Biomedical & Comparative Immunology Symposium

Florida International University

16th Annual Biomedical & Comparative Immunology Symposium

February 13th – 14th, 2014

Modesto Maidique Campus
MARC Pavilion
Miami, FL

16th ANNUAL BIOMEDICAL AND COMPARATIVE IMMUNOLOGY SYMPOSIUM
Florida International University, Modesto Maidique Campus
MARC Pavilion

Welcome

Dear Conference Participant,

On behalf of the Symposium Organizing Committee and the FIU Comparative Immunology Club, I enthusiastically welcome you to the 16th Annual Biomedical and Comparative Immunology (BCI) Symposium in Miami, Florida, February 13-14th, 2014 at Florida International University. The Committee and Club have put together a nice program featuring the hard work of students, faculty, and research staff.

This annual symposium will address recent advances in our understanding of diverse biological systems and phenomena. It will focus on cellular and molecular mechanisms of fundamental processes governing organismal development, reproduction, cellular differentiation, disease, growth and survival. We anticipate biologists and biomedical scientists working with a variety of vertebrate (including human and other mammals) and invertebrate organisms will find the plenary sessions and other scientific presentations informative and stimulating.

The meeting will provide an opportunity to learn, to see old friends, meet new friends, and explore current topics in biomedical and comparative immunology research. I invite you to participate in the symposium and activities we have planned throughout the next two days.

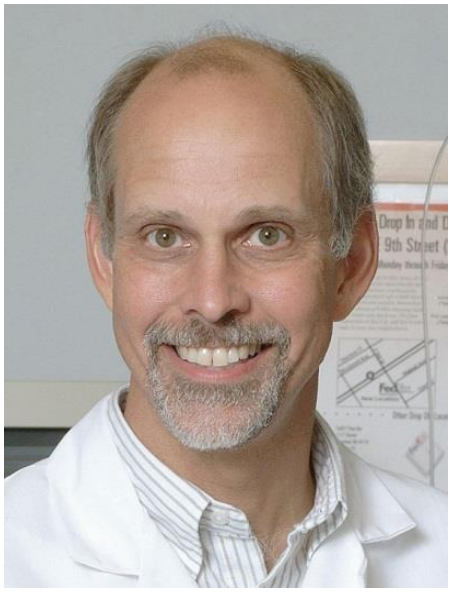
We look forward to welcoming you to South Florida where we hope you will also enjoy the weather and the wealth of cultural opportunities!

Sincerely,

Charles H. Bigger, Ph.D.
Symposium Chair Person
Florida International University

PLENARY SPEAKER BIOGRAPHIES

CHARLES L. BEVINS, M.D./PH.D.



Charles (Chuck) Bevins is a Professor of Microbiology and Immunology in the School of Medicine at the University of California, Davis. He received his M.D. and Ph.D. (biochemistry) from the University of Maryland. He completed his clinical training (pediatrics) at the University of California, San Francisco, and his postdoctoral studies with Dr. Michael Zasloff at the National Institutes of Health, Bethesda. His group studies innate immune defense mechanisms at mucosal surfaces, with a primary focus on defensin peptides. The Bevins laboratory is credited with the discovery of the first mammalian beta-defensin and the identification of HD5 and HD6, two alpha-defensins highly expressed in the human intestinal mucosa. Other investigations have focused on regulated expression of epithelial defensins, structure-function of defensin activity, the role of defensins in inflammatory bowel disease, and development of animal models to elucidate physiological activities of defensin peptides.

http://biosci3.ucdavis.edu/FacultyAndResearch/FacultyProfile.aspx?FacultyID=14231

DIANE M. ROBINS, PH.D.

Diane (Didi) Robins is a Professor in the Department of Human Genetics at the University of Michigan. She obtained her B.S. from Yale and Ph.D. from Stanford (NSF Fellowship), studying hormonally regulated gene expression in chick oviduct. In postdoctoral studies at Columbia University (Jane Coffin Childs Fellow), she defined hormone response elements using novel gene transfer methods. In her own lab, Didi developed a molecular genetic model system of androgen regulation, using mouse sex-limited protein (*Slp*). This has led to two current research areas. One focus uses mice with humanized androgen receptor genes to study initiation and progression of prostate cancer. A recent finding is that strength of the androgen axis predisposes response to therapy, providing a genetic paradigm to reveal biomarkers and novel treatments targeting human AR. The second focus is on sexually dimorphic gene expression, exemplified by *Regulator of sex-limitation* (*Rsl*) genes that modify *Slp* regulation. These rapidly evolving KRAB zinc finger repressors play intriguing roles in mammalian diversification via effects on reproduction, immunity and metabolism.

www.hg.med.umich.edu/faculty/diane-m-robins-phd

Dr. Robins' awards include an NSF ADVANCE from U-M, and she was recently inducted as an AAAS Fellow. She serves on several Editorial Boards and has been on numerous study sections (American Cancer Society, Department of Defense Breast and Prostate Cancer Panels). Didi is active on committees within the University and for the Endocrine Society, and served as Director of Graduate Studies in Human Genetics at U-M for 12 years, including oversight of a Master's program in genetic counseling as well as the Ph.D. program.

PLENARY SPEAKER BIOGRAPHIES

BILLIE J. SWALLA, PH.D.

http://faculty.washington.edu/bjswalla/

Billie J. Swalla is a Professor of Biology in the College of Arts and Sciences and also Interim Director at Friday Harbor Laboratories in the College of the Environment at the University of Washington. She is an expert in Invertebrate Development and Marine Genomics. She moved to the University of Washington from Penn State University in 1999 in order to work on the diversity of marine invertebrates that inhabit Puget Sound and the Salish Sea. The Swalla lab uses transcriptomics and genomics to investigate the evolution of animal body plans by comparing gene expression between different animal embryos. Through studies of metamorphosis, it became clear that tunicates are using innate immunity to remodel their body plan during metamorphosis. Specific interests are the Evolution and Development of innate immunity in ctenophores, tunicates and hemichordates.

PATTY ZWOLLO, PH.D.

http://wmpeople.wm.edu/site/page/pxzwol

Patty Zwollo is a Professor of Biology in the College of William and Mary. She received her B.A. in Biology (1980), M.A. in Biology (1984) and Ph.D. degrees (1992) at the University of Utrecht in the Netherlands. Dr. Zwollo completed a Postdoctoral Fellow at the Johns Hopkins University/HHMI in 1993 and became a Research Assistant Professor at Occidental College from 1993-1995. She was a visiting Assistant Professor at UC Berkeley in 1996 until moving to the College of William and Mary. Dr. Zwollo's laboratory studies the immune system of salmonids, which is remarkably similar to that of mammals with the presence of B and T lymphocytes and a highly diverse antibody repertoire. One of our projects investigates whether long-lived, immunoglobulin-secreting plasma cells (LLPCs) protect adult sockeye salmon against pathogens encountered upon return to their natal grounds for spawning. Recently, her laboratory has developed the Immunological Imprinting Hypothesis, linking immune memory in the form of LLPCs to chemical imprinting and the highly accurate return rates of adults to their natal streams. Dr. Zwollo's lab has also developed flow cytometric approaches to determine the frequency of developing and activated B cell subpopulations in the major immune tissues of trout, such as spleen, blood, anterior kidney, and posterior kidney. The resulting patterns, visualized in the form of contour graphs, can then be used to define the "B cell signature" of a given tissue, in a given fish. In collaboration with Dr. Greg Wiens (the National Center for Cool and Cold Water Aquaculture) and Dr. Steve Kaattari (Virginia Institute of Marine Science), her lab also investigates potential immunological differences in a strain of rainbow trout that was selected based on increased resistance to the fish pathogen Flavobacterium. Lastly, they are exploring the effects of plasticizers or "phthalates", including DEHP and MEHP, on the immune system of juvenile's fish.

16th ANNUAL BIOMEDICAL AND COMPARATIVE IMMUNOLOGY SYMPOSIUM

PROGRAM

THURSDAY, FEBRUARY 13th

8:00 – 9:00 a.m. **On-site Registration and Continental Breakfast**

9:00 – 9:15 a.m. **Welcome Remarks**
Lakshmi N. Reddi, Ph.D., Dean of the University Graduate School
Charles H. Bigger, Ph.D., Professor Emeritus, Department of Biological Sciences
Florida International University, Miami, FL

9:15 – 10:15 a.m. **Plenary Session I**
Defensins: Key Mediators of Host-Microbe Interaction at Mucosal Surfaces
Charles L. Bevins, M.D./Ph.D.
Department of Medical Microbiology and Immunology
University of California – Davis, CA

10:20 – 10:40 a.m. **Post-translational modification of *Leishmania* Aquaglyceroporin AQP1**
Mansi Sharma[a,b], Goutam Mandal[b], and Rita Mukhopadhyay[b]
[a]Department of Biology, College of Arts & Sciences; [b]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

10:40 – 11:00 a.m. **Endocytic trafficking mediates cell migration and cell proliferation in cancer cells.**
Porther N.[a] and Barbieri, M.A.[a]
[a]Department of Biological Sciences, College of Arts & Sciences, Florida International University, Miami, FL

11:00 – 11:20 a.m. **Morning Session - Break**

11:20 – 11:35 a.m. **Inhibition of quorum sensing by *Bucida buceras* in conjunction with clinically-relevant antibiotics to attenuate virulence in *Pseudomonas aeruginosa***
Liana Apolis[a], Rohan Batra[c], James Martin Quirke[b], Lisa Schneper[c], and Kalai Mathee[c]
[a]Department of Biological Sciences and [b]Department of Chemistry, College of Arts and Sciences; [c]Department of Molecular Microbiology and Infectious Diseases, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

11:35 – 11:55 a.m. **Characterizing the role of MifSR two-component system proteins in regulating *Pseudomonas aeruginosa* metabolism**
Gorakh D. Tatke[a], Kumari H[b], Eugenia Silva-Herzog[b], Lourdes Ramirez[a] and Kalai Mathee[a,b]
[a]Department of Biological Sciences, College of Arts & Sciences; [b]Department of Molecular Microbiology and Infectious Diseases, College of Medicine, Florida International University, Miami, FL

11:55 – 1:20 p.m. **Lunch Break - MARC Building Patio**

1:20 – 2:25 p.m. **Plenary Session II**
Function of the Androgen Receptor in Gene Regulation and Prostate Cancer
Diane M. Robins, Ph.D.
Department of Human Genetics
University of Michigan Medical School, Ann Arbor, MI

