00 A MORE DETAILED EXAMINATION OF THE INFLUENCE OF MAGNESIUM ION ON ALDH2 ENZYME ACTIVITY, Jordan Karlstad*‡, Thomas P. Gonnella‡, and Matthew J. Picklo [p.22]

10: 20 BREAK

10:40 THE INFLUENCE OF LARVAL GROWTH HISTORY AND EXOGENOUS THYROID HORMONE ON LIFE HISTORY ALLOCATION PATTERNS IN THE AXOLOTL (*AMBYSTOMA MEXICANUM*), Pamela C. Clarkson and Christopher K. Beachy [p.16]

11:00 AN ASSESSMENT OF PRESENCE OF CHYTRID FUNGAL INFECTION IN TIGER \ SALAMANDERS IN NORTH DAKOTA, Leah L. Crites, Charles Crites, Kenneth Cabarle, Heidi Super, and Christopher K. Beachy [p.17]

11:20 EFFECTS OF ZINC ON ROOT MEMBRANE POTENTIALS OF A HEAVY METAL INTOLERANT PLANT (*ARABIDOPSIS THALIANA*) AND A HEAVY METAL TOLERANT RELATIVE (*ARABIDOPSIS HALLERI HALLERI*), JoshuaE. Seil, Mitchell J. Meyer, Turner K. Fishpaw* and Christopher P. Keller [p.18]

11:40 ALPHA-1 ADRENERGIC RECEPTOR REGULATION OF SEIZURES AND NEURODEGENERATION, Brianna Goldenstein, Chris Jurgens, Chris Knudson, Jessica Lichter, Patrick Carr, Dianne Perez, Van Doze [p.19]

12:00 LUNCH (served in room 211 of the Norm Skalicky Tech Incubator)

AFTERNOON SESSION

1:00 DETERMINATION OF HEAVY METAL CONCENTRATIONS IN SOIL SEDIMENT AND MUSSELS OF EASTERN NORTH DAKOTA RIVER SYSTEMS, Andrew J. Hager, Louis M. Wieland, Andre W. DeLorme. [p.20]

1:20 ALPHA-2 ADRENERGIC RECEPTOR INHIBITION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY: COMPARISONS OF LIGAND EFFICACY AND POTENCY, Jenna M. Wald*, Brianna L. Goldenstein, Brian W. Nelson, Ke Xu, Jacquline A. Pribula, Jasmine J. O'Brien, Kylie L. Davis, Kristan M. Green, Sarah J. Boese, Jessica A. Lichter, James E. Porter, Van A. Doze [p.34]

1:40 SCANNING ELECTRON MICROSCOPY USED TO STUDY QUANTITATIVE VARIATION IN BIOFILMS AMONG DIFFERENT MUTANTS OF *ESCHERICHIA COLI*, Tanush Wadhawan* and Birgit M. Prüβ [p.33]

2:00 LEAF ATTACHMENT, WOUNDING, AND CONTROL OF LEAF EXPANSION IN ARABIDOPSIS BY THE PLANT HORMONE INDOLE-3-ACETIC ACID, Derek S. Lentz*, Morgan L. Grundstad, Michael Evanoff and Christopher P. Keller [p.23]

2:20 DIET OF TIGER SALAMANDERS IN NORTH DAKOTA, WITH IMPLICATIONS FOR FLOW OF HEAVY METALS THROUGH WETLAND FOOD WEBS , Heather Modrow [p.24]

2:40 BREAK

3:00 RGS PROTEIN SUPPRESSION OF Gα_o PROTEIN-MEDIATED α_{2A}-ADRENERGIC INHIBITION OF MOUSE HIPPOCAMPAL CA3 EPILEPTIFORM ACTIVITY Brian Nelson, Ke Xu, Brianna Goldenstein, Elizabeth Luger, Jacquline Pribula, Jenna Wald, David Weinshenker, Raelene Charbeneau, Xinyan Huang, Richard Neubig, Van Doze [p.26].

3:20 DOES ATRAZINE HAVE A SEASONAL AFFECT ON THE ENDOCRINE SYSTEM OF THE FLAT-HEADED MAYFLY (*STENACRON INTERPUCTATUM*)? Brittany Olson, Ryan Lorenz, Tiffany Ost, Rachel Stack, Louis M. Wieland, and Andre W. DeLorme, [p.28]

3:40 The X-Files: Molecular Regulation of Neuralepithelial Migration In Early Mouse Brain Development, Wilson, J.T., Drees, K. and Darland, DC [p.12]

4:00 α_{1A} ADRENERGIC RECEPTORS REGULATE NEUROGENESIS AND COGNITIVE FUNCTION, Danielle D. Schlosser, Sarah J. Boese, Chris A. Knudson, Patrick A. Carr, Dianne M. Perez, & Van A. Doze. [p.31]

4:20

EVENING

6:30 Banquet will be in the Hilton Garden Inn Dahl room. Cash bar open at 6:00.

UNDERGRADUATE SESSION #2 CONTINUED ON NEXT PAGE

SCHEDULE OF PRESENTATIONS - UNDERGRADUATE SESSION #2

Undergraduate session #2 talks will be in the Hilton Garden Inn Buckley room– session will be chaired by A. Rodger Denison Competition judges

MORNING SESSION

7:30 Registration desk open

8:00 Greetings from President Van Doze in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center

8:20 A COMPARISON OF METHODS FOR DETERMINING LITHIUM EXTRACTED FROM SOIL; ANALYSIS VIA FLAME ATOMIC ABSORPTION, Jack M Carraher and Bob Crackel [p.15]

8:40 APPLICATION OF THE LEUCKART REACTION TO 4 HYDROXYBENZALDEHYDE, Scott Mortensen [p.25]

9:00 DETERMINING THE SCALABILITY OF THE ONE-POT SYNTHESIS OF A NOVEL FORMAMIDE FUNGICIDE, Jeri Nurnberger and Mikhail M. Bobylev [p.27]

9:20 THE ACCELERATED LEUCKART REACTION IN THE SYNTHESIS OF PHARMACEUTICALS, Andrew Podrygula [p.30]

9:40 DEVELOPMENT OF TARGET-INDUCED FLUORESCENT NANOPARTICLES FOR THE DETERMINATION OF MERCURY, *Paul D. Selid, Song Liang, Hanying Xu, Julia Xiaojun Zhao** [p.32]

10:00 EXAMINING ATRAZINE LEVELS IN THE WILD RICE RIVER AND SHEYENNE RIVER, Tiffany J. Ost, Ryan Lorenz, and Andre W. DeLorme [p.29]

10: 20 BREAK

10:40 SCALING UP THE ACCELERATED LEUCKART REACTION FOR THE SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES, Dennis Ingold and Mikhail M. Bobylev [p. 21]

11:00

12:00 Lunch (served in room 211 of the Norm Skalicky Tech Incubator)

EVENING

6:30 Banquet will be at the Hilton Garden Inn Dahl room. Cash bar open at 6:00

ANALYSIS OF PROTEIN BINDING IN THE MYELOID-LYMPHOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT CLUSTER REGION

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The Myeloid-Lymphoid Leukemia (*MLL*) gene fuses to greater than 50 different loci as the result of reciprocal translocations associated with several subtypes of human acute leukemia. In every case, the fusion point in *MLL* is within the same 8.3 kilobase pair (kbp) region, called the *MLL* breakpoint cluster region (bcr). A number of studies indicate the *MLL* bcr is susceptible to DNA cleaving agents, such as topoisomerase II (topo II) and DNase I, but there have been no reports of direct binding of such proteins in this region. In fact, despite the conserved nature of the breakpoint region, no definitive translocation mechanism has been described. A major goal in understanding *MLL* rearrangement is the identification of proteins that recognize specific DNA sequence or chromatin structure in the *MLL* bcr. Therefore, the objective of this study is to determine the general protein binding characteristics of the *MLL* bcr. We have used an electrophoretic mobility shift assay (EMSA) to analyze protein binding within specific regions of the *MLL* bcr. Protein binding was noted in the extreme boundaries of this region, but not in an internal region previously described as both a topo II cleavage site and a DNase I hypersensitive site. The limited mobility observed with the protein-DNA complexes indicates large proteins or protein complexes may bind in these boundary regions. Binding occurred with nuclear proteins from hematopoietic cells, but not with nuclear proteins from fibroblast cells. Protein binding was sequence-specific as shown by competitor assays. Detailed analysis of the most 5' region of the *MLL* bcr shows that several discontinuous regions are important for protein binding. Our results suggest that specific protein-DNA interactions may define the limits of the *MLL* bcr. The finding that blood cell-specific proteins (but not fibroblast proteins) bind to this region suggests these protein-DNA interactions may be relevant to the formation of *MLL* rearrangements resulting in leukemia. The lack of detection of protein binding in the previously described topo II and DNase I cleavage region may suggest a more indirect role of these proteins in *MLL* rearrangements.

This research is supported by NIH Grant Number P20 RR016741 from the North Dakota INBRE Program of the National Center for Research Resources, and by a Minot State University Institutional Research Grant.

ASSESSING DNA TOPOISOMERASE BINDING IN THE MYELOID-LYMPOID LEUKEMIA GENE TRANSLOCATION BREAKPOINT REGION

Alysa Anderson *, Cheryl A. Lepp, Heidi J. Super
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Background/Objective: The mixed-lineage leukemia gene, (*MLL*), breaks and fuses to one of >50 different translocation partner genes in several subtypes of de novo human acute leukemia. All breaks in *MLL* occur within the same 8 kilobase (kb) region, (bcr) and create in-frame fusion mRNAs and fusion proteins with the translocation partner gene. *MLL* fusions are also observed in chemotherapy-related, secondary leukemias. These *MLL* fusions often follow treatment with drugs for cancer which interfere with the endonuclease DNA topoisomerase II (topo II) activity and implicate topo II in the translocation mechanism, in vivo.

To date, all evidence supporting topo II as a DNA cleaving agent in *MLL* translocations is indirect and includes the clinical cases discussed above as well as in vitro cleavage studies of topo II inhibitors. However, no one has shown direct binding of DNA topo II in the *MLL* bcr. Since it is possible that topo II could act at sites outside the *MLL* bcr and alter the accessibility of the bcr to other DNA damaging agents, it is important to show whether the *MLL* bcr directly binds topo II. The objective of this study, then, has been to assay the affinity of topo II for regions of the *MLL* bcr.

Methods: We are using chromatin immunoprecipitation (ChIP) to assay binding of topo II specifically in the *MLL* bcr. Human leukemia cell lines with normal *MLL* genes (intact *MLL* bcr) are treated with DNA topo II inhibitors and then with formaldehyde to cross-link and stabilize all protein-DNA interactions. (DNA topo II inhibitors covalently link topo II to DNA at sites of binding.) Cells are lysed and DNA is sheared into small, uniform fragments. Sheared chromatin is then immunoprecipitated with DNA topo II or control antibodies. Sheared immunoprecipitated chromatin is recovered and assayed for the presence of the genomic region of interest (specific regions of the *MLL* bcr) by polymerase chain reaction. Amplification of the region of interest indicates direct binding of the protein in question.

Results/ Conclusions: In preparation for ChIP analysis of the *MLL* bcr, we have developed a novel positive control for topo II binding. The human β-globin gene promoter has been shown by other methods to have a high affinity topo II binding site (1). We have shown by ChIP assay, amplification the β-globin gene promoter region following immunoprecipitation with topo II antibodies, but not with control antibodies or mock (no antibody) immunoprecipitation.

Analysis of 3 non-contiguous regions of the MLL bcr has shown no evidence of topo II binding in the ChIP assay in cells treated for 4 hours with topo II inhibitor, etoposide. One of the regions assayed includes a hot-spot for *MLL* breaks, and a reported in vitro topo II cleavage site. Although less than 10% of the MLL bcr has been assayed, the apparent lack of binding in this region is inconsistent with a direct role of topo II in MLL translocations in leukemia.

1. Lee Ge, Kim JH and Chung IK 1998 Mol Cells. 8(4):424-30.

Supported by NIH grant P20 RR016741 from the NCRR (North Dakota INBRE).

DETERMINING GENE EXPRESSION IN THE *Drosophila melanogaster* AFTER MULTIGENERATIONAL EXPOSURE TO CHLOROPHENOXY HERBICIDES

Marcie A. Bata*, Heidi M. Gienger, Aaron D. Dobmeier, Bridget M. Blunck and Hilde E. van Gijssel

Toxicology Laboratory, Science Department, Valley City State University, Valley City, ND, 58072

Recent studies have shown an increase in both respiratory and circulatory birth defects in wheat producing areas of the U.S. This risk is increased when the child, especially in males, are conceived during the application of herbicides. It is suggested that the exposure to chlorophenoxy herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), is responsible, but the mechanism remains unknown. Chlorophenoxy herbicides that have been used since the 1940's and multiple generations have been exposed. Therefore, it is our hypothesis that the risk of birth defects increases due to exposure of multiple generations. The goal of these experiments is to determine the effect of multigenerational exposure of 2,4-D and MCPA on development and identify and determine the changes in gene expression after exposure. The *Drosophila melanogaster* was chosen as a model organism to study the effect of multiple generations because of its short generation time and genetic homolog with human genes involved in development.

Drosophila embryos were collected for 20 minutes after a pre-lay period to ensure embryos of equal age. Embryos were added to a vials with food containing chlorophenoxy herbicides in the following concentrations (control, 1µM and 3mM 2,4-D or MCPA). Development was observed every 24 hrs for a period of 16 days. Concurrently, *Drosophila* adults (75 females and 50 males) were put into 8 bottles with food containing the same concentrations as above to produce the next generation. After 7 days the parents were removed stored and stored at -20°C until further use. After 14 days embryos were collected for the next developmental study and adults were used to produce the next generation. Development and growth were followed for 4 generations.

Adults were used to determine gene expression. After isolation of mRNA using Trizol, mRNA from two sources (for example 4th generation 2,4-D and 4th generation control) was labeled with Cy5 and Cy3 using PCR and hybridized to a *Drosophila* microarray. Subsequently the array was washed carefully and read with a microarray slide reader.

Results showed a significant delay in development of *Drosophila* exposed to 3mM 2,4-D and MCPA. This effect was most severe in flies treated for 4 generations with 2,4-D. Flies exposed to 1µM 2,4-D and MCPA did not show this delay; in contrast flies, seem to develop faster even though this was not significant. To our surprise flies exposed to 3 generations of ethanol and subsequently one generation of 3 mM 2,4-D showed a similar delay as exposure for 4 generations 2,4-D.

Multi-generational exposure to 2,4-D and MCPA significantly reduced survival in each generation. For control and 1 µM MCPA and 2,4-D it was possible to maintain 8 bottles in each generation, but only 2 bottles were left at the end of the 4th generation after exposure of 3mM 2,4 and MCPA. Also females produced fewer embryos during the 20 minute embryo collection.

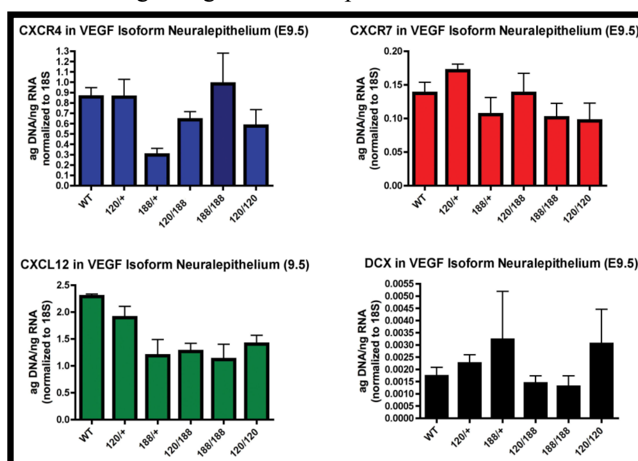
To determine gene expression *Drosophila* microarrays are being used. Currently, we are optimizing the microarray protocol for the *Drosophila* using test-arrays. We are seeing a signal but we would like to increase the strength of the signal.

The data showed that long term multi-generational exposure affects the development of the *D. melanogaster*. The effects change in each subsequent generation indicating that a multigenerational exposure changes the severity of exposure. We hypothesize that epigenetics plays a role. To identify the changes in gene expression linked to multigenerational exposure *Drosophila* microarrays are being used. Unfortunately, we have not been able to produce a reliable array, due to problems with mRNA isolation. The procedure for mRNA needs to be improved to create an increased and more reliable signal.

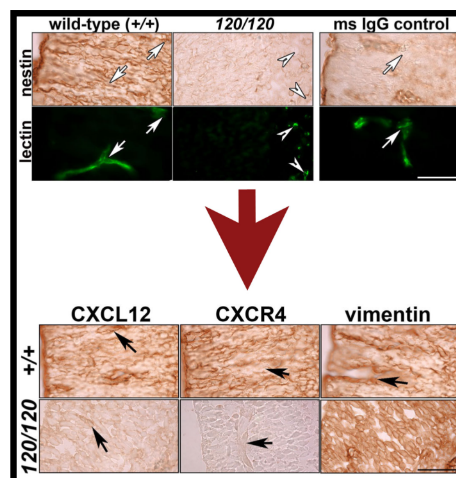
THE X-FILES: MOLECULAR REGULATION OF NEURALEPITHELIAL MIGRATION IN EARLY MOUSE BRAIN DEVELOPMENT

Wilson, J.T., Drees, K. and Darland, DC
Biology Department, University of North Dakota, Grand Forks, North Dakota, United States of America.

We investigate neurovascular interactions in the developing central nervous system, specifically examining the role that Vascular Endothelial Growth Factor (VEGF) plays in regulating cell migration. VEGF has been shown to promote neural, as well as vascular, migration, but VEGF's role in early neuronal migration in brain development is less clear ([1], [2]). Our overall goal is to investigate the role that VEGF plays in regulating neurogenesis. Specifically, how C-X-C chemokine receptor type 4 (CXCR4), C-X-C chemokine receptor type 7 (CXCR7), C-X-C chemokine ligand 12 (CXCR12), and doublecortin (DCX) affect migration of neural pathways in relation to VEGF. CXCL12 is a multipotent ligand impacting cells in the neural, vascular and immune systems. The CXCL12 ligand works through 7-transmembrane receptors CXCR4 and CXCR7 to affect changes in a variety of neural cells and alter migration. CXCR7 interacts antagonistically with CXCR4 and CXCL12. Whereas CXCR4/CXCL12 promotes migration, CXCL12 bound to CXCR7 inhibits migration. Our hypothesis is that normal VEGF induces CXCR4 and CXCL12 while suppressing expression of CXCR7 in neuralepithelium, allowing post-mitotic neurons to migrate to appropriate locations, thereby regulating neurogenesis. We are currently using a transgenic mouse model with mis-expressed VEGF to address the critical regulatory role of the CXC signaling axis in this process.



Total RNA was isolated from developing neuralepithelium of wild-type (E7.5, E9.5 and E11.5) and VEGF isoform (E9.5) mice ([3]) that lack the predominant VEGF isoform in the brain, VEGF164, but carry either VEGF120 or VEGF188. Gene-specific primers were used to generate product DNA (by reverse-transcription/PCR) for a standard curve in quantitative real-time (Q-RT/PCR). DNA values shown were calculated from Real-time C_T value relative to the standard curve run in parallel using AbsolutBlue™ Cybr green and normalized to 18S. The mean and standard deviation of 7-10 samples were graphed and Kruskal-Walis tests were run to determine statistical significance with an value set at 0.05 (see Figure to the right). We found that CXCR4 and CXCL12 were downregulated and CXCR7 upregulated when normal VEGF expression is disrupted. Parallel protein analysis for CXCR4 and CXCL12 showed similar changes in expression (Figure to the left). In summary, levels of both ligand and receptors vary during the critical window of radial glial development around E9.5. CXCR4 and CXCR7 vie for a limited amount of expressed CXCL12 to fuel developmental migration at stage E11.5. Disruption of normal VEGF isoform expression alters the pattern of neural precursor development leading to a cluster of cells near the ventricular zone and reduces expression of the CXCL12 signaling axis components. There is limited DCX expression prior to E11.5 suggesting this factor is active later in neuralepithelial development. Our working model is that CXCL12 and its 7-transmembrane G-protein coupled receptors, CXC4 and CXCR7, form a signaling unit that regulates neuronal migration during early epithelial development.



NOTES

[1] Greenberg, D. A. and K. Jin (2005). "From angiogenesis to neuropathology." Nature **438**(7070): 954-9.
[2] Ruhrberg, C., H. Gerhardt, et al. (2002). "Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis." Genes Dev **16**(20): 2684-98.
[3] VEGF isoform transgenic mice were generated by an international consortium of scientists and used with permission of Dr. P.A. D'Amore, Schepens Eye Research Institute and Harvard Medical School, Boston, MA.

GENETIC DIVERSITY AND SELECTION OF COMMON BEAN GENOTYPES FOR MAPPING GENES CONDITIONING MINERAL CONTENTS.

Erika Anderson[1*], Kayla Schmidt[1], Zahirul Talukder[1], Phillip Miklas[2], and Khwaja Hossain[1]
(1) Mayville State University, Mayville, ND (2) USDA-ARS, Prosser, WA, 99350.

Common bean (*Phaseolus vulgaris* L.) is an important source of protein and minerals for human diets worldwide. Mineral content is known to be variable among accession but genes conditioning such variability are unknown. Our ultimate goal is to identify genes conditioning genetic variation for zinc and iron content. To establish mapping populations for this objective we sought to measure genetic diversity among 29 common bean genotypes representing parents of established mapping populations, standard cultivars, and genotypes with high mineral content. Chemical analysis reveals significant genetic variability among the genotypes for both zinc and iron content of the seeds. Genetic diversity was evaluated with markers generated from 49 primer pairs, of which 23 amplified SSRs obtained from coding and non-coding regions, 16 were developed from tentative consensus (TC) sequences, and 10 were generated from eight coding sequences (CDs) of NBS-LRR type resistance genes of common bean. Genetic diversity was estimated by polymorphism information content (PIC) and genetic similarities between genotypes. Genetic similarities between genotypes ranged from 14.0 (‘Jalo EEP558’ and ‘DOR 364’) to 91.4 (‘MIB 152’ and ‘MIB 465’). Genotypes were selected for inter- and intra-gene pool crossing programs combining seed mineral contents and genetic similarity among the genotypes at the molecular level. This project will contribute to strengthening common bean genomic resources and generating mapping populations and molecular markers for mapping and tagging genes conditioning genetic variation for mineral content in common bean.

EFFECT OF FIBROBLAST GROWTH FACTORS AND CHLOROPHENOXY HERBICIDES ON CELL PROLIFERTION OF HUMAN LUNG FIBROBLASTS

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Toxicology Laboratory, Science Department, Valley City State University, Valley City, ND, 58072

Fibroblast growth factors (FGF) and it receptors is a large family of growth factors involved in angiogenesis, wound healing, embryonic development and many other processes. FGF's are heparin-binding proteins and interactions with cell-surface associated heparin sulfate have been shown to be essentials for FGF signal transduction. FGF's are key-players in the processes of proliferation and differentiation of cells. The goal of our experiment is to study the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) on the FGF induced proliferation of human lung fibroblasts. Our hypothesis is that 2,4-D and MCPA increase proliferation at low concentration in presence and absence of FGFs.

Human lung fibroblasts were exposed to various concentrations of 2,4-D, MCPA, FGF1, FGF6 and FGF10. Two controls were used: no cells, and solvent solution. The experiment was repeated three times. Cells were seeded on day -1 and left to attach and settle for 24 hours. At day 0, the media was replaced with media with no serum and the compounds of interest were added. After 24 hours of exposure, 70ul of MTT reagent was added to each well and plates were returned to the incubator. Four hours later, 700ul of MTT detergent was added to the wells and the plates were placed in a dark area overnight. Proliferation was measured by UV/VIS spectrometer as a wavelength of 570nm. Proliferation was normalized to the rate of proliferation in samples exposed to solvent solution only; proliferation is these wells were set to be 100%.

Human lung fibroblasts exposed to 2,4-D and MCPA showed a slight increase in proliferation at low concentrations (104 % and 112% at 3µM, respectively compared to control at 100%) although for 2,4-D this increase was not significant. No increase in proliferation was found after exposure to FGF-10, FGF-1 and heparin sulfate. A slight increase in proliferation was found (100 to 112%) after exposure to FGF-6.

In previous experiments we showed the fibroblast are serum dependent and have the FGF-receptors present at the membrane and, therefore, should be growth factor dependent. Therefore, it was surprising to see a only a slight increase in proliferation after exposure to FGF-6 which is a specific FGF for fibroblast. Because, FGF are heparin dependent, experiments that combine FGF-6 and heparin sulfate exposures need to be done. FGF-10 was used as a negative control because this growth factor does not induce growth in fibroblasts, which was supported by our results. The increase in proliferation after 2,4-D and MPCAP exposure supports our hypothesis that MCPA and 2,4-D stimulate growth in low concentration although this effects needs to be confirmed with a second method to determine proliferation. We also want to do longer exposure times to see if longer exposure enhances the effects on growth.

A COMPARISON OF METHODS FOR DETERMINING LITHIUM EXTRACTED FROM SOIL; ANALYSIS VIA FLAME ATOMIC ABSORPTION

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Introduction:
Approximately 10.4 million Americans over the age of 12 have used methamphetamine (meth) for non-medicinal reasons (ONDCP 2005). Meth is easily made, and Midwest states like North Dakota have the highest concentration of clandestine meth manufacturing sites (USDEA 2005). A common method of manufacturing meth is the Birch method, also known as the "Nazi" method (USDEA 2005). During the manufacturing process noxious solvents and gases like hydrogen chloride and phosphine gas are generated. For every kilogram of meth manufactured, 2 to 3 kilograms of toxic waste are left behind (Potera 2005). The waste and excess reagents are often discarded in ditches, rivers, or plumbing. Hazardous materials teams are necessary to clean the former manufacturing sites. During cleanup, crews typically wear hermetically sealed suits and self-contained breathing apparatuses for protection (Snell 2001). Cleanup is costly. California spent $7.65 million on cleanup of meth manufacturing sites in 1997 (Bergeron 1997). Li was chosen for analysis because it is commonly used in the manufacture of meth via the Birch method and its presence could be evidence of contamination from production or waste disposal. Li is not toxic; it is a possible indicator that meth waste could be present as other potential sources of Li are rare. Three methods were investigated for extraction of Li from soil: extraction with water (H_20), extraction with ammonium acetate (NH_4CH_3COO), and extraction with nitric acid (HNO_3). Flame AAS was chosen as the method of analysis because of its low detection limit of 2 ppm (Varian), and the availability of the instrumentation.

Method:
Li concentrations were determined using a Varian SpectrAA model 110 atomic absorption spectrometer with a lamp emitting light at 670.8nm. Three extraction methods were tested on two soil types (sandy and potting): extraction with 1M ammonium acetate (NH_4CH_3COO), extraction with 6M nitric acid (HNO_3), and extraction with water (H_2O). Known concentrations of Li containing solutions were added to known masses of soil, and the concentrations of the extract were determined by comparing the extract absorbencies to the absorbencies of a set of standards. The standards had K added to prevent ionization of the Li. Triplicate samples were prepared at 7 different concentrations, 21 samples, for each soil type, 42 samples total. A known mass of soil was weighed into a beaker and known concentrations of Li were added. The soil was then dried. Two grams were taken from each sample and 20mL of extracting solution was added. Samples sat overnight and were filtered then analyzed.

Results:
Extraction with NH_4CH_3COO resulted in the highest percent recovery for potting soil with an average of 95.82% Li recovered with a standard deviation of 10.62%. The percent of Li recovered was higher at the lower concentrations of added Li. Two of the 21 samples were more than one, but less than two standard deviations from the average. Both were samples with higher concentrations of Li. Extraction with HNO_3 resulted in the highest percent recovery for sandy soil with an average of 75.85% Li recovered with a standard deviation of 9.63%. Extraction with HNO_3 from the potting soil could not be determined because a precipitate formed in the burner head during analysis. The problem was not addressed because of time constraints. Paired t-tests show that at a 95% confidence interval there was no difference between extraction with H_2O and extraction with NH_4CH_3COO for the potting soil, but the two methods are statistically different at a 90% confidence interval. The tests also show that the sandy soil extraction with H_2O and NH_4CH_3COO are statistically the same at a 95% confidence interval.

Potting Soil			Sandy Soil		
Extraction Method	Average % extracted	Standard Deviation of the Average	Extraction Method	Average % extracted	Standard Deviation of the Average
H_2O	87.04	17.56	H_2O	65.54	10.77
NH_4CH_3COO	95.82	10.62	NH_4CH_3COO	63.26	13.47
HNO_3	Could not be determined		HNO_3	75.85	9.63

THE INFLUENCE OF LARVAL GROWTH HISTORY AND EXOGENOUS THYROID HORMONE ON LIFE HISTORY ALLOCATION PATTERNS IN THE AXOLOTL (*AMBYSTOMA MEXICANUM*)

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In vertebrates, growth and development are interacting systems that are regulated at different points along the hypothalamus-pituitary-thyroid axis (1,2). Amphibian metamorphosis is an excellent system for studying hormone-mediated interactions because of the ease of manipulating environmental variables, e.g., temperature, and because several endocrine-controlled events have morphological results that are easy to assay, e.g., metamorphosis (3). Regardless, what is known about these important events, i.e., development, growth, metamorphosis, sexual maturation, and storage, remains complicated because any given study usually examines only a single one of these assayable events. We assayed metamorphic development, sexual maturation, and storage in the paedomorphic salamander *Ambystoma mexicanum* (axolotl) by manipulating larval growth rates and the initiation of metamorphosis (via treatment with the thyroid hormone, thyroxine [T_4]) and observing consequent differential expression in days required to metamorphose (i.e., metamorphic timing), size and developmental status of gonads (i.e., maturation), and size of fat bodies (i.e., storage).

