2012 ABSTRACT BOOK

Summer Undergraduate Research Program

Graduate Programs
in the
Biomedical Sciences

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2012

Victoria H. Freedman, Ph.D.
Associate Dean for Graduate Studies
Director, Summer Undergraduate Research Program

2012 Summer Undergraduate Research Program

Student Name	Undergraduate School	Einstein Mentor
Hannah Aldeborgh	Vassar College	Dr. Wei-Li Liu
David Barris	Yeshiva University	Dr. Louis Hodgson
Rachel Blinick	Stern College for Women	Dr. Richard Gorlick
Seth Burack	University Of Miami	Dr. Bettina Fries
Omar Burris	SUNY at Oneonta	Dr. Ekaterina Dadachova
Tiffany Chan	University Of Florida	Dr. Paul Riska
Giles Chickering	Worcester Polytechnic Institute	Dr. Joshua Nosanchuk
Victoria Cipollone	Fordham College	Dr. Ana Maria Cuervo
Madeleine Coleman	Whitman College	Dr. Sunhee Lee
Leah Croll	Duke University	Dr. Julie Secombe
Alfonso DeLeon	Fairleigh Dickinson University	Dr. Myles Akabas
Chelsea Dieck	Bucknell University	Dr. Jonathan Lai
Russell Dulman	Duke University	Dr. Lloyd Fricker
Batya Edelman	Stern College for Women	Dr. Bettina Fries
Jordan Epps	University Of California-Los Angeles	Dr. Joan Berman
David Gaita	SUNY At Binghamton	Dr. Linda Jelicks
Caitlin Gilbert	Georgetown University	Dr. Jean Hebert
Sinead Gilmore	James Madison University	Dr. Myles Akabas
Mariusz Gorz	Johns Hopkins University	Dr. Bryen Jordan
Aaron Goshinska	Arizona State University	Dr. Nir Barzilai
Veronica Gray	College of William and Mary	Dr. William Jacobs
Michaela Greenbaum	Brandeis University	Dr. Hernando Sosa
Rebecca Greenstein	Carleton College	Dr. Johanna Daily
Bryanna Gulotta	Cornell University	Dr. Michael Lipton
Leah Gutstein	Yeshiva University	Dr. Sridhar Mani
Erica Hasten	Yeshiva University	Dr. Matthew Gamble
Christopher Hernandez	Fordham University	Dr. Bridget Shafit-Zagardo
Christyn Hicks	Fort Valley State University	Dr. Florence Marlow
Carly Hirschberg	Emory University	Dr. David Spray
Kellie Ifill	Manhattanville College	Dr. Thomas McDonald
Jesus Juarez	University Of California-Davis	Dr. Ertugrul Ozbudak
Mark Kaminetzky	Yeshiva University	Dr. Bernice Morrow
Daniel Lapidus	Cornell University	Dr. Pablo Castillo
Jaclyn Lee	Boston College	Dr. Harry Ostrer
Randi Mackler	University Of North Carolina-Chapel Hill	Dr. William Jacobs
Zachary Meinhart	Fordham University	Dr. Evripidis Gavathiotis
Tova Miller	Stern College for Women	Dr. Elyse Sussman
Eitan Novogrodsky	Yeshiva University	Dr. Vinayaka Prasad
Michael Plietz	University Of Virginia	Dr. Amy Fox
Jose Quiroz	University Of Arizona	Dr. Allan Wolkoff
Steven Reisman	Loyola University of Chicago	Dr. David Cowburn
Melanie Reschke	Macalester College	Dr. Chi-Wing Chow
Brian Sanders	Yeshiva University	Dr. Adam Kohn
Katelyn Scharf	University Of Michigan- Ann Arbor	Dr. Kami Kim
Brandon Schein	University Of Maryland- College Park	Dr. Rizwan Naeem
Naomi Schwartz	Yeshiva University	Dr.Cristina Montagna
Scott Shuldiner	Tufts University	Dr. Ian Willis
Joseph Soucy	University of Rochester	Dr. David Gritz
Adina Sperling	New York University	Dr. Xingxing Zang
Sannia Tauqeer	Iona College	Dr. Maria Alejandra Abadi
Bella Wolf	Yeshiva University	Dr. Ales Cvekl
Sara Wong	CUNY-Queens College	Dr. Nicholas Sibinga
Lucille Woodley	The University Of Alabama	Dr. Wei-Li Liu
Michael Zingman	Northwestern University	Dr. Louis Weiss

Real-time analysis of interactions between tumor suppressor p53 and target promoter DNA

Nikki Aldeborgh, Robert Coleman and Wei-Li Liu
Albert Einstein College of Medicine, Bronx NY

The tumor suppressor protein p53 is a transcription factor, deemed "guardian of the genome" due to its role in reacting to and preventing genome mutation. As a response to damaged DNA, p53 will induce multiple genes in a variety of pathways including cell growth arrest and apoptosis in order to maintain cellular integrity. It is in this process that p53 prevents tumor growth and proliferation, though it is not yet known how p53 discriminates amongst these genes to selectively activate transcription. We have developed a single-molecule assay to reveal how this is done.

Some target genes of p53, are only transcribed when cellular stress reaches critical levels. Meanwhile, others such as p53 regulatory feedback gene *hdm2* are sensitive to p53 concentrations and will be activated even under mild cellular stress. To determine how p53 distinguishes between these types of genes, we use a single-molecule microscopy assay for real-time detection of the interactions between p53 and the *hdm2* promoter. By fluorescently tagging these molecules, we are able to observe their binding frequency and duration.

We found that p53 has the ability to dynamically probe the promoter. Surprisingly, it binds to the *hdm2* mutant as many times as it does to a wild type promoter, however it stays bound to the wild type longer. In general, results suggest that the initial binding of p53 is nonspecific, but its dwell time on the promoter is dependent on the response elements.

Thanks to Albert Einstein SURP for the funding of this project

Spectral Modification to Photoactivated Rac1 Construct

David Barris[1], Yasmin Moshfegh[2], Louis Hogdson[2]

ABSTRACT:

The Rho family of GTPases is a class of regulator proteins that control many aspects of intracellular actin dynamics. These proteins play a key role in regulating cell proliferation, apoptosis and gene expression. Rac1, a member of the Rac subfamily of the Rho family of GTPases, is necessary for the formation of lamellipodia, a cytoskeletal protein actin projection on the mobile edge of the cell. Due to its role in regulating cell protrusions, Rac1 has been implicated the formation and function of invadopodia, a necessary step in the metastasis of caner.

Research has shown that specific spatiotemporal activation dynamics, control the signaling cascade of Rac1. In order to gain a greater understanding of this complex process, we use fluorescent biosensors based on fluorescence resonance energy transfer (FRET). Using these biosensors, we can determine the activation state of each respective protein. In order to selectively activate and deactivate these GTPases, Rac1 mutants were fused to the LOV (light oxygen voltage) domain from phototropin. Rac1 interactions were sterically blocked until irradiation led to the unwinding of the helix that connects Rac1 to LOV. This construct can be activated and deactivated using 458 or 473 nm of light. In order to use this construct, due to a spectral conflict with another red fluorescent protein, a spectral modification to the biosensor had to be performed. We used recombinant DNA technology to change the fluorescent protein from monomeric cherry (mCherry) to blue fluorescent protein (BFP).

ACKNOWLEDGEMENTS:

Yeshiva University, New York, NY 10033[1]
Gruss Lipper Biophotonics Center, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY[2]

Identification of an Osteosarcoma Progenitor Cell by Analysis of Differential Expression of Cell Surface Receptors of Human Mesenchymal Stem Cells and Mature Chondroblasts

Rachel Blinick[1], Sajida Piperdi[2], Amy Y. Park[2], Hua Yi Qu[2], and Richard Gorlick[2]

[1]Stern College for Women, Yeshiva University, New York, NY

[2]Department of Pediatrics Hematology/Oncology, Albert Einstein College of Medicine of Yeshiva University,The Children's Hospital at Montefiore, Bronx, NY

Osteosarcoma, the most common malignant bone tumor in children and young adults, is thought to originate from a cell at some point along the differentiation pathway of human mesenchymal stem cells (hMSC) to specific cells such as chondroblasts, osteoblasts, and adipoblasts. These three cell types are believed to differentiate from a single cell of origin along the differentiation pathway of hMSCs to specific cell types. Identifying changes in surface marker expression throughout the process of differentiation may help characterize the cell of origin as well as the intermediate stages in the differentiation pathway which is crucial to understanding the molecular pathogenesis of osteosarcoma. Looking at the differentiation pathways of hMSC to osteoblasts, chondroblasts and adipoblasts may help in identifying the cell of origin that unites all three pathways.