2:25 – 2:40 p.m. **Organization of centromeres within the human sperm nucleus**
Elizabeth Jordan, Nicole M. Millan, Dimitrios Ioannou, and Helen G. Tempest
Department of Human and Molecular Genetics, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

2:40 – 3:00 p.m. **X-Linked Immune Regulatory Genes Polymorphisms, Childhood Acute Lymphoblastic Leukemia Risk and Male Disadvantage**
Sandeep K. Singh[1], Ziyad Ben Taleb[2], Amy E. Kennedy[2], and Mehmet T. Dorak[2, 3]
[1]Department of Environmental and Occupational Health and [2]Department of Epidemiology, Robert Stempel College of Public Health and Social Work, Florida International University, Miami FL; [3]School of Health Sciences, Liverpool Hope University, United Kingdom

3:00 – 3:20 p.m. **Production of eumelanin and pheomelanin is regulated by Edn3**
Javier Pino and Lidia Kos
Department of Biological Sciences, College of Arts & Sciences, Florida International University, Miami, FL

3:20 – 3:35 p.m. **Afternoon Session – Break I**

3:35 – 3:55 p.m. **Synthesis of Methyl Indolylfulgimide with a Polymerizable Group and Incorporation into Polymers for Regulation of Biological Systems**
Chang-Jun Fan and Watson J. Lees
Department of Chemistry and Biochemistry, Florida International University, Miami, FL

3:55 – 4:10 p.m. **Ceramide Incorporated Nanodiscs: a Model for Understanding Ceramide-PKCζ Lipid-Protein Interactions**
Patricia Theard[a], Mark Kester[c], and John Flanagan[b]
[a]Department of Chemistry and Biochemistry, Department of Biological Sciences, Florida International University, Miami, FL; [b]Department of Biochemistry and Molecular Biology and [c]Department of Pharmacology, Penn State College of Medicine, Hershey, PA

4:10 – 4:30 p.m. **Enhancing Nanoparticle penetration in tumors via the use of Hyperthermia: An experimental and theoretical investigation**
Abhignyan Nagesetti[a], George Dulikravich[b] and Anthony.J.McGoron[a]
[a]Department of Biomedical Engineering, College of Engineering and [b]Department of Mechanical and Materials Engineering, College of Engineering, Florida International University, Miami, FL

4:30 – 4:40 p.m. **Afternoon Session – Break II**

4:40 – 6:00 p.m. **Poster Presentations & FIU Recruiter Fair**

Poster 1. **Elucidating the role of Sab during adipogenesis-induced mitophagy.**
Richard B. Barrios[b] and Jeremy W. Chambers[a]
[a]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine; [b]Department of Biological Sciences, College of Arts and Sciences, Florida International University, Miami, FL

Poster 2. **Comparative methods for isolation of Human Dendritic Cells**
Gloria Figueroa[a,b,c], Marisela Agudelo, PhD[a], and Madhavan Nair, PhD[a]
[a]Department of Immunology, Herbert Wertheim College of Medicine; [b]Department of Biological Sciences, and [c]Department of Chemistry, College of Arts & Sciences, Florida International University, Miami, FL

Poster 3. **TLR agonists differentially induce maturation of nicotine-exposed dendritic cell**
Saba Tamjidi[a], Erika Nourishirazi[b], Brittany Bible[a], Menghua Zeng[a] and Mahyar Nouri-Shirazi[a]
[a]Department of Immunology, Florida Atlantic University, College of Biomedical Sciences, Boca Raton, FL; [b] University of Miami, Miller School of Medicine, Miami, FL

Poster 4. **Effects of Elevated Prenatal Mesotocin on Social and Stress Behaviors in Northern Bobwhite Quail (*Colinus virginianus*)**
Brittany Yusko and Robert Lickliter
Department of Psychology, College of Arts and Sciences, Florida International University, Miami, FL

Poster 5. **Characterizing Immune Cells of Atlantic Bottlenose Dolphins**
Brittany Bible[a], Menghua Zeng[a], Saba Tamjidi[a], Gregory Bossart[b] and Mahyar Nouri-Shirazi[a].
[a]Department of Biomedical Science, College of Medicine, Florida Atlantic University, Boca Raton, FL; [b]Georgia Aquarium, Atlanta, GA

Poster 6. **Novel Polymer for Bioseparations of Complex DNA Mixtures using Capillary Electrophoresis**
Natalie Damaso, MS [a,b,c] and DeEtta Mills, PhD [b,c]
[a]Department of Chemistry and Biochemistry, [b]International Forensic Research Institute, and [c]Department of Biological Sciences, Florida International University, Miami, FL

Poster 7. **Breed designation for unknown equine case samples**
Ketaki V. Deshpande, MSFS[a,b,c], Natalie Leyva, MSFS [a,b] and DeEtta Mills, PhD[b,c]
[a]Department of Chemistry and Biochemistry, [b]International Forensic Research Institute, and [c]Department of Biological Sciences, Florida International University, Miami, FL

Poster 8. **Sab-mediated signaling initiates mitophagy *in vitro***
Aneysis D. Gonzalez[b] and Jeremy W. Chambers[a]
[a]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine; [b]Department of Biological Sciences, College of Arts and Sciences, Florida International University, Miami, FL

Poster 9. **Species specific post-transcriptional regulation of *Leishmania* aquaglyceroporin AQP1 via 3'UTR**
Srotoswati Mandal[a], Goutam Mandal[a], Mansi Sharma[a], Jose Orta[a], Barbara Papadopoulou[b] and Rita Mukhopadhyay[a].
[a]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine; [b]Laval University, Quebec, Canada

Poster 10. **Genetic background influences the NK recruitment and Th1 polarization in response to TLR agonists**
Menghua Zeng[a], Brittany Bible[a], Saba Tamjidi[a], and Mahyar Nouri-Shirazi[a]
[a]Department of Biomedical Science, College of Medicine, Florida Atlantic University, Boca Raton, FL

Poster 11. **Heme-Pocket Characterization of Neuroglobin using a fluorescent probe**
Adrian Rodriguez and Jaroslava Miksovska
Department of Chemistry and Biochemistry, Florida International University, Miami, FL

Poster 12. **Assessment of Gene Repositioning in Human Lymphocytes Due to Genotoxic Agents**
Amanda Vaccarella, Dimitrios Ioannou, Helen Tempest
Department of Human and Molecular Genetics, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

Poster 13. **Fluorescent Random Amplified Microsatellites (F-RAMS) Analysis of Mushrooms as a Forensic Investigative Tool**
Beatrice Kallifatidis[a*], Jan Borovička[c], Jana Stránská[d], Jiří Drábek[d], DeEtta K. Mills[a,b]
[a]Department of Chemistry and Biochemistry, International Forensic Research Institute, and [b]Department of Biological Sciences, Florida International University, Miami, FL; [c]Nuclear Physics Institute, v.v.i., Academy of Sciences of the Czech Republic, Řež, Czech Republic; [d]Institute of Molecular and Translational Medicine of the Faculty of Medicine and Dentistry of Palacký University in Olomouc, Olomouc, Czech Republic

Poster 14. **Age-Associated B Cells Are Enriched for Idiotype T15+ Phosphorylcholine Specific Antibodies**
Kelly McAvoy, Sarah Alter, and Richard L. Riley
Department of Microbiology and Immunology, University of Miami Miller School of Medicine, University of Miami, Miami, FL

6:15 p.m. **Shuttle Departs for Courtyard Marriot (Pick-up at MARC Building)**

FRIDAY, FEBRUARY 14th

8:15 – 9:15 a.m. **On-site registration and Continental Breakfast**

9:20 – 9:30 a.m. **Welcome Remarks**
Charles H. Bigger, Ph.D.
Department of Biological Sciences
Florida International University, Miami, FL

9:30 – 10:30 a.m. **Plenary Session III**
Evolution and Development of Innate Immunology
Billie J. Swalla, Ph.D.
Biology Department and Friday Harbor Laboratories, University of Washington, Seattle, WA

10:35 – 11:00 a.m. **Opsonic activity and immunohistochemical localization of SeC3, a complement component C3-like protein from *Swiftia exserta***
Lorenzo P. Menzel and Charles H. Bigger
Department of Biological Sciences, Florida International University, Miami, FL

11:00 – 11:15 a.m. **Morning Session - Break**

11:15 – 11:30 a.m. **The Impact of Over-Expressing the Mitochondrial Scaffold Protein, Sab, on Cellular Metabolism and Susceptibility to Chemotherapies**
Charles Robbins[b], Adriana Prado[a], Jeremy W. Chambers[a]
[a]Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL; [b]Medical Academy for Science and Technology, Homestead, FL

11:30 – 11:55 a.m. **Selective interactions between host immunity and adherent gut bacteria in *Ciona intestinalis***
Brittany Leigh[a], Asunta Liberti[b], Charlotte Karrer[a], Gail Mueller[a], John P. Cannon[a], Rosaria De Santis[b], Maria Rosaria Pinto[b], Gary W. Litman[a] and Larry J. Dishaw[a]
[a]University of South Florida, FL; [b]Stazione Zoologica Anton Dohrn, Napoli, Italy

11:55 – 1:25 p.m. **Lunch Break - MARC Building Patio**

1:25 – 2:35 p.m. **Plenary Session IV**
Fishing for B cells; going with (the) flow
Patty Zwollo, Ph.D.,
Department of Biology
The College of William and Mary, Williamsburg, VA

2:35 – 2:55 p.m. **Hematopoiesis and hemocyte replacement after repeated hemolymph withdrawals in *Pomacea canaliculata* (Mollusca, Gastropoda)**
Alice Accorsi, Davide Malagoli, and Enzo Ottaviani
Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

2:55 – 3:15 p.m. **BCR and TLR Signaling Converge on Btk to Regulate BAFF Receptors and T Cell Independent Antibody Responses in Marginal Zone B Cells**
Justin Boucher[a], Gianluca Carlesso[b], Eden Kleiman[a], Ellen Kuta[b], Emily Clark[a], Wasif Khan[a]
[a]University of Miami, Miller School of Medicine, Department of Microbiology and Immunology, Miami, FL; [b]MedImmune, Department of RIA, Gaithersburg, MD