We tested the hypothesis that variation in growth history and exogenous T_4 affects the vector of allocation variables (i.e., growth, metamorphosis, maturation, and storage) by using a full-factorial 4 X 2 randomized complete block design with four growth treatments (constant rapid growth, constant slow growth, rapid-then-slow growth, slow-then-rapid growth) and two T_4 treatments (no T_4, 5 nM T_4).

Adult axolotls were paired and resulting embryos were hatched and larvae were raised individually in plastic container in reverse-osmosis water. Hatchling larvae were fed freshly hatch brine shrimp. As larvae grew, they were fed tubificid worms. We examined growth by periodic weighings (to the nearest mg) using a top-loading balance. Metamorphosis was scored when tail fin and gill resorption were complete. Salamanders were then killed by prolonged immersion in MS-222, fixed in 10% formalin, and stored in 70% ethanol. Gonads and fat bodies were then dissected and weighed to the nearest milligram. Dissection also allowed us to determine sex in order to test the hypothesis that allocation was sex-dependent.

Feeding treatments had desired growth effects. Treatment with T_4 resulted in complete metamorphosis of all treated salamanders. In the T_4 treated salamanders, metamorphosis was independent of larval growth rate. Allocation differed among treatments and was dependent on sex. Females stored significantly less than males, while having significantly larger gonads than males. Furthermore, female gonad mass was significantly influenced by feeding treatment whereas male gonad mass was not; this indicates the expensive allocation cost of reproduction in females.

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Sources:

1) Denver, R.J., K.A. Glennemeier, and G.C. Boorse. 2002. *In* D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fahrback, and R.T. Rubin (eds.). Hormones, brain and behavior, pp. 469-513. Academic Press, San Diego.
2) Rose, C.S. TREE
3) Hickerson, C.A., E. Barker, and C.K. Beachy. 2005. Southeastern Naturalist 4:33-50.

AN ASSESSMENT OF PRESENCE OF CHYTRID FUNGAL INFECTION IN TIGER SALAMANDERS IN NORTH DAKOTA

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The chytrid fungus *Batrachochytrium dendrobatidis* is a widespread fungal infection affecting amphibians and has been linked to worldwide declines in amphibians worldwide (1). No formal analysis has focused on the presence of chytrid fungus in salamanders in North Dakota. In ongoing attempts to determine amphibian population abundances and xenobiotic influence on amphibians, we analyzed tail tissue and skin swabs (n = 80) for *B. dendrobatidis* from eight tiger salamander (*Ambystoma mavortium*) populations from North Dakota.

Tail clips were preserved in 70% ethanol and stored in a -80°C freezer. Total DNA was extracted using Qiagen DNEasy kits. Purified DNA was amplified with PCR following standard protocols. Product amplification was done using *B. dendrobatidis*-specific primers, forward CHYITS1F3 5'-ACAAAATT-TATTTATTTTTCGAC-3' (located in ITS1) and reverse CHYIST2R2 5'-CATGGTTCATATCTGT-CCAG-3' (located in ITS2) (2). Each PCR reaction contained 0.5 M of each primer, 10-100 ng of extracted DNA, 1X Taq buffer, 0.8 mM dNTP, 2.5 mM magnesium, nad 0.05 units/ l of Taq polymerase. This mix was thermocycled for 30 cycles. Cycle 1 was 95°C for 60 sec, 50°C for 45 sec, and 72°C for 90 sec, followed by 29 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 90 sec. The mix was held at 72°C for 7 min, then held at 4°C. The final PCR product was run on 2% agarose gels and analyzed in groups arranged by regional population. Populations represent areas in northwest, north-central, southwest and the turtle mountain regions of North Dakota (Fig. 1) where the Amphibian Growth Project has ongoing life history analyses.

At present, at least one individual from each sampling location has been analyzed. Ten individuals from each location will be assessed. Initial results have shown no indication of chytrid presence in analyzed populations.

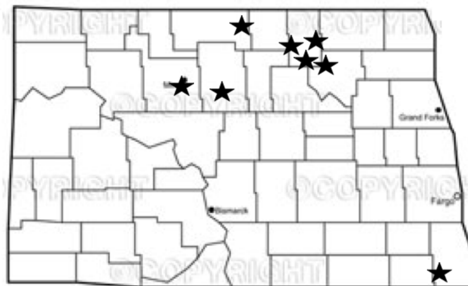

Fig. 1.—Locations of tiger salamander (*Ambystoma mavortium*) sampled for presence of chytrid fungus (*Batrachochytrium dendrobatidis*) infections.

This research was supported by NIH Grant Number P20 RR016741 from the INBRE Program of the National Center of Research Resources.

Sources

1) Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, R.A. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pessier, and J.P. Collins. 2006. Proc. Natl. Acad. Sci. 103:3165-3170.
2) Knapp, R.A., and J.A.T. Morgan. 2006. Copeia 2006:188-197.

EFFECTS OF ZINC ON ROOT MEMBRANE POTENTIALS OF A HEAVY METAL INTOLERANT PLANT (*ARABIDOPSIS THALIANA*) AND A HEAVY METAL TOLERANT RELATIVE (*ARABIDOPSIS HALLERI HALLERI*)

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Understanding the mechanisms of uptake of toxic heavy metals by plants is of obvious importance as heavy metal accumulation in of crop plants is the principle route of toxic metal entry into the human food-chain. Here we have studied two species of *Arabidopsis*: *Arabidopsis thaliana*, known to be intolerant of heavy metals in soil water and *Arabidopsis hallari halleri* , known to be tolerant of high concentrations of heavy metal ions like Cd^{2+} and Zn^{2+} and to hyperaccumulate these to high levels (2). While Cd^{2+} uptake is of greatest concern, we studied the effects of Zn^{2+} as the two elements are probably transported by the same mechanisms. Evidence for which includes the protective effect of excess Zn^{2+} against Cd^{2+} toxicity (3). Complementation tests of *Arabidopsis* genes in yeast mutants have suggested a role for an iron transporter (4) in plant Cd^{2+} and Zn^{2+} uptake while other work with leaf guard cells suggests a Cd^{2+} and Zn^{2+} permeable Ca2+ channels may be involved (5) but direct tests of root membrane Cd^{2+} and Zn^{2+} transport are lacking. As an initial foray into the study of the mechanisms of heavy metal uptake in plants, we have tested the effect Zn^{2+} on root cell membrane potential of our two Arabidopsis species. Our hypothesis is that differences in Zinc ion transport between these species should be evident in the membrane potential that ultimately drives ion uptake.

For this study, seeds of *Arabidopsis thaliana* var. Columbia (Col-0) were obtained from Lehle Seeds (Round Rock, TX) and seeds of *Arabidopsis halleri halleri* were collected from plants growing on highly zinc polluted soil new a smelter at Auby, France (a gift from Dr. Vincent Castric, Université des Sciences et Technologies de Lille, France). Both plants were grown on soil for 12-25 days. Selected seedlings (with roots 2 to 4 cm in length) were uprooted, rinsed clean of soil, and rested intact at least one hour in incubation medium including 0.1 mM KCL, 1 mM $CaCL_2$, and 1 mM Mes/Btp pH 6.0. After mounting in a perfusion chamber the plants were exposed to the incubation medium flowing through the chamber. Roots were pierced between 1 and 3 cm from the root tip with a conventional microelectrode. The membrane potential was monitored by an electrometer and recorded by a chart recorder. After a stable membrane potential recording was established of at least -130 mV for ten minutes, plants were exposed to $ZnCl_2$ by switching the perfusion stream to incubation medium augmented with $ZnCl_2$. Recordings were maintained for a minimum of 60 minutes or until failure of the recording.

Root cell membrane potentials of *A. thaliana* and of *A. h. halleri* were both found to respond to abrupt exposure to 1.0 mM $ZnCl_2$ with an initial transient hyperpolarization followed by a larger more sustained depolarization. With *A. thaliana*, the hyperpolarization (1-5 mV, maximal within 1-3 minutes) was evident in 10 of 14 recordings while the subsequent depolarization (10-27 mV, maximal within 6 to 24 minutes) was evident in all recordings. Six of 10 *A h. halleri* recordings showed an initial hyperplolarization (2-67 mV, maximal within 2-28 minutes). The depolarization (2-16 mV, maximal in 2-20 minutes) was evident in 9 of 10 recordings. Exposed to 5 mM $ZnCl_2$, *A. thaliana* initially hyperpolarized in only 1 of 11 recordings and the depolarization increased (37.3 mV +/- 4.2 s.e.) while 5 of 7 *A. h. halleri* recordings showed a hyperpolarization followed by a smaller depolarization (18.6 mV +/- 3.6 s.e.).

The results suggest that abrupt Zn^{2+} exposure alters two membrane conductances in the root membranes of both species of *Arabidopsis*, a rapidly initiating conductance change that hyperpolarizes the membrane and a more slowly initating change that depolarizes the membrane. The depolarizing conductance is greater in *A.thaliana* and, when induced by 5 mM Zn^{2+} exposure, appears to obscure the initial hyperpolarization. A planned patch-clamp based study of effects of $ZnCl_2$ on root cortex cells of *A.thaliana* and *A. h. halleri* will attempt to determine the molecular/ionic nature of the Zn2+ induced conductances.

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1) Pauwels M, Frérot H, Bonnin I, Saumitou-Laprade P. J. Evol. Biol. 19: 1838-1850 (2006)
2) Clemens S. Biochimie 88: 1707-1719 (2006)
3) Korshunova YU, Eide D, Clark WG, Guerinot ML, Pakrasi HB. Plant Mol. Biol. 40: 37-44 (1999)
4) Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C. Plant J. 32: 539-548 (2002)

ALPHA-1 ADRENERGIC RECEPTOR REGULATION OF SEIZURES AND NEURODEGENERATION

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Neurogenesis, the proliferation of new brain cells, has potential as a treatment for epilepsy and neurodegeneration. Norepinephrine (NE), an endogenous neurotransmitter and possessing antiepileptic effects, may be involved in promoting neurogenesis through the activation of α_1 adrenergic receptors (ARs). This project aims to obtain additional evidence for a possible role of α_1ARs in neurogenesis and seizures using transgenic mice genetically engineered to either overexpress α_{1A}ARs (CAM-1A) or α_{1B}ARs (CAM-1B), or having no functional (knockout) α_{1A}ARs or α_{1B}ARs. When treated with the epileptogenic agent flurothyl and compared to age-matched controls, mice overexpressing α_{1A}ARs had more hippocampal interneurons and showed an increase in latency periods preceding seizures while α_{1B}AR overexpressing mice had significantly fewer interneurons and exhibited an enhanced susceptibility to seizures. These findings suggest that α_{1A}AR stimulation is antiepileptic, and activation of α_{1B}AR is proepileptic. Electrophysiological recordings during application of an α_1AR agonist produced a concentration-dependent increase in action potential frequency in hippocampal interneurons in normal and α_{1B}AR knockout mice, but not in α_{1A}AR-knockout mice.

Immunohistochemistry was then used to visualize fluorescently labeled interneurons in the hippocampal CA1 region of transgenic mice overexpressing α_{1A} or α_{1B}AR. Overexpression of α_{1A}AR was associated with increase in numbers of interneurons. In contrast, decreased interneuron numbers were observed in mice overexpressing α_{1B}ARs. Control mice exhibited interneuron counts intermediate between the overexpressed α_{1A} and α_{1B}AR mice.

These findings are potentially very significant because they link α_{1A}AR-induced proliferation of interneurons to the possible antiepileptic, neuroprotective and neurogenic actions of NE. Insight into these mechanisms may lead to new treatment strategies for epilepsy and other neurodegenerative diseases.

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DETERMINATION OF HEAVY METAL CONCENTRATIONS IN SOIL SEDIMENT AND MUSSELS OF EASTERN NORTH DAKOTA RIVER SYSTEMS

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Introduction

In Eastern North Dakota, it is known that Cadmium, (Cd), and other heavy metals are naturally present within the soil possibly due to the glacial history of the area (Stoner et al., 1998). Cadmium is a known endocrine disruptor and often bioaccumulates within organisms having toxic side effects. We are examining the levels of Cd and other trace metals in river sediments from the Sheyenne and Pembina Rivers in eastern ND and mussel tissue samples collected from the Sheyenne River.

Methods

We collected 52 mussels that encompassed 8 native species. Mussels were chosen for a biological indicator because of the mussel's close proximity with stream substrate during their lifetime. They were also chosen for their large size and abundance within the study area. Soft tissues of these mussels were dissected after a 24 hour depuration period to allow gut contents to empty. Dissections were carried out using non-metallic instruments in order to eliminate contamination of the tissue samples with a foreign metal. Mussel tissues were then dried to a constant weight and digested according to EPA method 3052 while dried soil samples were digested with EPA method 3051A. Blanks were prepared using acid digestions of concentrated analytical grade nitric acid that was diluted to a constant volume. Controls for heavy metals were prepared using mussel tissues that have known concentrations of trace elements that were obtained from the National Institute of Standards & Technology. Samples were sent to Activation Laboratories in Ontario, Canada, to be analyzed by inductively coupled plasma mass spectrometry, (ICP-MS). Fifty-nine different elements were analyzed by this method.

Results

In general, heavy metals were slightly higher in the Pembina River sediments. For example, the Cd levels in sediment from the Pembina sampling sites were more than 3 times greater than the samples from the Sheyenne River. The average levels for the Sheyenne sediment samples were 0.247 ppm Cd (n=14) while the average for the Pembina was 0.847 ppm (n=13). This higher value for the Pembina is congruent with the data of Stoner et al. (1998). Other metals showed less of a difference, but were still slightly higher in the Pembina. The average for Zinc in sediment from the Sheyenne sites was 81.81 ppm (n=14), while for the Pembina it was 95.08 ppm (n=13). The Lead levels in the Sheyenne was 11.07 ppm (n=14), and 12.89 for the Pembina (n=13).

In examining the heavy metal concentrations for the mussel tissue there was one set of data that stood out. The White Heel Splitter, *Lasmigona complanata,* had higher Cd levels at a degree between 8 to 13 times greater than all other species. On average the Cd levels for the White Heel Splitter was 124.2 ppb (n=5). The average for Cd in the Plain Pocketbook (*Lampsilis cardium)* was 9.6 ppb (n=5), 9.92 ppb (n=15) for Three Ridge (*Amblema plicata*), 14.8 ppb (n=6) for Wabash Pigtoe (*Fusconaia flava*), and 12.41 ppb (n=10) for the Fat Mucket (*Lampsilis silquoidea*). Overall the average for all species other than the White Heel Splitter was 11.4 ppb (n=52).

Conclusions

The White Heel Splitter had much higher levels of cadmium in its' tissues than the other species of mussels. This would seem to indicate that this mussel can sequester Cd. The populations of this mussel are healthy within the Sheyenne River so this level of Cd does not seem to be having a negative effect on the White Heel Splitter. We would like to examine what physiological mechanisms, e.g. heat shock proteins and/or metallothionens, the mussels may be utilizing in sequestering Cd.

References

Stoner, J. D., D.L. Lorenz, R.M. Goldstein, M.E. Brigham, and T.K. Cowdery. 1998. "Water Quality in the Red River of the North Basin Minnesota, North Dakota, and South Dakota, 1992-95." U.S. Geological Survey Circular 1169.

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SCALING UP THE ACCELERATED LEUCKART REACTION FOR THE SYNTHESIS OF NOVEL FORMAMIDE FUNGICIDES

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The accelerated version of the Leuckart reaction was discovered by Bobylev et al during the work on novel formamide fungicides. The accelerated Leuckart reaction can be completed within minutes instead of hours and is especially valuable for combinatorial chemistry and automated parallel synthesis. Because the accelerated Leuckart reaction is accompanied by a highly vigorous evolution of gases, it was unclear if it could be safely scaled up. The goal of the current work was to investigate the scalability of the accelerated Leuckart reaction. It was found that the reaction can be safely scaled to a 0.1 mole level. The results of the research open the way to a wider application of the accelerated Leuckart reaction in both laboratory and industrial settings. The project is supported by NIH grant P20 RR016741 from the NCRR.

A MORE DETAILED EXAMINATION OF THE INFLUENCE OF MAGNESIUM ION ON ALDH2 ENZYME ACTIVITY †

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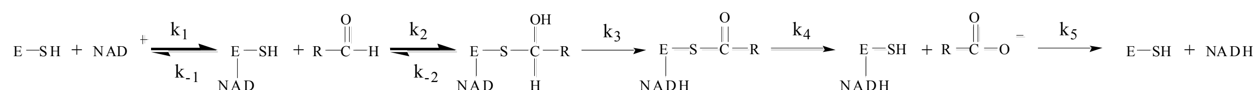
Within many mammalian systems aldehyde dehydrogenases (ALDH's) convert toxic aldehydes into more manageable carboxylic acids. Nicotinamide adenine dinucleotide (NAD^+) is an essential coenzyme for this enzymatic process to occur. Through the course of a five step reaction scheme[1], NAD^+ is converted to its reduced form (NADH) and released. The release of NADH from the ALDH is necessary for further turnover of the enzyme. The addition of magnesium ions to these ALDH systems modulates activity, in some cases increasing activity (ALDH2) and in others, like ALDH1, decreasing activity. The specific role of the magnesium ion**s** the reaction scheme of the different ALDH's is in the process of being resolved.[2]

Our research is focused on using the intrinsic fluorescence of NADH to provide fundamental information regarding factors that influence the enzyme-cofactor interactions. By applying time resolved fluorescence spectroscopy we have been able to distinguish between free NADH in solution (= 0.4 ns) and NADH bound to recombinant rat ALDH2 (τ = 6.0 ns) in the presence of magnesium ions. With such as dramatic difference in fluorescence lifetimes, we have been able to determine ALDH2 enzyme activity, the steady state NADH-ALDH2 complex concentration, the rate of rate of NADH displacement from NADH-ALDH2 complex and the dissociation constant for NADH with ALDH2 under a wide range of magnesium ion concentrations. From these results we have been able to more clearly identify the influence the magnesium ion has on individual steps in the overall five step reaction. Future studies will involve closely examining the influence other divalent metal cations have on this system.



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1 Hammen, P. K.; Allali-Hassani, A.; Hallenga, K.; Hurley, T.D.; Weiner, H. *Biochemistry* **2002**, *41*, 7156.

2 Ho, K. K.; Hurley, T.D.; Weiner, H. *Biochemistry* **2006**, *45*, 9445.

LEAF ATTACHMENT, WOUNDING, AND CONTROL OF LEAF EXPANSION IN ARABIDOPSIS BY THE PLANT HORMONE INDOLE-3-ACETIC ACID

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Indole-3-acetic acid (IAA) is a hormone responsible for controlling various aspects of plant growth including leaf expansion. Previous work has shown that increasing the IAA content of intact expanding leaves of *Arabidopsis* and *Phaseolus*, either through exogenous application or through trapping the endogenous hormone in leaves, results in inhibition of leaf growth (1). Paradoxically, other work has clearly shown that treatment of excised leaf strips from tobacco (*Nicotiana*) (2) and from beans (*Phaseolus*) (3) with IAA stimulates rather than inhibits growth.

Two hypothetical explanations might explain the apparent reversal of growth sensitivity to IAA by leaf tissues upon excision. It is possible that excision isolates the tissue from interaction with a second unstable growth controller that, in the intact plant, interacts with IAA to inhibit growth. Alternatively, wounding may induce a collapse in endogenous IAA levels in excised leaf tissues so that IAA levels, which are optimal for growth in the intact plant, become sub-optimal. To assess these possibilities, we have monitored growth responses to IAA of attached leaves, wounded attached leaves, excised leaves, and of excised leaf strips of the model plant system *Arabidopsis*.

For our experiments, stratified *Arabidopsis* seeds were sown in 288 well plug trays containing moist potting soil and grown in a growth chamber at 19°C, with continuous illumination (150 μM s^{-1} m^{-2}). After 10-14 days, plants were selected with both the first two true leaves 2.8-3.2 mm in diameter. For one set of experiments, scaled digital images of the intact attached leaves were prepared for subsequent determination of initial leaf area. For another set of experiments detached leaves were imaged. Initial images of excised leaf strips (0.7 mm wide cut transversely across the midpoint of the leaves) were prepared for a third set of experiments and for a fourth set of experiments wounded attached leaves (sliced transversely from leaf edge to near the midvein in three places) were imaged. For all experiments one of the first two leaves (selected randomly) from each plant served as the experimental (IAA treated) leaf and the other leaf served as a paired control. Treatment solutions included: full strength Murashige and Skoog media (Caisson Laboratories, Rexburg, ID), 10 mM KCl, 0.1 mM Mes/Btp (pH 6.0), +/- IAA at various concentrations (10 µM, 50 µM, 100 µM, 300 µM, and 1 mM). Following initial imaging attached leaves and wounded attached leaves received either a 5µl drop of a control treatment solution (minus IAA) or a 5 µl drop of the treatment solution containing IAA. Detached leaves and leaf strips were each incubated on 3 mL of the same solutions. One day (24 hours) later, the attached leaves and wounded leaves were detached from the plant and again imaged as were the detached leaves and excised leaf strips.

The results do not clearly differentiate between our two alternative hypotheses. As expected, growth of intact *Arabidopsis* leaves, though somewhat insensitive, is inhibited by IAA at higher concentrations. For example, at 300 µM the area of IAA treated leaves increased 51.7 +/- 10.1% compared to 77.2 +/- 6.3 % for the controls (n=12). Also, as expected, the growth excised leaf strips was increased by IAA treatment across a range of concentrations 10 µM and higher. The growth of wounded attached leaves was significantly inhibited by IAA applied at 50 µM and higher. This result argues against the possibility of wounding inducing lower leaf IAA levels and supports the possibility that detachment is a requirement for IAA-induced growth increase. Growth of detached leaves floated on solution, however, were inhibited by IAA with growth responding to as little as 10 µM IAA, indicating that detachment does not, by itself, reverse IAA sensitivity. It remains possible, however, that only substantial wounding, as experienced by leaf strip tissues, is sufficient to induce a decline in IAA level. We are currently collaborating with Jerry Cohen's lab at the University of Minnesota to test this possibility through analysis of the IAA content of variously treated leaf tissues.

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1) Keller CP, Stahlberg R, Barkawi L, Cohen JD (2004) Plant Physiology 134: 1217-1226.
2) Keller CP, Van Volkenburgh E (1997) Plant Physiology 113: 603-610
3) Keller CP (2007) Physiologia Plantarum 130: 580-589

DIET OF TIGER SALAMANDERS IN NORTH DAKOTA, WITH IMPLICATIONS FOR FLOW OF HEAVY METALS THROUGH WETLAND FOOD WEBS

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Tiger salamanders in North Dakota (*Ambystoma mavortium*) grow extremely rapidly. Furthermore, salamanders have very high assimilation efficiencies (1). In addition, due to their large collective biomass and metamorphic lifestyle, they may represent a significant vector for transport of energy and chemicals (e.g., heavy metals) between aquatic and terrestrial habitats (2). This species has a large literature concerning natural history attributes, however, there is not a thorough diet analysis of any population. I used a series of larval and transformed *A. mavortium* from a population in northwest North Dakota and inspected gut contents in order to test the hypotheses that age, size, sex, maturation status, and life history stage influence food preferences.

All sampled animals (n = 49) were collected from Swalls Lake, Ward Co. Stomachs were extracted, weighed, and prey content was keyed out to order. Number of each type of prey were counted. I used SVL, headwidth, sex, reproductive status (juvenile or sexually mature), and developmental stage (larval or transformed) as in a series of exploratory regression analyses (for SVL and headwidth) or ANOVAs (for sex, reproductive status, and development stage) in order to determine if any factor was associated with significant differences in prey type and abundance.

Headwidth correlated significantly with prey variation and abundance. This means that salamanders with larger heads eat larger prey and more of them. The other significant factor was developmental status: larval salamanders (both paedomorphs and juvenile larvae) at different prey than transformed salamanders. This is likely due to the different gape-and-suck capabilities between larvae and transformed salamanders: the gill slits close at metamorphosis, thus limiting the suction retention of prey items. Transformed salamanders rely more heavily on "ram" feeding.

Interestingly, all the prey in the guts of transformed salamanders were aquatic prey. This contrasts with the classic notion that transformed amphibians return to ponds only to breed. Supplemented with the observation that many of these transformed salamanders were juveniles, this suggests that salamanders return to pond for significant growth opportunities in addition to any reproductive potential.

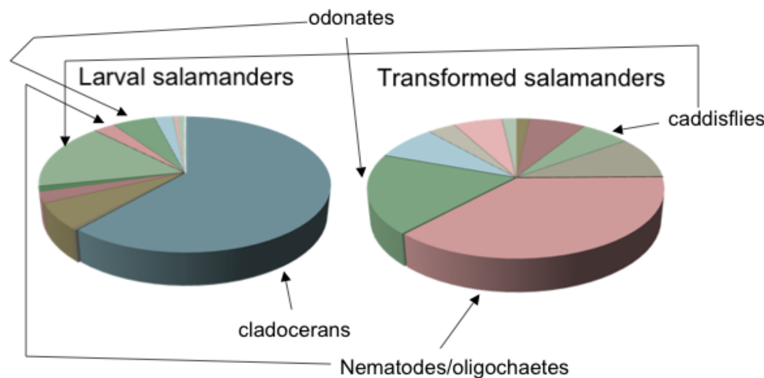

Fig. 1.—Pie charts representing diet differences between larval and transformed Ambystoma tigrinum sampled from Swalls Lake, Ward Co., North Dakota.

This research was supported by NIH Grant Number P20 RR016741 from the INBRE Program of the National Center of Research Resources.

Sources:

1) Bobka, M.S., R.G. Jaeger, and D.C. McNaught. 1981. Copeia 1981:417-421.
2) Regester, K.J., K.R. Lips, and M.R. Whiles. 2006. Oecologia 147:303-314.

APPLICATION OF THE LEUCKART REACTION TO 4-HYDROXYBENZALDEHYDE

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Aldehydes and ketones are valuable building blocks for chemical industry. Reductive amination is a fundamental chemistry process that dramatically expands the application of aldehydes and ketones by transforming them into amines. The Leuckart reaction is a unique one step method of reductive amination. It is a remarkably simple process that includes only two components: the carbonyl compound and formamide. The reaction is completed simply by heating the components at 160ºC to 185ºC for 6 to 25 hours. The long processing time seems to be the only shortcoming of the reaction. During their work with formamide fungicides, Bobylev et al developed an accelerated procedure for the Leuckart reaction. The accelerated Leuckart reaction could be completed in 30 minutes or less. As a highly intensive process, the accelerated Leuckart reaction has a potential of being successful in the areas where the traditional Leuckart reaction was not. Specifically, it was believed that the Leuckart reaction does not work on 4-hydroxybenzaldehyde and that 4-hydroxybenzylformamide cannot be obtained via the Leuckart reaction. The results of this work show that the accelerated Leuckart reaction can be successfully applied to 4-hydroxybenzaldehyde and that it produces 4-hydroxybenzylformamide in good yield. The project is supported by NIH grant P20 RR016741 from the NCRR.

RGS PROTEIN SUPPRESSION OF $G\alpha_o$ PROTEIN-MEDIATED α_{2A}-ADRENERGIC INHIBITION OF MOUSE HIPPOCAMPAL CA3 EPILEPTIFORM ACTIVITY

Brian Nelson[1*], Ke Xu[1], Brianna Goldenstein[1], Elizabeth Luger[1], Jacquline Pribula[1], Jenna Wald[1], David Weinshenker[2], Raelene Charbeneau[3], Xinyan Huang[3], Richard Neubig[3], Van Doze[1].

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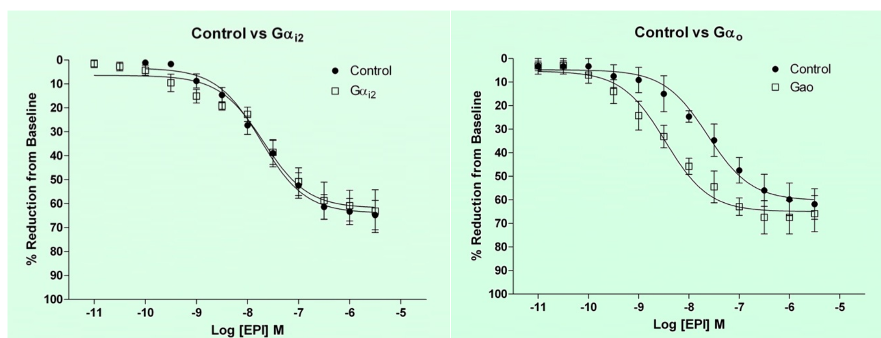
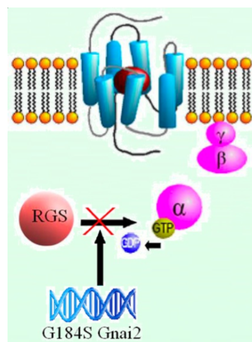
G-protein coupled α_2 adrenergic receptor (AR) activation by epinephrine (EPI) inhibits epileptiform activity in the mouse hippocampal CA3 region. The mechanism underlying this action is unclear. This study investigated which subtypes of α_2ARs and G-proteins ($G\alpha_o$ or $G\alpha_i$) were involved in this response using recordings of CA3 epileptiform bursts in mouse brain slices. First, we determined that this effect was mediated by the α_{2A}AR subtype as the inhibitory action of EPI on burst frequency was abolished in slices from α_{2A}AR, but not α_{2C}AR, knockout mice. Next, using transgenic mice with the G184S Gnai2 allele (knock-ins) which prevents inhibition by interruption of the G-protein alpha unit binding to regulators of G-protein signaling (RGS), we found enhanced α_{2A}AR effects in hippocampal slices from mutant $G\alpha_o$ mice but not $G\alpha_{i2}$ mice. These results indicate that the EPI-mediated inhibition of mouse hippocampal CA3 epileptiform activity is through an α_{2A}AR $G\alpha_o$ mediated pathway under inhibitory control by RGS proteins. This suggests a role for RGS inhibitors as a novel antiepileptic drug therapy.