In this study, RNA was extracted from hMSC and mature chondroblasts, and gene expression was measured using microarray on the Affymetrix Gene 1.0 ST array. A list of differentially expressed genes was generated but only genes for cell surface proteins were analyzed for this experiment. hMSCs and chondroblasts were cultured and chondroblasts were characterized using an Alcian Blue Stain. The gene list derived from the microarray will be corroborated with flow cytometry to confirm the differential expression of those surface markers. hMSCs will then be differentiated into chondroblasts, which will be harvested every few days to check for changes in surface marker expression to determine the intermediate stages of the differentiation pathway.

Seth Burack and Elizabeth Diago

Bettina Fries Lab

Characterization of Klebsiella pneumonia By PFGE, MLST, And Molecular Serotyping

Klebsiella pneumoniae is becoming a problem in hospitals because of its increasing resistance to antimicrobial reagents. To alleviate the pressures for new antibiotics we are characterizing 28 strains acquired from Montefiore hospital with the goal of developing an antibody for immunotherapy. We determined the phylogeny of the strains by performing a pulsed field gel electrophoresis (PFGE) and a multi-locus sequence typing (MLST). We found 11 different PFGE types (PT) within the first 22 strains. We also determined the molecular serotypes of many of the strains by performing a multiplex PCR, targeting genes constituting the capsular gene cluster (CPS). Many of our strains are ST 258 which account for 70% of the *Klebsiella pneumonia* carbapenemase (KPC) producing *K. pneumonia* in the USA. The non-carbapenem resistant strains were found to belong to ST 37. ST 111 ST 502 and ST 23. Strains 17 and 18 have the same allelic profiles that have not been recorded in the K*lebsiella pneumonia* MLST database. We performed a PCR of the MagA gene, which is only found in serotype K1, and we found that most of the strains are of serotype K1. This is significant because in the future when the lab attempts to develop an antibody against the *Klebsiella* capsule they can focus on the polysaccharide present in the K1 serotype. This project was funded by Albert Einstein College of Medicine and is part of the Summer Undergraduate Research Program.

Radiation of Melanized cells: *C. neoformans* and B16, A2058 and 293T cell lines

Omar Burris, Ruth Bryan, Ekaterina Dadachova, and Thomas Jandl

Albert Einstein College of Medicine, Department of Nuclear Medicine

Abstract:

Melanin pigments are very common among various microorganisms in nature. It is useful in the protection of UV and solar radiation in these microorganisms. Melanized microorganisms are found in very high level background radiation environment like the nuclear reactor cooling pool and the destroyed reactor in Chernobyl. Now, the resistance of melanized fungi to ionizing radiation proposes a role of this pigment in radioprotection. We have hypothesized that one, melanin will protect the cells from high doses of radiation; two, radiation will enhance the growth of melanized cells. We studied the radioprotective properties of melanin by exposing the human pathogenic fungi *Cryptococcus neoformans* in its melanized and non-melanized forms to sublethal doses of radiation of up to 530 cGy. For further investigation on the effect of radiation on melanized cells, we have included three cell lines: B16, a mice melanoma cell line, A2058, a human melanoma cell line, and 293T, a human embryonic kidney cell line. These cell lines were radiated at higher doses: 0, 4,8,16 Gys. The properties of melanin were investigated by electron paramagnetic resonance (EPR) or electron spin resonance (ESR), spectrophotometer, gamma radiator and x-ray radiator. There was a killing of melanized cells at different doses of radiation. We have obtained data that indicated that the B16 and the A2058 cell lines are similar and are more resistant to radiation than 293T cell line.

Acknowledgements:

1. SURP: Summer Undergraduate Research Program
2. DSSURP: Diversity Student Summer Undergraduate Research Program
3. Dr. Gary J. Gerfen at Albert Einstein College of Medicine
4. Dr. Igor Shuryak at Columbia College of Physician and Surgeons

Genotypic relatedness among epidemic strains of NAP-1/027 *Clostridium difficile* in New York City Hospitals

Tiffany Chan, Karachi Egbuta, and Paul Riska, M.D.

Department of Medicine (Infectious Diseases), Montefiore Medical Center-Albert Einstein College of Medicine, Bronx, NY

Abstract

Clostridium difficile, a spore forming bacteria, has grown in prevalence as a leading hospital-acquired infection. In 2005, *C. difficile* cases were nearly triple the number in 1996. The epidemic strain, North American Pulsotype 1 (NAP-1), is both the most commonly found strain in North America and the most virulent. There are questions whether C. difficile spreads mainly through hospital transmission or through other means like community reservoirs. Multiple-locus Variable-Number Tandem Repeat Analysis (MLVA) is a more sensitive technique than previously used methods. MLVA examines the variable number tandem repeats (VNTR) at various locations to identify the relatedness between samples; the patterns are stable for single isolates after passaging for over 1 month. With this technique, 58 epidemic strains from 10 hospitals around New York City were examined for similarity among isolates. Examining 4 different loci, multiplex PCR and a subsequent multicolored capillary electrophoresis gave numerical fragment lengths for MLVA. These *C. difficile* samples did not cluster by hospital location and few clonal isolates were identified, even among strains considered to be the epidemic type. This suggests that other sources of *C. difficile* transmission are becoming more common than hospital transmission. Further analysis with larger sample sets of epidemic isolates is needed to confirm this observation.

Acknowledgements:

Special thanks to David Reynolds, Bola Omotosho, and the Gorlick Lab for providing research support. This work was funded by grants from the Einstein SURP (YI) and supported by the CDC-AHRQ ERASE-Cdiff project.

Analyzing *Sporothrix*: Investigating the Virulence of this Emerging Pathogenic Fungus

Giles Chickering, Dr. Dayvison Francis Saraiva Freitas, Dr. Joshua D. Nosanchuk

Departments of Medicine and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York

Sporothrix species are dimorphic fungi known to cause an often-chronic mycosis called sporotrichosis, which typically results in lymphatic nodules and lesions forming on the skin of infected individuals [2]. The necessity for subcutaneous infection has kept rates of transmission relatively low; however, there has been a noticeable increase in the number and severity of cases seen in Rio de Janeiro, Brazil over the past 10-15 years [1]. The strong association of transmission by scratches and bites received from cats has resulted in sporotrichosis being classified as a zoonotic epidemic in Rio de Janeiro [6].

The goal of this project was to study several aspects of virulence of five isolates of *Sporothrix* collected from one chronic patient using a variety of techniques in vitro. We sought to determine whether alterations in virulence contributed to the chronic nature of the disease. Factors such as reactions to oxidative stress, differing temperatures, and susceptibility to a simulated immune response were tested using techniques frequently applied to a wide range of pathogens.

The results of the experiments indicated high levels of variation in the different isolates of *Sporothrix* and will be compared to genotyping research that is soon to take place. Drawing associations between these results and differences in the genotypes of the pathogenic fungus will lead to a clearer understanding of *Sporothrix* and may allow us to better combat both individual cases of sporotrichosis and the epidemic as a whole in Rio de Janeiro.

This research was performed under the direction of Dr. Dayvison Francis Saraiva Freitas and Dr. Joshua D. Nosanchuk as part of the Summer Undergraduate Research Program at the Albert Einstein College of Medicine.

Cellular Response to Chaperone-Mediated Autophagy Blockage

Victoria R. Cipollone, Jaime L. Schneider, and Ana Maria Cuervo
Department of Molecular and Developmental Biology
Albert Einstein College of Medicine, Bronx, NY

Autophagy is a catabolic process that degrades intracellular components in lysosomes for later recycling and resynthesis. Autophagic activity decreases with age, contributing to an abnormal buildup of damaged or old cellular components. Macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) are the three main types of autophagy that co-exist in cells. Currently, there is no available information on the effect that reduced CMA may have *in vivo*. Determining the cellular response to CMA blockage in vivo should elucidate the impact of the decline in CMA activity. This insight should aid in the development of systems to postpone or lessen the severity of aging and its related diseases. In this study, we intended to determine the cellular response to the blockage of CMA in vivo using novel mouse models knocked out for an essential CMA component. Our goal was to identify if CMA blockage in liver leads to (1) changes in the lysosomal composition, (2) stress of the endoplasmic reticulum, (3) upregulation of the proteasome, or (4) upregulation of macroautophagy.