3:15 – 3:30 p.m. **Afternoon Session – Break**

3:30 – 3:50 p.m. **Modulation of Kv4.3-KChIP3 Interactions by Ca^{2+} and NS5806**
Walter G. Gonzalez and Jaroslava Miksovska
Department of Chemistry and Biochemistry, Florida International University, Miami, FL

3:50 – 4:05 p.m. **Characterization of Potassium Channel Interacting Proteins with focus on KChIP-2**
Andres Arango, Walter G. Gonzalez, and Jaroslava Miksovska
Department of Chemistry and Biochemistry, Florida International University, Miami, FL

4:05 – 4:25 p.m. **Characterization of Interactions between Bovine Prion Protein and Wogonin, a Potential Antiprion Compound**
Elena Shersher and Xiaotang Wang
Department of Chemistry and Biochemistry, College of Arts and Sciences, Florida International University, Miami, FL

4:25 – 4:45 p.m. **Characterization and function of a sea star's leukocytes**
Charles H. Bigger
Department of Biological Sciences, Florida International University, Miami, FL

5:00 – 5:30 p.m. **Announcement of Student Awards & Closing Remarks**
Andres Gil, Ph.D., FIU Vice-President for Research, Division of Research
Charles H. Bigger, Ph.D., Professor Emeritus, Department of Biological Sciences
Florida International University, Miami, FL

5:40 – 7:00 pm **Reception in MARC Lobby**

7:15 pm **Shuttle Departs for Courtyard Marriot (Pick-up at MARC Building)**

ORAL ABSTRACTS

Plenary Session I

Defensins: Key Mediators of Host-Microbe Interaction at Mucosal Surfaces

Charles L. Bevins, M.D./Ph.D.
Department of Medical Microbiology and Immunology, University of California - Davis

The intestinal mucosa interfaces with a complex, dense community of microorganisms, including hundreds of species of resident microbiota and many transient microbes entering from food and water borne sources. The host relies on multiple immune defense mechanisms to maintain homeostasis and avert microbial disease. The epithelium serves as a key arm of the immune system, both by providing a physical barrier and by secreting various antimicrobial factors, including antimicrobial peptides. In the small intestine, specialized epithelial cells called Paneth cells produce abundant quantities of α-defensins and several other antibiotic peptides. Human Paneth cells make two α-defensins: HD5 and HD6. The mechanism by which these two α-defensins protect from enteric pathogens is quite distinct. HD5 is a potent antimicrobial that kills target microbes, whereas HD6 is newly discovered to self-assemble to form fibrils and nanonets that surround and entangle bacteria. Studies in humans suggest that reduced expression of Paneth cell defensins may contribute to the pathogenesis of inflammatory bowel disease.

Post- translational modification of *Leishmania* Aquaglyceroporin AQP1

Mansi Sharma[a,b], Goutam Mandal[b], and Rita Mukhopadhyay[b]
[a]*Department of Biology, College of Arts & Sciences;* [b]*Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL*

Leishmania aquaglyceroporin AQP1 is responsible for several important physiological functions such as, osmoregulation and osmotaxis. Additionally, AQP1 is an adventitious uptake system for trivalent metalloids such as arsenite and antimonite. Recently, we reported that Mitogen activated protein kinase 2 (MPK2) regulates AQP1 stability at the post-translational level through phosphorylation at threonine 197. Also, we found that lysine 42 (K42) is critical for LmMPK2 and we observed that cells co-expressing AQP1+MPK2K42A showed slower volume recovery compared to wild type cells. Ubiquitin-proteasome pathway and lysosomal proteolysis are two major pathways for intracellular protein degradation. In this project we explored the role of ubiquitination in AQP1 regulation. Ubiquitinated proteins are destined to be degraded by proteasomes. *Leishmania* has an E3 ubiquitin ligase called Anaphase Promoting complex/Cyclosome (APC/C) homologue in its genome. APC/C recognizes its substrates by RXXL motifs. We found three RXXL motifs at the N-terminus of AQP1 and a putative ubiquitination site specific lysine 12 upstream to the first motif in the cytosolic N-terminal end of the channel. Hence, we hypothesized that AQP1 is degraded by ubiquitination at K12. During cell volume regulation assay, upon hypo-osmotic shock, we observed that single mutants for $R^{20}XXL^{23}$ and $R^{27}XXL^{30}$ motif showed increased cell swelling with a higher drop in absorbance and slower volume recovery as compared to wild-type AQP1, suggesting lower expression of protein when compared to wild type. This data corroborated with the protein expression levels. When *L.donovani* promastigotes coexpressing AQP1K12A + MPK2K42A were exposed to a hypo-osmotic shock, swelling and recovery rates increased. Taken together, we conclude that AQP1 is stabilized in presence of MPK2K42A when K12 is mutated to K12A whereas it is degraded when the RXXL motifs are mutated. Therefore, we propose that K12 is the ubiquitination site of LmAQP1 which when removed allows the channel to be stabilized.

Endocytic trafficking mediates cell migration and cell proliferation in cancer cells.

Porther N.[a] and Barbieri. M.A.[a]
[a]*Department of Biological Sciences, College of Arts & Sciences, Florida International University, Miami, FL*

Cell invasion, proliferation, migration and angiogenesis are classic indicators of metastasis. Growth factors, which include epithelial growth factor (EGF), insulin growth factors I and II (IGFI and IGFII); while important for normal cellular processes, are also pro-metastatic as they have been implicated in most if not all aspects of this phenomena. We hypothesize that cancer metastasis may be regulated by the small GTPase, Rab5 in response to IGFI. Methods: Stable cells expressing GFP-Rab5 and its constitutive mutants were established and the Rab5 activity in response to IGFI stimulation was determined via immunoblots. We also ascertained the migratory and invasive potential of our cell lines using a Boyden chamber assay. Results: Our study has indicated that the invasive and migratory properties of breast cancer cells were abrogated in cell lines that only expressed the inactive (GDP-bound) form of Rab 5 irrespective of growth factor stimulation. The invasive potential of breast cancer cell lines expressing the wild type and active (GTP-bound) form of Rab 5 were noticeably greater when exposed to growth factors. In addition, we observed that Rab5 was activated in cells incubated with IGF-I in a time and concentration dependent. Therefore, we can tentatively conclude that Rab5 activation may play an integral role in cell invasion, proliferation, migration particularly in response to growth factor stimulus.

Inhibition of quorum sensing by *Bucida buceras* in conjunction with clinically-relevant antibiotics to attenuate virulence in *Pseudomonas aeruginosa*

Liana Apolis[a], Rohan Batra[c], James Martin Quirke[b], Lisa Schneper[c], Kalai Mathee[c]
[a]Department of Biological Sciences and [b]Department of Chemistry, College of Arts and Sciences; [c]Department of Molecular Microbiology and Infectious Diseases, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

Pseudomonas aeruginosa infections are often difficult to treat due to the organism's intrinsic resistance and ability to acquire antibiotic resistance. This necessitates the identification of novel therapeutic strategies that focus on other virulence mechanisms such as quorum sensing (QS). Two QS systems in *P. aeruginosa*, *las* and *rhl*, control virulence. Previous studies demonstrated that aqueous extracts from *Bucida buceras* leaves inhibited *P. aeruginosa* QS systems. In this study, the specificity of *B. buceras* aqueous extract, the combinatorial activity of *B. buceras* extracts with clinically-relevant antibiotics, and the identification of the active component were investigated. Direct effects on signaling were determined by measuring *las*- and *rhl*-dependent gene expression in heterologous host background in the presence and absence of *B. buceras* extract. The effects of interactions between *B. buceras* extract and azithromycin, tobramycin, ciprofloxacin, meropenem, imipenem and cefotaxime on *P. aeruginosa* growth were analyzed using Epsilometer tests. *B. buceras* aqueous extract significantly inhibited both *las* and *rhl* QS-dependent gene expression. Also, *B. buceras* was antagonistic with all the antibiotics tested. *B. buceras* extracts directly inhibit *las* and *rhl* QS-dependent gene expression and the mechanism of action is independent of acyl homoserine lactone synthesis. Although our data suggests the plant extract is antagonistic with current treatments, it is possible that the compound with the antibiotic-mitigating effect is not the same as the anti-QS compound. Current research is focused upon purifying the quorum-quenching compound in *B. buceras* extract through fractionation via reverse phase chromatography. The focus of future studies demands further identification of the active anti-QS compounds which may provide a potential solution to *P. aeruginosa*'s remarkable resistance to antimicrobial drugs.

Characterizing the role of MifSR two-component system proteins in regulating *Pseudomonas aeruginosa* metabolism

Gorakh D. Tatke[a], Kumari H[b], Eugenia Silva-Herzog[b], Lourdes Ramirez[a] and Kalai Mathee[a,b]
[a]*Department of Biological Sciences, College of Arts & Sciences;* [b]*Department of Molecular Microbiology and Infectious Diseases, College of Medicine, Florida International University, Miami, FL*

P. aeruginosa is a Gram-negative, metabolically versatile opportunistic pathogen. It is accountable for causing incapacitating infections in individuals with defective host defense mechanisms. *P. aeruginosa* infections are difficult to treat, particularly due to the expression of a multitude of virulence factors and its extraordinary intrinsic and acquired resistance to a gamut of clinically significant antibiotics. This ability, in part, is mediated by several two-component regulatory systems (TCS) that play a crucial role in regulating virulence mechanisms and metabolism. MifSR is one such TCS known to regulate an important virulence trait, biofilm formation. In addition, our preliminary data indicates the inability of MifSR deficient strains to utilize α-ketoglutarate (α-KG), a key tricarboxylic acid (TCA) cycle intermediate as a sole carbon source. This suggests the role of MifSR proteins in regulating metabolism by modulating TCA cycle. However, the reason for α-KG utilization defect in *mifSR* mutants was unclear and, it was unidentified if the phenotype exhibited by the mutants is restricted to a defective α-KG utilization or affects the TCA cycle at different junctures. This study aims to address the role of *mifSR* TCS in regulating *P. aeruginosa* metabolism, particularly focusing on TCA cycle and α-KG utilization. Growth profile of *mifSR* mutants in comparison to the wild-type *P. aeruginosa* PAO1 strain demonstrates no difference in growth pattern, in presence of sugars such as glucose, sucrose or arabinose and TCA cycle intermediates (except α-KG) as the sole carbon source. Furthermore, complementation of *mifSR* mutants with *P. aeruginosa* putative carboxylic acid transporter protein PcaT, that shares 83% similarity with *Escherichia coli* α-KG permease KgtP, restores their growth in media supplemented with α-KG. Thus, growth profile analysis and complementation studies together, clearly indicates the role of MifSR TCS proteins in regulating *P. aeruginosa* metabolism, specifically TCA cycle, by modulating α-KG transport through PcaT.