Supported by American Physiological Society, ND EPSCoR EPS-0447679, NSF 0347259, NSF 0639227, NIH P20RR0167141, NIH 5RO1DA17963 and NIH 5RO1GM039561.





DETERMINING THE SCALABILITY OF THE ONE-POT SYNTHESIS OF A NOVEL FORMAMIDE FUNGICIDE

Jeri Nurnberger and Mikhail M. Bobylev

Division of Science – Chemistry, Minot State University, Minot, North Dakota 58707

Novel formamide fungicides were designed by Bobylev et al as analogs of triazole fungicides were the triazole group was replaced with the formamide group. The Leuckart reaction was successfully used for the synthesis of the novel formamide fungicides. In the case of the lead candidate, N-[1-tert-butyl-3-(2,4-dichlorophenyl)-2-propenyl-1]formamide (I), it appeared to be the first successful application of the Leuckart reaction to an α,β-unsaturated ketone. Later on, a one-pot synthesis of I was developed that included a combination of the aldol condensation and the Leuckart reaction. The one pot method provided a fast and convenient method for the synthesis of I and its analogs. However, it was unclear if it could be safely scaled-up, because the Leuckart reaction is a high temperature reaction that is accompanied by a vigorous evolution of gases. The goal of the current work was to investigate the scalability of the one-pot aldol-Leuckart combination. It was found that the process can be safely scaled-up to a 0.1 mole level. The results of the research open the way to a wider application of the one-pot aldol-Leuckart combination in both laboratory and industrial settings. The project is supported by NIH grant P20 RR016741 from the NCRR.

DOES ATRAZINE HAVE A SEASONAL AFFECT ON THE ENDOCRINE SYSTEM OF THE FLAT-HEADED MAYFLY (*STENACRON INTERPUCTATUM*)?

Brittany Olson, Ryan Lorenz, Tiffany Ost, Rachel Stack, Louis M. Wieland, and Andre W. DeLorme,

Department of Biology, Macroinvertebrate Lab, Valley City State University, Valley City, ND.

BACKGROUND

Atrazine is a herbicide most commonly used to stop emergence of broadleaf and grassy weeds in crops such as corn. In recent years atrazine has been reported as an endocrine disrupter in numerous animals. An endocrine disrupter is a chemical that interferes with production, development and other hormonally regulated processes. Our hypothesis is that if atrazine is an endocrine disrupter, a delay or acceleration in the larval development of certain aquatic insects will be experienced. In addition, endocrine changes may also affect the male to female ration and body length of adults.

METHODS

To examine this, we compared the emergence rates of flat headed mayfly larvae (*Stenacron interpunctatum*) in various concentrations of atrazine, to an acetone control. All insects were collected as larva from the Sheyenne River and raised in aquariums. Each aquarium was equipped with rocks of different sizes which were covered with algae as a natural food source for the larvae. The temperature in each tank was monitored as well as the atrazine levels. The concentrations of atrazine in all experiments ranged from 1-100 parts per billion. The higher range of these levels of atrazine is higher than those that exist in natural conditions. In North Dakota rivers we have tested, the average concentration during the summer months ranges from below 1 to 4 ppb. Eight experiments were ran at several different times of the year.

RESULTS

In experiments run during summer months, no significant difference was shown in emergence rates, male to female ratios, or length of adults. However in experiments run during winter months, two of three experiments showed a delay in emergence. For example, in an experiment run between the late fall and winter months of 2007, we saw a consistent difference in the number mayfly adults emerging in each treatment. By the completion of the experiment the acetone control tank had 22 adult mayflys emerge; in larvae exposed to 5 ppb of atrazine 10 adult mayflys emerged; and larvae exposed to 20 ppb had 5 adults emerge. Each tank started with 25 larvae. When the experiment was completed, 2 living larva were taken from the 5 ppb tank and 11 living larvae were taken from the 20 ppb tank.

CONCLUSION:

Our experiments did not measure an affect of atrazine on the endocrine systems of *Stenacron* during summer months, when atrazine is more likely to be present. However during winter months a delay was shown in the atrazine treated organisms. This is interesting because at this time of year insects are normally experiencing a period of rest. In control treatments it would seem that raising the control temperature brought the insects out of the state of rest and into a phase of normal growth and development. In atrazine treated insects it appeared that they remained dormant in development, even though water temperature was conducive to growth. While this may not be applicable to natural systems it could illustrate interplay of hormonal processes. This interplay may be due to atrazine suppressing the endocrine system during winter months, even when the insect is placed in warmer than seasonal average water.

This work is supported by NIH grant P20 RR016741 from the NCRR.

EXAMINING ATRAZINE LEVELS IN THE WILD RICE RIVER AND SHEYENNE RIVER

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Introduction

Atrazine has been one of the most widely used herbicide in the United States and also has been the most frequently detected pesticide in ground and surface water. While originally considered to be nontoxic, recent work has shown atrazine to cause endocrine disruption in a variety of animals. Atrazine is used on a variety of crops, but here in North Dakota, the application of atrazine to corn is most common. The application process for atrazine is usually pre-emergence, or before the crop reaches a certain height. Since we know when atrazine is applied, we can expect there to be peaks in the levels of atrazine in the rivers near fields where run off has the opportunity to enter the river. Corn production has been in the spotlight recently with ethanol being used as an alternative energy source. More corn is being planted in North Dakota because of this higher demand. . Between the years of 2005 and 2006 there was a 17% increase in corn production and then between 2006 and 2007 that increase jumped to 68% more corn being harvested.

Methods

The Wild Rice River and Sheyenne River are in areas of highest corn production in the state so we chose four sites on each. Sampling was done weekly in the summer and monthly in winter months. A depth-width integrated method (DWI) was used. The water sample is collected, placed in a Teflon churn splitter for mixing and placed in amber glass bottles. These bottles are placed in a cooler, transported back to the lab and placed in the refrigerator until testing. In the lab the water is filtered and atrazine concentrations of the water are determined using an Atrazine ELISA kit that has a limit detection of 0.1 ppb.

Results

Summer 2005 atrazine levels in the Wild Rice River reached a maximum of 2.6 ppb in July and a level of 1.0 in the Sheyenne River in June. Atrazine levels in 2006 were much lower. Out of 32 samples of water taken that summer, zero had amounts of atrazine that was higher than 1.0 ppb. The Wild Rice showed an increase in 2007 while the Sheyenne River remained low in atrazine content. During this period in the Wild Rice we found 19 of the 32 samples to have at least 1.0 ppb and 13 of those to be higher than 2.0 ppb. Summer months were the only time we saw detectable levels of atrazine.

One factor that may have played a role in the low atrazine levels in 2006 was rainfall amounts and the subsequent affect on river flow. In 2005, our sampling season saw an above average rainfall amount. Then in 2006, we saw a below average rainfall amount. The summer of 2007 brought what we considered to be a normal rainfall summer.

Discussion

There was a substantial difference between the atrazine levels in 2006 to 2007 in the Wild Rice River. The 68% increase in harvested corn in North Dakota is the most likely contributing factor to this increased detection of atrazine. It is predicted that there will be more corn planted in the next few years as we continue to strive for alternative sources of energy. This could potentially create higher levels of atrazine in the rivers. Another contributing factor may be the precipitation and river flow patterns during the spring and summer. Both years 2005 and 2006 brought unusual flow. We plan on sampling the Wild Rice River this upcoming spring and summer to see if the trend of higher atrazine levels continues.

This work is supported by NIH grant P20RR016741 from the NCRR.

THE ACCELERATED LEUCKART REACTION IN THE SYNTHESIS OF PHARMACEUTICALS

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The Leuckart reaction is a unique one step method of reductive amination. It is a remarkably simple process that includes only two components: the carbonyl compound and formamide. The reaction is completed simply by heating the components at 160ºC to 185ºC for 6 to 25 hours. The long processing time seems to be the only shortcoming of the reaction. During their work with formamide fungicides, Bobylev et al developed an accelerated procedure for the Leuckart reaction. The accelerated Leuckart reaction could be completed in 30 minutes or less. This work comprises an attempt to use the accelerated Leuckart reaction for the synthesis of commercially important pharmaceuticals. Specifically, the accelerated Leuckart reaction was successfully applied to 1-(3-trifluoromethylphenyl)-2-propanone and produced N-[1-(3-trifluoromethylphenyl)-2-propyl]-formamide (I) in good yield. I and the analogs are immediate precursors of important weight reduction medicines. The project is supported by NIH grant P20 RR016741 from the NCRR.

α_{1A} ADRENERGIC RECEPTORS REGULATE NEUROGENESIS AND COGNITIVE FUNCTION

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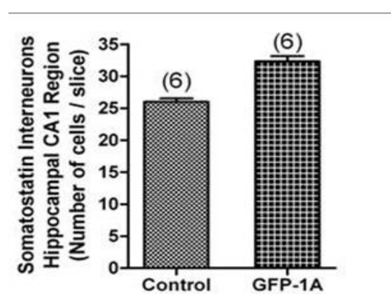
[a]Departments of Pharmacology, Physiology & Therapeutics and [b]Cell Biology & Anatomy University of North Dakota School of Medicine & Health Sciences, Grand Forks, ND 58202-9037 [c]Deparatment of Molecular Cardiology, The Cleveland Clinic Foundation, Cleveland OH 44195

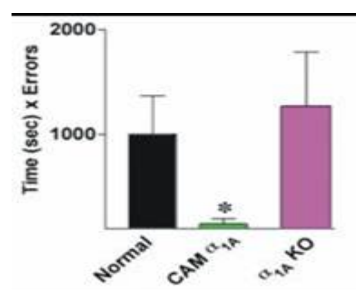
The adrenergic system is involved in many physiological functions including learning and memory, stress and anxiety, mood and appetite. A major target of this system is the hippocampus, a region that is critically important for learning and memory. Recent studies have suggested that alpha-1A adrenergic receptors (α1A-ARs) may regulate neurogenesis and neuronal differentiation (1, 2). However, our understanding of the function of α1A-ARs has been limited due to a lack of specific ligands and antibodies. To address this problem, transgenic mice were generated which over-express the α1A-AR with enhanced green fluorescent protein (EGFP) or constitutively active mutant (CAM) α1A-AR. Knockout (KO) α1A mice were also generated.

Neurogenesis continues in the mammalian brain proceeding birth. The most active neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus (3, 4). Immunohistochemistry showed that the CAM α1A-AR mice had increased BrdU incorporation in the SVZ compared to normal and KO α1A-AR mice. Increased numbers of hippocampal interneurons in CAM α1A mice compared to normal mice were also observed, demonstrated n the left figure.

An increase in the number of hippocampal interneurons may affect learning and memory. To explore this, normal, CAM α1A-AR, and KO α1A-AR mice were tested on a multi-component T-maze and the Morris water maze. As illustrated in the right figure, CAM α1A-AR mice displayed increased cognitive ability in the multi-component T-maze and the Morris water maze compared to normal mice. In both models, KO α1A-AR mice displayed decreased cognitive ability in comparison to both normal and CAM α1A mice. Treating normal mice with the selective α1A-AR agonist cirazoline also showed enhanced learning and memory processes, measured by the multi-component T-maze.

Implications of these results could have great significance through linking the α1A-AR to neurogenesis. Stimulation of α1A-ARs increased neuronal density and cognitive functions and may offer a new therapeutic strategy for increasing cognitive abilities and treating neurodegenerative diseases.





1) Papay R, Gaivin R, Jha A, McCune DF, McGrath JC, Rodrido MC, et al. (2006) J Comp Neurol, 497, 209-222.
2) Jin K et al. (2002) Proc Natl Acad Sci, 99, 11946-11950.
3) Lie, DC, Song, H, Colamarino, SA, Ming GL, & Gage FH. (2004) Annu Rev Pharmacol Toxicol, 44, 299-421.
4) Ming, GL, & Song H. (2005) Rev Neurosci., 28, 223-250.

Acknowledgements: Supported by North Dakota Experimental Program to Stimulate Competitive Research (ND EPSCoR) through National Science Foundation (NSF) Grant Number EPS-0447679 (VAD), ND EPSCoR AURA Award (DDS), NSF Doctoral Dissertation Award (CAK), NSF Faculty Early Career Development (CAREER) Award Grant Number 0347259 (VAD), NSF Research Experience for Undergraduates (REU) Site Grant Number 0639227 (VAD), National Institutes of Health (NIH) Grant Number RO1HL61438 (DMP), and NIH Grant Number 5P20RR017699 from the COBRE program of the National Center for Research Resources (PAC,VAD).

DEVELOPMENT OF TARGET-INDUCED FLUORESCENT NANOPARTICLES FOR THE DETERMINATION OF MERCURY

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Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

The reduction of ionic mercury (Hg^{2+}) by thiamine (Vitamin B_1) in conjunction with nanoparticles was studied. This research was focused on the enhancement of ionic mercury detection using thiamine-doped silica nanoparticles. The synthesis of thiamine-doped nanoparticles was confirmed by experimenting with the supernatant of the nanoparticle synthesis solution and elemental analysis using electron dispersive X-ray spectroscopy (EDS). The oxidation of thiamine to thiochrome was quantitatively measured by using fluorescence spectroscopy and atomic absorption spectroscopy techniques. Atomic absorption techniques clearly show the ability of the nanoparticles to reduce ionic mercury to elemental mercury. Data confirmed the nanoparticles were able to reduce ionic mercury at concentrations as low as 0.01ppb. Aggregation of the nanoparticles consistently proved to be a problem. Coating the nanoparticles with a surface-modified carboxyl group decreased the aggregation and enhanced the functionality of the thiamine-doped nanoparticles. Scanning electron microscope imaging of the nanoparticles confirms the presence of less aggregated nanoparticles. Fluorescence intensity and fluorescence imaging data are both currently being researched to obtain sensitive and selective results for the determination of ionic mercury. Optimal pH data is also currently being pursued.

SCANNING ELECTRON MICROSCOPY USED TO STUDY QUANTITATIVE VARIATION IN BIOFILMS AMONG DIFFERENT MUTANTS OF *ESCHERICHIA COLI*

Tanush Wadhawan* and Birgit M. Prüβ

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Biofilm is a community that forms on surfaces or interfaces due to proliferation of microbes, which are enveloped by extracellular polymers. Studies to date suggest that biofilm formation is a complex and highly regulated process (for a review, please, see 1). There are many genes involved in biofilm formation whose mutation would alter the morphological structure of the biofilm. We used *Escherichia coli* as a model to investigate the quantitative effects of several surface organelle and global regulator genes on biofilm formation. Wild-type *E. coli* and its six isogenic mutants were grown on Luria Bertani Broth (LB; 1% tryptone, 0.5% NaCl, 0.5% yeast) plates at 37ºC and then inoculated in liquid LB medium at 37ºC. From this overnight culture, we inoculated a six well plate, containing glass discs in tryptone broth (1% tryptone, 0.5% NaCl) and incubated at 37ºC for 40 h. To harvest the biofilms, each of the wells was washed with 1% PBS and dried for 10 min. The biofilms obtained on the glass discs were fixed in 2.5% glutaraldehyde and dehydrated using a graded alcohol series, then critical-point dried using an Autosamdri-810 critical point drier (Tousimis Research Corporation, Rockville MD) with liquid carbon dioxide as the transitional fluid. The samples were attached to aluminum mounts and silver paint and coated with gold/palladium using a Balzers SCD 030 sputter coater (Balzers Union Ltd., Liechtenstein). Images were obtained using a JEOL JSM-6490LV scanning electron microscope (Fig. 1).

Microscopic analyses of the mutants yielded quantitative and qualitative differences. For example, the *fimH* mutant strain that was unable to synthesize fimbriae exhibited a quantitatively less dense biofilm then the wild- type bacteria (Fig. 1). In contrast, the *fliA* and the *flhD* mutants that are unable to produce flagella produced a biofilm that was quantitatively similar to wild-type, but less well structured. The *ompR* and the *rcsB* mutants both overexpressed flagella and produced a denser biofilm than the wild-type bacteria. Obviously, flagella and fimbriae are both important attachment tools. Fiber-like structures were seen for some of the strains and correlate with the occurrence of flagella. We will identify these structures in the future.

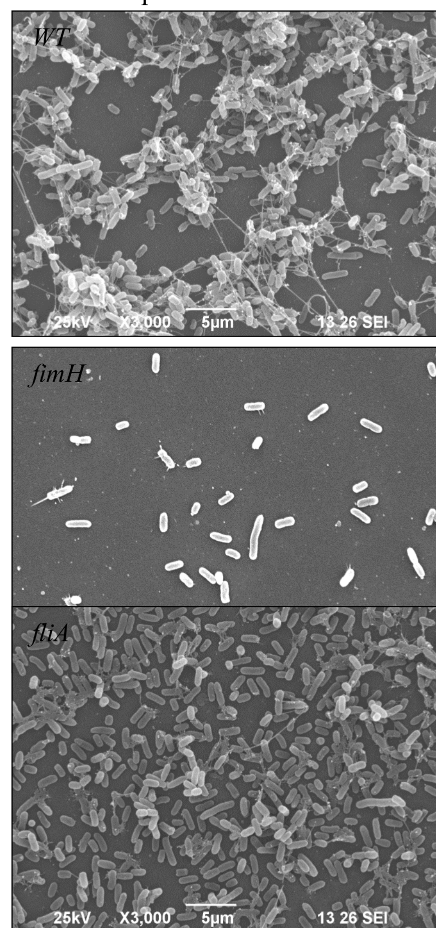


Fig. 1: Scanning electron micrographs for wild-type, *fimH* and *fliA* mutant.

Reference

1.) Birgit M. Prüß, Christopher Besemann, Anne Denton, and Alan J. Wolfe**.** A Complex Transcription Network Controls the Early Stages of Biofilm Development by *Escherichia coli*. J. Bacteriol. 2006. **188**: 3731-3739.

Acknowledgment

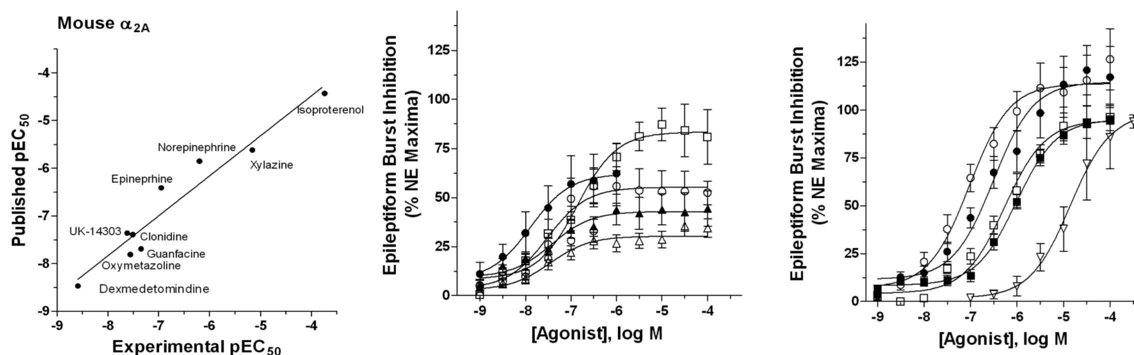
The project is funded by the NDSU Development Foundation.

ALPHA-2 ADRENERGIC RECEPTOR INHIBITION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY: COMPARISONS OF LIGAND EFFICACY AND POTENCY

Jenna M. Wald*, Brianna L. Goldenstein, Brian W. Nelson, Ke Xu, Jacquline A. Pribula, Jasmine J. O'Brien, Kylie L. Davis, Kristan M. Green, Sarah J. Boese, Jessica A. Lichter, James E. Porter, Van A. Doze

Department of Pharmacology, Physiology, and Therapeutics, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND 58202

Alpha-2 adrenergic receptors (ARs) are important regulators of many physiological processes (1). Although many AR ligands have been characterized on peripheral ARs, little is known about their actions in the central nervous system. This study focused on generating agonist ligand data for the alpha-2 AR which mediates the antiepileptic effects of the neurotransmitter norepinephrine in the hippocampus (2). We hypothesized that among the different types of agonists tested, the performance of catechcolamines on alpha ARs will be higher than that of the synthetic imidazoline and guanidine compounds because most of the catecholamines tested were endogenous or derivatives of the endogenous neurotransmitters in the brain. Using extracellular field potential recordings in the hippocampal cornu ammonis 3 (CA3) region of rodent brain slices, agonists of various chemical classes described above were studied, and their antiepileptiform actions were recorded and analyzed. The variations of the potency and efficacy of the chemicals are taken into consideration along with the parameters of their structural differences. The results indicated that the potency (pEC50 values) rank order was Dexmedetomidine > Guanabenz > *p*-aminoclonidine > UK-14304 > Oxymetazoline > Clonidine > Xylometazoline > Guanfacine > Naphazoline > (-)EPI > α-methyl-NE > 6-FNE > (-)NE > Deoxy EPI > Dopamine (deoxyNE) > Xylazine > (+)-NE > (-)-Phenylephrine > Isoproterenol. The rank order of potency correlated with the rodent alpha-2A-AR (r = 0.97) (left figure), suggesting that an alpha-2A-AR mediates this effect. Agonist classifications (full vs. partial) were based on their relative efficacy to the endogenous neurotransmitter norepinephrine, with a >80% relative efficacy being a full agonist. The catecholamines were all full agonists (center figure). In contrast, except for UK-14304, all of the imidazolines and guanidines were partial agonists in this system (right figure). Another interesting finding was that although the imidazolines and guanidines were partial agonists, their potency were significantly higher than that of the catecholamines. This makes intuitive sense because they are synthetic ligands that were initially developed for binding affinities. Since the differences of the structures between catecholamines were trivial while the structural differences among each class of agonist were significant, conclusions can be drawn that structural attributes of the catecholamines contribute to their efficacy and potency at inhibiting hippocampal epileptiform activity through an alpha-2A-AR.



1) Pupo AS and Minneman KP (2001) CNS Spectr, 6, 656-662.
2) Giorgi FS, Pizzanelli C, Biagioni F, Murri L and Fornai F (2004) Neurosci Biobehav Rev 28, 507-524.

Acknowledgments: Supported by: ND EPSCoR through NSF grant EPS-0447679, NSF CAREER Award 0347259, NSF REU Site grant 0639227, NIH grant P20RR017699 from the COBRE program, APS, ND EPSCoR AURA, UNDSMHS, and by NIH grant P20 RR016741 from the INBRE program.

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GRADUATE COMMUNICATIONS

IN THE

A ROGER DENISON COMPETITION

SCHEDULE OF PRESENTATIONS - `GRADUATE SESSION #1

Graduate session #1 talks will be in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center – session will be chaired by A. Rodger Denison Competition judges

MORNING SESSION

7:30 Registration desk open

8:00 Greetings from President Van Doze in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center

8:20 ZINC TRANSPORTER mRNA EXPRESSION IN THE RWPE-1 HUMAN PROSTATE EPITHELIAL CELL LINE, Amy L. Albrecht*, Seema Somji, Mary Ann Sens, Donald A. Sens, Scott H. Garrett [p. 44]

8:40 APP MODULATES ENDOTHELIAL PHENOTYPE WITHIN THE VASCULATURE: IMPLICATIONS FOR ATHEROSCLEROSIS, Susan A. Austin and Colin K.Combs [p.46]

9:00 CADMIUM INDUCED ALTERATION OF CADHERIN EXPRESSION IN THE MT-3 TRANSFECTED HUMAN PROXIMAL TUBULE CELL LINE HK-2, Chandra S Bathula; Scott H. Garrett; Maryann Sens; Donald A. Sens; Seema Somji [p.47]

9:20 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EARLY MOUSE BRAIN Development, Cain, JT, Odens, PW, Berosik, MA, and Darland, DC [p.48]

9:40 EXPRESSION OF KERATIN 6A AND KERATIN 16 IN ARSENITE AND CADMIUM TRANSFORMED UROTSA CELL LINES IS INDUCED IN VIVO AND IS ASSOCIATED WITH SQUAMOUS DIFFERENTIATION., Ling Cao, Xu Dong Zhou, Maryann Sens, Scott H. Garrett, Donald A. Sens and Seema Somji. [p.49]

10:00 KETOGENIC DIET'S EFFECTS ON LEVELS OF ENERGETIC BRAIN METABOLITES IN RAT, Jeremy W. Gawryluk, John F. Wagener, Jason Gockel, Daniel Coleman, Charles C. Swart, Susan A. Masino, Jonathan D. Geiger [p.50]

10:20 BREAK

10:40 ERK 1 PHOSPHORYLATES N-TERMINAL TAIL DOMAIN OF RAT DOPAMINE TRANSPORTER ON THREONINE-53 *IN VITRO*., Balachandra K Gorentla and Roxanne A Vaughan [p.51]

11:00 α_1-ADRENERGIC RECEPTOR STIMULATION MODULATES LPS INDUCED INFLAMMATION IN THP-1 CELLS, Laurel A. Grisanti* & James E. Porter [p.52]

11:20 TUMOR NECROSIS FACTOR ALPHA: UNCOVERING REGULATION MECHANISMS ON IONOTROPIC RECEPTORS IN THE CNS, J. H. Jara, B. B. Singh, S. LEI, C. K. Combs [p.53]

11:40 EVALUATION OF FECAL DNA PURIFICATION METHODS FOR THE DETECTION OF *E. COLI* O 157:H7 IN FECES OF NATURALLY INFECTED FEEDLOT CATTLE, Ebot S. Tabe*, James Oloya, Dawn K. Doetkott, Margaret L. Khaitsa. [p.65]

12:00 LUNCH (served in room 211 of the Norm Skalicky Tech Incubator).

AFTERNOON SESSION

1:00 HYALURONAN FRAGMENTS, AS PRODUCTS OF CARTILAGE DEGRADATION, ENHANCE CATABOLIC PROCESSES THROUGH ENHANCED MAP KINASE AND MMP ACTIVITY, Chang Liu, Danping Guo and Gene A. Homandberg [57]

1:20 THE N-TERMINAL TAIL OF THE DOPAMINE TRANSPORTER IS PHOSPHORYLATED AT MULTIPLE SITES IN VITRO AND IN VIVO, A. E. Moritz*, B. K. Gorentla and R. A. Vaughan [p.58]

1:40 PODOCYTE LOSS IN AGING OVE26 TRANSGENIC DIABETIC MICE, Jennifer Teiken*, Janice Audette, Donna Laturnus, Shirong Zheng, Paul Epstein, and Edward Carlson [p.66]

2:00 A REQUIREMENT OF CAVEOLIN-1 IN THE REGULATION OF TRPC1 MEDIATED Ca^{2+} ENTRY, Biswaranjan Pani*, Kristina Rauser and Brij B Singh [p.60]

2:20 BRANCHED *E. COLI:* ROLE OF PENICILLIN-BINDING PROTEINS IN CELL SHAPE MAINTENANCE, Lakshmiprasad Potluri* and Kevin D. Young [p.61]

2:40 BREAK

3:00 EOSINOPHILIA AND IMMUNOGLOBULIN A IN EXPERIMENTAL ALLERGIC ASTHMA, Amali Samarasinghe*, Scott Hoselton, and Jane Schuh [p.62]

3:20 GENOTYPIC RELATEDNESS OF BISON AND BOVINE FECAL ISOLATES OF *ENTEROBACTER SAKAZAKII* TO ENVIRONMENTAL, CLINICAL, AND FOOD ISOLATES BY PULSED-FIELD GEL ELECTROPHORESIS. , Tracy A. Solseng*, Tara Johnson, Lillian M. Nangoh, Heather Vinson, Margaret Khaitsa and Penelope S. Gibbs [p.63]

3:40 CHARACTERIZING THE GROWTH OF *ESCHERICHIA COLI* O157:H7 ON THE SURFACE OF BEEF., Preeti Sule, Catherine M. Logue, Birgit M. Prüβ [p.64]

4:00 LIPID RAFT MEDIATED LYN KINASE ACTIVATION REGULATES HOST INNATE IMMUNITY, Shibichakravarthy Kannan*, Aaron Audet, Huang Huang, Weidong Zhang and Min Wu [p.68]

4:20 AN ASSESSMENT OF HEAVY METAL TOXICITY IN EMBRYONIC AND LARVAL AXOLOTL (*AMBYSTOMA MEXICANUM*) , Kenneth C. Cabarle and Christopher K. Beachy [p.70]

4:40 *NEROICKETTSIAL* ENDOSYMBIONTS OF TREMATODES IN NORTH DAKOTA, Jay Schroeder, Vasyl V. Tkach, Jefferson A. Vaughan [p71]

EVENING

6:30 Banquet will be at the Hilton Garden Inn Dahl room. Cash bar open at 6:00

GRADUATE SESSION #2 CONTINUED ON NEXT PAGE.