Our results show that (1) current mouse models used to study CMA inactivity are effective, (2) CMA inactivity alters cytosolic and organelle homeostasis, and that (3) various autophagy pathways are interrelated and compensate for one another *in vivo*.

Based on our findings we conclude that the transgenic mice with compromised CMA are good models for the future study of the contribution of CMA malfunctioning to aging and its related diseases.

Acknowledgments: I would like to express my deep appreciation to the members of the Cuervo lab, particularly to Jaime L. Schneider for her technical assistance, explanations, time, and patience. I would like to thank SURP 2012 for providing me with this invaluable research experience. This work was supported by NIH/NIA grant P01 AG031782-01 (AMC).

Inflammasome Signaling and IL-1 Production in Mouse Glial Cells

Madeleine Coleman, Leonid Tarassishin, Sunhee C. Lee

Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY

Interleukin-1 (IL-1) is a crucial cytokine involved in neuroinflammation and neurodegeneration. In the periphery, macrophages are the main producers of IL-1β, but the production of IL-1β by glial cells such as astrocytes has not been clearly elucidated. The goal of this study was to examine whether astrocytes can produce IL-1β and to determine the types of immunologic triggers that activate IL-1β synthesis and release. Primary mouse astrocytes and microglial cell line BV2 were stimulated with Toll-like receptor (TLR) ligands, lipopolysaccharide (LPS) and polyinosinic-polycytidylic acid (Poly IC) and the effects of ATP and bacterial toxin nigericin on IL-1β release were examined by ELISA and western blot. The results indicate that mouse astrocytes are capable of producing IL-1β at levels comparable to macrophages. Furthermore, ATP and nigericin, which have been shown to mediate cleavage of pro-IL-1β (35kDa) to active IL-1β (17kDa) through activation of macrophage inflammasome, are also highly effective in astrocytes. These results suggest that astrocytes, together with microglia and macrophages, may play a role in the CNS immune responses through production of bioactive IL-1. Ongoing studies are determining the types of inflammasomes in astrocytes and the molecular machinery that mediates LPS responses in astrocytes. Supported by the NIH grant MH55477, Einstein SURP, and the Whitman College summer internship grant.

Transcriptional regulation of stress resistance and genomic instability by Lid and Myc

Leah Croll, Christina Greer, Xingyin Liu, Julie Secombe
Department of Genetics, Albert Einstein College of Medicine
Bronx, NY

Myc is a transcription factor that regulates cell growth and development. The histone demethylase Little Imaginal Discs (Lid) is a rate-limiting cofactor for Myc-induced growth. Our lab is interested in the function of the Lid-Myc complex in addition to their independent functions. My research projects have focused on the functions of Myc and Lid that are independent of each other: Lid-mediated induction of the stress activated gene Heat shock protein 22 (Hsp22) and characterization of a Lid RNAi transgenic fly strain, and Myc-induced genomic instability. Hsp22 mediates cellular response to oxidative stress and is activated by Lid. To understand how Lid regulates Hsp22 we made use of a Hsp22 reporter transgene. Lid overexpression activated the Hsp22 reporter in larval wing imaginal discs, while expression of Lid mutants that affect Lid's ability to bind specific chromatin modifications did not. This suggests that these domains facilitate Lid's regulation of Hsp22. I also characterized two inducible RNAi transgenes to determine how efficiently they reduce Lid mRNA levels, as these will be useful tools for further characterization of Lid. Using real-time PCR, I determined that both RNAi lines are useful genetic tools in females. My final project involved Myc, a known oncogene that plays a role in genomic instability. To determine whether Myc requires its ability to bind DNA to induce double-stranded breaks and subsequent genomic instability we generated two mutations that prevent Myc from binding DNA. This work was funded by grants from the Einstein SURP (LC) and CFAR #A1051519.

Characterization of Histidine Residues Responsible for pH Sensitivity in Purine Uptake of Plasmodium vivax Equilibrative Nucleoside Transporter 4

Alfonso De Leon, I.J. Frame, Avish Arora, and Myles Akabas

Department of Physiology & Biophysics, Neuroscience, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

Abstract:

Organisms of the genus *Plasmodium* are known to be causative of malaria. They obtain purines from the host using Equilibrative Nucleoside Transporters (ENTs). *P. vivax* possesses four different ENTs referred to as PvENT1-4. Previous research demonstrated that PvENT4 purine uptake was sensitive to pH, similar to the pK_a of histidine. This lead to the hypothesis that one or more of the four histidine residues (H4, H30, H219, H302) may be accessible to the environment and might be responsible for pH sensitivity. The main focus of these studies was to determine which histidine residues are responsible for the pH sensitivity of PvENT4 by mutating the four native histidine residues and examining expression of the protein in mRNA injected *Xenopus laevis* oocytes. Oocytes were injected with synthetic mRNA coding for PvENT4. After 3-4 days of expression, uptake of radioactive ^{14}[C]-2'deoxyadenosine at pH 5.5 and pH 7.4 over a period of 1 hour was measured. Triple mutants line 1 (H4S, H219S, H302S) and line 6 (H4N, H219S, H302S), along with individual mutants H30A, H4S, and H219S showed expression of the transporter and uptake of the labeled deoxyribonucleoside relative to control. A histidine-free knock out will be created using the triple mutants plus the H30A mutation, then each histidine will be added back individually to test which one or combination of more than one is responsible for the pH sensitivity.

Acknowledgments

Special thanks to the Summer Undergraduate Research Program and the Diversity Student Summer Research Opportunity Program for funding the project. Also, thanks to Dr. Myles Akabas for allowing me to be part of his lab and Assistant Dean of Diversity Enhancement Nilda Soto for her help and guidance. Most importantly, I would like to thank those who struggled everyday with me I.J. Frame, Avish Arora, Roman Deniskin, Sinead Gilmore and Dr. Moez Bali.

Identification of a Cyclic Peptide that Targets Maf1 by Phage Display

Chelsea L. Dieck, Chelsea D. Higgins, and Jonathon R. Lai
Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, USA 10461

Maf1 is a phosphoprotein known to regulate RNA polymerase III transcription. Studies have shown that Maf1 knockout mice have a lifespan similar to wild type mice, but do not develop cancer and are resistant to obesity. In an effort to understand the mechanism behind the physiological effects of Maf1, phage display technology was used to identify a peptide sequence that interacts with Maf1. One Maf1 binding peptide, R4H8, was obtained after four rounds of phage selection and monoclonal ELISAs of 600 isolated colonies. A phage ELISA was then used to confirm R4H8's Maf1 binding ability. The R4H8 peptide was synthesized and its identity confirmed with MALDI MS. Further characterization and experimentation is required, but we hope this peptide will be valuable as a research tool to help better understand the relationship between Maf1 and obesity. In addition, we plan to test this peptide for potential Maf1 inhibition to help determine whether Maf1 is a viable druggable target.

Title: Effects of Proteasome Inhibitors Clasto-Lactacystin β-Lactone and MG262 on the Peptidome of Human Cell Lines

Authors: Russell S. Dulman, Sayani Dasgupta, Lloyd D. Fricker
Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

Abstract: Proteasome inhibitors can be effective anti-cancer agents, as indicated by the drug bortezomib. Further research into other proteasome inhibitors is needed to elucidate what is unique about bortezomib and develop other anti-cancer drugs. Previously, bortezomib was shown to have a markedly different effect on the peptidome than an established proteasome inhibitor epoxomicin. In this study, we treated HEK293T and SH-SY5Y cells with the proteasome inhibitors clasto-lactacystin β-lactone (hereafter lactacystin) and MG262, extracted and labeled the peptides with isotopic labels, and conducted mass spectrometry for peptidome analysis. This peptidomics approach can detect hundreds of peptides without specific targets and allows for comparisons amongst treatment and controls between other peptidomics studies. A trimethylammonium-butyrate labeling scheme was utilized to directly compare treated and control groups within the same sample. Upon comparing results to past similar studies, the peptidome of MG262 was found to resemble that of bortezomib while lactacystin's peptidome resembled that of epoxomicin. The peptide decreases seen in lactacystin and epoxomicin align with our predictions of a proteasome inhibitor's peptidomic effect while the peptide increases seen in bortezomib and MG262 require further explanation and exploration. We note the presence of a boronate within the proteasome inhibitor structure and inhibitor reversibility as features shared by bortezomib and MG262, but not with lactacystin or epoxomicin, that could explain the large increases observed in the peptides. Future studies will look into other reversible boronate proteasome inhibitors to further explore this hypothesis as well as other possible downstream effects of bortezomib and MG262.