Plenary Session II

Function of the Androgen Receptor in Gene Regulation and Prostate Cancer

Diane M. Robins, Ph.D.
Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI

The androgen receptor (AR) is a hormone-activated transcription factor and orchestrates male physiology, from development to disease, by regulating genes in a context-dependent manner. Prostate cancer relies on AR for progression and responds to androgen ablation therapy, but disease ultimately recurs, still driven by AR. We study the function of AR in prostate cancer using mouse models, cell lines and human tumors. In mice in which we have replaced the mouse gene with human sequences, genetic variation of AR affects tumor progression and therapy response. In both mouse and human tumors, somatic AR mutations arise selected by treatment and reveal multiple mechanisms to resist therapy, including altered target gene selectivity, chaperone interaction and nuclear localization.

To more accurately reflect human disease, we developed a mouse model coupling humanized AR alleles of varying strength with overexpression of ETV1 (a key prostate oncogene) and loss of PTEN (a critical tumor suppressor). ETV1 effects on gene expression were evident prior to notable pathology, supporting the idea that ETV1 overexpression is an early event priming tumorigenesis. Loss of PTEN cooperated to increase neoplasia, leading to a lethal phenotype at late age in a subset of mice that had more active AR alleles and ETV1 overexpression. Gene expression profiles from deep sequencing of early and late disease samples revealed a strong antagonism between AR and ETV1 at AR target genes, and this antagonism was abrogated upon loss of PTEN.

These studies indicate that antagonism between AR and ETV1 at the molecular level leads to synergy in oncogenesis and may vary with strength of the androgen axis. Further knowledge of mechanisms underlying these regulatory interactions may lead to better predictors for therapy response and development of novel treatments that avoid resistance.

Organization of centromeres within the human sperm nucleus

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In a wide variety of cell types there is a distinct evolutionarily conserved higher order organization of chromatin within the interphase nucleus. We hypothesize that chromatin organization within the sperm nucleus may be crucial for fertilization and normal embryogenesis. However, the organization of the paternal genome has been hitherto poorly investigated. In this study, we investigate whether nonrandom organization of centromeres exists in the human sperm nucleus. Semen samples were collected from nine normozoospermic males and analysis of the organization of centromeres for chromosomes 18, X & Y and a pan-centromeric probe (staining all human centromeres) was performed using fluorescence in-situ hybridization. Centromere organization was assessed via two means: radial (interior-peripheral) and polar (apical-basal). 100 cells/chromosome/subject/analysis were analyzed (n=10,800). Chi-squared goodness-of-fit test was used to assess randomness in each analysis. All individual centromeres tested exhibited significantly non-random organization for both radial and polar analyses. A preferential distribution of centromeres for chromosome 18, X & Y was observed in the interior. Polar analysis showed a head region preference in X centromeres, and a mid region preference for 18 & Y centromeres. Furthermore, we observed that centromeres preferentially form clusters with an average of 9.26 centromere clusters within each sperm nuclei. Our data suggests that the organization of centromeres within the sperm nucleus is non-random and demonstrates a preferential co-localization of individual centromeres to form clusters. Paternal chromosomes are hypothesized to be withdrawn from the nucleus in a sequential order allowing gradual exposure of chromosomes to the ooplasm, which may be critical for fertilization and early embryogenesis.

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X-Linked Immune Regulatory Genes Polymorphisms, Childhood Acute Lymphoblastic Leukemia Risk and Male Disadvantage

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The X chromosome contains a disproportionately high number of immune-related genes. The difference in X chromosome numbers (females two copies and males one copy) results in immunological differences in males and females. Mutations or polymorphisms of an X chromosome-linked gene will be phenotypically expressed in males, who may then show functional impairment of the respective proteins. Childhood acute lymphoblastic leukemia (ALL) incidence rate shows a consistent gender effect with male to female ratio of 1.3. We hypothesized that functional polymorphisms of X-linked immune regulatory genes modify childhood ALL risk in males. Newly diagnosed childhood (age ≤18 yr) ALL cases (n=161) and healthy controls (n=231) were recruited at Baylor College of Medicine. Six X-linked immune regulatory gene polymorphisms (*FOXP3* rs2280883, rs2232365; *IRAK1* rs1059702; *BTK* rs700; *XIAP* rs5956583 and *SH2D1* rs2239481) were selected by a thorough analysis of population and bioinformatics data, and genotyped by TaqMan allelic discrimination assays. *FOXP3* SNPs showed an association differing in direction between two genders, but none of the results for gender differential were statistically significant, and statistical interaction analysis by gender did not yield any statistical significance. Stratified analysis by ethnicity for *XIAP* rs5956583 showed a statistically significant association for non-Hispanic whites (OR = 2.07; CI = 1.06 to 4.07) and a trend towards protection for Hispanic (OR = 0.59; CI = 0.34 to 1.04) without gender differential. The interaction with ethnicity reached statistical significance (P_{int} = 0.005). We did not identify any gender-specific risk marker in this preliminary study. The result for rs5956583 was modified by ethnicity, and showed a statistically significant risk association in non-Hispanic whites. In this preliminary study, we explored only six SNPs from five X-linked immune regulatory genes. Exploration of more X-linked immune regulatory genes may unravel gender-specific risk markers for childhood ALL.

Production of eumelanin and pheomelanin is regulated by Edn3

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Melanocytes depend on various signaling molecules for proper development and efficient pigment production. α-Melanocyte Stimulating Hormone (α-MSH) controls the switch between the production of eumelanin (black/brown) and pheomelanin (yellow/red) while *Endothelin 3* (*Edn3*) is essential for melanocyte development. Doxycycline (dox) inducible transgenic mice that express Edn3 under the *Keratin 5* (*K5*) promoter showed hyperpigmentation of both skin and coat. The goal of this study is to understand the role of Edn3 in pigment production. Lethal yellow mice (A^y), that have a non-functional pathway downstream of α-MSH, carrying the *K5-Edn3* transgene were considerably darker than A^y mice. In order to test if continuous *Edn3* transgenic expression is required for the maintenance of a dark pigmentation phenotype in A^y mice, dox was administered to 1-day old pups, deactivating the transgene. After a period of 6 weeks the coat color of A^y;*K5-Edn3* mice became lighter and was similar to those of A^y mice. The comparative analysis of melanin content of hairs from A^y, A^y;*K5-Edn3* and wild type mice showed that transgenic *Edn3* expression significantly increased the amounts of both eumelanin and pheomelanin. These results indicate that the paracrine expression of *Edn3* from keratinocytes is capable of bypassing the requirement for a functional α-MSH pathway for the generation and maintenance of dark coat color. Different than α-MSH, Edn3 does not appear to regulate the switch between eumelanin and pheomelanin, but can up-regulate both simultaneously.

Synthesis of Methyl Indolylfulgimide with a Polymerizable Group and Incorporation into Polymers for Regulation of Biological Systems

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Indolyfulgimides, which are one class of organic photochromic compounds, have at least two forms, including an open form and a closed form, which can be interconverted by different wavelengths of light. Indolyfulgimides have numerous promising properties, such as thermal stability, hydrolytic stability, and enhanced photochemical fatigue resistance. Indolylfulgimides with polymerizable groups can be incorporated into polymers, which can amplify the effects of changing between the two forms and provide enhanced stability. When enzymes are embedded into photochromic polymers, we expect that the enzyme's activity can be regulated by light. Switching between the two photochromic forms will change the polymer's conformation, which in turn will alter the substrate's accessibility to the active site. Therefore, water-soluble photochromic copolymers with good thermal and photochemical stability could serve as promising photoswitches and biosensors in biological systems. Herein, a new indolyfulgimide with a pendant styrene group and a methyl group at the bridging position was prepared. Co-polymerization with poly(acrylamide) (PAA) successfully produced a water soluble fulgimide-PAA copolymer. The fulgimide and copolymer were found to be photochromic and their photochemical stability and thermal stability were followed using UV-Vis spectroscopy. For photochemical stability, the fulgimide degraded 20% after 1100 cycles back and forth between the open and closed forms in toluene and the copolymer degraded 20% after 92 cycles in buffer. In toluene at 80 °C, the *E*-form fulgimide showed very good stability with no decomposition after 400 h and the *C*-form fulgimide was less stable and degraded 38% after 391 h. In both water and buffer at 37 °C, the *E*-form copolymer was more stable than the *C*-form copolymer. The thermal stability of the *E*-forms copolymer has been significantly improved by replacing the trifluoromethyl group with a methyl group at the bridging position. However, the thermal stability is still limited by the least stable *C*-form.

Ceramide Incorporated Nanodiscs: a Model for Understanding Ceramide-PKCζ Lipid-Protein Interactions

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Membrane proteins must adopt precise conformations to carry out their functions within the membrane of cells. Determining these native structures to atomic resolution has proved challenging for lack of a planar lipid-membrane mimetic environment. Current methods to determine native structures of membrane proteins using detergent micelles and lipid-based liposomes have proven inaccurate due to their shape, size, and high radius of curvature. By contrast, nanodiscs provide a stable, flat, membrane-like environment that is potentially ideally suited to studying membrane protein structure and function using aqueous conditions. Nanodiscs are discoidal phospholipid bilayers in which the hydrophobic tails of the lipids are stabilized and enclosed by a belt of amphipathic α-helical protein, MSP-1, derived from the lipid carrier protein Apo-AI. This novel system has been increasingly important not only in determining structure of membrane proteins, but also in protein-protein and lipid-protein interactions. The goals of this research project were to determine whether nanodiscs containing ceramide, a key signaling lipid involved in a wide variety of processes including inflammation, were able to be reconstituted; and whether a ceramide-activated protein, PKCζ, a key downstream target of this lipid involved in vascular permeability and angiogenesis of vascular tissue, could functionally interact and be activated by this model membrane. Nanodiscs self-assembled and comprised of MSP1, 1,2-dioleolyl-sn-glycero-3-phosphocholine (DOPC, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) and N-palmitoyl-D-*erythro*-sphingosine (CER, C16-ceramide) were created and optimized to determine the appropriate lipid-protein ratio for reconstitution (40:1) and for varying quantities of ceramide (1%, 8%, 10%). A kinetic assay tests serine/threonine phosphorylation by kinase, analyzing the effect of PKCζ activation in the presence of ceramide incorporated nanodiscs. The optimal component ratio, characterization of protein activation and protein activity demonstrate how ceramide-incorporated nanodiscs increase the binding activity and rate of phosphorylation of a substrate by PKCζ compared to previously reported kinase interaction with ceramide as a free lipid.