SCHEDULE OF PRESENTATIONS `GRADUATE SESSION #2

Graduate session #2 talks will be in the Hilton Garden Inn Buckley room – session will be chaired by A. Rodger Denison Competition judges

MORNING SESSION

7:30 Registration desk open

8:00 Greetings from President Van Doze in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center

12:00 Lunch (served in room 211 of the Norm Skalicky Tech Incubator). We will also conduct our business meeting (open to all members) during the lunch hour

1:00 SILICA NANOPARTICLE-BASED ZINC SENSOR FOR *IN VITRO* STUDY OF INTRACELLULAR ZINC, Carrie L. Amiot, Yuhui Jin, David T. Pierce, Julia Xiaojun Zhao [p.45]

1:20 EFFECT OF NANOSCALE MATRIX ON FLUORESCENCE QUANTUM YIELD , Song Liang, David T. Pierce, Julia Xiaojun Zhao [p.56]

1:40 SYNTHESIS OF SILICA NANOPARTICLE-SUPPORTED TiO2 PHOTOCATALYSTS AND THE APPLICATION FOR GAS PHASE METHANOL OXIDATION, Aize Li, David T. Pierce, Julia X. Zhao [p.55]

2:00 PREPARATION OF LUMINESCENT SILICA NANOPARTICLES WITH CONTROLLABLE SIZES, Julia Xiaojun Zhao, Yuhui Jin [p.54]

2:20 TEACHING GEOSPATIAL TECHNOLOGIES IN THE 2007 NDVIEW GEOGRAPHIC EDUCATION OUTREACH PROGRAM , Laura B. Munski, Gregory Vandeberg, Douglas C. Munski, William Wetherholt, and Darrel Nucech [p.59]

2:40 BREAK

3:00 USING REPEAT PHOTOGRAPHY FOR AN ANALYSIS OF CHANGE IN ANTLER, NORTH DAKOTA, William Wetherholt, Douglas C. Munski, and Gregory Vandeberg [p.67]

3:20 PALEOBIOGEOGRAPHIC ANALYSIS OF UPPERMOST CRETACEOUS VIVIPARIDAE (CLASS GASTROPODA) FROM INFRATRAPPEAN SEDIMENTS OF THE DECCAN PLATEAU, INDIA, Marron Bingle* and Joseph H. Hartman [p.69]

EVENING

6:30 Banquet will be in the Hilton Garden Inn Dahl room. Cash bar open at 6:00

ZINC TRANSPORTER mRNA EXPRESSION IN THE RWPE-1 HUMAN PROSTATE EPITHELIAL CELL LINE

Amy L. Albrecht*, Seema Somji, Mary Ann Sens, Donald A. Sens, Scott H. Garrett
University of North Dakota

The prostate accumulates and stores the highest amount of zinc of all soft tissues within the body, yet during prostatic neoplasia, the zinc content of this organ consistently and persistently falls to much lower levels. There are specialized membrane bound transporters that help to regulate the zinc homeostasis of the prostate. These transporters have been categorized into two families: ZnT (*SLC30*) and Zip (*SLC39*). Many of these genes have been newly discovered and full function and physiological role is not yet completely known. The ZnT family is responsible for pumping zinc out of the cell or into intracellular vesicles, decreasing the intracellular levels of zinc. In contrast, the Zip family is responsible for increasing the zinc concentration by transporting the metal into the cell or out of the vesicles. The goal of the present study was to determine if the immortalized human prostate cell line (RWPE-1) could serve as a model system to study the role of zinc in prostate cancer. The study examined the expression of mRNA for 19 members of the zinc transporter gene family in normal prostate tissue, the prostate RWPE-1 cell line, and the LNCaP, DU-145 and PC-3 prostate cancer cell lines. Real-time PCR was used to quantitatively measure the amount of mRNA present in normal prostate tissues, the normal prostate cell line, RWPE, and commonly utilized prostate carcinoma cell lines. The study demonstrated that the expression of the 19 zinc transporters was similar between the RWPE-1 cell line and the in situ prostate gland. Of the 19 zinc transporters, only 5 had levels that were different between the RWPE-1 cells and the tissue samples; all five being increased (ZnT-6, Zip-1, Zip-3A, Zip-10, and Zip-14). The response of the 19 transporters was also determined when the cell lines were exposed to 75 μM Zn^{+2} for 24h. It was shown for the RWPE-1 cells that only 5 transporters responded to Zn^{+2} with mRNA for ZnT-1 and ZnT-2 being increased while mRNA for ZnT-7, Zip-7 and Zip-10 transporters were decreased. It was shown for the LNCaP, DU-145 and PC-3 cells that Zn^{+2} had no effect on the mRNA levels of all 19 transporters except for an induction of ZnT-1 in PC-3 cells. Overall, the study suggests that the RWPE-1 cells could be a valuable model for the study of the zinc transporter gene family in the prostate.

SILICA NANOPARTICLE-BASED ZINC SENSOR FOR *IN VITRO* STUDY OF INTRACELLULAR ZINC

Carrie L. Amiot, Yuhui Jin, David T. Pierce, Julia Xiaojun Zhao
University of North Dakota

Optimization of surface modified silica nanoparticles has been done for enhanced Zn(II) determination. The molecule (carboxymethyl-{4-{10-(4-dicarboxymethylaminobenzyl)anthracen-9-ylmethyl}phenyl}amino) acetic acid dipotassium salt (CDAPAP) has shown a strong preference for Zn(II) with the interference of other metal ions. The reaction conditions, including buffer selection and pH, have been carefully studied. The reaction time has been compared for the detection of Zn(II) by the free CDAPAP molecule vs. the CDAPAP surface modified nanoparticles, this was measured by detecting the fluorescence intensity using a spectrofluorometer. When Zn(II) is introduced to the sensor it gives an increase in the emission peak at a wavelength of 430 nm, when excited at 377 nm. Although there is a high preference for Zn(II), the binding of the metal to the sensor is easily reversible for repeated use.

Zn(II) sensors are very popular because of its diverse role within biological systems. For instance, it is a necessary component in DNA synthesis and apoptosis. It also plays an important role in protein function. Zinc is also related to some diseases it has been found that the zinc concentration is very high for Alzheimer's patients.

Nanotechnology presents exciting and truly revolutionary approaches to address global challenges in the scientific world. A unique property of the nanoparticles is their size, resulting in two useful features, high mobility and high surface area to volume ratio. These features provide nanoparticles with very high sensitivity as a signaling reagent when combined with target-induced fluorescent compounds. By binding about 15,000 CDAPAP molecules to a single nanoparticle, the fluorescence signal can be localized for enhancement of the signal of low levels of Zn(II) present. This is particularly useful when using the fluorescence microscope to image cellular samples, and to monitor change over time.

APP MODULATES ENDOTHELIAL PHENOTYPE WITHIN THE VASCULATURE: IMPLICATIONS FOR ATHEROSCLEROSIS

Susan A. Austin and Colin K. Combs
University of North Dakota

The function of the amyloid precursor protein (APP) is unclear. However, due to its ability to bind components of the extracellular matrix and propagate signaling responses via its cytoplasmic phosphotyrosine, $_{682}$YENPTY$_{687}$, binding motif, it has been suggested to function in cell-cell or cell-matrix adhesion. Endothelial APP expression is upregulated by activating stimuli and overexpression can be toxic to these cells. Based upon these data and our prior work demonstrating that APP participates in tyrosine kinase-based proinflammatory signaling in immune cells, we hypothesized that endothelial APP may also be involved in generating a response to activating stimuli.

To begin addressing this hypothesis we utilized a well defined atherosclerotic mouse model of disease, apolipoprotein E deficient mice, and human atherosclerotic tissue to model vascular inflammation. First, we examined aortic immunoreactivity for APP and phosphorylated APP at Tyr residue 682 (pAPP). Staining revealed strong immunoreactivity for APP and pAPP in human and mouse atherosclerotic aortas, which colocalized with the endothelial marker, vonWillebrand factor. The cerebrovasculature of atherosclerotic mice also revealed strong endothelial immunoreactivity for APP and pAPP. Further, Western blot analyses from atherosclerotic brains showed statistically higher protein levels of APP and pAPP and increased association with the tyrosine kinase, Src. Lastly, utilizing a modified Stamper-Woodruff adhesion assay, we demonstrated that adhesion of monocytic cells to brain endothelium is at least partially APP-dependent. To address the role of APP in vitro, we utilized primary aortic endothelial cells. Using an N-terminal APP antibody, 22C11, we mimicked ligand binding-induced multimerization of APP to examine the proinflammatory consequences of such multimerization. Multimerization of APP led to a Src kinase dependent increase in phosphorylation of APP along with increased levels of numerous proinflammatory proteins, including VCAM, Cox-2 and iNOS. Taken together these data suggest that APP expression and/or signaling are involved in the endothelial dysfunction associated with atherosclerosis and defining endothelial APP-mediated signaling events may offer molecular targets for therapeutic drug design.

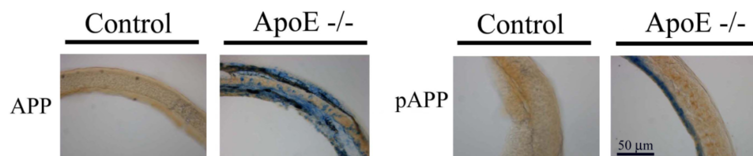
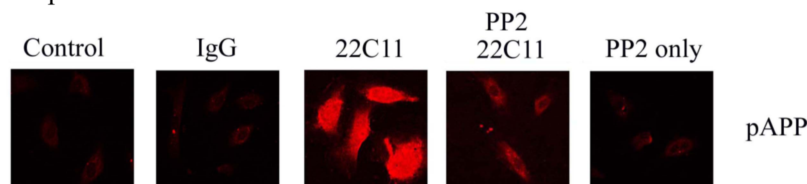


Figure 1. ApoE$^{-/-}$ abdominal aorta demonstrated increased immunoreactivity for APP and phosphorylated APP (pAPP) compared to control animals.



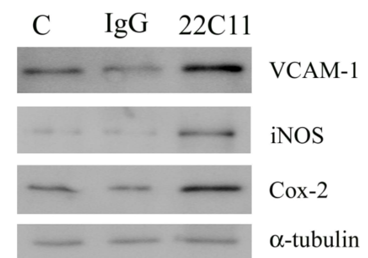


Figure 3. Cross-linking APP with agonist 22Cll antibody stimulates increased endothelial VCAM-1, iNOS, and Cox-2.

Figure 2. Cross-linking APP with agonist 22Cll antibody stimulates endothelial APP phosphorylation that is attenuated by the Src family inhibitor, PP2.

CADMIUM INDUCED ALTERATION OF CADHERIN EXPRESSION IN THE MT-3 TRANSFECTED HUMAN PROXIMAL TUBULE CELL LINE HK-2

Chandra S Bathula; Scott H. Garrett; Maryann Sens; Donald A. Sens; Seema Somji

Department of Pathology, School of Medicine and Health Sciences,
University of North Dakota, Grand Forks, ND 58202

Cadmium (Cd^{+2}), a heavy metal, is known to induce nephrotoxicity in the kidney involving inflammation and necrosis of the proximal tubule cells. A group of proteins known as metallothioneins can bind heavy metals and can provide protection against their toxic effects. Previous work from this laboratory has implicated the expression of the third isoform of metallothionein (MT-3) in the maintenance of proximal tubular vectorial active ion transport as evidenced by the formation of domes. It was shown that, an immortalized human proximal tubule cell line HK-2 does not form domes and further studies had indicated that this cell line also does not express the Metallothionein-3 isoform that is normally expressed in the proximal tubules of the kidney *in-situ* as well as in cell cultures. Transfection of the MT-3 gene into HK-2 cell line resulted in dome formation by confluent cell monolayers. This property seems to be specific for the MT-3 isoform and is not a general property of all the MT isoforms. In the current study, this association was further explored by determining the effect of MT-3 expression on the expression levels of E-,P-,N-.K-, and Ksp- Cadherins. Significant increase in the expression levels of mRNA and protein for the E-, P-, and Ksp-Cadherins were observed in HPT cells and HK-2 MT-3 cells compared to that of HK-2 cells transfected with the blank vector. In contrast, the HK-2 (blank vector) cells had significantly elevated expression of N- and K- cadherin compared to that of HPT and HK-2 MT-3 cell lines. In addition, we also determined the influence of Cd^{+2} exposure on dome formation, trans epithelial resistance and expression levels of MT-3 in proximal tubular cells and HK-2 MT-3 cells. It was shown that exposure to Cd^{+2} eliminated vectorial active transport, reduced trans epithelial resistance but did not reduce the expression levels of MT-3 in proximal tubular cells. The studies on the expression levels of cadherins have implicated that MT-3 might be involved in epithelial to mesenchymal transition (EMT) that is postulated to occur during several disease states and in the mesenchymal to epithelial transition (MET) that occurs during normal kidney morphogenesis.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EARLY MOUSE BRAIN DEVELOPMENT

Cain, JT*, Odens, PW, Berosik, MA, and Darland, DC
Biology Department, University of North Dakota, Grand Forks, North Dakota, United States of America.

We investigate neurovascular interactions in developing central nervous system, specifically examining the role that VEGF plays in regulating radial glia differentiation and developing pial blood vessels. VEGF in mouse has three isoforms, VEGF 120, 164, and 188, that differ in their ability to bind heparan sulfate proteoglycans on the cell surface and in the extracellular matrix (ECM) ([4], [5]). Our hypothesis is that diffusible isoforms of VEGF mediate vascular cell-radial glia interactions, including radial glia differentiation and end feet formation during developmental neurogenesis and angiogenesis.

Total RNA was isolated from developing neuralepithelium of wild-type (E7.5, E9.5 and E11.5) and VEGF188 (E9.5) mice ([6]). Gene-specific primers were used to generate product DNA (by reverse-transcription/PCR) for a standard curve in quantitative real-time (Q-RT/PCR). DNA values shown were calculated from Real-time C_T value relative to the standard curve run in parallel using AbsolutBlue™ Cybr green and normalized to 18S. The mean and standard deviation of 7-10 samples were graphed and Kruskal-Walis tests were run to determine statistical significance with an a value set at 0.05 (Figure 1). E11.5 brain sections were immunolabeled for RC2 (radial glia), laminin and lectin (blood vessles) in VEGF 188/188 (right) and wild type (left) mice (Figure 2).

Immunolabeling VEGF188 and wild-type neuralepithelium with the radial glia marker, RC2, revealed altered radial glia formation and disrupted end feet (Figure 2). Furthermore, fibronectin, laminin, and α6 integrin showed increased labeling throughout the neuralepithelium, particularly along vessels (data not shown). Comparative analysis of wild-type and VEGF isoform mice revealed that transcript levels for the stem cell marker, nestin, and vimentin (radial glia cytoskeleton) were downregulated in the absence of a normal VEGF isoform profile. In contrast, the Laminin C3 extracellular matrix transcript and the notch 3 transcripts were upregulated when only VEGF188 isoform was present. The transcriptional regulators FoxM1 and Hhex were largely unchanged by the change in isoform profile. Transcript levels for 18S were consistent across samples indicating baseline equivalency for comparison (Figure 1). VEGF protein levels (ELISA) remained constant in isolated neuralepithelium regardless of isoform profile (data not shown).

Absence of diffusible VEGF isoforms (120 and 164) resulted in increased deposition of ECM around developing neuralepithelium and invading vasculature. We conclude that the absence of both VEGF120 and the predominant brain isoform, VEGF164, leads to altered cell-matrix interactions that result in distorted radial glia process and end feet formation as well as disrupted differentiation.

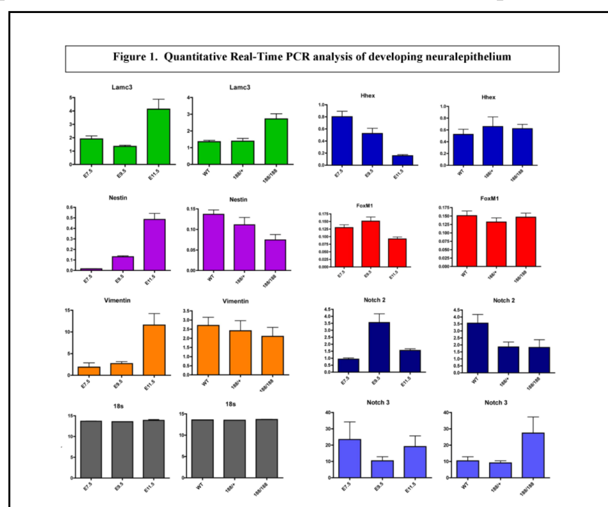


Figure 1. Quantitative Real-Time PCR analysis of developing neuralepithelium

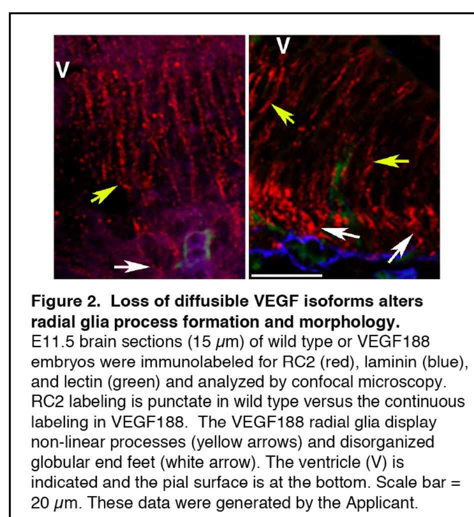


Figure 2. Loss of diffusible VEGF isoforms alters radial glia process formation and morphology. E11.5 brain sections (15 μm) of wild type or VEGF188 embryos were immunolabeled for RC2 (red), laminin (blue), and lectin (green) and analyzed by confocal microscopy. RC2 labeling is punctate in wild type versus the continuous labeling in VEGF188. The VEGF188 radial glia display non-linear processes (yellow arrows) and disorganized globular end feet (white arrow). The ventricle (V) is indicated and the pial surface is at the bottom. Scale bar = 20 μm. These data were generated by the Applicant.

([4]) Houck KA, DW Leung, et al. (1992). *J. Biol. Chem*. 257: 26031
([5]) Ferrara N. (1999). *J Mol Med* 77: 527-543.
([6]) VEGF isoform transgenic mice were generated by an international consortium of scientists and used with permission of Dr. P.A. D'Amore, Schepens Eye Research Institute and Harvard Medical School, Boston, MA.

EXPRESSION OF KERATIN 6A AND KERATIN 16 IN ARSENITE AND CADMIUM TRANSFORMED UROTSA CELL LINES IS INDUCED IN VIVO AND IS ASSOCIATED WITH SQUAMOUS DIFFERENTIATION.

Ling Cao, Xu Dong Zhou, Maryann Sens, Scott H. Garrett, Donald A. Sens and Seema Somji.

Department of Pathology, University of North Dakota, Grand Forks, ND.

This laboratory has previously demonstrated that cadmium (Cd^{+2}) and arsenite (As^{+3}) transformed UROtsa cells grown on serum-containing growth medium and the derived heterotransplants all overexpressed keratin 6a mRNA and protein compared to parental UROtsa cells grown in serum-containing growth medium. Growth factor deletion and addition studies indicated that the level of keratin 6a expression was regulated by the presence of both insulin and epidermal growth factor. In contrast, growth factors had no effect on the elevated levels of keratin 6a expression found in transformed UROtsa cells. Subsequently, this laboratory generated independently, additional As^{+3} and Cd^{+2} transformed cell lines. In the present study, the expression of keratin 6a and 16 was determined in these transformed UROtsa cell lines grown in serum containing media and the tumor heterotransplants. Real time PCR and western analysis was used to determine gene and protein expression in the transformed cell lines and heterotransplants. Immunohistochemistry was performed on the tumor heterotransplants. The data obtained indicates that parental UROtsa cells express low levels of keratin 6a and 16. However, the process of transformation induces the expression of keratin 6a and 16 in some of the As^{+3} and Cd^{+2} transformed cell lines. The tumor heterotransplants generated from these cell lines all expressed keratin 6a and 16. Immunohistochemical analysis of these tumors indicated that the expression of keratin 6a and 16 was localized to the area of the tumor with squamous differentiation. In conclusion, our data suggests that there is a gain in expression of keratin 6a and 16 when the transformed cell lines are grown as heterotransplants, and the expression is associated with squamous differentiation.

KETOGENIC DIET'S EFFECTS ON LEVELS OF ENERGETIC BRAIN METABOLITES IN RAT

Jeremy W. Gawryluk *[1], John F. Wagener [1], Jason Gockel [4], Daniel Coleman [3], Charles C. Swart [2,3], Susan A. Masino [2], Jonathan D. Geiger [1]

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[2] Biology Dept., Trinity College, Hartford, CT
[3] Neuroscience Dept., Trinity College, Hartford, CT
[4] Psychology Dept., Trinity College, Hartford, CT

Ketogenic diets (KD) have been used for close to a century to facilitate medically intractable epilepsy. The anticonvulsant effects of KD develop slowly with chronic ingestion but the molecular mechanisms that underlie its actions remain unidentified. We have previously reported that KD significantly increased the phosphocreatine to creatine ratio (PCr/Cr) in hippocampus of seizure-naïve rat (1). After further analyses, we hypothesize that KD increases levels of high-energy metabolites in discrete rat brain regions. We measured by HPLC levels of adenine nucleotides, adenosine, phosphocreatine and creatine. Using a head-focused high-energy microwave system to quickly kill rats and instantly snap-inactivate brain enzymes, we observed significantly elevated levels of high-energy brain metabolites in rats fed KD. We observed increased PCr/Cr ratios, levels of ATP, and adenylate energy charge values in these discrete brain regions. KD-induced increases in levels of adenosine did not reach statistical significance in any of the tested regions. Electrophysiological recordings from KD-fed rats demonstrated changes in synaptic transmission as well as the influence of purines on synaptic transmission that were consistent with the alterations in adenylate energy charge and purine metabolism observed neurochemically. These anticonvulsant and neuroprotectant effects of KD may be owed to increased PCr/Cr ratios and a superior ability to maintain elevated levels of ATP. (Supported by NCRR P20RR017699 & MH065431 (JDG) & NS 29173 (SAM) & CT Space Grant (SAM & CCS))

(1) Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ.: Annuals of Neurology. 2006 Aug; 60 (2) : 223-35.

ERK 1 PHOSPHORYLATES N-TERMINAL TAIL DOMAIN OF RAT DOPAMINE TRANSPORTER ON THREONINE-53 *IN VITRO*.

Balachandra K Gorentla and Roxanne A Vaughan

University of North Dakota

N-terminal phosphorylation of DAT is involved in dopamine efflux and subcellular distribution, but the DAT amino acid residues, protein kinases and protein phosphatases involved in this process are not known. In this study, we showed that a variety of protein kinases including PKCα, PKA, CaMKII, and ERK1/2 phosphorylate the recombinantly expressed N-terminal tail of DAT (N-DAT) *in vitro*. The phosphoamino acid analysis specifically showed that PKCα phosphorylates on serine(s) and ERK1 phosphorylates on threonine(s) of N-DAT. Protein phosphatases (PP1 and PP2B) were able to dephosphorylate the PKCα phosphorylated N-DAT; however, none of the tested protein phosphatases dephosphorylated the ERK1 phosphorylated N-DAT. Further, we have identified T53 as ERK1 phosphorylation site on N-DAT *in vitro* and localized the threonine phosphorylation in an N-terminal portion of striatal DAT *in vivo*. These studies implicate the role of multiple kinases or phosphatases in differential phosphorylation or dephosphorylation respectively of DAT N-terminus *in vivo*.

Funding support: RO 1 DA13147 to R.A.V, Red river Neuroscience initiative and ND EPSCoR Doctoral Dissertation Fellowship to B.K.G.

α_1-ADRENERGIC RECEPTOR STIMULATION MODULATES LPS INDUCED INFLAMMATION IN THP-1 CELLS

Laurel A. Grisanti* & James E. Porter

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University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND

Catecholamine release from the sympathetic nervous system is an important modulator of immune regulation. As a response to stress or injury, levels of catecholamines are increased, which impacts the expression of inflammatory cytokines. Adrenergic receptors (AR) are G protein-coupled receptors that mediate the effects of epinephrine and norepinephrine. They have been shown to be expressed on various immune cell populations, but have not been well characterized and their functions are virtually unknown. THP-1 cells, a human monocytic cell line, and phorbol 12-myristate 13-acetate (PMA), which differentiates THP-1 cells into macrophages were used to measure expression of the inflammatory mediator interleukin-1β in response to α_1-adrenergic receptor (AR) activation with or without lipopolysaccharide (LPS). We hypothesized that α_1-ARs are present on THP-1 cells and that stimulation will result in changes in cytokine production. Radioligand binding studies characterized a homogenous population of α_{1b}-ARs on THP-1 cells, which changed to a heterogeneous population of α_{1a}-, α_{1b}- and α_{1d}-ARs upon differentiation into macrophages. IL-1β production in response to LPS stimulation decreased in response to α_1-AR activation on THP-1 cells. A function which was not observed when THP-1 cells were differentiated by PMA into macrophages. This demonstrates that α_1-ARs are present on both monocytic cells and monocyte derived macrophages and represents a mechanism where modulation of AR input can be used to regulate an immune response.

TUMOR NECROSIS FACTOR ALPHA: UNCOVERING REGULATION MECHANISMS ON IONOTROPIC RECEPTORS IN THE CNS

J. H. JARA[1], B. B. SINGH[2], S. LEI[1], C. K. COMBS[1]

Excitotoxic activation of *N*-methyl-D-aspartate (NMDA) receptors is a mechanism of neuron death commonly hypothesized to occur in various neuroinflammatory paradigms including Alzheimer's disease. Although changes in glutamate release or uptake may precipitate this excessive receptor activity, it is possible that other factors also contribute to excitotoxic activation. For example, tumor necrosis factor alpha (TNFα) is a pleotropic cytokine that is elevated in the central nervous system in a diverse set of neuroinflammatory conditions where it may contribute to death or protection through binding to either TNFRI or TNFRII receptors. Previously, we demonstrated that TNFα stimulated neuronal NMDAR activity resulting in an ERK-dependent death mechanism mediated via TNFRII since agonist antibody for TNFRII but not TNFRI stimulated NMDA receptor-dependent Ca^{2+} influx and death. We hypothesize that elevated concentrations of TNFα may potentiate neuron death even in the absence of excitotoxic glutamate levels. In order to further define the effect of TNFα on NMDAR-dependent action we stimulated primary mouse cortical neuron cultures at 14 days *in vitro* with TNFα and TNFRI/TNFRII agonist antibody. Agonist antibody for TNFRII but not TNFRI were toxic alone and potentiated NMDA receptor-dependent toxicity. This correlated with the ability of TNFα and TNFRII agonist antibodies to stimulate increased NMDA receptor activity as observed by increased intracellular Ca^{2+} levels and NMDA receptor-evoked currents. In addition, using two different techniques to assess surface expression of receptors (biotinylation assays and immunocytochemistry with non-permeabilizing conditions); we observed an increase in the surface localization of NMDAR1 and GluR1 subunits after transient TNFα or TNFRII stimulation. Preliminary data suggests that the TNFRII-stimulated change in NR1 localization is mediated by PKC activity since the PKC inhibitor, Gö6976, blocks this response. These data suggest a novel mechanism whereby elevated levels of proinflammatory cytokines, particularly TNFα, may contribute to neuron loss during disease via potentiation of excitotoxicity.

Treatment	NMDA (μM) 0	10	15	20	50	100
	14 days *in vitro*					
control	98.13± 3.79	81.88±4.00	81.54±4.36	45.71±2.91*	30.00±2.04*	6.25±1.80*
TNFα	80.83±3.58	83.13±3.95	61.25±3.15*#	16.88±2.18*#	11.25±2.02*#	5.00±1.83*
TNFRI	82.5±2.81	82.19±3.38	87.50±7.26	40.00±3.16*	42.5±4.61*	22.50±3.35*
TNFRII	56.88±4.63*	45.63±2.88*§	22.73±5.06*§	25.63±2.58*§	10.63±2.50*§	5.63±1.57*

Neuronal survival is expressed as the % normalized against control and represents the mean ± SEM, *n* = 16. Statistical differences among groups are indicated as follow: * any condition versus control (NMDA 0 μM); # TNFα + NMDA versus NMDA alone; §TNFRII+NMDA versus NMDA alone. p<0.001 for all comparisons; except p<0.01 TNFα +NMDA 15μM vs. NMDA 15μM, and TNFRII+NMDA 20μM vs. NMDA 20μM; and p<0.05 TNFα+NMDA 50 μM vs. NMDA 50 μM and TNFRI+NMDA50 μM vs. control (NMDA 0 μM). Values are representative of at least 3 experiments.

Fig 1. TNFα and TNFRII agonist antibody potentiated NMDA receptor-dependent death in mature primary cortical neurons

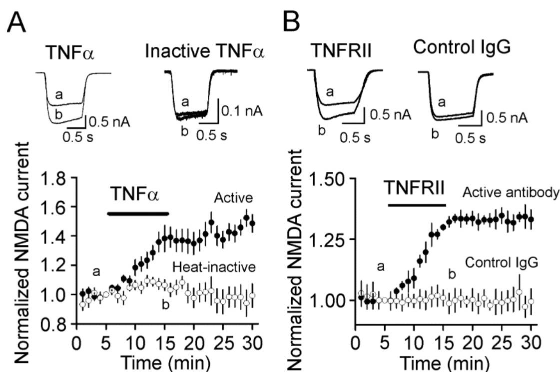

Fig 2. TNFα and TNFRII agonist antibody stimulation increased NMDA receptor-evoked currents in mature primary cortical neurons. Whole-cell patch-clamp recordings were used to record NMDA-evoked currents from primary cortical neurons. Application of TNFα or TNFRII significantly increased NMDA currents after 15 min (*p*<0.01).