Acknowledgements: This work was funded by grants from the Einstein SURP (YI) and the NIH (RO1-DA-004494).

Investigation on the Replicative Life Span of Early and Late Clinical Isolates of C. neoformans

Batya Edelman[1], Tejas Bouklas[2], Bettina C. Fries[2,3]
[1]Stern College for Women of Yeshiva University, [2]Department of Microbiology and Immunology, [3]Department of Medicine - Division of Infectious Diseases, Albert Einstein College of Medicine of Yeshiva University, New York, NY

C. neoformans (*Cn*) is a fungal pathogen that causes fatal chronic meningoencephalitis in immunocompromised individuals. Like other fungi, *Cn* undergoes asymmetric cell divisions in a process of replicative aging, and the sum of these divisions is its replicative life span (RLS). Consequently, aging "mother" cells are distinguishable from "daughter" cells by size. We have shown that aging *Cn* cells manifest phenotypes that could promote their selection *in vivo*. Our goal is to investigate whether the host can select for cells with a variable RLS. We have established for the first time the RLS of *Cn* by micro-dissection of sequential isolates. Median RLS of some sequential isolates was similar (9 v. 10.5 generations in isolate 8, 29 v. 36 generations in isolate 9), but was significantly different in other isolates (isolates 7 and 12). In isolate 8, glucose starvation resulted in a higher RLS (39 v. 9; 18 v. 10.5 generations). Strains divided every 2 hours, but slowed closer to their death. Cell size varied among the isolates, but increased until death. Growth curves of the isolates were performed in low glucose, at a concentration relevant to the host spinal fluid; however, growth was more rapid in high glucose. In summary, important information about strain variability and the effect of glucose starvation on RLS was successfully determined in sequential isolates. Since *Cn* grows in the host under selection pressures that include glucose starvation, our results indicate that the RLS of *Cn* may be relevant to the outcome of cryptococcal infection.

Acknowledgment: R21 AI087564, R01 A059681, Roth Scholars Program, and Summer Undergraduate Research Program

The Effect of Buprenorphine and CCL2 on Human Brain Microvascular Endothelial Cells: A model of therapy for HIV infected drug abusers

Jordan K. Epps,* Lillie Lopez,* Jacqueline Coley,* Joan W. Berman*,¥
Department of *Pathology and ¥Microbiology and Immunology, The Albert Einstein College of Medicine, Bronx, New York

Drug abuse mediates a significant portion of new HIV infections every year in the United States. Buprenorphine is a therapeutic treatment for opiate drug abusers. Its effect on the permeability of the blood brain barrier (BBB) to leukocytes is undetermined. Chronic inflammation of the CNS in people infected with HIV is one of the major causes of HIV associated neurocognitive disorders (HAND). Despite successful antiretroviral therapy, more than 50% of HIV infected people exhibits HAND. CCL2, a chemoattractant for monocytes, a type of blood leukocyte, is highly expressed in the CNS of people with HAND. This chemokine mediates, in part, the inflammation that causes HAND. We hypothesize that buprenorphine will alter the permeability of the BBB to leukocytes by altering junctional and signaling proteins of brain microvascular endothelial cells (BMVEC). Human BMVECs were treated with CCL2, to model the neuroinflammation of HAND, and buprenorphine for 15, 30, and 45 minutes, 4, and 24 hours. Protein levels of JAM-A, Erk1/Erk2, Occludin, and PECAM-1 and phosphorylation of JAM-A and Erk1/Erk2 were measured using western blotting. Quantitative real-time PCR was performed for mRNA analysis of JAM-A and PECAM-1. We show that phosphorylation of JAM-A and Erk1/Erk2 decreases and increases, respectively, in response to treatment with buprenorphine and CCL2. Total JAM-A decreases with CCL2 + buprenorphine for 24 hours. These preliminary data suggest that buprenorphine + CCL2 renders the BBB less permeable to monocytes than CCL2 alone and may help to limit the neuroinflammation caused by HIV infection.

Distribution of Selected SPECT Tracers in Conscious Goldfish

David Gaita, Wade Koba, Linda Jelicks
MRRC, Albert Einstein College of Medicine, Bronx NY

Interest in the use of goldfish in biomedical research has been growing recently, particularly in the field of neurophysiology. Although the goldfish blood-brain barrier is poorly understood, evidence suggests that it is much less selective in its permeability than that of mammals. Thus, the possibility arises for the use of brain radiotracers in goldfish that would not normally cross the intact blood-brain barrier in other species. Tc^{99m} pertechnetate, and Tc^{99m} diethylenetriame pentaacetic acid (DTPA), are radiotracers that are or have been used to image the brain of humans with compromised blood-brain barriers. Our aim was to elucidate the distribution of these radiotracers as well as that of Tc^{99m} macroaggregated albumin (MAA), a lung perfusion agent, in conscious goldfish by performing three SPECT/CT acquisitions for each tracer using using a Siemens Inveon scanner and a fish immobilizer aquarium. Pertechnetate and DTPA did not distribute selectively enough for the images to be of any statistical significance. The MAA distributed selectively to the gills, the suprabranchial chamber, which is involved in hearing enhancement, and to a lesser extent to the brain. Despite the vascular occlusion and being restrained for 30 to 80 minutes, the fish survived. There are many other radiotracers that have yet to be tested on goldfish.

Upregulation of Cerebral pERK Expression in NKCC1-Deficient Mice

Caitlin Gilbert[1,2], Michelle Antoine[2], and Jean Hébert [2,3]

[1] Graduate Programs in Biomedical Sciences Summer Undergraduate Research Program, [2]Dominick P. Purpura Department of Neuroscience, and [3]Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461

The formation and maintenance of functional neural circuits require a balance between excitatory and inhibitory signaling. A disruption in this balance can result in psychiatric disorders, like schizophrenia. Recent research has shown that single-nucleotide polymorphisms (SNPs) in the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) affect risk for schizophrenia. Expression of NKCC1 typically allows for GABA-induced innervation of neurons, but this chloride transport is altered in schizophrenia, thereby affecting normal neurotransmission. Mice deficient in NKCC1 exhibit a distinct anxious-depressive phenotype, which includes impaired sensorimotor gating, a feature common in schizophrenia. We have also seen similar depressive and anxious behaviors.

To investigate the molecular basis for these behavioral phenomena, we used a Cre/loxP system to generate the following tissue-specific NKCC1 knockout mouse models:

1. Emx-Cre: Hippocampus/Cortex KO
2. Nestin-Cre: CNS KO
3. FoxG1-Cre: Striatum/Cortex KO

Out of various markers associated with proper glutamatergic signaling, including DARPP and GluR1/R2, only phosphorylated ERK (pERK) was found to exhibit a difference in expression between control and mutant mice, across multiple models. Further immunohistochemical analyses suggest that this difference is a mutant-specific upregulation of pERK, localized in the stria terminalis, superior colliculli, and locus coeruleus. Functionally, these regions are associated with previously observed anxious-depressive and hallmark schizophrenic behaviors.