Enhancing Nanoparticle penetration in tumors via the use of Hyperthermia: An experimental and theoretical investigation

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The penetration of nanoparticles into the tumor tissue is severely hindered by the tumor extracellular matrix. This limits their successful application as diagnostic or therapeutic probes *in-vivo*. Tumor spheroids recapitulate the histomorphological and functional complexity of avascular tumors, features that are absent in current mono-layer cultures. Therefore, spheroids are suitable as in-vitro models to study the penetration of nanoparticles. We hypothesize that hyperthermia at 43ºC can be used to increase the penetration of nanoparticles in tumor spheroids by changing the uptake rate constants of cells and by increasing the porosity of tumor tissue. *Methods:* Ovarian carcinoma (Skov-3) and Uterine Sarcoma (MESSA/Dx-5) spheroids are created using the liquid overlay method. Spheroids are characterized using scanning electron microscopy (SEM) and histology. Silica nanoparticles loaded with Fluorescein-Isothiocyanate are created using the reverse microemulsion technique and coated with polyethylene glycol to improve aqueous stability. A 2-dimensional mathematical model is prepared based on SEM sections of tumor spheroids. *Results*: Spheroids with volumes 0.342 ± 0.036 mm^3 (Dx-5) cells and 0.083 ± 0.012 mm^3 (Skov-3) cells (mean ± stdev; n=5) are obtained. Histology sections show stratified organization of cell layers inside the spheroid. SEM images show that Skov-3 and Dx-5 cells form spheroids with very different organization of cells. Silica nanoparticles with the final size of 58 ± 6 (n = 3) and surface charge of -5.88 ± 0.8 mv are obtained. The particles show high fluorescence when excited at 480 nm's wavelength. Fluorescent imaging and cell uptake results show that surface modification of silica particles has a significant impact on the uptake of nanoparticles into the cells. Furthermore, hyperthermia treatment alters the uptake of nanoparticles into the cells. *Conclusions:* Hyperthermia affects the delivery of nanoparticles to cells. Further implications in tumor spheroids and preliminary results from the mathematical model will be presented.

Plenary Session III

Evolution and Development of Innate Immunology

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Innate immunity has been shown to be involved in developmental processes in several animal systems. Genome sequencing has shown that most bilaterians and cnidarians possess a complement system. My lab has shown that the complement system is involved in ascidian larval settlement and metamorphosis. Recent transcriptome analyses have shown that the ascidian metamorphosis program begins much earlier in molgulid ascidians and may be responsible for the radically different larval body plans of tailless *M. occulta* larvae. Tailed *M. oculata* embryos, like most solitary ascidians, have 40 notochord cells that are converged and extended in the tadpole larvae. The larvae also have tail muscle cells flanking the notochord in the tail, and, in the head, an otolith, a gravity sensory organ. The tailless *M. occulta* does not form a tail in their larval stage, and have only 20 notochord cells that do not converge and extend during larval development. This radical heterochronic shift in innate immunity expression has been documented in another tailless ascidian, *Molgula tectiformis*, and is now reported for both the tailed, *Molgula oculata* and tailless *Molgula occulta*. Furthermore, both species have already formed siphons at the time of hatching, so morphologically metamorphosis has began as well. Functional data is necessary to determine if this pronounced heterochrony is the necessary preadaptation for tailless tadpole to evolve in molgulid ascidians. We are especially excited about using these studies to understand how innate immunity interacts with the metamorphic signal in ascidian tadpole larvae, which is still currently unknown.

Opsonic activity and immunohistochemical localization of SeC3, a complement component C3-like protein from *Swiftia exserta*

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The third Complement component (C3) is the central protein of the complement cascade linking the three activation pathways (the antibody-dependent classical pathway, the lectin-dependent pathway, and the alternative pathway) with the lytic cascade. C3 is an evolutionarily ancient molecule involved with host defense: C3 genes have been found in many metazoan phyla. In fact our lab published the first report of C3 from a cnidarian, the octocoral *Swiftia exserta*.

Functional studies of complement cascade activity began with Bordet and the burgeoning field of immunology, investigating mammalian complement systems. These studies include the end-of-cascade lytic pores developed by the membrane attack complex (MAC), opsonic functions of C3b (a fragment of C3 covalently bound to targets via a thiol-ester bond), and chemo-attraction assays of C3a (as well as C4a and C5a fragments from C4 and C5, respectively). Opsonization is the labeling of particles for phagocytosis, causing opsonized particles to be ingested faster than non-opsonized particles.

Several groups have expanded functional studies of the lectin-dependent complement cascade to protochordates (T Fujita and M Nonaka) and echinoderms (LC Smith), but, to date, no functional assays have been published from more basal metazoan animals. Here we demonstrate: the production of the SeC3 protein and identify some of the C3b chains by western blot analysis; the localization of C3 in the basal metazoan *Swiftia exserta*, by cryo-immunohistochemistry; and that opsonized zymosan particles are phogytosed more rapidly by phagocytic cells from *Swiftia exserta* and by RAW 237.1 cells (a mouse macrophage cell line).

The Impact of Over-Expressing the Mitochondrial Scaffold Protein, Sab, on Cellular Metabolism and Susceptibility to Chemotherapies

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Chemotherapy is readily employed for many types of cancer; however, chemotherapeutic associated toxicity remains a concern in the administration of these therapies. To decrease the dose of a chemotherapeutic agent (and lower toxicity), chemotherapeutic sensitizers are employed to enhance the effects of chemotherapeutic drugs. One chemosensitizer, LY294002, has been shown to increase the expression of the mitochondrial scaffold protein Sab in HeLa cells. Signaling on Sab has been associated with mitochondrial dysfunction and programed cell death (apoptosis) in cancer cells. We propose that the increased Sab induced by LY294002 "primes" mitochondria for cell death, contributing to the chemosensitizing effect of LY294002. Further, we hypothesize that Sab concentrations indicate the susceptibility of individual cancers to conventional chemotherapeutic approaches. To test our hypothesis, we overexpressed Sab in the HeLa cell (human cervical carcinoma) line through DNA transfection. We then validated our overexpression using Western Blot analysis to detect Sab concentrations in mock, AKAP1 overexpressed, and Sab overexpressed groups of the HeLa cell line. To complete the experiment, we seeded and treated each group in a 96-well plate with the chemotherapeutic agent Paclitaxel for 24 hours. We were able to determine the effectiveness of the treatment by analyzing the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of each group with the Seahorse XF-96 Extracellular Flux Analyzer. The Western Blot analysis showed that Sab was overexpressed in the transfected group of HeLa cells compared to the mock control. In the metabolic analysis, cells over-expressing Sab had reduced oxygen consumption compared to controls. Furthermore, there was a dramatic decrease in cell viability (shown as a decrease in OCR and ECAR) in the Sab overexpressed group as compared to control groups for the 96-well plate treated with Paclitaxel. Increasing the concentration of Sab on the mitochondria causes the cancerous HeLa cells to become more susceptible to chemotherapeutic treatment. This research suggests that Sab is a candidate biomarker for cancer cell susceptibility to conventional therapeutic methods. This is important because simple noninvasive assays for Sab can be used to indicate whether a patient's tumor cells are responsive to treatment options.

CR is a high school participant in the F.I.U. Summer Research Internship, 2012 & 2013.

Selective interactions between host immunity and adherent gut bacteria in *Ciona intestinalis*

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The animal gut is a habitat for microbes that can serve essential roles in both health and disease. Unraveling the molecular dialogue between host immunity and complex microbiomes (like in the gut) is of immense biomedical interest. Our group is developing an invertebrate chordate, *Ciona intestinalis*, as a model that provides unique technical advantages in the study of the host-microbe interface in the gastrointestinal tract. Previously, we demonstrated the existence of a stable but complex community of bacteria that inhabit the *Ciona* gut. Because this core community is preserved in starved animals, many are likely adherent and reside in the mucus-rich layer adjacent to the epithelium. To study the interplay between these bacteria and the host epithelium, sections of snap-frozen *Ciona* stomach and intestines were stained for bacterial biofilms as well as for Variable region-containing Chitin-Binding Protein C (VCBP-C), an Ig-like protein previously shown to be expressed and secreted into the lumen by gut epithelium, to bind exogenous bacteria, and demonstrates opsonic activity with resident phagocytes. Here we show that VCBPs are expressed in distinct regions of the gut, respond to features of outer membrane coatings (e.g., Gram staining), and at least one (VCBP-C) is trapped in the mucus layer of the gut and may serve a role in modulating the formation of biofilms. Surprisingly, *Ciona* VCBP-C can also influence the chemotactic mobility of certain bacterial species; several gut-adherent bacterial isolates are currently being analyzed for similar VCBP-mediated effects. We hypothesize that secreted Ig-like molecules play an essential role in modulating the formation of biofilms in the chordate gut and that multiple convergent systems for biofilm regulation have emerged during the evolution of chordates.

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Plenary Session IV

Fishing for B cells; going with (the) flow

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Our lab aims to understand the complex developmental and activation pathways of B cells in salmonids. Because insufficient reagents are currently available to study these processes in salmonids, we developed an approach that uses differentially expressed transcription factors as developmental and activation markers. We combined this with flow cytometry, which allows us to characterize individual B cell populations in any vertebrate species. I will illustrate the strength and the broadness of flow cytometry to study humoral (antibody) immune responses in fish, through four different projects. In the first project, we use this approach to determine whether resistance to a fish pathogen (*Flavobacterium p*.) is associated with changes in B cell developmental and/or activation pathways in rainbow trout (*Oncorhynchus mykiss*). A second project uses expression of alternatively spliced forms of the transcription factor Pax5 to detect a small population of early developing B cells in trout blood and spleen. A third project investigates changes in the immune system of sockeye salmon (*Oncorhynchus nerka*) during their return journey from the ocean to their Alaskan spawning site. The last project illustrates how flow cytometry can be used to detect changes in salmonid B cell proliferation as a result of exposure to a pollutant, in this case, the plasticizer DEHP. In summary, flow cytometric analysis using transcription factors as markers can be used to explore and dissect the pathways of B cell development in any species for which reagents are lacking. As such, it provides an important new tool to assess immune health and immune responsiveness, both in trout and other vertebrate species.