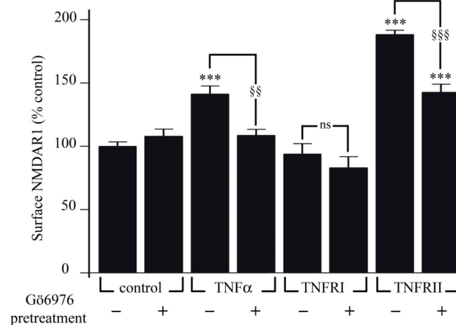

Fig 3. Quantification of immunostaining demonstrating that surface expression of NMDAR1 decreases after stimulation with TNFα and TNFRII agonist antibody previous pre-treatment with the PKC inhibitor, Gö6976. (***p<0.001 versus control or control+Gö6976, §§p<0.01 and §§§p<0.001 for each particular comparison)

PREPARATION OF LUMINESCENT SILICA NANOPARTICLES WITH CONTROLLABLE SIZES

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Luminescent silica nanoparticles are considered promising materials for labeling and luminescence probes. They have been widely studied; from toxicity to the applications of detecting trace amount of biochemical molecules. However, the performance of the luminescent silica nanoparticles is greatly affected by many parameters. One of these parameters is their size. Nanoparticles with larger diameters generally produce higher luminescent signals than the smaller ones. Smaller particles show a quicker rate of penetrating cell membranes, consequently, the toxicity of smaller nanoparticles is more severe than for larger ones. Therefore, different sized silica nanoparticles are seriously required for various research and application needs.

In this work, different sized luminescent silica nanoparticles have been successful prepared through a reverse microemulsion method. In this experiment, the concentration of surfactant and the ratio of water to surfactant were kept as constant. Choice of solvent was the only parameter that was altered. By changing the solvent from cyclohexane to pentane and other n-alkanes, different sized luminescent silica nanoparticles have been synthesized with a highly reproducible results and a uniformed size distribution. These nanoparticles cover a broad range of sizees from 20 nm to 110 nm. Some other factors, such as adjusting the temperature, and the using a mixture of solvents have been also studied to further explore the synthesis possibilities for different sized silica nanoparticles. The luminescent intensities of these nanoparticles vary due to the difference of their sizes. Finally, the basic biochemical behaviors of these nanoparticles were investigated, such as their toxicities and the rate of endocytosis by cells.

SYNTHESIS OF SILICA NANOPARTICLE-SUPPORTED TiO2 PHOTOCATALYSTS AND THE APPLICATION FOR GAS PHASE METHANOL OXIDATION

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Supported-titanium dioxide (TiO_2) particles have received increasing attention because of their great potential in photocatalysis. A newly developed template method was used to prepare silica nanoparticle (NP)-supported TiO_2. A controlled amount of H_2O was required for the synthesis of TiO_2 due to concerns of its fast hydrolysis process. By introducing SiO_2 NPs in absolute ethanol, an ultra-thin TiO_2 layer was coated on silica surfaces via the reaction between titanium precursors and the hydroxyl groups on SiO_2. The following alternating addition of titanium precursor and water results in a well-controlled TiO_2 shell growth. The final crystalline TiO_2 catalysts were achieved after being annealed at high temperatures. To investigate the catalytic performance of the SiO_2 NP-supported TiO_2, the reaction of gas phase methanol oxidation was exploited. This catalyst demonstrated better catalytic ability than TiO_2 alone. The effects of annealing temperatures and Ti: Si ratios on the catalytic properties, such as TiO_2 crystalline sizes and the catalytic activities are also discussed herein. The future work will focus on the study of nanoscale effects of silica substrates on the formation of TiO_2 and their catalytic properties.

EFFECT OF NANOSCALE MATRIX ON FLUORESCENCE QUANTUM YIELD

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A fundamental study of quantum yields of fluorescent silica nanoparticles is described. A reported dye-doped silica nanoparticle has been selected as a study model for the effects of a nanometer-sized matrix on other molecules encapsulated in it. Two types of classic dye molecules have been employed to construct the study model: an organometallic compound, tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate ($Ru(bpy)_3^{2+}$), and an organic compound, tetramethyl rhodamine (TMR). The quantum yields of the dye molecules in the nanomatrix and in bulk solutions were measured. Nanoparticles doped with various amounts of $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$ doped nanoparticles with different sizes were synthesized and studied. The results showed that the network nanostructured silica matrix significantly affects fluorescence quantum yields of the doped dye molecules. Quantum yield are also affected by the amount of the dye molecules in each nanoparticle and the size of the nanoparticles. A possible mechanism was proposed to explain how the nanostructured matrix affects the quantum yields of encapsulated dye molecules.

HYALURONAN FRAGMENTS, AS PRODUCTS OF CARTILAGE DEGRADATION, ENHANCE CATABOLIC PROCESSES THROUGH ENHANCED MAP KINASE AND MMP ACTIVITY

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Objective: Osteoarthritis (OA) is a degenerative disease that results in damage to the extracellular matrix through alteration of down- stream signaling pathways. Our laboratory is actively investigating the role of fibronectin fragments (Fn-fs), liberated from the degraded matrix, on their ability to upregulate catabolic processes by disturbing the normal signaling pathway of native fibronectin (Fn) receptors. A second system, that of type II collagen fragments (Col-fs), also enhances catabolic processes. We have now expanded to studies of a third matrix degradative system, that of hyaluronan fragments (HA-fs), which are also elevated in damaged matrix and likely in OA.

Methods: HA-fs of various masses were added to chondrocytes and cartilage cultures and effects on matrix metalloproteinase (MMP) production, cartilage damage and activation of intracellular kinases studied.

Results: We report that low mass 4.5-kDa HA-fs enhance MMPs, including MMP -3 and 13 but not an ADAMTS-5 in chondrocytes and cartilage explant cultures, to a lesser extent than a Fn-f, included as a positive control, but enhance cartilage matrix damage to a similar extent as Fn-f. The MMP upregulating activities were inversely correlated with HA mass when HA-fs of 4.5, 20, 60, 200 and 800-kDa were compared, suggesting that native HA is inactive. Studies of MAP kinase and Nf-kB activation with comparison with the Fn-f, suggested that HA-fs activated Erk1/2, p38 and JNK much more weakly and much more slowly and in some cases required 8 hours as compared with 1 hour for Fn-f, consistent with the possibility that HA-fs activate kinases only indirectly. As a possibility, HA-fs might upregulate cytokines such as IL-1 which in turn enhance kinase activation. This was tested and IL-1 was found to be elevated in HA-f treated cartilage media. To test whether the pathways for Fn-fs and HA-fs were similar furthest upstream, we tested effects of HA-fs on FAK and PYK2, and found only a weak effect on FAK.

Conclusion: We propose the low mass HA 4.5-kDa, as products of cartilage matrix degradation, may be a third matrix fragment system of relevance to OA. Most of the effects on catabolic parameters, such as MMPs and MAP kinases, were weaker or slower than those for the Fn-fs, although HA-fs still very potently damaged cartilage. Thus, the HA-f pathway may be different than the Fn-f and be damaging indirectly through MAP kinases or the MMPs studied here. Future work will focus on the role of cytokines and effects of cytokine blockers on HA-f mediated MMP upregulation.

THE N-TERMINAL TAIL OF THE DOPAMINE TRANSPORTER IS PHOSPHORYLATED AT MULTIPLE SITES IN VITRO AND IN VIVO

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The dopamine transporter (DAT) is an integral plasma membrane phosphoprotein that mediates the presynaptic re-uptake of dopamine. DAT is the main site of action for the psychostimulants cocaine and methamphetamine. DAT activity and surface expression are regulated by many kinases through unknown mechanisms. The sites of phosphorylation of DAT have been localized to the intracellular N-terminal tail (primarily serines), although the exact residues undergoing phosphorylation are currently unknown. In order to further study the phosphoregulation of DAT, our lab has recombinantly expressed the N-terminal tail of DAT (NDAT) (residues 1-65). We have demonstrated that NDAT is an *in vitro* substrate for numerous kinases including protein kinase C (PKC), extracellular signal-regulated kinase-1/2 (ERK1/2), protein kinase A (PKA), calcium-calmodulin-dependent protein kinase II (CaMKII), and casein kinase, indicating that multiple kinases may play a role in the phosphoregulation of DAT *in vivo*. Phosphoaminoacid analysis of NDAT phosphorylated by PKC, PKA or CaMKII revealed that these kinases phosphorylate NDAT exclusively on serines. The regulation of DAT by PKC is well-established, although the exact phosphorylation sites of PKC are unknown. In order to characterize these sites, the first five serines (S2, S4, S7, S12, and S13) of NDAT were individually mutated to alanine. Phosphorylation of some of these point mutants by PKC was reduced, but not abolished, indicating that PKC phosphorylates NDAT at multiple residues. Phosphorylation of NDAT by PKA was abolished by the S7A mutation, and phosphorylation by CaMKII was abolished by the S13A mutation. Two-dimensional thin layer chromatography (2D TLC) peptide mapping was conducted to help further identify the PKC phosphorylation sites. Samples of wild type (WT) NDAT were phosphorylated with either PKA, CaMKII, or PKC. They were then digested with either trypsin, thermolysin, or a combination of the two and run on 2D TLC plates. The spots obtained from the PKC-phosphorylated sample overlapped with those obtained from the PKA and CaMKII phosphorylated samples, which implies that PKC phosphorylates NDAT on serines 7 and 13. A similar peptide mapping approach was employed with rat WT striatal DAT. The spots obtained from WT NDAT samples phosphorylated with either PKA or CaMKII overlapped with those for the rat WT striatal DAT. This implies that the *in vivo* PKC phosphorylation sites are also serines 7 and 13. The possibility that other residues are also phosphorylated has not been ruled out.

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TEACHING GEOSPATIAL TECHNOLOGIES IN THE 2007 NDVIEW GEOGRAPHIC EDUCATION OUTREACH PROGRAM

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When a 501(c)3 science education organization works with a higher education institution to provide services and materials to K-12 teachers and their students, there is a synergy that is mutually beneficial. Furthermore, such activity helps to satisfy teacher preparation in science education accreditation that incorporates Interstate New Teacher Assessment and Support Consortium Principle 10, i.e., forming and engaging in partnerships among the stakeholders of an educational community (1,2). The Dakota Science Center of Grand Forks, North Dakota, and the University of North Dakota's Department of Geography are partners in the USGS-funded NDView, a program whose mission is diffusing public usage of geospatial technologies.

This three-year partnership began in 2006. The summer activities of that year consisted of a GEOG 900 workshop at Mayville State University in Mayville, North Dakota. It operated through the Division of Continuing Education of the University of North Dakota. Because the workshop developers and presenters used a creative approach to traditional teacher education, there was support for that model to be replicated and brought to a new site, Lake Region State College in Devils Lake, the next year.

The 2007 program was to be a three-pronged project. The first phase was a two-day, K-12 teacher workshop (GEOG 900) at the new site in mid-June; it highlighted pedagogy and activities using GPS, GIS, and remote sensing. The second phase was a half-day program in early August for students in grades 5-9 students attending the University of North Dakota's Young Scientists Academy; the emphasis was upon applying geospatial technologies in geocaching as opposed to traditional map and compass techniques. The third phase, working with home-school populations of parents and children, had to be reconsidered when anticipated logistics changed; that facet of the programming is being planned for implementation in the summer of 2008.

GEOG 900 was held June 13, 2007 and June 20, 2007. The first day's activities included reviewing the National Geography Standards in depth, introducing basic tools of remote sensing, conducting two GPS exercises, presenting the fundamentals of GIS using selected parts of ***Mapping Our World*** (3), and providing a take-home activity to be completed by the next workshop session. Because most of the teachers were unfamiliar with geospatial technologies, the week interval was quite appropriate. The workshop's second day activities included reviewing the previous session, completing Module 1 of ***Mapping Our World***, making the transition into an activity using remote sensing imagery, introducing a Google Earth Exercise, and handling Module 2 of ***Mapping Our World.***

Post-workshop evaluation data indicated that the most valuable aspects of the workshop were the hands-on learning with the GPS equipment (2.5 hours) plus the structure lab component when using the computer software for both GPS and GIS activities (6.5 hours). According to the participants, the workshop met its objectives, the materials were useful, and the instruction effectively handled; an overall rating of 'strongly agree' (80%) was marked for each of the aforesaid workshop attributes being evaluated**.** The teachers also agreed overwhelmingly that they preferred the week interval between class meeting days.

Following the GEOG 900 workshop, the project's second phase emerged from partnering with The Young Scientists Academy (YSA) of the UND School of Engineering and Mines, on August 1, 2007. Consequently, 25 students from grades 5-9 had a half-day program involving geospatial technologies. The youngsters were introduced to geospatial concepts, participated in a traditional latitude and longitude exercise with globes, became familiar using GPS units, engaged in a basic geocaching activity **(**1.5 Hours**)**, tied the outdoor activity with Google Earth data in an indoor computer lab activity, and were sent home with a generic geocaching exercise.

The 2007 program's overall results have justified continuation of the K-12 teacher workshop in the 2008 summer but at a different site and to expand the commitment of the program planners to the University of North Dakota's YSA program. Although the 2007 project's third phase had to be postponed to the next summer, working with home-school populations is anticipated to be feasible and another means to expand NDView's geographic education outreach programs for a currently underserved K-12 constituency. Thus, the Dakota Science Center and the University of North Dakota's Department of Geography is working together with completing its three-year cycle in 2008 and establishing new venues to promote geospatial technologies for K-12 education in North Dakota.

1) Interstate New Teacher Assessment and Support Consortium http://www. ccs.o.org/projects/Interstate New_Teacher_Assessment and Support Consortium
2) National Council for Accreditation of Teacher Education,. http://www.ncate.org
3) Malone, L., Palmer, A. M., Voigt, C. Napoleon, E., and Feaster, L. 2005. ***Mapping Our World: GIS Lessons for Educators.*** Redlands, CA: ESRI Press.

A REQUIREMENT OF CAVEOLIN-1 IN THE REGULATION OF TRPC1 MEDIATED Ca^{2+} ENTRY

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AIM: To investigate the regulation of TRPC1 by Caveolin1 and lipid rafts.

RATIONALE: "Caveolae" are cholesterol rich specialized plasma membrane domains with Caveolin1 (Cav1) as their major structural protein. These membrane domains constitute a major cellular compartment that facilitates various signal transduction events, Ca^{2+} influx being one (1). In submandibular gland (SMG) cells TRPC1 (Transient receptor potential canonical 1), a store operated Ca^{2+} entry (SOCE) channel, associates with caveolar microdomains, interacts with and requires Cav1 for its functioning (2). Recently, however STIM1 (Stromal interaction molecule 1, an endoplasmic reticulum (ER) Ca^{2+} sensor) has been identified to regulate Ca^{2+} entry into cells by interacting with plasma membrane channels that mediate SOCE (3). Whether or not the functional interaction of TRPC1-STIM1 requires caveolar microdomains is yet to be determined. In our present study we reasoned that membrane targeting of TRPC1 will be regulated by Cav1, which would therefore determine the TRPC1-STIM1 interaction and activation of SOCE. Thus, in SMG cells from Caveolin1 knockout (*cav1-/-*) mice, localization of TRPC1 with caveolar domains will be disrupted and hence TRPC1 mediated SOCE will be compromised. Consequently, physiological processes which are regulated by TRPC1 function such as saliva secretion (4), activation of Ca^{2+} dependant transcription factor NFκB (5) will be attenuated.

EXPERIMENTS/RESULTS: Using biochemical and molecular techniques, we demonstrate the association between TRPC1-Cav1 and identify their interacting domains. In mice SMG, TRPC1 and STIM1 associate with caveolar rafts as opposed to their non-raft partitioning in *cav1-/-* tissue. Interaction of TRPC1-STIM1 was preferentially caveolar and was regulated by the status of ER Ca^{2+} (co-immunoprecipitation studies).
Silencing of Cav1 limits TRPC1 membrane targeting (as seen by cell surface labeling). In Cav1 depleted cells, activation dependant recruitment of TRPC1 to membrane rafts and its functional interaction with STIM1 was also disrupted, leading to attenuation of SOCE. As a physiological readout for impaired TRPC1 function, we measured pilocarpine (a muscarinic receptor agonist) stimulated saliva secretion. Agonist stimulated saliva secretion was severely reduced in *Cav1-/-* mice. Additionally, TRPC1 mediated activation of NFκB was also repressed in Cav1 silenced cells.

CONCLUSION: Our findings demonstrate a requirement of Cav1 for TRPC1 function and saliva secretion. Additionally, our study suggests an impairment of salivary gland function associated with Cav1-/- mice, which adds to it's previously know cardiomayopathic and pulmonary hypertensive phenotypes.

REFERENCE:

1. Anderson, R., G. (1993) Caveolae: where incoming and outgoing messengers meet. *Proc Natl Acad Sci U S A.* Dec 1; 90(23):10909-13.
2. Lockwich, T. P., Liu, X., Singh, B. B., Jadlowiec, J., Weiland, S., and Ambudkar, I. S. (2000) *J. Biol. Chem.* 275, 11934-11942.
3. Ong, H.L., Cheng, K.T., Liu, X., Bandyopadhyay, B.C., Paria, B.C., Soboloff, J., Pani, B., Gwack, Y., Srikanth, S., Singh, B.B., Gill, D.L., and Ambudkar, I.S. (2007) *J. Biol. Chem.* 282, 9105-9116.
4. Liu, X., Cheng, K.T., Bandyopadhyay, B.C., Pani, B., Dietrich, A., Paria, B.C., Swaim, W.D., Beech, D., Yildrim, E., Singh. B.B., Birnbaumer, L., and Ambudkar, I.S. (2007) *Proc. Natl. Acad. Sci. U S A.* 104, 17542-17547.
5. Pani, B., Cornatzer, E., Cornatzer, W., Shin, D.M., Pittelkow, M.R., Hovnanian, A., Ambudkar, I.S., and Singh, B.B. (2006) Up-regulation of transient receptor potential canonical 1 (TRPC1) following sarco (endo) plasmic reticulum Ca2+ ATPase 2 gene silencing promotes cell survival: a potential role for TRPC1 in Darier's disease. Mol Biol Cell.17, 4446-4458.

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BRANCHED *E. COLI:* ROLE OF PENICILLIN-BINDING PROTEINS IN CELL SHAPE MAINTENANCE

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Bacterial cell shape is defined by an exoskeleton-like structure composed of peptidoglycan (PG). Polymerization and maturation of PG requires certain enzymes called penicillin binding proteins (PBPs). One of these, PBP 3, is recruited to the cell division site by the scaffold-like protein, FtsZ, to synthesize septal PG. FtsZ is the earliest known protein to move to the cell division site, where the protein polymerizes and forms a dynamic ring structure, the Z ring. The Z ring recruits other cell division proteins, leading to the formation of new cell poles of separated daughter cells.

In some bacteria altering the FtsZ concentration, results in morphological anomalies such as branches, buds or swellings. Similar morphological abnormalities are observed in *E. coli* mutants lacking certain low molecular weight PBPs. These abnormalities are more prominent in strains lacking DD-carboxypeptidase (PBP 5) along with endopeptidases (PBPs 4 and 7). To test whether these morphological abnormalities could be enhanced in PBP mutants, FtsZ was overexpressed from plasmids under control of its constitutive promoter(s). When FtsZ was overexpressed in mutants lacking PBPs 4, 5 and 7, the number of abnormalities increased, with cells producing multiple branches. Approximately 90% of the mutant cells branched. This frequency was far higher than the branching that appeared when FtsZ was overexpressed in the parent strain (~ 30 %). When the muropeptide composition of strains overproducing FtsZ was analyzed by HPLC, no significant change was observed. However, time-lapse microscopy of the triple mutant, which was overproducing FtsZ, revealed that branches arose from abnormal septation events. To investigate the role of PBP 5 in branching phenotype, we performed GFP localization studies using a torA-gfp-dacA translational fusion. Preliminary observations suggest that this fusion localizes to the septum. We hypothesize that branching in *E. coli* arises from abnormal septation events, instead of being caused by a change in the muropeptide composition.

EOSINOPHILIA AND IMMUNOGLOBULIN A IN EXPERIMENTAL ALLERGIC ASTHMA

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Eosinophil infiltration is considered a hallmark of Th2-mediated diseases, such as allergic asthma and gut-dwelling parasitic infections. Typically, immunoglobulin (Ig) E is considered the major inducer of eosinophil activation. Allergen-bound IgE is in turn bound to the surface of eosinophils by the Fc RI, causing degranulation and perpetuating inflammation. Eosinophil degranulation is believed to help rid the gut of a parasitic worm, but in the allergic lung it may lead to fibrotic remodeling of the airway. Fibrosis accounts for much of the airway dysfunction observed in chronic asthma. It is an irreversible outcome seen in many, but not all, cases of chronic asthma. Currently, there are few treatment options available. Understanding eosinophil activation may provide effective therapeutic targets for chronic asthma that are currently lacking.

While IgA's main function is to neutralize potential pathogens on mucosal surfaces, it can also activate eosinophils via the Fc R. *Hypothesis:* We hypothesize that eosinophil degranulation in the airway lumen may be triggered by allergen-specific IgA that is actively pumped to this area. The first step in testing this hypothesis was to analyze IgA levels and eosinophil numbers in the context of allergic airways disease (asthma), to determine whether IgA was produced at the appropriate time to activate eosinophils. *Method:* C57BL/6 mice were sensitized with *Aspergillus fumigatus* fungal antigens and challenged with live fungal spores to illicit allergic airways disease. Eosinophils from bronchoalveolar lavage (BAL) were counted following cyto-centrifugation and staining at days 1, 3, 7, 14, and 35 after fungal spore challenge. IgA levels in the BAL fluid were analyzed via enzyme-linked immunosorbent assay at the same time points. *Results:* Eosinophils were progressively recruited to the airways, and BAL eosinophil numbers peaked at day 7 after allergen challenge (Figure 1). IgA levels in the BALF were increased significantly in response to the allergen and also peaked 7 days post allergen challenge (Figure 2). Data from each time point was compared to naïve controls using the Welch's corrected *t* test (GraphPad InStat software).

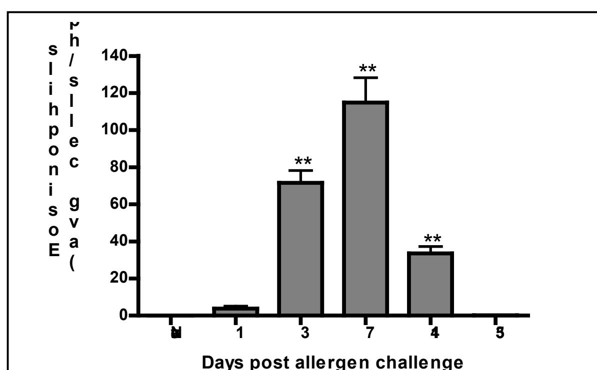

Figure 1. Eosinophilia in the lumen peaks at day 7 after allergen challenge. ** p<0.001

Conclusions: Eosinophil activation is part of the Th2 immune response against extracellular pathogens. Since eosinophilic degranulation within the tissue is harmful to the host and ineffective against a perceived pathogen in the gut or airway lumen, eosinophil activation within the lumen itself is vital but cannot be achieved with IgE which cannot cross the epithelium. Eosinophil activation and degranulation calls for a combination of appropriate signals that are available at the right time and place. The results reported in the present study support a potential role for IgA in the activation and degranulation of eosinophils in allergic airways disease. The spatial and temporal correlation between IgA and eosinophils within the lumen of the allergic lung provides an attractive mechanism for eosinophil activation in response to allergen challenge.

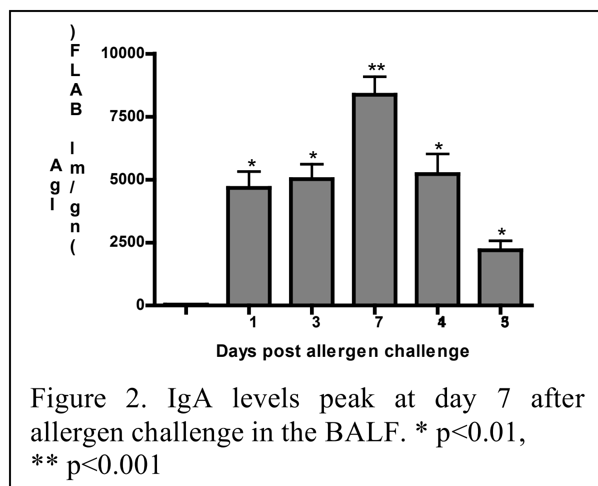

Figure 2. IgA levels peak at day 7 after allergen challenge in the BALF. * p<0.01, ** p<0.001

GENOTYPIC RELATEDNESS OF BISON AND BOVINE FECAL ISOLATES OF *ENTEROBACTER SAKAZAKII* TO ENVIRONMENTAL, CLINICAL, AND FOOD ISOLATES BY PULSED-FIELD GEL ELECTROPHORESIS.

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Enterobacter sakazakii continues to be implicated in neonatal infections such as meningitis and necrotizing enterocolitis. Powdered infant formula has been reported to be the most common source of contamination for infected neonates, but the natural habitat of this opportunistic pathogen has been elusive. In addition, the epidemiology and pathogenicity are currently not well understood (1, 2). Research in our laboratory at North Dakota State University has determined that *E.sakazakii* is present in bison and bovine feces; however, it has not been proved that this is the primary source of *E.sakazakii* contamination in powdered infant formula. The primary objective of this study was to determine the relatedness of *E. sakazakii* from various sources, including food, clinical settings, the environment, or from bovine or bison feces using Pulsed-field gel electrophoresis.

Methods. *Enterobacter sakazakii* isolates obtained from the American Type Culture Collection, North Dakota bison and bovine feces, and one clinical isolate from a neonatal meningitis case were used from our laboratory. Several food, environmental, and clinical isolates were obtained from Cornell University. All isolates were digested with the restriction enzyme *Xba*I and subjected to pulsed-field gel electrophoresis (PFGE). Analysis was performed using methods from the Fingerprinting II Informatix manual, and combined dendrograms were obtained and compared.

Results. The 40 isolates tested contained anywhere from 9 to 30 DNA fragments indicating a high degree of diversity among the isolates. The Cornell isolates F6-049 and F6-051 had the highest degree of similarity; both of these isolates were from a clinical source. The isolates from bison and bovine feces, designated 52 and N72, respectively, had only 25% similarity to each other. The bison isolate (52) displayed 65% similarity to an isolate from a food source. The bovine isolate (N72) had a 55% similarity to an isolate from a clinical source.

Discussion. Previous studies have found that PFGE is a good measure of genotypic relatedness between *Enterobacter sakazakii* isolates (3). The results of this study show that there is a high degree of diversity among *Enterobacter sakazakii* from multiple sources. This also indicates that there is a great deal of genetic diversity among species of *Enterobacter sakazakii*. However, this does not definitively prove that the isolates from bovine and bison feces are definite pathogens of neonates or the cause of powdered infant formula contamination. Comparison of additional isolates will aid in determining whether the bovine and bison isolates are a potential source of powdered infant formula contamination.

References:

1. Restaino L., E. Frampton, W. Linoberg and J. Becker. 2005. A chromogenic plating medium for the isolation and identification of *Enterobacter sakazakii* from foods, food ingredients and environmental sources. J. Food Prot. 69, 315-322.
2. Mutjens, H., and L. Kollee, 1990. *E. sakazakii* meningitis in neonates: causative role of formula. Pediatr. Infect. Dis. J. 9, 372-372.
3. Proudy, I., D. Bougle, E. Coton, M. Coton, R. Leclercq, and M. Vergnaud. 2008. Genotypic characterization of *Enterobacter sakazakii* isolates by PFGE , BOX-PCR, and sequencing of the *fliC* gene. J. Appl. Micro. 104:26-34.

CHARACTERIZING THE GROWTH OF *ESCHERICHIA COLI* O157:H7 ON THE SURFACE OF BEEF.

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Escherichia coli O157: H7 has emerged as a major public health concern over the past few years. The pathogen belongs to the enterohemorrhagic group of *E. coli* (EHEC) and is considered to be the predominant cause of hemorrhagic diarrhea. The organism is acid tolerant, hence classical treatment with organic acid sprays such as lactic acid and acetic acid have a limited effect in preventing their growth (Brackette et al., 1994). Food borne outbreaks are associated with ingestion of contaminated beef, though other contaminated foods have also been implicated. Infection generally leads to watery diarrhea, blood in the stool, nausea, vomiting and fatigue, in some cases (especially children) it can cause hemolytic uremic syndrome (HUS) resulting in acute renal failure (Gransdsen et al., 1986)

The experiment that we discuss here is ground work for future experiments, where we would like to investigate differential expression of genes associated with virulence upon treatment with various sprays (metabolic intermediates), on the surface of beef.

Meat (Beef) surface was sterilized by brief immersion in boiling water followed by removal of the top layer of meat. The sterilized meat was cut into approximate pieces of approximately 20 gm each. Each piece was then spiked with *E. coli* O157:H7 diluted to an OD_{600} of 0.10. Control samples were collected in a similar manner. All samples were placed in trays and wrapped with food grade cling films and stored at 10 ºC.

Two samples each from the spiked and the control meat were collected on day 0 and every alternate day till day 10. 10 gm of the first sample was homogenized in 90 ml of maximum recovery diluent (MRD) in a stomacher. The sample was then serially diluted as required. 100 µl of the diluted sample was plated on STAA agar, Plate Count Agar, Sorbitol MacConkey agar, Violet Red Bile Glucose agar, APT agar and the PS agar. The second sample was homogenized in 30 ml MRD and cells were pelleted out for RNA extraction.

At day 10 the Pseudomonas count (PS Agar) in the spiked sample was 8.3 Log_{10} CFU/gm. The heterofermentative bacterial count (APT agar) was 10.5 Log CFU/gm as compared to 9.5 Log_{10} CFU/gm in the control sample. The *E. coli* O157: H7 count showed a 6 log increase over the period of 10 days (Fig1).The total number of colonies (Plate Count Agar) in the treated and the spiked sample were however the same (9 Log_{10} CFU/gm).

RNA was extracted from samples and ongoing work is directed towards studying the gene expression pattern. The completion of the study shall enable us to develop a spray that would reduce the virulence of the organism and limit its spread in meat.