Acknowledgments:
The Hébert Lab (Michelle Antoine, Frank Diaz, Grigoriy Gutin, Jean Hébert, Wenfei Kang, and Nicholas McKeehan)
Albert Einstein College of Medicine Summer Undergraduate Research Program
Tourette Syndrome Association Research Award

GABA Induces a Conformational Change of the $GABA_A$ Receptor Resulting in Movement at the αM3-βM1 Interface

Sinead L. Gilmore, Moez Bali and Myles H. Akabas
Department of Physiology and Biophysics, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

The $GABA_A$ receptor is the target of general anesthetics and many other drugs. When agonist binds to the receptor in its quiescent, closed state, it induces opening of the channel gate followed by its desensitization. Little is known about the structural conformation of the $GABA_A$ receptor in these three potential states. In this study, we investigated movement occurring at the membrane level of the αM3-βM1 interface in the $\alpha_1\beta_2\gamma_2$ receptor subtype. We measured the reaction rates of the cysteine specific reagent p-chloromercuribenzenesulfonate (pCMBS) with cysteine substituted mutants in the presence and absence of GABA. Reaction progression was monitored by periodically pulsing with submaximal [GABA]. Resulting currents were plotted as a function of cumulative exposure to pCMBS and fitted with a monoexponential curve to yield the rate constants. Differences in reaction rates in the absence and presence of GABA imply GABA-induced conformational movement. We demonstrate that mutant β_2G219C shows no detectible movement between the closed and activated states. The results for β_2Q224C suggest that the two cysteines present in the receptor react at two different rates, potentially due to the influence of the positive charge α_1R274 in the vicinity. Future experiments measuring the rates of reaction with a mutated R274Q will be done. These results differ from the rates measured at the aligned position in α_1, suggesting a structural asymmetry in the subunit interfaces.
I thank Moez Bali, Myles Akabas, and members of the Akabas lab for their guidance with this project. I acknowledge Einstein SURP for funding this work.

Characterizing a Synaptic Role for AIDA-1 in the Trafficking of AMPA Receptors

Mario Gorz, Jaafar Tindi, Bryen Jordan
Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY USA

Abstract

The amyloid precursor protein (APP) intracellular domain associated-1 (AIDA-1) protein was previously identified in a mass spectrometric screen of rat postsynaptic densities.[1] It has been shown that AIDA-1 contains a PDZ binding motif which is required for direct interaction with the synaptic protein PSD95 and is in a complex with NMDA-type glutamate receptors.[2] Because AMPA- and NMDA-type receptors mediate most of the excitatory neurotransmission in the brain and play crucial roles in synaptic plasticity, we speculated whether AIDA-1, given its localization, may be involved in regulating the expression of these receptors. We found that knockdown of AIDA-1 by lentiviral transduction of AIDA-1 targeting shRNAs led to a decrease in the dendritic expression of the GluA1 subunit of AMPA receptors (AMPARs) by imaging. Additionally, electrophysiological recording of miniature excitatory postsynaptic currents (mEPSCs) from these neurons showed a decrease in amplitude but not frequency, a finding consistent with the imaging results. Surface biotinylation showed no change in total surface GluA1 abundance, indicating that the observed changes in GluA1 expression are limited to the synapse. It has been shown that AIDA-1 binding to ubiquitinated EphA8 receptors regulates EphA8 expression by preventing their degradation.[3] We ask whether a similar mechanism plays a role in AMPAR expression at synapses and may therefore account for the above findings.

References:

1.Jordan, et al. (2007). Activity-dependent AIDA-1 nuclear signaling regulates nucleolar numbers and protein synthesis in neurons. Nature Neuroscience, 10(4): 427—35.

2.Jacob et al. (2010). Organization of amyloid-beta protein precursor intracellular domain-associated protein-1 in the rat brain. Journal of Computational Neuroscience, 518(16): 3221—26.

3.Kim, et al. (2010). The SAM domains of Anks family proteins are critically involved in modulating the degradation of EphA receptors. Molecular and Cellular Biology, 30(7): 1582—92.

Effect of central IGF-1 on peripheral glucose metabolism requires intact IGF-1 receptor signaling

Aaron J. Goshinska[1,3], Ardijana Novaj[1,3], John Lofrese[1,3], Nir Barzilai[1,2,3], and Derek M. Huffman[1,3]

[1]Departments of Medicine, [2]Genetics, and [3]Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461

IGF-1 can act centrally to improve insulin sensitivity, but how these effects are mediated is unknown. In order to gain greater mechanistic insights, we determined the role of IGF-1 receptors (IGF-1Rs) in the brain on these effects, and expression of specific targets in the mediobasal hypothalamus (MBH) and liver. We performed 6hr hyperinsulinemic-euglycemic clamp studies in 4 month old FBN male rats to assess insulin sensitivity. During the clamp, rats received simultaneous intracerebroventricular (ICV) infusion of either vehicle, IGF-1 and/or the IGF-1R antagonist, JB-1. Animals were then anesthetized and the MBH and liver were rapidly collected and frozen in liquid nitrogen. Gene expression was performed in liver for glucose-6-phosphatase (Glu-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) and in MBH for pro-opiomelanocortin (POMC), agouti-related peptide (AgRP), and neuropeptide Y (NPY). In response to ICV IGF-1, there was an increase in glucose infusion rate and glucose disposal. Furthermore, central IGF-1 suppressed hepatic glucose production ($P<0.05$) and reduced Glu-6-Pase expression in liver ($P<0.05$). However, these effects on metabolism and expression were completely blocked by central JB-1. In the MBH, there was no significant effect of IGF-1 on neuropeptide expression, though a slight tendency for reduced AgRP and NPY was observed. In summary, these data show that the central effects of IGF-1 on peripheral metabolism require intact IGF-1R signaling. While these preliminary data do not support the hypothesis that NPY, AgRP, or POMC are involved in these effects, more sophisticated studies are still needed to definitely demonstrate the potential role of these neuronal populations.

Characterization of TM4-resistant *Mycobacterium tuberculosis* mutants

Veronica Gray*, Randi Mackler*, Kevin Mu, Oren Mayer

Mentor: William R. Jacobs, Jr., Ph. D
Department(s) of Microbiology & Immunology, Genetics
Albert Einstein College of Medicine of Yeshiva University
Bronx, New York

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis,* kills millions of people worldwide each year. Since the discovery of antibiotics, multidrug and extensively drug-resistant TB have made treatment more problematic. *Mycobacterium smegmatis*, a closely-related non-pathogenic bacterium, is studied as a model for *M. tuberculosis* and other mycobacterial species.

The goal of my project was to create and characterize TM4-resistant *Mycobacterium smegmatis* mutants. Using the mycobacteriophage phAE180 containing a transposon, I generated and pooled a transposon library of 2,500 *M. smegmatis* mutants and exposed the cells to the mycobacteriophage TM4. I isolated 40 potential TM4-resistant colonies and tested to confirm TM4-resistance in 30. Future directions include using molecular genetics to map the location of the transposon to specific genes correlating to phage resistance. This will include DNA harvesting, digestion, & ligation; electroporation into *E. coli*; miniprep, DNA sequencing, and analysis. Additional screening of the transposon library will also test for cross-resistance in other phages that infect *M. smegmatis*.

I would like to give a special thanks to Torin Weisbrod, the Jacobs' Lab, and the 2012 Summer Phage Phinders. This work was funded by grants from the Exceptional Research Opportunities Program (EXROP) of the Howard Hughes Medical Institute and the Summer Undergraduate Research Program (SURP) of Albert Einstein College of Medicine.

A Novel Microtubule Binding Site for Kinesin-13 is Important for Spindle Structure

Michaela Greenbaum , Ana Asenjo, Dong Zhang, Hernando Sosa
Albert Einstein College of Medicine, Department of Physiology and Biophysics

Kinesin-13 is different from other members of the kinesin superfamily in that it functions to depolymerize microtubules (MT), undergoing one dimensional diffusion along them. Previous studies have used electron microscopy (EM) to show that constructs containing the kinesin-13 motor domain (MD) form rings and spirals around MTs *in vitro*. This ring formation has been linked to the existence of three positively charged lysines conserved among the kinesin-13s and located on the opposite side of the conventional kinesin MT binding sites. We therefore wanted to investigate how these positively charged residues contribute to the cellular function of kinesin-13. The three lysines of the MD of KLP10A, a kinesin-13 protein from the Drosophila melanogaster genome, were mutated to alanines and EM successfully showed that while the WT formed rings around MTs, the mutant formed rings only off to the sides. In order to study these proteins *in vivo*, RNAi and rescue of KLP10A MD in drosophila cells was performed using either a wild type or mutant rescue. Live cell imaging of the WT and M3K mutant KLP10A rescued cells showed that while the WT was similar to the pre-RNAi state, the M3K rescue was unable to restore proper spindle and kinetochore alignment. Finally, a bundling assay using either WT or mutant protein was used to determine whether MT bundling could account for the differences seen *in vivo*. In fact, MTs incubated with the WT kinesin-13 MD were found to bundle more than do those in the M3K mutant condition, suggesting that MT bundling mediated by these charged residues is important for spindle function. Future experiments will continue to investigate the cellular function of M3K and its influence on MT bundling.