Hematopoiesis and hemocyte replacement after repeated hemolymph withdrawals in *Pomacea canaliculata* (Mollusca, Gastropoda)

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Pomacea canaliculata is a freshwater snail attracting growing interest as an invasive pest. *Pomacea* is native of South America but diffused in South-East Asia and North America. Recently, the EU Parliament banned *P. canaliculata* import and diffusion within the Union. Information about the immune system of *P. canaliculata* may be useful to efficiently prevent the diffusion of the specimen. The principal cellular components of the innate immune response are the hemocytes. Hematopoiesis is the process supporting the hemocyte turnover, but very little information is available on molluscan hematopoiesis.
Pomacea canaliculata is an excellent model for studying molluscan hematopoiesis since it is possible to analyze hemocyte profile and potential hematopoietic organs after repeated hemolymph withdrawals. The hemolymph was collected daily from the same animals and the hemocyte population was morphologically evaluated. After the 4^{th} withdrawal a significant increment of large hemocytes versus small hemocytes was noted, but the cell density was stable. In order to better understand this observation we analyzed though light microscopy the components situated in the pericardial cavity, *i.e.*, heart, aortas, ampulla and veins. The ampulla, which is internally organized in hemocyte-containing islets, was the only component showing changes after 4 daily hemolymph collections. The stocked hemocytes were released and the ampullar internal organization was lost. In order to determine whether the ampulla is a hematopoietic organ, immunohistochemical staining with the anti-p-H3(Ser10) antibody, targeting mitotic cells, was performed. Positive nuclei were observed along the external side of the veins inside the pericardial cavity both in controls and in the 4-time withdrawn samples. Positive cells were never evidenced in ampulla, heart or aortas. These results suggest that in *P. canaliculata* the ampulla acts as hemocyte reservoir, while the hematopoietic tissue is localized within the pericardial cavity along the external side of the veins.

BCR and TLR Signaling Converge on Btk to Regulate BAFF Receptors and T Cell Independent Antibody Responses in Marginal Zone B Cells

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Mutations in the gene encoding Bruton's tyrosine kinase (Btk) cause X-linked agammaglobulinemia (XLA), characterized by a severe B cell deficiency and a failure to mount a T cell-independent (TI) antibody (Ab) response. Previous work by our lab and others has shown Btk is critical in BCR and TLR signaling pathways. We hypothesized TI responses were dependent on Btk mediated TLR and BCR signaling. To evaluate this we used genetically modified mouse models to dissect Btk's role in surface expression of calcium-modulator and cyclophilin ligand interactor (TACI) and B cell activating factor receptor (BR3). Our results show Btk-dependent BCR and TLR signaling in primary mouse and human B cells is essential for increased surface expression of TACI and BR3. Increased TACI and BR3 provide a mechanism for TI Ab production regulated by BAFF and APRIL. This Btk function is particularly important in MZ B cells as they serve as the primary source of Ab production in response to TI antigens. In addition, we found the TLR/Btk pathway in MZ B cell also induced pro-inflammatory cytokines. These data reveal that Btk plays an important role in BCR and TLR regulation of TI responses. First, by sensitizing MZ B cells to BAFF receptor/ligand system and second, by pro-inflammatory cytokine mediated activation of innate cells that may facilitate BAFF and APRIL production. In conclusion these findings define Btk-dependent mechanisms of TI antibody production as well as explain why XLA patients respond poorly to encapsulated bacteria.

Modulation of Kv4.3-KChIP3 Interactions by Ca^{2+} and NS5806

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Here we report how the interactions between Kv4.3 T1 domain and KChIP3 are modulated by Ca^{2+} binding KChIP3 as well as by the recent fast K^+ current activator NS5806. The hypothesis is that the observed K^+ current gating regulation due to KChIP3 association with Kv4 channels is due to structural rearrangement of the protein complex which is triggered by calcium, and that the mechanism of action of the novel drug NS5806 is mediated by modification of these structural changes. We implemented fluorescence techniques and isothermal calorimetry to study the interaction between recombinant KChIP3 and Kv4.3 T1 domain constructs and how they depend on calcium and NS5806. We show that binding of KChIP3 to Kv4.3 is calcium dependent, whereas NS5806 functions by eliminating this calcium dependency. Moreover, we show that NS5806 enhances the sequestering of the hydrophobic N-terminus of Kv4.3, which has been associated with the fast K^+ current inactivation, and correlates well with previous electrophysiological studies. We also show using FRET that the Kv4.3-KChIP3 complex attains a different structural organization in the presence of calcium as well as in the presence of NS5806. Overall, the studies shown here support the idea that calcium plays an active role in K^+ current regulation and that KChIP3 may provide for a novel drug target.

Characterization of Potassium Channel Interacting Proteins with focus on KChIP-2

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Potassium channel interacting proteins (KChIPs), belong to the family of neuronal calcium sensors and are expressed in brain and heart tissue. These proteins bind to alpha-subunits of Kv4 channels and regulate channel trafficking, membrane association, and current kinetics. Among them, KChIP-3 was shown to interact with other intracellular partners (presenilin, calmodulin, and DNA), and thus be involved in Alzheimer's disease and pain sensing. KChIP-2 is expressed in brain, heart, and lung tissue however less is known about its structural properties compare to other KChIP prpoteins. The objective of this study is to over-express KChIP-2, characterize it's interaction with Ca^{2+} and Mg^{2+}, structural change associated with metal binding, and ascertain its dimerization state via time resolved anisotropy, also any additional interacting partners will be identified. KChIP-2 was overexpressed in *Escherichia coli* cells. Upon isolation, the protein was purified via affinity and Ion exchange chromatography. Subsequently the interaction of KChIP-2 with Ca^{2+} and Mg^{2+} was characterized in terms of affinity constant and associated secondary and tertiary structural changes using steady-state and time resolved spectroscopic techniques. Finally, the oligomerization state was examined using time resolved fluorescence anisotropy. KChIP-2 has shown to readily form inclusion bodies during overexpression. Optimal conditions for refolding were found (LDAO/High Glycerol/Sucrose). KChIP-2 was shown to readily form aggregates in the absence of surfactants like LDAO. No tryptophan transition was readily observed in apo and Ca^{2+} bound forms. KChIP-2 did not readily bind to Ni-NTA, which is likely due to dimer formation. Further characterization of KChIP-2 could potentially lead to a better understanding of the role that KChiP-2 plays in neuronal cells in the heart, brain, and lungs, which could lead to a better understanding of neuronal as well as cardiac related diseases.

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Characterization of Interactions between Bovine Prion Protein and Wogonin, a Potential Antiprion Compound

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Transmissible spongiform encephalopathies are fatal neurodegenerative disorders resulting from accumulation of the pathogenic prion protein (PrP^{Sc}) in the brains of affected individuals. PrP^{Sc}'s secondary structure is predominantly comprised of beta sheets, which makes it prone to aggregation. The conformational conversion of the prion protein from its cellular form (PrP^{C}), which is mostly alpha-helical, into the scrapie form, is the hallmark of such diseases as bovine spongiform encephalopathy ('mad cow disease') in cows, scrapie in sheep, and Creutzfeldt-Jakob disease in humans. Evidently, inhibition of PrP^{Sc} formation would provide an attractive method for therapeutic intervention. Our research is based on the hypothesis that molecules, capable of binding to PrP^{C} to increase its stability and/or to PrP^{Sc} to prevent or slow down its aggregation, could have the potential to serve as drug candidates for the treatment of prion diseases. Previously, we have identified wogonin (4H-1-benzopyran-4-one, 5,7-dihydroxy-8-methoxy-2-phenyl) as a potential antiprion compound. Upon binding to bovine PrP (bPrP), it induces favorable conformational changes in beta-monomer form of the protein by significantly increasing its alpha-helical content, which in turn decreases the propensity of the protein to aggregate. Here, we further characterize the interactions between bPrP and wogonin using such spectroscopic techniques as UV/Vis spectrophotometry and circular dichroism. We have shown that wogonin affects not only the protein secondary structure but the tertiary structure as well. Calculations of bPrP binding constant indicate intermediate binding between bPrP and the ligand. Wogonin-bPrP binding simulations have been performed using Autodock Vina software and several possible wogonin binding sites have been identified. PyMol software is used for the protein-ligand complex visualization. Therefore, we have further investigated the effects of wogonin on bPrP conformation, calculated the binding constant, and identified possible wogonin binding sites.

Characterization and Function of a Sea Star's Leukocytes

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While the Echinoderms, members of the deuterostome linage, continue to receive immunological research interest, most of the recent attention has focused on the sea urchins rather than sea stars. While sea stars share the basic phyletic characteristics, their anatomy and circulating cell populations are quite different from sea urchins. The sea star *Dermasterias imbricata* has been the subject of some older immunologic experiments regarding allogeneic tissue grafting and has the advantage of being relatively large and easy to bleed. However, the animals have three fluid filled systems that contain "circulating" cells and the relationships of the cells in the systems has not been clear. The study to be reported used a combination of experimental conditions and light, transmission electron and scanning electron microscopy to investigate the leukocytes of the coelomic, water vascular and perihemal systems. While the same three cell types (large granular leukocytes, LGL, large hyaline, LHL and small leukocytes, SL) are found in all three systems and the total number of cells is similar, there are significantly more small leukocytes in the axial sinus. At least some of the large granular leukocytes are phagocytic. The large granular leukocytes are also highly adherent and under the appropriate conditions will aggregate. As will be discussed, the apparent Mg^{++} sensitivity of LGL adherence and aggregation would provide a mechanism for the rapid sealing of wounds to maintain homeostasis.

POSTER ABSTRACTS

P1. Elucidating the role of Sab during adipogenesis-induced mitophagy.