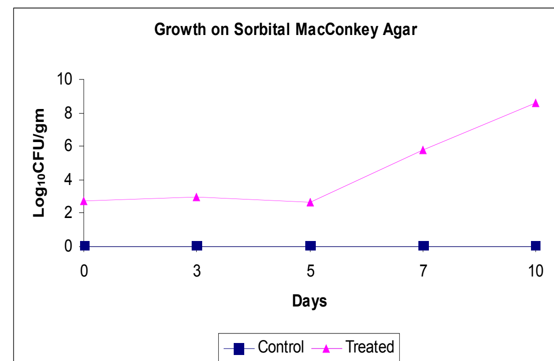

Figure1: Growth pattern of *E. coli* O157: H7 on beef surface at 10 ºC.

References

Brackett, R., Y. Hao, and M. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *J. Food Prot*. **57**;198-203

Gransden, W. R., M. A. Damm, J. D. Anderson, J. E. Carter, and H. Lior. 1986. Further evidence associating hemolytic uremic syndrome with infection by verotoxin-producing *Escherichia coli* O157:H7. *J. Infect. Dis.* **154**:522-524.

Acknowledgements.

Dr Scott Minnich- for the gift of *E. coli* O157:H7 strain
SBARE (State Board of Agricultural Research and Education)- for funding this research.

EVALUATION OF FECAL DNA PURIFICATION METHODS FOR THE DETECTION OF *E. COLI* O 157:H7 IN FECES OF NATURALLY INFECTED FEEDLOT CATTLE

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Several protocols have been used to purify DNA from animal feces for PCR; direct DNA extraction from feces, DNA extraction with previous enrichment in selective media, and the use of commercial kits that involve the use of spin columns. The objective of this study was to compare the accuracy of the QIAamp DNA Stool Mini Kit (QIAamp Kit) in detecting *E. coli* O157:H7 in enrichment and non-enrichment cattle feces from naturally infected feedlot cattle.

Using conventional culture methods (gold standard), 456 fecal samples were analyzed for detectable levels of *E. coli* O157:H7. QIAamp Kit was used to purify fecal DNA (from enrichment and non-enrichment feces) and *E. coli* O157:H7 genes (*Stx*1 and *Stx* 2) were targeted and amplified by PCR.

A total of 199/456 (43.6%) fecal samples were positive for *E. coli* O157:H7 by culture. Of the 456 enrichment fecal samples, *E. coli* O157:H7 Shiga toxin-like genes were detected from 159/456 (34.6%) enrichment fecal samples compared to 43/456 (9.4%) targeted from non-enrichment samples. There was a substantial agreement (*Kappa* > *0.61*) between the gold standard and QIAamp Kit (enrichment) as opposed to a fair agreement (*Kappa* > *0.21*) for non-enrichment samples. Positive agreement rates were greater for the enrichment samples (83.6%) compared to the non-enrichment samples (65%). Agreement rates were lower (86.4%) for QIAamp Kit for enrichment samples compared to non-enrichment samples (98.5%). These data indicate that the use of QIAamp DNA Stool Mini Kit as the select protocol for *E. coli* O 157:H7 detection in cattle feces should take into account the respective accuracy of the test for enriched and non-enriched fecal samples in comparison to the conventional culture method.

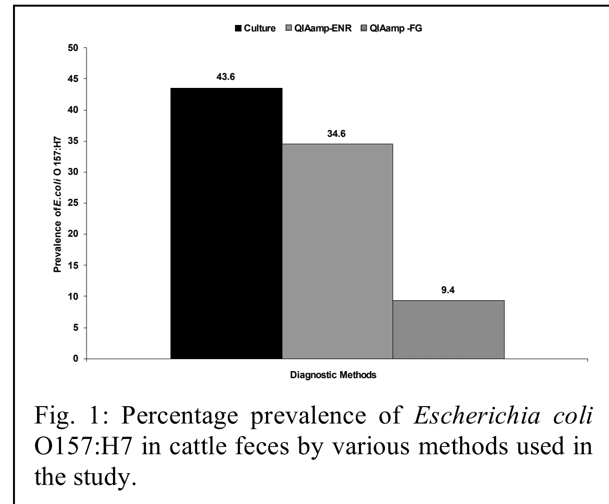

Fig. 1: Percentage prevalence of *Escherichia coli* O157:H7 in cattle feces by various methods used in the study.

Acknowledgments: We are grateful to USDA-APHIS (Agrosecurity: Disease Surveillance and Public Health) and USDA-CSREES (Food Safety Risk Assessment Grant) for providing funding for this project.

References:

1. Khaitsa, M. L., M. L. Bauer, P. S Gibbs, G. P Lardy, D. K. Doetkott, and R. B. Kegode. 2005. Comparison of two sampling methods for *Escherichia coli* O157:H7 detection in feedlot cattle. *J. Food Prot.* 68:1724–1728.
2. Gioffré, A., L. Meichtri, M. Zumarraga, R. Rodriguez, and A. Cataldi. 2004. Evaluation of QIAamp DNA stool purification kit for Shiga-toxigenic *Escherichia coli* detection in bovine fecal swabs by PCR. *Revista Argentina de Microbiologia*. 36:1-5.

PODOCYTE LOSS IN AGING OVE26 TRANSGENIC DIABETIC MICE

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The podocyte is a component of the blood-urine barrier of the kidney. Recent studies show that podocyte nuclear density (N_V) and numbers of renal podocytes per glomerulus (N) are altered in experimental and spontaneous diabetes mellitus. N_V and N are generally reduced, and it has been hypothesized that these morphological changes may relate to the loss of glomerular permselectivity in diabetic nephropathy (DN). In the current study, OVE26 transgenic diabetic mice and age-matched (FVB) controls (60, 150, or 450 days) were fixed by vascular perfusion and renal cortical tissues were prepared for morphometric analyses. ImageJ software and point counting analyses were carried out on light and transmission electron micrographs to determine glomerular volume (V_G), N_V, and N.

As expected, mean VG in OVE26 mice increased substantially (~134%) over the course of the study and was significantly increased over FVB mice at all ages. At 60 days, N_V and N were not statistically distinguishable in OVE26 and control mice, while at 150 days, N_V was significantly reduced in diabetics but not N. In 450-day-old OVE26 animals, however, N_V and N were both significantly decreased (~231% and ~99%, respectively) relative to age-matched FVB mice. These data suggest that in the OVE26 model of diabetes, significant podocyte loss occurs relatively late in the course of the disease. Moreover, it seems possible that these podocytic changes could play a role in sustaining the increased permeability of the blood–urine barrier in the later stages of diabetic renal decompensation.

USING REPEAT PHOTOGRAPHY FOR AN ANALYSIS OF CHANGE IN ANTLER, NORTH DAKOTA

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The northern Great Plains' physical and human landscapes have changed over time (1, 2). Much of the interest in studying this part of North America has emphasized a macro-scale of inquiry as part of general regional studies with emphasis upon looking at major trends in social geography and environmental change that often has centered on the controversial concept of "The Buffalo Commons" (3, 4, 5). Yet, the macro-scale of changes for a region as vast as the northern Great Plains is the product of a number of complex, interrelated alterations of the physical and human landscape at the micro-scale. While the concept of population decline in the abstract is well documented in North Dakota through census materials (6), the localized consequences of depopulation on the built environment of communities often is relegated to generic concerns of historic preservation (7). In order to bring the micro-scale to the foreground of the picture of the dynamic spatial aspects of North Dakota's settlement geography, a case study was conducted of the built environment of Antler, a declining rural community in the northwestern part of the state.

Central to this research on micro-scale landscape transformation is the method of repeat photography, a technique well-established in studies of cultural and physical environmental change (8, 9). Repeat photography is the technique of identifying the location of an earlier photograph, reoccupying the original camera position, and creating a new photograph of the exact same scene. A set of historical photographs of Antler was obtained from the State Historical Society of North Dakota and from residents of the community (10, 11).

The research question focused upon how have the images of Antler changed between when the original photographs were taken in the early 20th century compared to today. The 12 sites of the historical photos were revisited during a field trip taken on November 4, 2007. During this fieldwork, a number of repeat photographs were taken of the modern built environment in the same locations portrayed in those earlier images of Antler. Geographic coordinates, camera orientation, and noted disturbances at each scene were documented. Coordinates were obtained with a Garmin GPSMAP 60.

After the imagery was obtained, Adobe Photoshop was utilized to analyze the comparisons over time. First, each historical photograph had to be layered over by the appropriate revisited scene using the software's semi-transparency tool . Then, each pair of photographs had to be to aligned and cropped so that the overlapping imagery could be compared evidence of changes, Among the identifiable features in the photo pairs noted were buildings, roads, rail lines, poles (carrying telephone or power lines), fences, agriculture, grazing, and vegetation cover.

When the results were tabulated, the greatest change was observed in vegetation. There were extensive increases noted at nine of 12 sites. However, not all sites could be properly revisited due to various barriers, particularly overgrown vegetation, uncertain/improper orientation, and any missing elements from the historical picture that would have been useful guidepoints in acquiring the specific repeat photograph. Yet, while there were limitations of the study, sufficient data could be interpreted to obtain a strong sense of what has been happening to the built environment of Antler between the early 1900s to the time of the field work in 2007.

In conclusion, the technique of repeat photography was a relatively successful in analyzing environmental change in Antler, particularly of the changing presence of vegetation in its built environment. This methodology could be employed for similar studies in other geographic locations. Indeed, plans are underway for determining how best to implement it in studies of more urbanized locations across North Dakota with the focus of such future research in the historic central business districts of communities such as Grand Forks and Fargo.

1) Borchert JR (1987) America's Northern Heartland: An Economic and Historical Geography of the Upper Midwest, Minneapolis, MN: Univ. of MN Press.
2) Rathge, R and Highman P (1998) Rural Dev. Perspectives 13 (1), pp. 19-26.
3) Popper D and Popper FJ (1987) Planning 53 (12), pp. 12-18.
4) Popper D and Popper FJ (1999) Geog. Rev. 89 (4), pp. 491-510.
5) Popper D and Popper FJ (2004) in WorldMinds: Geographical Perspectives on 100 Problems (Janelle D, Warf B. and Hanson K eds.) , Norwell, MA: Kluwer, pp. 245-249.
6) North Dakota State Data Center, http://www.ndsu.edu/sdc/data/populationtrends.htm
7) Presevation North Dakota, http://www.prairieplaces.org
8) Nusser M (2001) Land. & Urb. Plan, 57, pp. 241-255.
9) Zier J and Baker w (2006) For. Ecol. & Mgt., 228, pp. 251-262.
10) State Historical Society of North Dakota Photographic Collections, Bismarck: ND
11) Schell EJ (1975) Antler, N. Dak. The Gateway to the U.S. 1882-1916, Antler, ND: np.

LIPID RAFT MEDIATED LYN KINASE ACTIVATION REGULATES HOST INNATE IMMUNITY

Shibichakravarthy Kannan*, Aaron Audet, Huang Huang, Weidong Zhang and Min Wu
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Introduction: Pseudomonas aeruginosa (PA) is a common nosocomial pathogen primarily affecting immunodeficient individuals and in genetically susceptible diseases such as Cystic Fibrosis and Chronic Granulomatous Disease. PA causes clinical problem in treating the infection as it is resistant to multiple antibiotics and potential to form biofilms by colonizing the airways that are difficult to eradicate. Understanding the host innate immune response to gram negative opportunistic infections like PA will help to combat this type of infection by developing newer therapeutic strategies. We studied the host – pathogen interactions and discovered the role of lipid raft mediated host signaling that enables the immune detection and elimination of PA in the immunocompetant host. Recently the alveolar epithelium has been identified to possess immune functions like signaling immune cells to attack the invading pathogens.

Background and Significance: Lipid rafts are cholesterol rich membrane microdomains that bring innate immune receptors like Toll like receptor (TLR) together with signaling molecules like Src family tyrosine kinases to transmit the extracellular events into the cytoplasmic cascade of signaling proteins activation. We identified a member of Src family, Lyn tyrosine kinase, as a major signaling component in this mechanism. Lyn has been previously well studied in B cell receptor (antibody mediated) signaling pathway. This is a novel report on innate immune function (no antibody involved) of Lyn and also first report to associate Lyn with PA infection. Lyn seems to be a very potent signaling mediator in both epithelial and macrophage cell types and orchestrates many downstream pathways that regulate cytoskeletal changes for effective phagocytosis and increased expression of pro-inflammatory cytokines.

Methods: We have used both in vitro (cell lines) and in vivo (mice and rats) approach to understand the host response to PA infection. Lipid raft isolation using sucrose density gradient method enabled us to study the signaling proteins involved in this process. Protein interaction studies and immunohistochemistry methods were employed to identify the sequence of signaling molecules recruitment to the raft domains. Phagosome isolation by detergent free method was used to study role of Lyn in phagocytosis. We have used dominant negative strategy, siRNA mediated gene silencing and Lyn knock out mice model to firmly establish the importance of Lyn in host innate immunity.

Results: We found that PA infection of alveolar epithelial type II (AEC II) cells triggered TLR2 mediated Lyn activation within lipid raft domains. Lyn was found to be important for NFκB mediated increased expression and release of macrophage chemotactic factors (MCP-1 secretion) that recruit alveolar macrophage (AM) to the site of infection. We also found that Lyn plays a major role in phagocytosis by recruiting PI3K and Akt to phagosome membranes in activated AM. Lyn also regulates superoxide release within phagosomes by inducing NADPH oxidase enzyme complex assembly and function. Thus we demonstrate multiple roles for Lyn in host immune response.

Conclusion: Lipid raft mediated Lyn kinase activation seems to be a common mechanism in both epithelial and macrophage cell types but the outcome is different. In epithelial cell type it stimulates the release of pro-inflammatory and chemotactic cytokines where as in macrophage it stimulates phagocytosis and respiratory burst activity. Targeting Lyn function will enable to devise novel strategies to boost innate immune response in susceptible individuals that will help in eliminating PA infection.

PALEOBIOGEOGRAPHIC ANALYSIS OF UPPERMOST CRETACEOUS VIVIPARIDAE (CLASS GASTROPODA) FROM INFRATRAPPEAN SEDIMENTS OF THE DECCAN PLATEAU, INDIA

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INTRODUCTION

Toward the close of the Cretaceous, the India protosubcontinent was an isolated landmass making its way northward across the Indian Ocean on a collision course with Asia. During this time, India was a landscape periodically ravished by volcanism. Numerous flood basalt flows spread west to east, covering most of western and central India. Today, these flows, known as the Deccan Traps, are delimited and interbedded by a sequence of sediments referred to as infratrappean (before trap), intertrappean (between trap), and supratrappean (after trap). In spite of frequent habitat destruction (many flows over very short periods of time, with intertrap sediment thicknesses often less than a meter), end-Cretaceous Indian environments still supported a relatively diverse continental biota. Mollusks inhabited the lakes and rivers of the terrain both before and after volcanism, but also during. The continental molluscan fauna of the Deccan Plateau included aquatic caenogastropod families (e.g., Viviparidae, Hydrobiidae, and Pleuroceridae), terrestrial taxa (e.g., Stylommatophora), and freshwater mussels (Unonoidea).

ORIGINS AND SURVIVORSHIP

The survival, recovery, and rediversification of continental mollusks in lake and river systems after episodes of volcano-induced extirpation is an exciting story at a time when the Deccan flows are considered in Cretaceous-Paleogene (K/Pg) global extinction scenarios. This consideration aside, the origin of the Deccan molluscan fauna represents its own mystery. The continental molluscan species preserved in the infra- and intertrappean beds (~67 Ma) appear no earlier than the Upper Cretaceous, but at this time, proto-India was well isolated from other landmasses. This study considers the potential paleobiogeographic origin for the Deccan molluscan fauna on the basis of statistically assisted comparative morphology. Identifying a possible origin for Deccan fauna may better constrain the geographic position of the Indian subcontinent near the end of the Cretaceous and the phylogeny of viviparids in the eastern hemisphere.

METHODS AND DISCUSSION

To begin this study, three species of "*Viviparus"* from the infratrappean locality Pijdura (InL009) in the Nand-Dongargaon Basin in central India were analyzed. Pijdura locality sediments (Maastrichtian), located about 60 km south of Nagpur in the state of Maharashtra, are composed of distinctive red-colored lacustrine fine clastics. The Pijdura species ("*Paludina*" *normalis*, "*Paludina*" *conoidea*, and "*Paludina*" *wapsharei* [genera of Hislop, 1860]) were compared to 13 modern viviparid species from four continents (*Bellamya japonica*, *B. bengalensis, B. unicolor, B.* sp. [from Egypt]*, B. angularis, Viviparus sclateri, V. contectus, Lioplax cyclostomatiformis, L. sulculosa, Filopaudina sumatrensis, Idiopoma ingallsiana, Mekongia pongensis,* and *Neothauma tanganyicense* in the collections of the University of Michigan Museum of Zoology, Academy of Natural Sciences of Philadelphia, and National Museum of Natural History, Smithsonian Institution).

Several character traits were used to create a numerical representation of the species morphology in order to assess variability and potential relationships. The degree of variability in morphology between the species was assessed by measuring basic shell parameters and then converting them into ratios to lessen any size dependent bias. Measures included maximum length, maximum width, length of final whorl, length of the spire, apertural length and width, sutural angle, and number of whorls.

A paired-group cluster analysis (R=0.9) incorporating modern and fossil taxa established a possible African origin for the Deccan fauna based on a close similarity between "*Paludina*" *normalis* (Deccan Plateau fossil) and *Bellamya unicolor* and *Bellamya* sp. (modern northern and eastern Africa). The high correlation coefficient suggests the relationships displayed could be robust and worthy of continued study. The results from this study may also lend itself to taxonomic reclassification. The placement of the Deccan viviparids into the genus "*Paludina*" is a relict of a previous taxonomic system that is no longer valid. Its current equivalent is the genus *Viviparus*. However, the occurrence of these species in India might suggest placement into the Gondwanan genus *Bellamya*. Further research will compare other fossil Deccan viviparids to a geographically broader range of modern viviparid species.

1) Hislop RS. (1860) Quart. Jour. Geolog. Soc. Lond., Proc., v. 16 (1), 154–181, 188, 189, pls. V–X.

AN ASSESSMENT OF HEAVY METAL TOXICITY IN EMBRYONIC AND LARVAL AXOLOTL (*AMBYSTOMA MEXICANUM*)

Kenneth C. Cabarle[1,2] and Christopher K. Beachy[2]

[1]Department of Biology, University of North Dakota, Grand Forks, ND 5, USA; [2]Department of Biology & Amphibian Growth Project, Minot State University, Minot, ND 58707, USA

Embryos and 75 day old larvae of the model organism *Ambystoma mexicanum* were treated with differing concentrations of the heavy metals Cadmium ($CdCl_2$) and Methyl mercury ($HgCH_3$). Each experiment exposed embryos and larvae to 8 treatment levels including controls. Treatment levels for cadmium were 0ug/L, 15ug/l, 50ug/l, 150ug/l, 1500ug/l, 15,000ug/l, 150,000ug/l and 1.5million ug/l. Treatment levels for methyl mercury were 0ug/l, 1.5ug/l, 5ug/l, 15ug/l, 50ug/l, 150ug/l, 1500ug/l and 15,000ug/l. All treatment groups were observed every 12hrs during the 96hr treatment for mortality, physical anomalies and behavioral anomalies. Tissue samples were collected from deceased individuals and organisms were fixed in 10% formalin and preserved in 70% ethanol for future analysis. Embryonic LC-50 values for cadmium are shown in Figure 1. Embryos were scored for development (figure 2.) according to Bordzilovskaya et al (1).

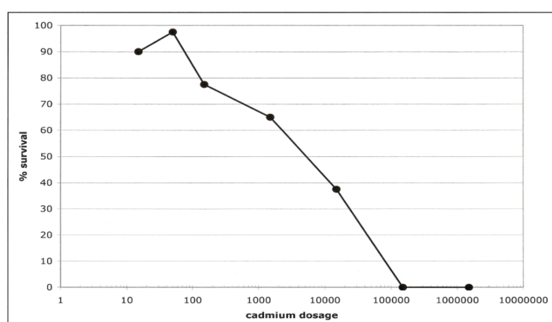

Figure 1: Embryonic LC-50 values for cadmium treated *Ambystoma mexicanum*.

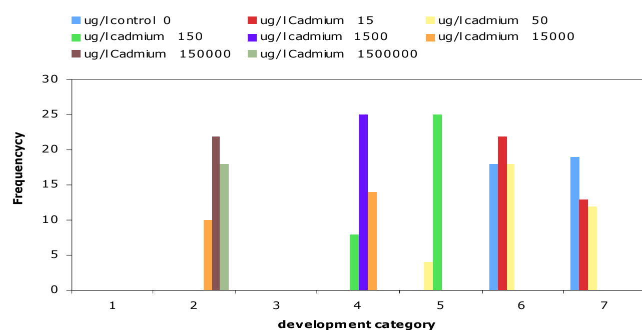

Figure 2: Developmental stage frequencies for cadmium treated *Ambystoma mexicanum*.

We present the embryonic and larval LC-50 values as well as developmental staging and percent survivorship. Toxicologically, cadmium LC-50 concentrations were higher than mercury. Additionally, mercury may have a greater effect on survival beyond exposure period at lower ecologically relevant doses.

1) Bordzilovskaya, N.P., T.A. Dettlaff, Susan T. Duhon, and George M. Malacinski. 1989. Developmental-stage series of axolotl embryos. In Developmental Biology of the Axolotl edited by J.B. Armstrong and G.M. Malacinski. Oxford University Press, New York, pp. 201-219.

NEROICKETTSIAL ENDOSYMBIONTS OF TREMATODES IN NORTH DAKOTA

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Department of Biology, University of North Dakota.

Neorickettsia are a genus of intracellular bacterial endosymbionts of parasitic trematodes (digeneans). are the only known genus of bacterial endosymbionts to use a trematode as a vector. *Neorickettsia* cause several diseases including: Sennetsu fever of humans, Potomac horse fever, and salmon dog poisoning. The lifecycle of the trematode host is complicated, usually having two intermediate hosts; cycling first through a mollusk (almost always a snail), then through a second intermediate host (fish, another snail, etc.) and finally infecting the definitive host (a vertebrate). Little is known about how the bacterium is transmitted between individual trematodes, or between trematode lineages. Our research has three main goals: 1st is to identify a local source of *Neorickettsia* infected digeneans, 2nd is to establish a *Neorickettsia* infected lineage of trematodes in the laboratory, and 3rd is to investigate the manner of transmission, both in the asexual and sexual phases of the trematode life cycle, as well as in between lineages of digeneans. In 2007, aquatic snails were sampled from a local pond. Snails were maintained in individual containers and examined daily for the presence of larval trematodes (=cercariae). Cercariae were extracted for DNA and PCR's were performed for detection of *Neorickettsia* (16S rRNA gene), and for identification of digenean species (i.e., amplification and sequencing of the digenean 28S rRNA gene). A total of 5,158 snails were examined, of which 967 produced cercariae, resulting in an overall cercarial infection rate throughout the season of 18.7%. Of 752 cercarial samples screened, 24 (ca. 3%) were found positive for neorickettsial DNA (Table 1). Considering the cercarial infection rate in the snails was 18.7%, this means that (0.187) x (0.032) or 0.6% of the snails (*i.e*., 1 in every 175) harbored *Neorickettsia* endosymbionts. There were no significant differences in *Neorickettsia* infection rates among the different cercarial types (Yate's Chi-square tests, p's>0.05), however there was a significantly higher rate of neorickettsial infection in the cercarial population during May (10%) than later in the season (1 to 2%) (Yate's Chi square values>8.0, p<0.01). We sequenced the 16S rRNA gene amplicons from 7 of the *Neorickettsia*-positive echinostome samples and aligned them against sequences published on GenBank. Alignment analyses indicate that the neorickettsial species circulating within Sweitzer's Pond were all *N. risticii* – agent of Potomac horse fever. We have identified a local source of *Neorickettsia* in North Dakota.

Table 1. Seasonal *Neorickettsia risticii* infection rates in the 4 major cercarial types shed by *Stagnicola elodes* snails from Sweitzer's Pond, 2007. Numbers in parenthesis indicate the number of cercaria-infected snails tested.

Date of Collection	Echinostome	Furcocercous	Plagiorchid	Amphistome	**TOTAL**
29 May	11.2% (116)	6.2% (32)	0% (1)	None present	**10.0% (149)**
26 June	0% (135)	0.8% (122)	None present	2.6% (154)	**1.2% (411)**
25 July	3.3% (30)	0% (37)	3.4% (87)	0% (38)	**2.1% (192)**
TOTAL	**5.0% (281)**	**1.6% (191)**	**3.4% (88)**	**2.1% (192)**	**3.2% (752)**

NOTES

NOTES

PROFESSIONAL COMMUNICATIONS

SCHEDULE OF PRESENTATIONS

Professional talks will be in room 211 of the Norm Skalicky Tech Incubator – session will be chaired by *(to be determined)*

MORNING SESSION

7:30 Registration desk open

8:00 Greetings from President Christopher Keller in the James C. Ray Idea Lab in the Ina Mae Rude Entrepreneur Center

9:40 OBSERVATIONS ON *SYMPHORICARPOS OCCIDENTALIS* (BUCKBRUSH) AS FORAGE IN BILLINGS COUNTY, NORTH DAKOTA, 2005 – 2006, James M[c]Allister* and Jay Johnson [p.81]

10:00 MEMBRANE RAFT ASSOCIATION AND POST-TRANSLATIONAL PALMITOYLATION OF THE DOPAMINE TRANSPORTER, James D. Foster*, Steve D. Adkins and Roxanne A. Vaughan [p.86]

10:20 BREAK

10:40 ENGINEERING OF SIO_2-AU-SIO_2 SANDWICH NANOAGGREGATES USING A BUILDING BLOCK: SINGLE, DOUBLE AND TRIPLE CORES FOR ENHANCEMENT OF NEAR INFRARED FLUORESCENCE, Shuping Xu, Shay Hartvickson, Julia Xiaojun Zhao* [p.85]

11:00 RHODIOLA INTEGRIFOLIA: HYBRID ORIGIN AND MEDICINAL ANCESTRY Ursula Schittko and Jocelyn Grann [p.84]

11:20 RECRUITING AMERICAN INDIAN PARTICIPANTS FOR A GENETIC EPIDEMIOLOGIC STUDY, Melanie A. Nadeau*, Lyle G. Best [p.83]

11:40 SOCIAL GEOGRAPHY DIMENSIONS OF AGING-IN-PLACE IN LARIMORE, NORTH DAKOTA, Tina Billups, Devon Hansen and Douglas C. Munski [p.82]

12:00 LUNCH (served in room 211 of the Norm Skalicky Tech Incubator) We will also conduct our business meeting (open to all members) during the lunch hour

AFTERNOON SESSION

1:00 EFFECT OF THYROID HORMONE CONCENTRATION ON THE TRANSCRIPTIONAL RESPONSE UNDERLYING INDUCED METAMORPHOSIS IN THE MEXICAN AXOLOTL (*AMBYSTOMA*), Robert B. Page, S. Randal Voss, Amy K. Samuels, Jeramiah J. Smith, Srikrishna Putta, and Christopher K. Beachy [p.77]

1:20 GENETIC THROMBOPHILIA VARIANTS AND RISK OF PRE-ECLAMPSIA AMONG AMERICAN INDIANS, Melanie Nadeau, Sheri T. Dorsam, Jacob Davis, Lyle G. Best* [p.78]

1:40 THE “GREENER” LEUCKART REACTION, Mikhail M. Bobylev [p.79]

2:00 COMPARATIVE ANALYSIS OF FUNCTIONALITY AND SPECIES DIVERSITY OF ON-SITE / OFF-SITE MITIGATED WETLANDS, Mike Davis, Dan Ackerman, Nicole Kumkel and Sheri Lares [p.80]

2:20 ALTERED TRAFFICKING OF THE LEISHMANIA PLASMA MEMBRANE ADENINE NUCLEOTIDE TRANSLOCATOR BY TOR, Rania Elsabrouty and Siegfried Detke [p.87]

EVENING

6:30 Banquet will be in the Hilton Garden Inn Dahl room. Cash bar open at 6:00

EFFECT OF THYROID HORMONE CONCENTRATION ON THE TRANSCRIPTIONAL RESPONSE UNDERLYING INDUCED METAMORPHOSIS IN THE MEXICAN AXOLOTL (*AMBYSTOMA*)

Robert B. Page[1], S. Randal Voss[1], Amy K. Samuels[1], Jeramiah J. Smith[1], Srikrishna Putta[1], and Christopher K. Beachy[2]

[1]Department of Biology and Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY 40506, USA; [2]Department of Biology and Amphibian Growth Project, Minot State University, Minot, ND 58707, USA

Thyroid hormones (TH) induce gene expression programs that orchestrate amphibian metamorphosis (1,2). In contrast to frogs, many salamanders do not undergo metamorphosis in nature. However, metamorphosis can be induced by exposure to thyroxine (T_4). We induced metamorphosis in Mexican axolotls (*Ambystoma mexicanum*) using 5 and 50 nM T_4, collected skin from the head at four time points (days 0, 2, 12, 28) and used microarray analysis to quantify mRNA abundances.

All animals exposed to 50 nM T_4 initiated morphological and transcriptional changes earlier and completed metamorphosis by day 28. Initiation of metamorphosis was delayed in animals exposed to 5 nM T_4 and none of these animals completed metamorphosis by day 28. We identified 402 genes that were statistically and two-fold differentially expressed between T_4 treatments at one or more non-day 0 sampling times (Fig. 1). In addition, we used linear and quadratic regression to identify 542 and 709 genes that were differentially expressed by greater than two-fold in the 5 and 50 nM T_4 treatments, respectively.