Defining changes in *Plasmodium falciparum* virulence under oxidative stress

Rebecca L. Greenstein[1], Kris Subramaniam[1], Elizabeth Lawrence[2], Laura Kirkman[2], Vasiliki Pappa[1], Catherine Manix Feintuch[1], Johanna P. Daily[1]

[1] *Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY*
[2]*Department of Microbiology and Immunology, Weill Cornell Medical College, New York, NY*

Infection with *Plasmodium falciparum* is responsible for the most severe cases of malaria, killing approximately 600,000 people yearly [1]. Malaria-infected patients with high levels of inflammation have more severe disease outcomes than those with low inflammation [2]. *Var* genes are upregulated in patients with high inflammation [3], and are involved in virulence and sequestration [4]. Data in model organisms *Arabidopsis* and *S. cerevisiae* show that reactive oxygen species, an important component of inflammation, enhances genetic recombination, resulting in antigenic diversification [5, 6]. We thus examined the effect of ROS on *var* gene recombination and parasite growth. We defined lethal and sub-lethal concentrations of xanthine oxidase (XO), a reactive oxygen inducing agent. XO was added in varying concentrations to *in vitro* cultures. Parasites treated with high concentrations of XO (20 mU/mL) demonstrated extracellularity, sexual stages, and death. Using a sub-lethal dose of XO, 0.25 mU/mL, we identified an increase in parasite division and parasite growth. This is consistent with previous transcriptome work showing that parasites exposed to oxidative stress upregulate DNA replication and repair gene sets. Investigations as to whether the *var* genes demonstrate enhanced recombination under reactive oxygen are ongoing and will be presented.

Literature Cited

1. Huang H, Mackeen MM, Cook M, *et al.* Proteomic identification of host and parasite biomarkers in saliva from patients with uncomplicated Plasmodium falciparum malaria. Malar J 2012; 11(1):178.
2. Mohammed AO, Elghazali G, Mohammed HB, Elbashir MI, Xu S, Berzins K, Venge P. Human neutrophil lipocalin: a specific marker for neutrophil activation in severe Plasmodium falciparum malaria. Acta Trop 2003; 87(2):279-85.
3. Zhang Q, Zhang Y, Huang Y, *et al.* From in vivo to in vitro: dynamic analysis of Plasmodium falciparum var gene expression patterns of patient isolates during adaptation to culture. PLoS One 2011; 6(6):e20591.
4. Chen DS, Barry AE, Leliwa-Sytek A, *et al.* A molecular epidemiological study of var gene diversity to characterize the reservoir of Plasmodium falciparum in humans in Africa. PLoS One 2011; 6(2):e16629.
5. Brennan RJ, Schiestl RH. Free radicals generated in yeast by the Salmonella test-negative carcinogens benzene, urethane, thiourea and auramine O. Mutat Res 1998; 403(1-2):65-73.
6. Kovalchuk I, Filkowski J, Smith K, Kovalchuk O. Reactive oxygen species stimulate homologous recombination in plants. Plant Cell Environ 2003; 26(9):1531-9.

Differential Psychological Outcomes after Mild Traumatic Brain Injury:

The mTBI Personality

Bryanna Gulotta[1], Sara Rosenbaum[1], Tova Gardin[1,2], Jeremy Smith[1,2], Namhee Kim[1,2], Michael Lipton[1,2,3,4]

[2]Departments of Radiology, [3]Psychiatry and Behavioral Sciences, and the [4]Dominick P. Purpura Department of Neuroscience
[1]Gruss Magnetic Resonance Research Center, Albert Einstein College of Medicine, Bronx, NY, United States

Mood dis-regulation and personality changes after mild traumatic brain injury (mTBI) are serious concerns for patients and their families. Depression, anxiety, and other psycho-emotional problems that may result adversely impact productivity, cognition, social interaction, and may strain caregivers. While mTBI is associated with psycho-emotional disturbances, and damage to white matter structures implicated in mood disturbances have been identified in mTBI patients, specific associations between imaging abnormalities and psycho-emotional outcomes are less clear.

Traumatic axonal injury (TAI) results from shearing forces subsequent to head injury, and is revealed by Diffusion Tensor Imaging (DTI). Typically, DTI renders TAI as low fractional anisotropy (FA) and high mean diffusivity (MD). High FA on DTI has recently been reported, but its pathologic correlate remains unclear.

This study aimed to identify associations between DTI abnormalities and psycho-emotional function after mTBI, and to characterize the nature of the significant relationships. 40 mTBI patients were compared to 40 controls to identify FA and MD white matter abnormalities. Spearman correlations were performed to assess the association of abnormal FA and MD values and a battery of psycho-emotional outcome assessments. Associations were significant for both whole brain summary measures and localized measures at specific brain regions. Low FA and high MD, consistent with TAI, were generally associated with poorer psycho-emotional function. High FA was generally associated with better psycho-emotional function. This latter finding may indicate a neuroplastic response to mTBI, which supports better outcomes.

Future research can further refine the links between TAI location, compensatory responses, and psycho-emotional performance.

Acknowledgements: Thank you to SURP, Dr. Michael Lipton and the wonderful Lipton Lab, and the MRRC for granting me this wonderful summer research opportunity.

Optimizing an Experimental Design to Study PXR Interactions with mRNA using HITS-CLIP

Leah Gutstein, Madhukumar Venkatesh, Sridhar Mani,

Department of Genetics, Albert Einstein College of Medicine, New York, NY

The Nuclear Pregnane X Receptor, PXR, is an essential component of the body's ability to eliminate toxic xenobiotics and endogenous metabolites. Its canonical mode of action occurs through binding DNA at discrete nuclear receptor repeats, thus promoting gene induction/repression. More recently, our laboratory has uncovered a possible new association of this receptor with RNA. Preliminary evidence suggests that PXR binds the 3' UTR of TLR4 mRNA in a region that is AU-rich and likely interactions with other RNA stabilizing protein (e.g., HuR). Since this was unexpected for PXR, and the possible role of mRNA binding in xenobiotic metabolism, cancer, drug resistance and inflammation is not known, we designed a more extensive (high- throughput) project to decipher the extent of binding to RNA by PXR. The first step entailed transfecting cells with a plasmid construct that contains both Green Fluorescence Protein (GFP) and PXR and then sorting for only the cells that express GFP. The next step employed the novel method for studying RNA Binding Proteins, High Throughput Sequencing Crosslinked Immunoprecipitation (HITS-CLIP). Thus far, the immunoprecipitation protocol for isolating PXR from live cells has been optimized. GFP-Trap®, followed by Western Blotting with anti-PXR and GFP primary antibodies, has yielded clear, distinct bands at molecular weights corresponding to the PXR and GFP proteins. In the near future, cells transfected with PXR will be treated with xenobiotics and studied with HITS-CLIP, in an attempt to identify any PXR-RNA associations, and its role in pathophysiology.

Acknowledgements:

We thank the Damon Runyon Cancer Research Foundation, the National Institute of Health (NIH), and the Summer Undergraduate Research Program of Albert Einstein College of Medicine for supporting this research.

We thank the Flow Cytometry Core Facility of Albert Einstein College of Medicine for their assistance with this project

Exploring the role of Huntingtin in regulation of macroH2A1

Erica Hasten, Leonid Novikov, Matthew J. Gamble

Department of Molecular Pharmacology, Albert Einstein College of Medicine Bronx, New York

ABSTRACT

Huntington's disease (HD) is an autosomal dominant genetic disorder that involves an expansion of a polyglutamine (polyQ) track near the N terminus of huntingtin protein (HTT), which leads to the aggregation of this protein in the cells. While HTT is ubiquitously expressed, HD manifests clinically as a progressive CNS neurodegenerative disorder. A recent study has linked increased expression of the histone variant macroH2A1 to HD progression. MacroH2A1 incorporation into nucleosomes has been linked to both positive and negative regulation of transcription and, indeed, several links between HD and transcriptional disregulation have been suggested. Here, we explored the possible mechanisms by which mutant HTT might regulate macroH2A1 expression. First we determined the level of macroH2A1 in mouse neuronal precursor cells with wild type or mutant humanized HTT exon 1. To explore the possible role of wild type HTT in macroH2A1 expression we depleted HTT from IMR90 human fibroblast cell lines using lentiviral shRNA constructs. To determine the role of the polyQ track in triggering macroH2A1 overexpression, we expressed N-terminal HTT-GFP fusion proteins harboring different numbers of glutamines in A549 and HEK cell lines. We used immunofluorescence to monitor macroH2A1 levels in the transfected cells. Together, our findings suggest that HTT polyQ track length may indeed play a role in regulating the expression of macroH2A1. While our results need to be confirmed, they leave open the possibility that macroH2A1 may play a role in the molecular pathology of HD.