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Mammalian cells degrade mitochondria by a process known as mitophagy. PINK1, Parkin, and the autophagy-related (Atg) proteins are involved in the regulation of mitophagy. Mutation of PINK1 or Parkin results in the accumulation of dysfunctional mitochondria, which is characteristic of Parkinson's disease. Recent preliminary data from our lab demonstrates that Sab, a mitochondrial scaffold protein that can regulate apoptosis, may also be involved in the regulation of mitophagy. Pre-adipocytes reduce mitochondrial density during differentiation into adipocytes and for this reason adipogenesis has great potential as a system for the study of mitphagy. After inducing pre-adipocytes to differentiate, it was discovered that at the onset of differentiation there was an increase in Sab expression followed by a gradual decrease as differentiation progressed. Subsequently, there was an increase in Atg following the increase in Sab levels. We propose that Sab-mediated signaling destabilizes mitochondrial membrane potential, which results in the upregulation of mitophagy components, including the Atg family of proteins. Based on the current data, we plan to examine our hypothesis that Sab functions in conjunction with PINK1, Parkin, and Atg to regulate mitophagy.

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P2. Comparative methods for isolation of Human Dendritic Cells

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Dendritic Cells (DCs) are antigen presenting cells of the immune system that play a role in lymphocyte responses, host defense mechanisms, pathogenesis of inflammation, and recently they have been linked in the development of cancer immmunotherapies. Because of their distinctive feature, the isolation and study of DCs have been important in biological research. Although extremely important, DCs are very rare in blood, accounting for approximately 0.1% of all leucocytes. Therefore, different alternatives for isolation methods rely in the differentiation of DCs from more accessible CD34+ progenitor cells and monocytes isolated from peripheral blood mononuclear cells (PBMCs). Since choosing a proper isolation technique combining simplicity, affordability, high purity and yield of cells is important to consider; in the current study, we identify three distinct methods for isolation of DCs: isolation of monocytes derived dendritic cells (MDDCs) by adhesion and culturing with IL-4 and GM-CSF, isolation of monocytes by negative selection using the EasySep magnetic procedure and culturing with IL-4 and GM-CSF, and direct isolation of DCs from human blood also using EasySep. We hypothesize that the EasySep procedure use to isolate monocytes will give the highest percent yield and viability of MDDCs since purity has been shown to be above 93% ± 3.8% with 65.6% ± 16.2% yield. In addition, the characterization and function of isolated DCs were also studied. Surface markers and co-stimulatory molecules such as CD11c, CD40, DC-SIGN, CD80, CD83, and CD86 were analyzed by flow cytometry. Viability was tested with trypan blue and cell proliferation with XTT method. Surprisingly, MDDCs markers were modulated in MDDCs differentially isolated. Further, MDDCs isolated by all three methods were functional and had proliferative capacity. The method that provided the highest purity was the EasySep. However, the yield was compromise. Our results provide an insight into reliable methods for isolation of human DCs.

P3. TLR agonists differentially induce maturation of nicotine-exposed dendritic cell

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Vaccines aid in saving lives from infections and biological warfare attacks. They should be effective in all target populations otherwise the likelihood that an unprotected person will transmit disease to a vulnerable individual is greatly increased. There is compelling evidence that smokers are less responsive to vaccination. We have reported that both therapeutic and prophylactic vaccines fail to protect and cure animals from disease due to negative effects of nicotine in biological activities of DCs. Using in vitro mouse culture system we have identified an appropriate TLR agonist capable of correcting the defects in DCs exposed to nicotine. In order to translate these studies to human, we tested the hypothesis that appropriate TLR agonist(s) will also correct the degrading effects of nicotine on human DCs and consequently DC-NK cross talk and T cell polarization. Monocyte-derived DCs were generated in culture media containing growth factors GM-CSF and IL-4 with or without nicotine treatment. DCs were activated with indicated TLR agonists and their phenotypes and cytokine profiles were analyzed by flow cytometry and ELISA, respectively. Among the TLR agonists tested, we found that nicotine has less effect on human DC maturation in response to TLR4 plus TLR7/8 agonists as evidenced by expression levels of their costimulatory (CD80/83/86/40) and antigen-presenting (HLA-DR) molecules as well as inflammatory cytokines (IL-12, IL-10,TNF-α and IL-1β) production. We are currently investigating whether these TLR agonists also augment human DC-NK bidirectional signals essential for T cell differentiation in a nicotinic environment.

P4. Effects of Elevated Prenatal Mesotocin on Social and Stress Behaviors in Northern Bobwhite Quail (*Colinus virginianus*)

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Oxytocin (OT) plays a key role in the mediation of social and stress behaviors across many species; however, the mechanism is still unclear. This study investigated the influence of prenatal levels of mesotocin (MT; avian homologue of OT) on postnatal social and stress behavior in Northern bobwhite quail. Experiment one determined endogenous levels of MT in the brains of 40 quail embryos during prenatal development using an enzyme-linked immunoassay kit. Experiment two examined the influence of increased MT during prenatal development on chick's individual recognition ability and stress response to a novel environment. Experiment one showed MT levels increased significantly throughout embryonic development. Experiment two showed significant differences in stress behavior for chicks with increased MT during prenatal development, however, no significant differences were found for social behavior. Overall, this study suggests MT may serve different functions depending on the stage of embryonic development and that increasing MT levels affects postnatal stress behavior, but not social behavior.

P5. Characterizing Immune Cells of Atlantic Bottlenose Dolphins

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Marine mammals are ideal sentinel species for human health due to exposure to the same oceans and consumption of the same foods. There have been many studies which demonstrate that wild Atlantic Bottlenose Dolphins are exposed to high levels of contaminants which lead to a suppressed immune system and are therefore more susceptible to opportunistic infections, many of which are zoonotic diseases. However, nearly no research has been done on determining defects in the immune cell population of dolphins, especially DCs that are essential for initiating an immune response. We hypothesize phenotypic and functional differences in the PBMC, including DC precursors, of wild dolphins as compared to managed dolphins. Specifically in this study, we have used terrestrial-specific antibodies and growth factors to characterize immune cells in PBMC and to generate monocyte-derived DCs. We have identified cross-reactive terrestrial antibodies that could detect immune cell subsets within PBMC, including B cells, T cells, NK cells, monocytes and APCs. Interestingly, using these antibodies we found significant changes in immune cell subsets within PBMC of wild and managed dolphins. Finally among the terrestrial DC growth factors tested we found rat GM-CSF and IL-4 generated DCs expressing higher levels of CD11c, CD14, CD40, CD80, CD86, MHC I and MHC II. Our findings allow us to further study defects in the immune cells, especially DCs, in response to environmental contaminants.

P6. Novel Polymer for Bioseparations of Complex DNA Mixtures using Capillary Electrophoresis

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Microbial communities are diverse arrays of organisms that have complex interactions, genes, and gene functions. There are different levels of resolution commonly used to study microbial communities. Currently, DNA sequencing/metagenomic analyses are the highest resolution used to characterize communities. However, not every analysis needs that depth of resolution, so often community profiling via amplicon length sequence heterogeneity is employed. However, the true diversity is underestimated because analyses are based on number of bases in the amplicon versus the sequence polymorphisms. Taxonomically unrelated organisms can produce the same length amplicon but have different nucleotide sequences. A critical need exists to develop a method that can rapidly analyze community profiles not only by length, but also based on inherent sequence polymorphisms without the need for metagenomic sequencing. The commercial polymer (POP-4) and the novel polymer F-108, were compared using an ABI 310 Genetic analyzer and capillary electrophoresis (CE) to assess the best matrix for separating and detecting the obscured sequence diversity within length-based amplicons in microbial populations. Four model organisms that display the same length amplicon for hypervariable domain V3 within the 16S rRNA gene, but have variable nucleotide content within the amplicon were amplified by PCR using 16S rRNA universal primers and separated by capillary electrophoresis. In POP-4, only one amplicon was produced for all four taxonomically unrelated organisms. With F-108, four amplicons, reprenting the four different taxa were seen. A complex mixture was then analyzed from a natural community—a cyanobacteria-dominated microbial mat from Hunter Hot Springs in Lakeview, Oregon. F-108 did not underestimate the true diversity of the microbial mat community. Metagenomic analyses confirmed that the number of peaks produced in the profile represented the number of species identified by NexGen sequencing. Therefore, F-108 gel matrix was able to depict the true amplicon diversity in the natural community.

P7. Breed designation for unknown equine case samples

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The many unsolved horse slaughter cases in the Miami-Dade and Broward counties in Florida is a testimony to the gravity of these illegal and inhumane crimes. In equine forensic there is a tremendous need to develop methods that will supplement individual identification with breed designation in the identification of unknown samples. Often, with confiscated horsemeat, any physical description of the horse is lost and only DNA can link the horsemeat to evidence and hopefully to a suspect. Therefore, in order to more strongly correlate the evidence to the slaughtered horse, it is crucial to also identify the breed of the horse as well as obtain individual equine profile. The current case study illustrates the use of population genetic statistical software STRUCTURE 2.3.1 for breed identification of equine samples of a slaughter case in Broward County, Florida. Prior to this cluster-based analysis, there was no substantial information regarding breed-specific allele frequencies to use for calculating the Random Match Probability (RMP). Simply assuming the victimized horse was one of the more common breeds found in South Florida, resulted in an RMP of 1.84×10^{9}. However, by performing admixture analyses using comprehensive breed allele frequencies, the presumptive breed of the horse was narrowed down to Standardbred, Lipizzaner, Arabian or Tennessee-Walker. Using the published allele frequencies for those breeds, resulted in an improved RMP (1.63×10^{25}, 1.27×10^{20}, 2.88×10^{23} and 8.77×10^{21}). We will present a case study as an example to recommend the use of admixture software to determine the breed of horses for forensic match calculations.

P8. Sab-mediated signaling initiates mitophagy *in vitro.*

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Mitophagy is the process by which mammalian cells remove dysfunctional mitochondria. Similar to other degenerative processes (i.e. proteosomal degradation of proteins), mitophagy involves a series of protein-to-protein interactions, which facilitate the destruction of mitochondria. Specifically, it has been previously demonstrated that PINK1 aggregation in the mitochondrial inner membrane space promotes a Parkin-induced engulfment of impaired mitochondria. Another hallmark of mitophagy is the coordination of autophagy-related proteins (Atg), necessary for lysosomal removal of damaged organelles. Various disorders, namely Parkinson's disease, have been linked to malfunctioning mitophagy. However, despite the relevance of this biochemical pathway to human disease, little is known about the signaling mechanism used by cells to target damaged mitochondria for removal. Preliminary results from our lab suggest the involvement of signaling on the mitochondrial scaffold protein, Sab, in the initiation of mitophagy. We hypothesize that signaling components organized on Sab mark mitochondria for destruction and induce mitophagy. We propose that Sab-mediated signaling is responsible for disruption of mitochondrial membrane potential, and consequentially, the induction of gene expression required for mitophagy. By elucidating the mechanism responsible for inducing mitophagy, our work will identify new drug targets to treat human disease.