We found that T_4 concentration affected the timing of gene expression and the shape of temporal gene expression profiles. However, essentially all of the identified genes were similarly affected at both dosage levels. Our results indicate that while many common genes underlie the transcription profile during metamorphosis in salamanders and frogs, characterized in *Xenopus laevis* (3), there are a substantial number of differences. These similarities and differences provide new insights about the physiology and evolution of amphibian metamorphosis.

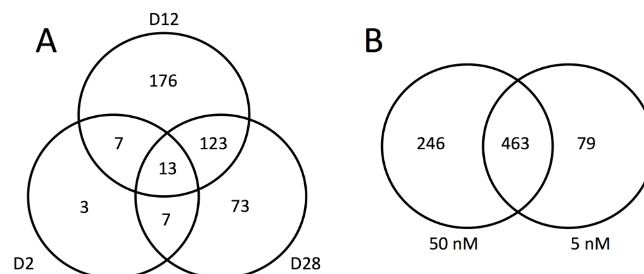

Fig. 1. -- Venn diagrams showing (A) the number of differentially-expressed genes (DEGs) at specific time points by contrasting the two T_4 concentrations and imposing fold-change criteria, and (B) depicting the relationship between DEGs that were identified via the 5 and 50 nM regression analyses relative to day 0. D2 = day 2, etc.

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Sources:

1) Larras-Regard, E., A. Taurog, and M. Dorris. 1981. Gen. Com. Endocrinol. 43:443-450.
2) Tata, J.R. 1966. Dev. Biol. 13:77-94.
3) Shi, Y.B. and D.D. Brown. 1993. J. Biol. Chem. 268:20312-20317

GENETIC THROMBOPHILIA VARIANTS AND RISK OF PRE-ECLAMPSIA AMONG AMERICAN INDIANS

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The clinical condition characterized by an increased propensity to pathologic clot formation is termed "thrombophilia". In 1994 Bertina([7]) and Greengard([8]) independently reported that a genetic variant of the human Factor V gene, known as Factor V Leiden (FVL), causes activated protein C resistance and is often found in patients with thrombophilia. An FVL allelic frequency of about 2% was initially reported in the Dutch population.(7) Another thrombophilic genetic variant, prothrombin 20210 (PT20210), was discovered in 1996, which is reported to have an allelic prevalence of 1.2%.(9)

Pre-eclampsia (PE) is a condition developing after the 20th week of pregnancy and manifested clinically by hypertension, nephropathy and in severe cases, seizures and consumptive coagulopathy. While the etiology of PE is unknown, one theory is that placental ischemia causes the release of various inflammatory and vaso-active substances that create these secondary effects. In 1996 investigators began to study the possible role of thromophilic variants in the development of this placental ischemia.([10],[11]) Meta-analyses have found significant pooled odds ratios (OR 1.81 95% CI 1.14-2.87) for pre-eclampsia in those with one or more FVL alleles([12]); but some other studies have not found significant associations.([13])

As part of an effort to enhance biomedical research capabilities at a tribal college, we investigated the possible etiologic role of FVL among PE patients in this American Indian community. A case-control study has enrolled 93 cases and 196 control participants to date. An additional 76 prospective cohort participants have been genotyped. Genotyping results are summarized in the following table:

Variant	Case	Hetero	Controls	Hetero	Signif**	Cohort	Hetero	Prevalence**
FVL	93	6	196	7	0.42	71	4	2.3% (1.2-3.4)
PT20210	93	1	196	2	0.57	76	1	0.5% (0.0-1.1)

* Number with a heterozygous genotype for this variant, from among the total number in the affected status group in the preceeding column. **The statistical significance of differences between cases and controls was tested by the Chi square statistic and given as a p value. **The population allelic frequency is calculated from the entire set of genotypes and given with 95% confidence intervals

The literature contains 4 references to FVL variants among American Indians or related populations. Only 3 FVL heterozygous individuals were found among a total of 1014 Oji-Cree and Pima Indian participants in Ontario, Canada and southwestern United States, respectively.([14],[15]) Two other studies among a total of 167 Greenland Inuit and American Indians residing in California found no FVL alleles.([16],[17])

Although the current study has limited power for detecting associations between PE and these relatively rare variants, no additional support is provided for the role of these two thrombophilic variants in this particular genetic background.

Supported by NIH grant P20 RR016741 from the NCRR.

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[7] Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, deRonde H, van der Velden PA, Reitsma PH. (1994) Nature.,369,64.
[8] Greengard JS, Sun X, Xu X, Fernandez JA, Griffin JH, Evatt B. (1994) Lancet., 343,1361.
[9] Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88:3698-703.
[10] Dizon-Townson DS, Nelson LM, Easton K, Ward K. (1996) Am J Obstet Gynecol.,175,902.
[11] Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, Martinelli P, Montanaro S, Pavone G, Di Minno G. (1997) Thromb Haemost.,77,1052.
[12] Lin J, August P. (2005) Obstet Gynecol.,105,182.
[13] GOPEC Consortium. (2005) Am J Hum Genet.,77,127.
[14] Hegele RA, Harris SB, Cao H, Hanley AJ, Zinman B. (1998) Diabetes Care.,21,1203.
[15] Kohler HP, Boothby M, McCormack L, Knowler WC, Grant PJ. (1997) Thromb Haemost.,78,961.
[16] Gregg JP, Yamane AJ, Grody WW. (1997) Am J Med Genet.,73,334.
[17] deMaat MPM, Kluft C, Jespersen J. (1996) Lancet.,347, 58.

THE "GREENER" LEUCKART REACTION

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The Leuckart reaction is a unique one step method of reductive amination. It is a remarkably simple process that includes only two components: the carbonyl compound and formamide. The reaction is completed simply by heating the components at 160ºC to 185ºC for 6 to 25 hours. The long processing time seems to be the only shortcoming of the reaction. However, it is associated with a number of serious practical problems. First, the prolonged exposure of the reaction mixture to high temperatures inevitably leads to significant thermal decomposition of the components, and, consequently, to lower yields of the products and large amounts of waste. Second, maintaining high temperatures for a long period of time means high consumption of energy and increasing production costs which make the Leuckart reaction unattractive to the chemical industry.

In this work a fast non-microwave procedure for the Leuckart reaction was developed. The new procedure can be completed in minutes instead of hours. It minimizes the use of heat and practically eliminates any thermal decomposition of the reaction mixture. The specific examples of the reactions will be presented. The project is supported by NIH grant P20 RR016741 from the NCRR.

COMPARATIVE ANALYSIS OF FUNCTIONALITY AND SPECIES DIVERSITY OF ON-SITE / OFF-SITE MITIGATED WETLANDS

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Within the current framework of federal rules, regulations, and policies, the North Dakota Department of Transportation (NDDOT) is required to mitigate all wetlands impacted as a result of construction projects completed either on site or within a mitigation site. Mitigation sites are located throughout the interior of North Dakota on property which has been previously drained and is leased or owned by the NDDOT. Assessing functionality of wetlands is somewhat problematic and available methodology for determining and measuring functionality are numerous and diverse. During the 2007 field season the NDDOT, using the Minnesota Routine Assessment Method (MnRAM), measured a total of 36 wetlands within mitigation sites in Nelson County and 37 wetlands mitigated on-site along US Highway 2. These wetlands were constructed between 2003-2006. Specifically, the wetlands were monitored for their hydrology, water quality, wildlife habitat structure, amphibian habitat, vegetative communities, as well as a brief invertebrate survey. The vegetative communities were measured through species count and documentation on transects taken across the greatest length of each wetland every 20 feet within a 3X 3 foot Daubenmire quadrat. The remaining characteristics were entered into the MnRAM algorithms, resulting in a value ranging from zero to one. The vegetative data was used to produce Shannon Diversity indices within each quadrat and then averaged for each wetland. The invertebrates were collected or noted when found. It was determined that those wetlands mitigated off site were achieving a higher degree of functionality in a shorter period of time. Those wetlands which were mitigated on-site were determined to be functioning at a satisfactory level within three years. With additional monitoring scheduled for the 2008 field season, the NDDOT will continue to build its dataset, giving an increasingly clear view of how these wetlands develop through time.

OBSERVATIONS ON *SYMPHORICARPOS OCCIDENTALIS* (BUCKBRUSH) AS FORAGE IN BILLINGS COUNTY, NORTH DAKOTA, 2005 – 2006

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Introduction. Dickinson State University maintains a small study area (approximately 0.75 x 0.75 x 0.45 km) in the badlands of western North Dakota east of the town of Medora. The study area is bounded to the north by I-94 and to the southeast by Business 94. A small grassland butte is located to the northeast and a dry woodland draw is to the southwest. In 1987 a deer exclosure was constructed under the direction of Professor Myron Freeman west of the present woodland draw. A 30 m transect line (transect D) bisects this moderately wooded exclosure into northeast and southwest. This transect extends 15 m to the west (transect E) and 15 meters to the east (transect C) into unenclosed grassland. The west endpoint of a 15 m east-west woodland transect (transect B) is located 51.3 m from the northeast cornerpost of the exclosure along an 168º line of azimuth (the woodland edge occurs 26.8 m from this post). The north endpoint of a north-south woodland transect (transect A) is located 10 m east of transect B. Over the course of one year (September 2005 - August 2006) monthly observations were made of *Symphoricarpos occidentalis* (buckbrush) along these transects. These observations provide a record that deer and cottontail utilized buckbrush as forage during fall and winter.

Observations/Discussion. The woodland draw is a *Fraxinus pennsylvanica/Prunus virginiana* (green ash/chokecherry) habitat with buckbrush extending from the woodland into the surrounding grasslands. The dominant grasses associated with the buckbrush are *Poa pratensis* (Kentucky bluegrass) and *Bromus inermis* (smooth brome). Observations include bones and antlers of *Odocoileus hemionus* (mule deer) as well as antler rubs, bedding sites, fecal pellets, and tracks in both the grassland and woodland draw. *Sylvilagus floridanus* (cottontails) were observed as well as their fecal pellets, tracks, and trails in both the grassland and woodland draw (including the exclosure).

Fieldwork October 15 and 22, 2005 consisted of identification and counts of vegetation intersecting each transect line. 30 buckbrush samples from each transect were randomly selected for morphometric data. Transect B had the lowest berry/flower counts (A: mean berry 4.61, 8.24 SD; mean flower 1.29, 2.34 SD; B: 0.34, 1.62 SD, 0.06, 0.35 SD; C: 3.72, 5.42 SD; 1.42, 3.38 SD; D: 2.84, 6.97 SD, 4.75, 7.57 SD; E: 6.44, 10.30 SD; 18.11, 16.74 SD). Transect B was highest in frayed and truncated foliage stem counts (A: 11, 5; B: 18, 3; C: 5, 1; D: 1, 4; E: 1, 3). Frayed stem evidence of deer browsing coincides with observations of deer fecal pellets, tracks, and a bedding site within 2 meters of B. Foliage browsing would result in a decrease of berries/flowers. Grassland (C, E) and lesser or no browsed similar woodland (A, D) provide partial control for potentially confounding factors (competition, nutrients, and sunlight).

On January 22, 2006 the study area was covered by approximately 10-15 cm of packed snow with 1 cm unpacked snowcover from the previous 24 hours. Cottontail fecal pellets and tracks (oriented toward sheared stems) were present on the new snow. The stems were sheared at 45º angles (± 15º) to within 5 cm of the snow. Number and condition of buckbrush were evaluated on May 16, 2006 as total #, frayed #, and sheared # (A: 35, 10, 19; B: 22, 0, 15; C: 25, 11, 4; D: 117, 0, 78; E: 38, 18, 10). Stem shearing is attributed to cottontails. The frayed upper stems attributed to deer browsing do not occur in the exclosure and these numbers are presumably reduced in this May transect data due to the cottontail shearing. Overstory density was estimated on June 22, 2006 with a spherical densitometer at mid-transect (A: 17; B: 46; C: 0; D: 79; E: 0%). Evidence indicates feeding preference in dense overstory transects by cottontails (stem shearing - A, B, D), and deer (October fraying of foliage stems – A & B).

Conclusion. Herbaceous plants dominate the preferred diet of deer and cottontails during the growing season. The transition to woody plants occurs as seasonal weather conditions worsen. At extreme conditions, snow cover severely limits foraging opportunities. The most parsimonious interpretation of the October observations at transect B (frayed stems, fecal pellets, tracks, and bedding impressions) suggest deer browsed the upper foliage, and reduced the berry/flower count by mid-fall (although other factors may contribute – growing conditions, bird foraging). January observations (stems sheared at an angle to near snow-level, fecal pellets, and tracks) provide a record that cottontails fed on entire buckbrush (B and D). Both herbivores preferentially foraged in the denser overstory transects in these observations. Cottontails utilized stems of the plant as winter progressed.

SOCIAL GEOGRAPHY DIMENSIONS OF AGING-IN-PLACE IN LARIMORE, NORTH DAKOTA

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Elderly population cohorts are increasing in the United States with quality of life implications at national, regional, and local levels (1). An important attribute of this phenomenon is aging-in-place, or the residential stability of growing old in a familiar location (2). The Great Plains states especially are experiencing this process, and North Dakota is not an exception to this phenomenon.

Aging-in-place studies are important for planning in a variety of economic sectors. Legislators, medical service providers, local governments, and businesses will be impacted greatly by a large elderly population and will need information to make the best decisions possible about a wide range of issues for the future regarding these senior citizens. One study suggests that states with a growing elderly population, due to aging-in-place, will experience a decreasing tax base and an older population more dependant on medical services (3). There are many studies focusing on the elderly population at the national and regional level (4, 5, 6). However, there is a lack of research focused solely on North Dakota, and specifically rural North Dakota.

Thus, the community of Larimore was selected as a case study to create a baseline from which to start to determine the extent to which that place in North Dakota is aging-in-place. To begin to understand how that process might be affecting the general quality-of-life in this community, the study was focused upon the following four research questions: 1. What are the social and demographic characteristics of the elderly population of Larimore?; 2. Why do the elderly residents of Larimore choose to reside there and does it have anything to do with attachment-to-place, homeownership, and/or close proximity to family members?; 3. Is Larimore aging-in-place, and if yes, why?; and 4. What are the implications for Larimore due to aging-in-place?

Case study data was gathered by conducting surveys and personal interviews with senior citizens plus exchanging e-mail correspondence with community leaders. After making personal contacts, most surveys were conducted at the Larimore Senior Center. The interview methodology, the snowball sampling method, was undertaken for efficiency in expanding the sample of participants. Data collection was designed to determine Larimore's specific elderly demographics, identify reasons for ongoing local residence, indicate if Larimore can be characterized as an aging-in-place location, and identify what are possible community implications.

Larimore certainly would be considered aging-in-place based on the responses from the surveys and interviews. One significant indicator was that all interview respondents stated that Larimore is to be their retirement location. Even those still working foresaw remaining in Larimore for their retirement. Many survey participants were well into their retirement years and already had lived in Larimore for a substantial amount of time, thus a strong indication that Larimore is aging-in-place. Further evidence of aging-in-place includes the majority of responses that convey attachment-to-place, including close proximity to family and friends, strong ties to the community, and homeownership.

The phenomenon of aging-in-place definitely will have an impact on the community of Larimore. The nursing and medical facilities must continue to expand and meet the needs of this senior citizen population. For example, one survey respondent expressed in a comment that there is a need for more smaller-sized, more manageable apartments. Responses from community leaders indicate that such construction is in the process of taking place. In addition, these facilities will continue to employ the needed staff, thus resulting in jobs for the community. While the community may be meeting the needs of the elderly in this aspect of the issue, there is still a need for a ride or taxi service within Larimore and to Grand Forks. Senior citizens and community leaders alike expressed a willingness to plan for improving Larimore in general. Interviewees provided suggestions regarding what these senior citizens desired to be developed in Larimore for improving their quality of life.

Consequently, this study contributes uniquely to the literature on elderly populations. Such a study in social geography helps reduce the lack of research on rural-oriented North Dakota communities. It is anticipated that this work will be useful to medical and social service providers, economic developers, and government leaders in Larimore and elsewhere in the Great Plains because of the increasing significance of aging-in-place to quality of life.

1) Conway KS, and Houtenville A (2003) Soc. Sci. Qrtly. 84, 309-327.
2) Graff TO and Wiseman RF (1978) Geog. Rev. 68, 379-393.
3) Rogers A and Woodward J (1988) Prof. Geog. 40, 450-459.
4) Longino CF Jr. (1990) Fam. Rel. 39, 38-43.
5) Plane D (1992) AAG Annals, 81, 64-85.
6) Graff TO and Wiseman RF (1990) Geog. Rev. 80, 239-251.

RECRUITING AMERICAN INDIAN PARTICIPANTS FOR A GENETIC EPIDEMIOLOGIC STUDY

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Due to previous negative experiences, some American Indian (AI) communities are distrustful of biomedical research in general and genetic research in particular. The Turtle Mountain Community College was awarded an NIH grant to study possible genetic influences on pre-eclampsia, to encourage tribal college students to consider a biomedical career and to develop the local research infrastructure. Pre-eclampsia is a serious medical condition among both Caucasian and AI populations. The prevalence in AI populations of genetic polymorphisms that may increase the risk of pre-eclampsia is unknown. In addition, the influence of these polymorphisms, given the AI genetic background, may differ from majority populations.

This analysis is based on recruitment of cases, controls, and prospective study participants. Approval for the study has been given by relevant Institutional Review Boards. Cases/controls are identified using the electronic records system at the local Indian Health Service hospital. Potential participants were given a brief description of the project and if agreeable, met for formal informed consent. Much effort has been made to publicize the project to the community.

To date, 76 cases, 90 controls, and 42 cohort participants have been recruited. During the past 28 months, 393 individuals have been identified and contact information was adequate for 309 (78.6%), 246 were given basic information, 212 have had full consent interviews, and of these 1.4% opted not to participate. Travel was required to recruit 75.3% of retrospective participants.

When recruiting potential participants in Indian Country, traveling to their homes appears more effective and efficient. Advertising efforts have been questionable in their effectiveness. Having local individuals as recruiters seems to be a very important component of the process.

RHODIOLA INTEGRIFOLIA: HYBRID ORIGIN AND MEDICINAL ANCESTRY

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Objective. The plant genus *Rhodiola* is predominantly Asian, but three species are native to North America: *Rhodiola integrifolia*, *Rhodiola rosea*, and *Rhodiola rhodantha*. *R. rosea* is known as a medicinal plant and has a long tradition of use in Asia. *R. integrifolia* has historically been considered a subspecies of *R. rosea* and has later been proposed to be of hybrid origin with the potential parent taxa being *R. rosea* and *R. rhodantha*. This hypothesis was mainly based on morphological similarity and chromosome counts, but has never been tested through sequence analysis. The objective of our work was to investigate the ancestry of *R. integrifolia* through DNA sequence analysis and thereby clarify some of the taxonomic discrepancies that exist in the literature that describes the traditional use of *R. integrifolia* and *R. rosea* in North America.

Methods. Sequence analysis of the chloroplast glutamine synthetase gene, a single-copy nuclear gene, was used to infer relationship between the *Rhodiola* species.

Results. Glutamine synthetase sequences obtained from *R. integrifolia* plants were either identical to *R. rosea* sequences or were recombinant sequences that were partly identical to *R. rosea* and partly to *R. rhodantha*, but not to *R. semenovii* or *R. algida*, the two closest Asian relatives of *R. rhodantha*.

Conclusions. Our data support the hybrid origin of *R. integrifolia*. The parental taxa are most likely *R. rosea* and *R. rhodantha*. This ancestry of *R. integrifolia* may strengthen the role of *R. integrifolia* as a medicinal plant. Pharmacological or phytochemical studies have not been done. Comparative analysis with the medicinally used parental species *R. rosea* would reveal how much of the parental properties have been retained in the hybrid species *R. integrifolia*.

ENGINEERING OF SiO_2-AU-SiO_2 SANDWICH NANOAGGREGATES USING A BUILDING BLOCK: SINGLE, DOUBLE AND TRIPLE CORES FOR ENHANCEMENT OF NEAR INFRARED FLUORESCENCE

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The need for sensitive determinations of trace amount of analytes has driven a rapid development of various novel nanomaterials. The photoactive nanomaterials is one of the most promising signaling reagents for achieving high detection sensitivity, such as quantum dots (QDs), dye-doped nanoparticles, gold or silver nanoparticles, *etc.* The photoactive nanomaterials provide direct intensive signals for the determination of trace analysis. However, the signal intensity of these nanomaterials is intrinsic and limited by their maximum value. To raise the limit of their intrinsic intensities in order to obtain higher signals, signal amplification is needed. Noble metallic nanostructures can generate an enlarged localized electromagnetic field through the surface plasmon resonance to enhance the optical signals of the photoactive molecules existed in this electromagnetic field (1-5). The aggregates of metallic nanoparticles provide a higher signal enhancement than well dispersed nanoparticles (6,7). However, the chemical synthesis of orderly metallic nanoaggregates is a challenge. Here we present a simple and flexible chemical method to orderly synthesize metallic sandwich nanoaggregates using a designed building block. The number of the building blocks in a nanoaggregate is tunable from one to three. The irregular poly core aggregates are greatly eliminated through adsorption of PVP molecules on the building block surface. These aggregates can be separated based on their weights. Different sized aggregates exhibited distinct surface enhancement for amplifying NIR signals when an NIR dye was placed in the electromagnetic field of the nanostructures. This work may open a new door for applications of surface enhancement in the sensitive detection of biological samples in NIR region. It also provides a new viewpoint for fundamental understanding plasmon properties of metallic aggregates.

1) Stuart DA, Haes AJ, Yonzon CR, Hicks EM, and Van Duyne RP (2005) IEEE Proc. Nanobiotech., 152(1), 13-32.
2) Haes AJ, Haynes CL, McFarland AD, Schatz GC, Van Duyne RP, and Zou S (2005) MRS Bull., 30(5), 368-375.
3) Willets KA, and Van Duyne RP (2007) Annu. Rev. Phys. Chem., 58, 267-297.
4) Xia Y, and Halas NJ (2005), MRS Bull., 30(5), 338-348.
5) Lakowicz JR (2006), Plasmonics, 1(1), 5-33.
6) Hao E, and Schatz GC (2004) Chem. Phys., 129, 357-366.
7) Quidant RA, Zelenina S, and Nieto-Vesperinas M (2007), Appl. Phys. A-Mater., 89(2), 233-239.

MEMBRANE RAFT ASSOCIATION AND POST-TRANSLATIONAL PALMITOYLATION OF THE DOPAMINE TRANSPORTER

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Clearance of synaptic dopamine (DA) by the dopamine transporter (DAT) is essential for the termination of dopaminergic neurotransmission. Therefore, alterations in transporter activity via intrinsic and trafficking mediated events are important factors in the temporal and spatial control of synaptic neurotransmitter levels and subsequent neural signaling. DATs undergo several types of post-translation modifications including glycosylation, ubiquitylation, and phosphorylation and it is hypothesized that these modifications play a role in DAT function, trafficking and regulation. DAT phosphorylation is stimulated in response to PKC activation via phorbol ester stimulation but the effects of phosphorylation on DAT function remain unclear.

We have previously demonstrated the presence of DAT in cholesterol- and sphingolipid-rich membrane subdomains isolated from a heterologous cell system and rat striatal tissue using a detergent-resistant method with 0.1% Triton X-100. We have also demonstrated the preferential phosphorylation of DATs within these membrane microdomains. To confirm that DATs associate with membrane rafts in intact cells and that our previous findings are not the result of the clustering of proteins and lipids in our detergent extraction procedure we prepared membrane rafts using a simplified detergent-free isolation method. We found that DATs are distributed between raft and nonraft membranes with more than 60% of DAT protein localized in raft fractions. DAT co-migrates with the raft associated tyrosine kinase Lyn in raft fractions that were well separated from nonraft fractions containing transferrin receptor, a well-accepted nonraft membrane marker. This raft association suggested the possible post-translational modification of DATs with lipids, a characteristic common to many raft associated proteins.

In this regard, we have discovered that DAT is a palmitoylated protein. Immunoprecipitation of DAT from heterologously expressing cells and rat striatal tissue metabolically labeled with [9,10-^{3}H]-palmitic acid demonstrated the presence of palmitoylated DAT, which was absent when non-transfected cells or preimmune serum was employed. Palmitate is attached to proteins through cysteine residues via a reversible thioester linkage. We have identified five DAT cysteine residues as potential sites of palmitoylation and have individually mutated these residues to alanine. A C580A rat DAT mutant exhibited near complete loss of [^{3}H]palmitate incorporation while C6A, C135A, C341A, and C522A mutants retained significant levels of [^{3}H]palmitate incorporation. We have begun to examine the consequences of DAT palmitoylation on its function and regulation, and have observed that the C580A palmitoylation deficient mutant exhibits a significant increase in both basal and phorbol ester stimulated DAT phosphorylation. Post-translational palmitoylation has important implications in the neuronal subcellular localization, trafficking, and activity of DAT.

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ALTERED TRAFFICKING OF THE LEISHMANIA PLASMA MEMBRANE ADENINE NUCLEOTIDE TRANSLOCATOR BY TOR

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Leishmania are flagellated protozoa belonging to the family Trypanosomatidae. The parasite is transmitted to humans by the bite of sand fly and is found in the tropical and subtropical regions on every continent except for Australia and Antarctica. Hundreds of thousands to millions of individuals are infected each year worldwide. Newer therapies are being sought as the older anti *Leishmania* compounds are too toxic or expensive and in some areas have become ineffective as the parasite has become resistant.

While investigating how *Leishmania* becomes resistant to toxic purine nucleosides, we identified and cloned the TOR gene [1,2]. TOR is an atypical multidrug resistance protein that is found in at least two regions in *Leishmania*: the mitochondria and the Golgi/trans Golgi network. In the Golgi/trans Golgi network, TOR acts as a traffic regulator and influences the amount of purine transporters which reach the plasma membrane [3]. As part of an ongoing project to identify other proteins which may be affected by TOR or to affect TOR itself, we examined the adenine nucleotide translocator (ANT), a mitochondrial transporter that moves ADP and ATP across the inner mitochondrial membrane. Subcellular fractionation, immunohistochemical localization with anti ANT antibodies and surface biotinylation show that the mitochondrial adenine nucleotide translocator is also present in the plasma membrane of both promastigotes and amastigotes [4]. However, unlike several other parasites with plasma membrane ANT, *Leishmania* uses its plasma membrane ANT not to take up ATP from its host but rather as part of a chemotaxis response. ATP is a chemorepellent for *Leishmania* and is released by neutrophils (the initial host of the parasite in mammals) at their leading edge which reinforces their movement in that direction. Movement by *Leishmania* away from an extracellular ATP source may be a defensive mechanism allowing this parasite additional time to adapt to the toxic environment in the phagolysosome prior to their initial phagocytosis by neutrophils.

High levels of intracellular recombinant ANT were able to abrogate the altered trafficking of the NT1 adenosine/thymidine permease elicited by TOR indicating that ANT interacted with TOR. This ability resided in ANT's N terminus and loop V extra-membrane domains. These two peptides could form a single determinant recognized by TOR based on the x-ray crystallographic structure for ANT reported in the literature. Co-immunoprecipitation and far western blots verified that ANT interacted with TOR. Unlike the mitochondrial ANT whose level was not noticeably altered by high levels of TOR, the level of plasma membrane ANT decreased to barely detectable levels, a fate similar to that of the NT1 adenosine/thymidine permease. The parasite also lost its negative chemotactic response to ATP with the disappearance of ANT from the plasma membrane.

References:

1. Detke, S. (1997) Identification of a transcription factor like protein at the TOR locus in *Leishmania mexicana amazonensis.* Mol. Biochem. Parasitol. 90: 505-511.
2. Kerby, B.R., and Detke, S. (1993) Reduced purine accumulation is encoded on an amplified DNA in *Leishmania mexicana amazonensis* resistant to toxic nucleosides. Mol. Biochem. Parasitol. 60: 171-185.
3. Detke, S. (2007) TOR-induced resistance to toxic adenosine analogs in Leishmania brought about by the internalization and degradation of the adenosine permease. Exp. Cell Res. 313: 1963-1978.
4. Detke, S., and Elsabrouty, R. (2008) Leishmania mexicana amazonensis: plasma membrane adenine nucleotide translocator and chemotaxis. Exp. Parasitol. 118: 408-419.

NOTES

CONSTITUTION of the NORTH DAKOTA ACADEMY OF SCIENCE

Founded 1908, Official State Academy 1958

ARTICLE I - *Name and Purpose*

Section 1. This association shall be called the NORTH DAKOTA ACADEMY OF SCIENCE.

Section 2. The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

ARTICLE II - *Membership*

Membership in the Academy shall be composed of persons who share the stated purpose of the Academy and who are active or interested in some field of scientific endeavor.

ARTICLE III - *Council*

The officers of the Academy shall be a President, a President-Elect, and a Secretary-Treasurer. The Council, consisting of the officers, the retiring President, and three elected Councilors, shall be responsible for the fulfillment of the scientific and business obligations of the Academy.

ARTICLE V - *Dissolution and Limits of Action*

Section 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for education and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.

Section 2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in or intervene in, any political campaign on behalf of any candidate for public office.

Section 3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

ARTICLE VI - *Amendments*

Section 1. This Constitution may be amended at any annual Business Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary-Treasurer who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.

Section 2. Bylaws may be adopted or repealed at any regular business meeting by a two-thirds vote.

BYLAWS

BYLAW 1. *Meetings*

Section 1. *Scientific Meetings*. The Academy shall hold at least one annual scientific meeting each year at a time and place determined by the Council. Other scientific meetings, regional, state, or local, may be held at times and places determined by the Council. The Council shall establish regulations governing the presentation of papers at Academy sessions. Such regulations shall be made available to members at least three months before any meeting at which they are to apply.