Acknowledgments

SURP 2011 at Albert Einstein College of Medicine and Roth Scholars Program

Lab of Dr. Eric Snapp for the neuronal precursor cell lines and for the HTT-GFP constructs

Thank you to Leo Novikov and Matthew Gamble for their patience and help with this project.

Christopher Hernandez
SURP 2012

AKT3 contributes to the protection of mice against MOG-induced experimental autoimmune encephalomyelitis (EAE).

Christopher Hernandez, Ross C. Gruber, Rebecca Bauer, Bridget Shafit-Zagardo
Albert Einstein College of Medicine
[1] Department of Pathology, [2] Department of Microbiology and Immunology,
Forchheimer Building, 1300 Morris Park Avenue, Bronx, NY 10461.

Abstract:

Multiple Sclerosis (MS) is a debilitating neurologic disease affecting young adults. In the United States there are an estimated 250,000-350,000 individuals diagnosed with MS. A central issue for the treatment of MS is how cell signaling pathways regulate the survival of resident central nervous system (CNS) cell populations during and after the inflammatory response. One such signaling pathway is the AKT survival pathway. The AKT serine/threonine family of protein kinases (AKT1, AKT2, AKT3) is important for proper cell growth, survival and proliferation. AKT3 is the major AKT isoform in the CNS representing 30% of the total AKT expressed in spinal cord, and 50% in the brain. AKT3 is also expressed in the immune system. Using MOG-induced EAE, a mouse model of MS, we have determined that mice devoid of AKT3 expression have a more severe phenotype. The AKT3-/- mice had higher clinical scores than wild type (WT) mice. By immunohistochemical analysis we observed that AKT3 -/- mice had more severe demyelination in the lumbar spinal cord during the acute and chronic phases of disease than wild type mice.

Acknowledgements:

I would like to thank Bridget Shafit-Zagardo Ph.D for taking me into her lab and giving me excellent guidance throughout the summer. I am very appreciative of Ross Gruber Ph.D, Kathy O'Guin Ph.D, and Rebecca Bauer for assisting me in my experiments and providing data for the poster. I would also like to thank the Summer Undergraduate Research Program (SURP) for providing me with the opportunity to conduct research at Albert Einstein College of Medicine.

Assessing the contribution of Kinesin-based transport to neurodegenerative pathology in zebrafish

Christyn A. Hicks, Philip D. Campbell, Florence L. Marlow
Department of Developmental and Molecular Biology
Albert Einstein College of Medicine, Bronx, New York

A key barrier to neurodegenerative disease (ND) research has been the inability to monitor the cellular and molecular basis of neurodegeneration *in vivo*. To develop diagnostics and therapeutics targeted at early pathology, it is imperative to understand the early mechanisms of NDs. Fundamental features of the nervous system are conserved between zebrafish and humans; therefore, we can take advantage of the optically transparency, rapid development, and genetic accessibility of the zebrafish embryo which allows for *in vivo* imaging of neurons in disease models. We propose to take advantage of the power of the zebrafish system in order to gain insights into ND mechanisms.

In order for polarized cells to maintain proper structure and function, they require precise control of the spatial distribution of proteins and organelles. A major method cells use to regulate this distribution is polarized intracellular transport. Evidence from AD models has implicated aberrant neuronal transport in NDs. To investigate the contribution of Kinesin-based transport to NDs, we are analyzing the genetic contribution of the molecular motors *kif5*'s and *kif1b* to pathology in a zebrafish transgenic tauopathy model. We are analyzing the expression patterns of these genes in the zebrafish embryo by *in situ* hybridization and using TALEN-mediated mutagenesis to disrupt individual *kif5b* genes. Finally, we are crossing *kif5*'s and *kif1b* mutations into a transgenic tauopathy model and assessing the embryos for cell death, primary motor neuron axon length, and pathologic Tau accumulation. These studies will shed light on ND mechanisms and potentially open new avenues for therapeutics.

Glycocalyx Thickness and Continuity in the Vasculature of Atherosclerotic Mice with Drug Induced Apoptosis Reduction

C.I Hirschberg (D.C. Spray, J.M. Tarbell, L.M. Cancel and E.E. Ebong)
Albert Einstein College of Medicine, Department of Neuroscience, New York, NY
The City College of New York, Department of Biomedical Engineering, New York, NY
Emory University, Department of Neuroscience and Behavioral Biology, Atlanta GA

Certain vessel geometries are prone to endothelial cell (EC) dysfunctions such as increased rates of apoptosis, and prone to lipid and macrophage accumulation to form atherosclerotic plaque. Studies suggest that in atherosclerotic disease states ECs may lose their protective glycocalyx (GCX) coat. No previous study has visualized GCX morphology overlying atherosclerotic lesions or compared the role of GCX degradation to the role of apoptosis in atherosclerotic plaque development.

We hypothesize that GCX shedding occurs in vessel areas of increased apoptosis and coincides with plaque formation. We expect GCX shedding to be secondary to apoptosis in mediating lipid and macrophage infiltration. To test our ideas, an atherosclerotic apolipoprotein E (ApoE) knockout mouse was fed a high fat diet and treated with the apoptosis-inhibiting drug Q-VD-Oph (QVD) or with vehicle control. Frozen vessel sections extracted from these animals after 10 weeks of treatment were stained for lipids, macrophages, and GCX.

Our results show that GCX continuity is disrupted in plaque areas compared to non-plaque areas. Unexpectedly, the results also demonstrate that our vehicle, DMSO, disrupts the GCX, yet QVD, which contains the same amount of DMSO, does not. The impact of QVD is still under investigation. In future studies we will use an alternative vehicle to DMSO, verify that QVD reduces apoptosis, visualize ECs to determine their integrity in the absence of GCX, and test drugs that protect the GCX. This work will help us to understand GCX and apoptosis mechanisms involved in atherosclerotic plaque formation.

Acknowledgements: Einstein SURP (YI), NIH HL094889.

Analysis of a Novel SCN5A Mutation: LQTS or Brugada Syndrome?

Kellie Ifill, Renjian Zheng, Thomas V. McDonald
Departments of Medicine & Molecular Pharmacology
Albert Einstein College of Medicine, Bronx, NY

Defects of the SCN5A gene encoding the cardiac sodium channel are associated with both the long QT-3 subtype of long-QT syndrome and Brugada syndrome (BrS). They result in increased risk of ventricular arrhythmias and sudden cardiac death, and in some reports cardiomyopathy. We discovered a novel SCN5A missense point mutation, I849N in a family that presented with the sudden death of a 21 month old child in during sleep. Initial evaluation of the family revealed that the father had ventricular tachyarrhythmia 7-years prior accompanied by a severe dilated cardiomyopathy. These findings resolved with adrenergic and angiotensin receptor blocking drugs and an implanted cardiac defibrillator/pacemaker. Mutagenesis modeling prediction programs strongly suggested that the mutation was deleterious. Either LQTS or BrS were possible phenotypes. Although the symptoms of the two disorders are the same, the mechanism in which it causes is different; as a result, different treatments are recommended for each. Accordingly, it is important to distinguish between the two phenotypes by functional expression of mutant protein. We analyzed the I849N novel mutation in two different WT backgrounds (hH1C1 and hH1C3) on the SCN5A gene, expressed in HEK 293 cells and characterized using immunoblot and whole-cell patch clamp procedures. Preliminary functional analysis of the SCN5A mutation I849N expressed in HEK 293 cells suggests Brugada Syndrome rather than LQTS.

I would like to thank Dr. Thomas V. McDonald and the Summer Undergraduate Research Program at Albert Einstein College of Medicine for giving me the opportunity to expand my research experience. I would especially like to thank Dr. Renjian Zheng, for his phenomenal mentorship and thorough guidance throughout my project, as well as Marika Osterbur and John Creag for their willingness to help in my research efforts. The authors thank NIH/NHLBI and AHA for providing the necessary funding.