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P9. Species specific post-transcriptional regulation of *Leishmania* aquaglyceroporin AQP1 via 3'UTR

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Antimonial compounds are the first line of treatment against leishmaniasis. We reported that *Leishmania* aquaglyceroporin 1 (AQP1) is responsible for important physiological functions such as volume regulation and osmotaxis. AQP1 also transports trivalent antimony [Sb(III)] and its expression levels positively correlated with antimony sensitivity. We observed that different *Leishmania* species inherently differ in their antimony sensitivity. We hypothesize that species specific AQP1 regulation is responsible for this species specific antimony sensitivity. Using QPCR we quantitated the AQP1 mRNA levels in 7 species of *Leishmania*. AQP1 mRNA levels showed a positive correlation with Sb(III) sensitivity and accumulation of the metalloid in different species. Chasing experiments with Actinomycine D revealed that half-life of AQP1 mRNA differs between the species: longer halve lives corroborated to higher basal level expression and vice versa. Next we explored the mechanism of species specific regulation of AQP1. In the absence of definitive promoter and transcriptional control *Leishmania* relies on post-transcriptional and/or post-translational control for gene regulation. In other systems 3'untranslated region (3'UTR) plays important roles in mRNA stability. We cloned 3'UTR of AQP1 mRNA from these seven species. Sequence analyses revealed that AQP1 mRNA has a long 3'UTR (~1.8 kb), however, primary sequences differ significantly between the species. To study the role of 3'UTR in the species specific regulation of AQP1, we cloned 3'UTR from each species downstream to the luciferase (Luc) open reading frame. We predicted that if the sequence of 3'UTR regulates the AQP1 mRNA stability, the same UTR will also regulate Luc mRNA stability and thereby modulating the activity of the luciferase enzyme. Indeed we report that levels of Luc activity was modulated by the species specific nature of the 3'UTR. Collectively we concluded that the sequence of 3'UTR of AQP1 mRNA was responsible for species specific regulation of AQP1 expression in *Leishmania*.

P10. Genetic background influences the NK recruitment and Th1 polarization in response to TLR agonists

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In response to vaccines, DCs (Dendritic cells) do not act in isolation but potentiate their efficiency by interacting with NK (Natural Killer) cells. The recognition of PAMPs (Pathogen-associated molecular Pattern) by TLRs (Toll-like receptors) triggers DC maturation which is essential for their migration and T-cell priming. Maturing DCs release IL-12 which regulates the function of NK cells and drives the Th1 cells differentiation. In turn, NK cells which are activated by IL-12 and PAMPs through their own TLRs, provide IFN-γ necessary for enhancing stable IL-12 production by DCs and maintaining Th1 cell polarization. Studies using Balb/c strain showed that TLR agonist R848 induces NK cell recruitment into lymph nodes and augments Th1 polarization through unknown mechanisms. However, it needs to be elucidated whether the genetic background will influence the NK cell recruitment and subsequent Th1 polarization in response to TLR agonists. In this study, we immunized Balb/c and B6 mice with OVA protein mixed with selected TLR agonist (TLR4, TLR7, TLR7/8, TLR9) or alhydrogel as a control. we found that immunization with OVA protein mixed with TLR agonist R848 among adjuvants tested, increased up to 3-fold recruitment of IFN-g producing $CD27^{+}CD11b^{+}$ NK cell expressing CXCR3 and CD62L in lymph nodes and spleen of Balb/c but to a much lesser extent in B6 mice. Interestingly, however, the increased in NK cell recruitment had limited impact on Th1 polarization in Balb/c as compared to Th1-prone B6 mice. These data suggest that for optimal Th1 polarization, adjuvant or adjuvant combination should be selected based on their ability to both recruit and maximize DC-NK bidirectional signaling.

P11. Heme-Pocket Characterization of Neuroglobin using a fluorescent probe

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Neuroglobin (Ngb) is a member of the hexacoordinate hemoglobin family and has been implicated in the protection of neuronal tissue under hypoxic and ischemic conditions. Additionally, Ngb plays an active role in cell survival by inhibition of the intrinsic pathwayof cell death by inhibiting the activation of pro-caspase 9 by interaction with cytochrome c. A prominent feature of human neuroglobin (hNgb) aside from hexacoordination of the heme iron, is the presence of a disulfide bond between Cys46 and Cys55 in human protein Here we attempt to characterize the effect of the internal disulfide bond on the conformation of the heme pocket in human and rat neuroglobin b using a fluorescent probe—zinc protoporphyrin IX (ZnPPIX). Both human and rat proteins are not natively fluorescent due to highly effective Trp emission quenching by the heme, iron protoporphyrin IX (FePPIX). ZnPPIX analogues of neuroglobin were prepared by extracting the native heme reconstituting the resulting apoprotein with the fluorescent ZnPPIX. Reconstituted protein was re-purified using size-exclusion chromatography. UV-Vis absorption spectroscopy was then used to verified a correct insertion of ZnPPIX into the heme pocket. Fluorescence properties of the reconstituted protein were characterized using a steady-state and time-resolved fluorescence. The reconstituted rat neuroglobin exhibits three fluorescence lifetimes (τ1: 0.151ns, τ2: 1.55ns, τ3: 6.15ns) whereas ZnPPIX in solution has only two lifetimes. This difference in number of lifetimes indicates structural heterogeneity within the heme pocket.

P12. Assessment of Gene Repositioning in Human Lymphocytes Due to Genotoxic Agents

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Recent discoveries have shown that genes occupy preferential positioning within the nucleus. Gene positioning has been associated with gene expression. Active genes tend to be more centrally localized, while inactive genes are more peripherally located. The specific aims of this project are assessing whether genes associated with epigenetic modifications and genomic imprinting are non-randomly organized within human lymphocytes, and if repositioning of genes occur as the result of genotoxic exposure to Hydrogen peroxide (H_2O_2) and UV. Human lymphocytes were obtained from six healthy human subjects and were cultured in the presence and absence of H_2O_2 and UV. Gene positioning for two genes located on chromosome 15 was assayed (UBE3A, involved in Angelman syndrome, and PML) using fluorescence in situ hybridization. Nuclear position was determined by assessing the radial position within the nucleus using software that divides the nucleus into five equal areas and measures the distribution of fluorescence within each area, providing also the median gene position. A total of one hundred cells per gene per treatment per human subject were analyzed. Non-random organization within the nucleus was determined by the chi-squared goodness-of-fit. Additionally, the localization of each gene was compared between the different conditions from the same individual. The results from this study show the median position of PML to be more interior than that of UBE3A (1.96 vs. 2.5 respectively). PML was non-randomly organized in all experimental conditions, while UBE3A demonstrated a random organization in three samples. When comparing cells exposed to genotoxic agents within individuals, two subjects demonstrated a significant difference in nuclear organization in UV exposed cells. In both cases the position of the tested genes moved to a more peripheral location than that of the control cells. Our preliminary findings provide the first evidence of nuclear organization of imprinted regions of the genome within human lymphocytes. Initial data suggest that altered organization may be observed after UV exposure in at least two subjects. This study will be expanded through the inclusion of additional genes including XIST and SNPRN and the potential effect of gene repositioning after inducing DNA damage with genotoxic agents will be explored.

P13. Fluorescent Random Amplified Microsatellites (F-RAMS) Analysis of Mushrooms as a Forensic Investigative Tool

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Under most forensic circumstances the standard method for proving illegal distribution of hallucinogenic mushrooms is chemical analysis of the indole alkaloid contents. However, this method does not provide any species and sub-species-specific information about the evidence that could aid forensic investigations. In this study, we tested the capability of a DNA based approach, termed Fluorescent Random Amplified Microsatellites (F-RAMS), to profile mushroom evidence in criminal cases to species and sub-species level. Thirty seven hallucinogenic and non-hallucinogenic mushrooms of the genera *Amanita* and *Psilocybe,* including 15 samples of the species *Amanita rubescens* were profiled using two fluorescently labelled degenerate primers, 5´DD $(CCA)_5$ and 5´DHB$(CGA)_5$, which target different microsatellite repeat regions. The generated amplicons were separated using a 310 Genetic Analyzer and analysed using the Genemapper® software version 4.0. Among the two primers, 5´ DHB $(CGA)_5$ provided more reliable data for identification purposes, by grouping samples of the same species and clustering closely related species together in a dendrogram based on amplicon similarities. A high degree of intra-specific variation between the 15 *A. rubescens* samples was shown with both primers and the amplicons generated for all *A. rubescens* samples were organized into three classes of amplicons (discriminant, private, and marker) based on their individualizing potential.

P14. Age-Associated B Cells Are Enriched for Idiotype T15+ Phosphorylcholine Specific Antibodies

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Old mice (~2 years of age) have reduced humoral immunity to bacteria including S*treptococcus pneumoniae.* In young adult BALB/c mice, antibodies specific for the dual bacterial antigen /self antigen phosphorylcholine and which bear the germ-line encoded T15 idiotype are required for immunity to *S. pneumoniae*. In old BALB/c mice, immunity to phosphorylcholine (PC) is characterized by less T15+ antibody. The B cell composition within the spleens of aged mice differs significantly from that of young mice. Old mice show a ~10-fold increase in B cells characterized by negligible expression of CD21/35 and CD23. These aged B cells are called Age-associated B Cells (ABC). Here we ask whether PC-reactive antibody is altered in ABC versus follicular (FO) B cells in old BALB/c mice. ABC from both young mice and aged mice responded to LPS in vitro by proliferation and antibody production. The IgM antibodies produced were enriched in PC reactivity compared to young adult FO B cells as measured by ELISpot assay. Moreover, in aged mice FO B cells also showed high anti-PC reactivity. ABC-derived anti-PC IgM from both young adult mice and aged mice was enriched for the T15 idiotype (80-90%) while FO B cell anti-PC IgM was ~40-50% T15+. We propose that 1) follicular B cells generate ABC via PC antigen stimulation with T15+ B cells being preferentially directed into the ABC compartment and 2) in old mice there is a sequestering of most T15+ anti-PC B cells in the immunologically less reactive ABC compartment. This in turn may compromise the T15+ anti-PC response required for elimination of *S. pneumoniae* in old mice.

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