Section 2. *Business Meetings*. A Business Meeting of the membership shall be scheduled at the regular, annual scientific meeting of the Academy. Ten percent of the active members shall constitute a quorum at the annual

business meeting.

Section 3. *Special Meetings*. Special meetings shall be called by the President upon the request of ten percent of the active members and require twenty percent of the active members for a quorum. Notice of the time and place of such meetings shall be sent to all members of the Academy at least four weeks in advance of the meeting. Only matters specified in the call can be transacted at a special meeting.

Section 4. *Procedure*. Parliamentary procedures to be followed in all business meetings shall be those specified in "Standard Code of Parliamentary Procedure" by Alice F. Sturgis.

BYLAW 2. *Financial*

Section 1. *Fiscal year*. The fiscal year shall run concurrently with the calendar year from January 1 to December 31.

Section 2. *Dues and Assessments*. The annual dues and assessments may be changed from time to time by the Council, subject to approval by a two-thirds vote of the members at an annual Business Meeting. These dues are payable by January 31 for the current fiscal year or by the Annual North Dakota Academy of Science Meeting for those registering for the meeting

Section 3. *Supporting Members*. Council shall maintain a program to encourage members to voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

Section 4. *Sustaining Members*. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

Section 5. *Audit and Reports*. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year. The Secretary-Treasurer shall report on the financial affairs of the Society, including the results of an annual audit, as may be requested by the Council.

BYLAW 3. *Membership*

Section 1. *Membership Categories*. Classes of membership shall include the following: (a) Regular, (b) Student, (c) Emeritus, (d) Honorary, (e) Supporting, (f) Sustaining, and (g) Lifetime Members.

Section 2. *Eligibility and Procedure for Membership*. Candidates for membership, except Sustaining Member, may be proposed by any regular or emeritus member of the Academy by submitting the candidate's name to the chairman of the Membership Committee.

(a) *Regular Members*. Any person who is active or interested in some field of scientific endeavor shall be eligible for regular membership. A majority vote of Council shall elect to regular membership.

(b) *Student Members*. Any student who is an undergraduate or graduate student in some field of science shall be eligible for student membership. A majority vote of Council shall elect to regular membership.

(c) *Emeritus Members*. Any member in good standing upon formal retirement is eligible for emeritus membership. A majority vote of Council shall elect to emeritus membership.

(d) *Honorary Member*s. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. A two-thirds vote of members attending the annual business meeting shall elect to honorary membership.

(e) *Supporting Members*. Regular or student members may voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

(f) *Sustaining Associates*. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

(g) *Lifetime Members*. Any regular member in current good standing for at least one year may become a

Lifetime Member by paying an assessment equal to 18 times the current annual dues in one lump sum or in two equal payments over the current and following year.

Section 3. *Privileges of Membership*.

(a) Voting at the annual business meeting is permitted of regular and emeritus members.

(b) Members of all categories may attend business meetings of the Academy.

(c) The Secretary-Treasurer and members of Council must be regular members in good standing.

(d) Regular, student, and emeritus members may submit abstracts or communications for scientific meetings of the Academy.

(e) Emeritus and Honorary Members shall be exempt from payment of dues.

(f) A Sustaining Member is provided a display area at the annual scientific meeting of five linear feet per $50 donation up to a maximum of 20 linear feet.

(g) Every member in good standing shall receive a printed copy or an electronic copy (if available and of equal or lesser cost than the printed copy) of the annual *Proceedings of the North Dakota Academy of Science*, the form to be determined by the member.

(h) Special offices such as Historian may be created by the unanimous vote of the regular members at the annual Business Meeting.

(i) All student research participants shall receive a properly inscribed certificate.

Section 4. *Forfeiture of Membership*.

(a) *Nonpayment of dues*. Members shall be dropped from the active list on 31 November following the nonpayment of dues during the membership year commencing the previous 1 December. A member may return to the active list by paying the current year dues.

(b) *Expulsion for Cause*. Membership may be terminated for conduct injurious to the Academy or contrary to the best interests of the Academy. The accused member shall be given an opportunity for a hearing before the Council. If a majority of the Council votes to expel the member, the action must be ratified by at least two-thirds of the members present at the next annual business meeting of the Academy. An expelled member shall forfeit all paid dues and assessments.

BYLAW 4. *Duties and Responsibilities of the Council and Council Members*

Section 1. *Council*. The Council shall meet, at the call of the President, at least twice a year. The Council shall:

(a) be the governing board of the Academy, responsible only to the membership.

(b) arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the Academy.

(c) determine the location of the annual meeting three years in advance.

(d) annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science.

(e) shall appoint and may compensate a Secretary-Treasurer.

(f) shall appoint and may compensate an Editor of the PROCEEDINGS and other publications.

(g) shall be empowered to charge a publication fee of authors on a per page basis.

(h) shall control all activities of the Academy including grant applications.

Section 2. *President*. The President shall preside at meetings of the Council and over the annual business meeting of the Academy at the close of the regular term of office. The President shall vote only to break a tie. Unless otherwise specified, the President shall, with the approval of the Council, appoint members to serve on Standing Committees and *ad hoc* Committees, designate the chair of each Committee, and appoint representatives to other organizations. The President serves as Coordinator of the Local Arrangements Committee for the annual meeting that occurs at the end of the President's term.

Section 3. *President-Elect*. The President-elect shall be considered a vice president and shall serve as such in the absence of the President.

Section 4. *Past-President*. The retiring President shall serve as Past-President and chair of the Nominating Committee. The Past President shall serve ex officio on those committees designated by the President and shall serve in the absence of the President and President-elect.

Section 5. *Secretary-Treasurer*. The Secretary-Treasurer shall:

(1) Assist Council in carrying on the functions of the Academy including the receipt and disbursement of funds under the direction of Council.

(2) Manage the Academy Offices under Council's general supervision.

(3) Serve as Managing Editor of the *Proceedings of the North Dakota Academy of Science*.

(4) Prepare a summary of the most recent audit and a report of the Academy's current financial status. This information shall be shared with the membership at the annual business meeting and published in the PROCEEDINGS following the business meeting.

(5) Perform all other duties of the Secretary-Treasurer listed in the Bylaws.

(6) Serve as archivist and be responsible for all official records, archives, and historic material which shall be in reposit with the Secretary-Treasurer.

BYLAW 5. *Appointment, Nomination and Election of Members of Council*

Section 1. *Eligibility for Office*. All candidates for election or appointment to the Council must be regular members in good standing. Nominees for President-elect must be members who reside within easy commuting distance of the site of the annual meeting selected by the Council that occurs when the President-elect serves as President.

Section 2. *Nomination Procedures*. The Nominating Committee shall be responsible for all nominations to elective office, shall determine the eligibility of nominees, shall ascertain that nominees are willing to stand for office, and shall be required to advance to the Secretary-Treasurer at least two names for each open position as needed. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report.

Section 3. *Election Procedures*. Election shall be by secret mail ballot. The Secretary-Treasurer shall prepare a printed ballot that bears all names submitted by the Nominating Committee, that contains a brief biography of each candidate, and that has space for write-in candidates for each office. This ballot is to be mailed to all members no later than 1 November. Each member wishing to vote must return the marked ballot in a sealed signed envelope to the Secretary-Treasurer postmarked not more than thirty days after the ballots were mailed out to members. The President shall appoint tellers, who shall count the ballots that have been received by the Secretary-Treasurer and the tellers shall present the results in writing to the President. A plurality of the votes cast shall be necessary to elect and in the case of a tie vote, the President shall cast the deciding vote. The results of the election shall be announced at the annual Business Meeting.

Section 4. *Term of Office*. A President-Elect shall be elected annually by the membership and the following years shall succeed automatically to President and Past President to constitute a three-year nonrenewable term. Three Councilors shall be elected by the membership to three-year, non-renewable terms on a rotating basis. All elected Council members shall take office at the end of the next annual Business Meeting following election and shall continue until relieved by their successors. Council is empowered to appoint and compensate a Secretary-Treasurer to successive three-year terms that commence with the beginning of the fiscal year.

Section 5. *Removal from office or position.* If for any reason any elected member of Council is unable to fulfill his/her duties, the Council member may be removed from office by two-thirds vote of Council. If for any reason the Secretary-Treasurer is unable to fulfill his/her duties, the Secretary-Treasurer may be relieved of all duties by a majority vote of Council.

Section 6. *Interim vacancies*. Should a vacancy occur in the Presidency, the Council by a majority vote shall appoint a member of the Academy able to coordinate the next annual meeting to fill the unexpired term. A retiring interim President shall succeed automatically to Past President. Should a vacancy occur in the Presidency-elect, the Council shall reassess and change the location of the coinciding annual meeting as necessary and then call for a special election by mail ballot. An interim vacancy in the Past-Presidency shall be filled by the most recently retired Past-President able to fill the duties of the Past-President. Persons appointed to fill the unexpired term of Secretary-Treasurer are expected to remain in the position for a minimum of three years. A vacancy in the office of Councilor shall be filled by a majority vote of Council until the following election at which time the interim Councilor may stand for a full three year nonrenewable term.

BYLAW 6. *Committees*

Section 1. *Standing Committees*. Standing committees shall include but not be limited to, the following: Editorial, Education, Denison Award, Necrology, Nominating, Resolution, Membership, and Audit Committees. The President shall appoint members of committees other than the Nominating and Audit Committees.

Section 2. *Editorial Committee*. The Editorial Committee shall consist of three regular members appointed to three year terms. The duties are explained in BYLAW 7 (Publications).

Section 3. *Education Committee*. The Education Committee shall consist of five regular members and two high school teachers appointed to five year terms. The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.

Section 4. *Denison Awards Committee*. The Denison Awards Committee shall consist of six regular members appointed to three year terms. The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors.

Section 5. *Necrology Committee*. The Necrology Committee shall consist of three regular members appointed to three year terms. The Necrology Committee shall report to the annual meeting on those deceased during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

Section 6. *Nominating Committee*. The Nominating Committee shall consist of the five most recent past-presidents. The major duties of the Nominating Committee are listed in BYLAW 5 (*Appointment, Nomination and Election of Members of Council*). The Nominating Committee will also administer the selection process, develop a separate funding source for a monetary award, and develop, for Executive Committee approval, the criteria for the North Dakota Academy of Science Achievement Award.

Section 7. *Resolution Committee*. The Resolution Committee shall consist of three regular members appointed to three year terms. The Resolution Committee shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.

Section 8. *Membership Committee*. T he Membership Committee shall consist of unlimited numbers of regular members appointed annually.

Section 9. *Audit Committee*. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year.

Section 10. *State Science Advisory Committee*. The State Science Advisory Committee (SSAC) shall consist of five regular or emeritus members appointed to four year terms. The SSAC shall serve to direct questions of a scientific nature to the appropriate expert as requested, shall inform regional granting agencies and state and national science policymakers of its expertise and availability and shall counsel those agencies and persons upon their request. The SSAC shall adhere in particular to the guidelines described in Article V, Section 2 of the Constitution.

Section 11. *Ad hoc Committees*. The President may appoint such additional committees as may be needed to carry out the functions of the Academy. Ad hoc committees serve only during the tenure of the president who appointed them. Reports of ad hoc committees shall be presented to Council or to the annual meeting.

BYLAW 7. *Publications*

Section 1. *Editorial Committee*. Three regular members are appointed to the Editorial Committee for renewable three year terms. The Editorial Committee shall develop and recommend the Academy publication program and policies to the Council. It will assist the Editors of each official publication in reviewing manuscripts for those publications that include the *Proceedings*. Chairs of symposia will review manuscripts written for relevant symposia.

Section 2. *Managing Editor*. The Secretary-Treasurer shall serve as the

Section 3. *Editor*. Editors shall serve three year terms. The Editors shall edit all official publications of the Academy including the *Proceedings*.

BYLAW 8. *Memorial Fund*

The Council of the Academy shall establish a J. Donald Henderson Memorial Fund and administer this fund so that the proceeds will be used to promote science in North Dakota.

BYLAW 9. *Fiscal Year*

The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December.

BYLAW 10. *Achievement Award*

The Academy establishes the North Dakota Academy of Science Achievement Award to be given periodically to an Academy member in recognition of excellence in one or more of the following:
a. Nationally recognized scientific research.
b. Science education.
c. Service to the Academy in advancing its goals.
The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Council approval, the criteria for the award.

BYLAW 11. *Research Foundation*

The **North Dakota Science Research Foundation** is established as an operating arm of the Academy. The purposes of the Foundation are:
(1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota. Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the Academy and shall be separately accounted for annually. The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President of staggered five year terms. The chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral report to the membership of the Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

BYLAW 12. *Affiliations*

The Academy may affiliate itself with other organizations which have purposes consistent with the purposes of the Academy. Such affiliations must be approved by the Council and by a majority of those attending a regularly scheduled business meeting of the membership.

BYLAW 13. *Indemnification*

Section 1. Every member of the Council or employee of the North Dakota Academy of Science shall be indemnified by the Academy against all expenses and liabilities, including counsel fees, reasonably incurred or imposed upon him/her in connection with any proceedings to which he or she may be made part, or in which he or she may become involved, by reason of being or having been a member of the Council, or employee at the time such expenses are incurred, except in such cases wherein the member of the Council or employee is adjudged guilty of willful misfeasance or malfeasance in the performance of his or her duties. Provided, however, that in the event of a settlement of the indemnification herein shall apply only when the Council approves such settlement and reimbursement as being for the best interests of the Academy. The foregoing right of indemnification shall be in addition to and not exclusive of all other rights to which such members of the Council or employee may be entitled.

MINUTES (UNAPPROVED) OF THE NORTH DAKOTA ACADEMY OF SCIENCE ANNUAL BUSINESS MEETING

Valley State University, Valley City, North Dakota, April 27, 2006, 12:00 pm

MINUTES (UNAPPROVED) OF THE NORTH DAKOTA ACADEMY OF SCIENCE ANNUAL BUSINESS MEETING

Minot State University, Minot, North Dakota, April 12, 2007, 4:30 pm

The first order of business was to approve the minutes of the previous business meeting from the April 2006 annual meeting in Valley City, North Dakota. The minutes, however, were not available so this was postponed until the 100th Annual Meeting in April 2008.

A brief financial report was presented by Secretary-Treasurer Detke. At this time, the Academy is financially sound. Secretary-Treasurer Detke noted, however, that a large fraction of the membership that did not register for the 2007 meeting failed to pay dues for 2007 despite repeated reminders by email.

Birgit M Prüß, North Dakota State University volunteered for President-Elect in 2008. She was nominated and elected without opposition by voice vote.

Douglas Munski, University of North Dakota, tenure as Councilor ended in 2008. He has volunteered to serve a second third year term. He was nominated and elected without opposition by voice vote.

Jon Jackson, University of North Dakota, volunteered to serve as the third Councilor. He was nominated and elected by voice vote.

Changes to Amendments in By-Laws:

A) A motion was made to create a new section in BYLAW 2. (*Financial Section)* which will be referred to as section 1 and to renumbered the other sections accordingly. This section to state:

"The fiscal year shall run concurrently with the calendar year from January 1 to December 31."

A discussion was held and the amendment was approved unamously

B) A motion was made to change Section 1. *Dues and Assessments* in BYLAW 2. (*Financial*) as follows:

Delete "~~These dues are payable 1 December of each year."~~ and replace it by "These dues are payable by January 31 for the current fiscal year or by the Annual North Dakota Academy of Science Meeting for those registering for the meeting".

A discussion was held and the amendment was approved unamously

C) A motion was made to change Section 1. *Dues and Assessments* in BYLAW 2. (*Financial*) as follows:

Delete ~~"The student member dues shall be one-third (to nearest dollar) of the regular member dues."~~

A discussion was held and the amendment was approved unamously

Change in dues to become effective January 1, 2008.

A motion was made to increase the Student's dues from $10 to $12 and Professional from $25 to $30 dollars.

A discussion was held and the amendment was approved unamously

Meeting statistics: 81 Registered attendees 30 professional, 51 student
0 Guests

We had 6 professional talks and 29 Denison papers presented, of which 11 were graduate and 18 were

undergraduate.

A. Rodger Dennison Award winners:

Graduate category:	Chris Jurgens
Undergraduate category:	Ashley Olander

A. Rodger Dennison Award runner ups:

Graduate category:	Sunitha Bollimuntha (1st runner-up) John Goering (2nd runner-up)
Undergraduate category:	Brianna Goldstein (1st runner-up) Brent Keller (2nd runner-up)

The 100th Annual meeting was set to be held in middle of April 2008 on a day determined by Van Doze.

Christopher Keller (Minot State University) officially ended his duties as President by introducing Van Doze (University of North Dakota). President Doze discussed preliminary plans for the Academy's 100th Annual Meeting, over which he will preside in Grand Forks on April 24, 2008.

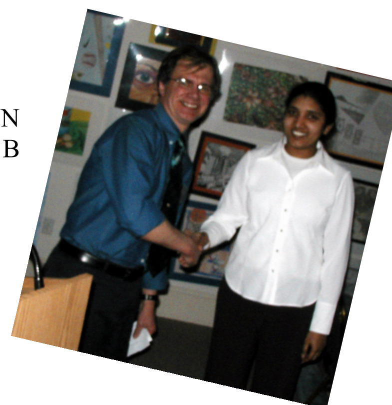
The business meeting was adjourned at 5:15 PM.

2007 Dennison Award Winners
Graduate Division

Winner: Chris Jurgens

ADRENERGIC RECEPTOR MEDIATED SUPPRESSION OF SEIZURES: α_2 ADRENERGIC RECEPTORS INHIBIT EPILEPTIFORM ACTIVITY VIA A SYNAPSE SPECIFIC MECHANISM. Chris WD Jurgens* and Van A Doze

1st runner-up: Sunitha Bollimuntha



ROLE OF THE C3 TRANSIENT RECEPTOR POTENTIAL CHANNEL IN RAT SUPRA OPTIC NUCLEUS. Sunitha Bollimuntha*, John A Watt, Brij B Singh

2nd runner-up: John Goering

N-/ISO-BUTANE ON ANATASE (001) THIN FILM: A SURFACE CHEMISTRY STUDY. J. Goering*, E. Kadossov, U. Burghaus (Picture not available)

2007 Dennison Award Winners
Undergraduate Division

Winner: Ashley Olander

DNA CLEAVAGE IN VITRO BY DNA TOPOISOMERASE II IN THE *AF4* GENE TRANSLOCATION BREAKPOINT REGION. Ashley L. Olander *, Cheryl A. Lepp, Heidi J. Super

1st runner-up: Brianna Goldstein

ALPHA-2 ADRENERGIC RECEPTOR INHIBITION OF HIPPOCAMPAL CA3 NETWORK ACTIVITY: AGONIST STRUCTURE-ACTIVITY RELATIONSHIPS. Brianna L. Goldenstein*, Ke Xu, Kristan M. Green, Jacquline A. Pribula, Jasmine J. O'Brien, Kylie L. Davis, Sarah J. Boese, Jessica A. Lichter, Brian W. Nelson, Melissa N. Austreim, James E. Porter, Van A. Doze

2nd runner-up: Brent Keller

MICROWAVE-ASSISTED SYNTHESIS OF N-VANILLYLFORMAMIDE. Brent D. Keller* and Mikhail M. Bobylev

AGENDA/NOTES

Notes for the 100th Annual Business meeting

The first order of business was to approve the minutes of the 98th Meeting in Valley City, North Dakota, in 2006 and the 99th Meeting in Minot, North Dakota, in 2007.

The minutes: were | were not approved
as printed in the Proceedings | as amended.

A brief financial report was presented by Secretary-Treasurer Detke.

(Person to be name) has volunteered to be President in 2009. He | She was elected without opposition by voice vote.

Heidi Super's tenure as counselor ended in 2008. (Person to be name) has volunteered and has been elected without opposition by voice vote.

Meeting statistics: _104__ Registered attendees (34) professional, (70) student
_____ Guests

We had (11) professional talks and (54) Denison papers presented, of which (28) were graduate and (26) were undergraduate.

A. Rodger Dennison Award winners:

Graduate category:

Undergraduate category:

A. Rodger Dennison Award runner ups:

Graduate category:

Undergraduate category:

Van Doze (University of North Dakota) officially ended his duties as President by introducing Birgit M Prüß (North Dakota State University). President Prüß discussed preliminary plans for the Academy's 101st Annual Meeting, over which she will preside in Fargo on April ( ), 2009.

DRAFT
FOR REVIEW ONLY

Executive Committee Membership

President
Past-President
President-Elect
Secretary-Treasurer
Councilors (three-year terms)

President
Van Doze
Department of Pharmacology Physiology & Therapeutics
University of North Dakota
Grand Forks, ND 58203
(701)777-6222
vdoze@medicine.nodak.edu

President-Elect
Birgit M Pruess (2005-2008)
Department of Veterinary and Microbiological Sciences
North Dakota State University
Fargo, ND
(701)231-7848
Birgit.Pruess@ndsu.edu

Past-President
Christopher Keller
Department of Biology
Minot State University
Minot, ND
(701)858-3067
christopher.keller@minotstateu.edu

Secretary-Treasurer
Siegfried Detke (2005-2008)
Department of Biochemistry & Molecular Biology
University of North Dakota
Grand Forks, ND 58203
(701)777-3202
sdetke@medicine.nodak.edu

Councilors
Douglas Munski (2004-2007)
Department of Geography
University of North Dakota
Grand Forks, ND 58203
(701)777-4246
douglas_munski@und.nodak.edu

Heidi Super (2005-2008)
Department of Biology
Minot State University
Minot, ND
(701)858-3079
heidi.super@minotstateu.edu

Jon Jackson
Department of Anatomy
University of North Dakota
Grand Forks, ND
(701) 777-4911

COMMITTEES OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Executive Committee
Editorial Committee*
Education Committee*
Denison Awards Committee*
Necrology Committee*
Nominating Committee
Resolution Committee*
Membership Committee*
North Dakota Research Foundation Board of Directors*

* indicates available openings

PAST PRESIDENTS AND THE LOCATIONS OF THE ANNUAL MEETING OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Year	President	Location
1909	M A Brannon	Grand Forks
1910	M A Brannon	Fargo
1911	C B Waldron	Grand Forks
1912	L B McMullen	Fargo
1913	Louis VanEs	Grand Forks
1914	A G Leonard	Fargo
1915	W B Bell	Grand Forks
1916	Lura Perrine	Fargo
1917	A H Taylor	Grand Forks
1918	R C Doneghue	Fargo
1919	H E French	Grand Forks
1920	J W Ince	Fargo
1921	L R Waldron	Grand Forks
1922	Daniel Freeman	Fargo
1923	Norma Preifer	Grand Forks
1924	O A Stevens	Fargo
1925	David R Jenkins	Grand Forks
1926	E S Reynolds	Fargo
1927	Karl H Fussler	Grand Forks
1928	H L Walster	Fargo
1929	G A Talbert	Grand Forks
1930	R M Dolve	Fargo
1931	H E Simpson	Grand Forks
1932	A D Wheedon	Fargo
1933	G C Wheeler	Grand Forks
1934	C I Nelson	Fargo
1935	E A Baird	Grand Forks
1936	LR Waldron	Fargo
1937	J L Hundley	Grand Forks
1938	P J Olson	Fargo
1939	ED Coon	Grand Forks
1940	J R Dice	Fargo
1941	F C Foley	Grand Forks
1942	F W Christensen	Fargo
1943	Neal Weber	Grand Forks
1944	E A Helgeson	Fargo
1945	W H Moran	Grand Forks
1946	J A Longwell	Fargo
1947	A M Cooley	Grand Forks
1948	R H Harris	Fargo
1949	R B Winner	Grand Forks
1950	R E Dunbar	Fargo
1951	A K Saiki	Grand Forks
1952	Glenn Smith	Fargo
1953	Wilson Laird	Grand Forks
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	2007	1/1/2008-3/31/2008	
ASSETS			
Operating Accounts			
Checking	$ 5,792.95		
Trust Accounts			
Scholarship (Savings)	$11,395.47		
Scholarship (Stocks)	$65,540.48		
Research Foundation (Savings)	$ 4,542.67		
Total	$87,271.58		
DUES			
Reinstatements	$ 25.00		
Current year	$1,420.00	$2,675.00	
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Total	$1,445.00	$2,675.00	
INSTITUTIONAL SUPPORT			
NDUS TOTAL	$0.00		
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Registration fees	$3,960.00	$4,745.00	
AWARDS PROG RAM			
Scholarship Dividends	$0.00		
NDAS Research Foundation	$0.00		
Total	$0.00		
PUBLICATION SALES	$390.00		
MISCELLANEOUS INCOME			
Interest	$ 30.93		
Dividend Income (Reinvested)	$288.61		
Total	$319.54		
MEMBERSHIP			
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Professional	40	41	
Delinquent	70+		
Withdrew	3	1	
ANNUAL MEETING			
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Meals/Refreshments	$2,912.40		
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SCIENCE RESEARCH FOUNDATION			
CASH INCOME	$0.00		
Donations from Members	$20.00	$30.00	
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Total	$20.00		
CASH EXPENSE			
Grants	$0.00		
Total	$0.00		
SCHOLARSHIP FUND			
CASH INCOME	$ 0.00		
Sempra Energy (Dividend)	$295.50		
Alliant Energy (Dividend)	$ 78.36		
Total	$373.86		
ASSETS			
Sempra Energy (purchased as ENOVA			
Number of shares 250 (1983)	979.586		
Price 18.50	$56.14		
Value $4,625.00	$55,082.13		
IEC/Alliant Energy (purchased as IES Industries			
Number of shares 120 (1990)	254.461		
Price 31.63	$41.09		
Value $3,795.60	$10,458.35		
Total Investment Value	$65,540.48		

Agenda for Business meeting
Thursday April 24, 2007

1) Approval of the minutes for the 2006 98th NDAS Annual Meeting in Valley City and 2007 99th Annual Meeting in Minot

2) Old business

3) Election of President-elect

4) Election of Councilor(s)

5) Other new business

6) Adjourn

ALVEOLAR MACROPHAGE PHAGOCYTOSIS AND RESPIRATORY BURST ACTIVITY IS REGULATED BY LYN -PI3K - AKT PATHWAY.

Shibichakravarthy Kannan*, Aaron Audet, Huang Huang, and Min Wu.

Department of Biochemistry and Molecular Biology, University of North Dakota.

Introduction:

Alveolar macrophages (AM) form the first line of defense in the lung alveoli (1). Pseudomonas aeruginosa (PA) is a common hospital acquired infectious agent that can cause life-threatening infections in susceptible individuals. We hypothesize AM to have important function in early stages of PA infection.

Rationale:

Recently, we have shown that Lyn tyrosine kinase is critically involved in alveolar epithelial cell invasion with PA through lipid raft mechanism (2). Lyn is known to be involved in mast cell motility and generation of superoxide free radicals in neutrophils. Thus we propose a role for Lyn in AM function during PA infection. PI3K is essential for phagocytosis (3). PA infection has been linked to PI3K activation (4).

Methods:

Akt activity was detected by immunoblotting with phospho Akt (Ser 473) antibody and by in vitro kinase assay. Lyn, PI3K and Akt protein interactions were analyzed by co-immunoprecipitation and Lyn-GST pull down assays. Phagosome formation and localization of signaling proteins were studied by immunostaining with fluorescent antibodies followed by confocal microscopy. Lyn-YFP and PH-Akt-GFP co-transfected MHS cells were used for studying spatio-temporal association of Lyn and Akt during PA phagocytosis by live cell confocal microscopy. Phagosomes from infected cells were isolated by sucrose density gradient centrifugation for biochemical characterization.

Results:

PA infection induced Akt activity in MHS cells depends on Lyn function. Lyn, PI3K and Akt were actively recruited to phagosome fractions in PA infected cells. Both Lyn-YFP and PH-Akt-GFP were found to co-localize in the lamellipodium and phagocytic cup of PA infected MHS cells from live cell imaging studies. GST pull down assay confirmed Lyn - Akt interaction. Respiratory burst activity depends on Lyn function. Inhibiting Lyn function decreased PA phagocytosis and reduced superoxide release.

Conclusions:

Our data indicates that Lyn-PI3K-Akt pathway is crucial for regulating phagocytosis and respiratory burst activity of AM. So Lyn mediated host cell signaling plays a major role in combating PA infection and thereby contribute to lung innate immunity.

Abbreviations: AM, alveolar macrophages; PA, Pseudomonas aeruginosa; GST, glutathione S transferase; YFP, yellow fluorescent protein; GFP, green fluorescent protein; PH, pleckstrin homology domain, Lyn, v-yes yamaguchi sarcoma related gene / protein; PI3K, phosphoinositol -3- kinase; Akt, protein kinase B; MHS, murine alveolar macrophage cell line from ATCC.

References:

1. Cheung D.O., Halsey K. and Speert D.P. (2000) Role of pulmonary alveolar macrophages in defense of the lung against Pseudomonas aeruginosa. Infect Immun, 68, 4585-4592
2. Kannan S., Audet A., Knittel J., Mullegama S., Gao G.F. and Wu M. (2006) Src kinase Lyn is crucial for Pseudomonas aeruginosa internalization into lung cells. European J Immunol, 36, 1739-1952.
3. Araki N., Johnson M.T. and Swanson J.A. (1996) A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. J Cell Biol, 135, 1249-1260.
4. Kierbel A., Gassama-Diagne A., Mostov K. and Engel J.N. (2005) The phosphoinositol-3-kinase-protein kinase B/Akt pathway is critical for Pseudomonas aeruginosa strain PAK internalization. Mol Biol Cell, 16, 2577-2585