Cited3 regulates morphogenesis of the heart through Mef2c

Jesus E. Juarez, G. Sheela Devakanmalai, Ertuğrul Özbudak
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

Heart development is regulated by various transcription factors in a coordinated manner. Cited3 is a transcriptional coactivator. Previously, we demonstrated that *cited3* is expressed in the skeletal muscle and heart and it regulates skeletal muscle differentiation by activating the expression of *Myocyte enhancer factor 2c* (*mef2c*). However, the functional role of *cited3* in the heart development remained to be investigated. In this study, we investigated the function of *cited3* in heart development and its regulatory relationship with mef2c- a crucial transcription factor for the heart development.

Expression of *cited3* was knocked down in zebrafish embryos by injecting antisense morpholino oligonucleotides at the one cell stage; this blocked the translation of *cited3*. The phenotypic effects on heart development in embryos were investigated at 18 hpf (hour post fertilization), 30 hpf, and 48 hpf stages. Knockdown of *cited3* resulted in enlargement of the atrium and pericardium. Furthermore, heart morphology was affected, where the heart failed to loop. Lastly, expression of *mef2c* was drastically reduced.

Our results demonstrated that *cited3* is expressed in the heart and it regulates the morphogenesis of the heart through *mef2c*.

I would like to express my gratitude to the members of the Özbudak lab, and in particular, to Sheela for the technical training, support and guidance. I would also like to thank the SURP for providing me with this invaluable research opportunity. We also thank Florence Marlow for providing the transgenic embryos.

Identification of Candidate Causative Genes for Velo-Cardiofacial Syndrome Using Whole Exome Sequencing Data

[1]Mark Kaminetzky, [1]Tingwei Guo, [1]Jinlu Cai, [1]Maria Delio, [1]Donna McDonald-McGinn, and [1]Bernice Morrow

[1]Department of Genetics at Albert Einstein College of Medicine, New York, NY

Velo-cardio-facial syndrome (VCFS)/DiGeorge syndrome (DGS) is a congenital disorder that results in a variety of abnormal phenotypes such as tetrology of fallot, structurally atypical right aortic arch, behavioral problems, and cleft palate. For most patients with the syndrome, the genetic basis is a 3-million base pair deletion on chromosome 22. Interestingly, a family presented in which a child had many of the phenotypes of the disease, but did not have a deletion. The father of the child has a structurally abnormal right aortic arch, but is otherwise normal. The mother is normal, but she has a niece with a structurally abnormal right aortic arch. Whole exome sequencing, an innovative technique that can be used to determine the complete sequence of a patient's genes, was used to sequence the exomes of the father, mother, and proband. A total of 49 single nucleotide mutations in the DNA from the proband (child) were found on the basis of a dominant model of inheritance. Integrative Genome Viewer (IGV) was used to visualize the selected mutations, their modes of inheritance, and the amino acid change that resulted from the mutation. Among the 49, twenty-five were selected for PCR amplification, and Sanger sequencing is being used to verify the selected sequence. *HOXA13*, *TMEM8*, *ZFAT*, and *USP34* are four known genes, which may be responsible for the abnormal phenotypes observed in the patient. Other genetic models are possible, and they will be analyzed when the DNA samples from the extended family are available.

Investigating the role of endogenous serotonin on signal transmission in the juvenile rat dentate gyrus

Daniel M. Lapidus, Andrés E Chávez, Pablo E. Castillo
Dominick P. Purpura Department of Neuroscience
Albert Einstein College of Medicine, Bronx, NY

The connections between neurons are plastic, in that they are constantly changing. This plasticity is responsible for our ability to adapt to our environment and is the mechanism of short-term memory formation in the hippocampus. Endogenous serotonin (5-HT) has been shown to play a presynaptic role in the induction of long-term depression in corticostriatal synapses. Using the selective serotonin-reuptake inhibitor, citalopram, we investigated the role of endogenous 5-HT in the rat dentate gyrus and its ability to induce a long-term depression in the medial perforant pathway (MPP). We hypothesized that slides incubated in citalopram (2 μM) would demonstrate an induced LTD due to elevated levels of 5-HT in the synapse. Acute transverse hippocampal slices (400 μm thick; Fig 1A) were prepared from Wistar rats, postnatal day 15 (P15) to P32 and perfused in a chamber with artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2 and 5% CO_2 (pH 7.4) and supplemented with the $GABA_A$ receptor antagonist picrotoxin (100 μM).The slides were stimulated by paired-pulses (200 μs duration, 5-7 V) in order to record changes in paired-pulse ratio and determine whether the mechanism is pre- or post-synaptically mediated. Hippocampal slides bathed in citalopram had significantly depressed EPSP slopes, compared to control slides ($p=0.001$), following 5-Hz stimulation. While this research suggests a role of endogenous 5-HT in the induction of MPP LTD, further research into the mechanism behind the LTD is necessary.

This research would not be possible without the generous funding from the Summer Undergraduate Research Program at Albert Einstein College of Medicine.

Gain-of-function Mutations in MAP3K1 that Regulate Human Gonadal Development

Jaclyn Lee, Johnny Loke, M.S., Harry Ostrer, M.D.

Albert Einstein College of Medicine of Yeshiva University; Bronx, New York

This study explored cases of 46, XY disorder of gonadal development, specifically focusing on mutations in an autosomal gene, MAP3K1, which accounts for up to 18% of all cases identified through linkage analysis. One familial case (c.634-8A) and two sporadic cases (p.Leu189Pro and p.Leu189Arg) were studied, all of which are located on exons 2 and 3 of MAP3K1.

Transfections were performed on NT2 (embryonic Sertoli-like) cells using wild-type and three mutant versions of the gene to examine the effects on various MAP3K1 downstream signaling targets that are markers for either the male or female gonadal developmental pathway. The expressions of these markers were assessed by real-time PCR and Western blot analysis. The results showed changes in regulation at both transcriptional and post-translational levels. An elevated level of expression of factors that promote the WNT/β-catenin signaling for ovarian development and reduced expression in factors along the SOX9, testis-promoting marker were observed in the mutant cell lines when compared to wild-type MAP3K1. This supports the hypothesis that these mutations in MAP3K1 have caused a shift of balance from the chromosomal designations of male gonadal development to allow for dominance of the female pathway.

Further potential studies include recreation of the mutations in transgenic mice, to see if offspring will develop the observed gonadal disorders. Acknowledgments: Alex Pearlman for his previous work and insight regarding this project and the SURP Program of AECOM.

D29: How Does it Infect *M. tuberculosis*?

Randi Mackler*, Veronica Gray*, Kevin Mu, Oren Mayer, William Jacobs Jr.

Howard Hughes Medical Institute, Albert Einstein College of Medicine. Bronx, NY

Tuberculosis continues to be a major world-wide pandemic. Although many drugs have been developed to combat this disease, tens of millions of people, particularly in third world countries, are suffering from it. Coupling this with the HIV epidemic, the prevalence of tuberculosis rises, as do multi drug-resistant (MDR) and extensively drug-resistant (XDR) strains. My goal is to contribute to a larger project developing a novel mycobacteriophage cocktail to complement or replace drugs as a tuberculosis therapy.

Mycobacteriophages are viruses that infect mycobacteria like *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Because of their ability to kill mycobacteria upon infection, they make ideal tools to use as a therapy. I am using *M. smegmatis* as a model organism for *M. tuberculosis* because it has behaves similarly but grows faster.

I created a transposon library of *M. smegmatis* using the temperature-sensitive mycobacteriophage phAE180 to insert a piece of DNA (transposon) randomly into the bacteria's genome. I screened this library for transposon mutants that are resistant to mycobacteriophage D29. These colonies will have their DNA sequenced to find the gene(s) responsible for D29 susceptibility and will be screened for cross-resistance to other mycobacteriophages that infect *M. tuberculosis*. An ideal phage therapy includes phages with different genes responsible for their susceptibility so that one mutation will not render the whole cocktail useless.

Thanks to Torin Weisbrod, the Jacobs lab and the Summer 2012 Phage Phinders. Funding from the Howard Hughes Medical Institute and the Summer Undergraduate Research Program, Albert Einstein College of Medicine of Yeshiva University.

Dissecting the Mechanism of Proapoptotic BAX inhibition by ARC

Zachary Meinhart, Thomas P. Garner and Evripidis Gavathiotis

Department of Biochemistry, Department of Medicine, Wilf Family Cardiovascular Research Institute, Albert Einstein Cancer Center, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Apoptosis is a controlled cell death process that rids the body of old, diseased and malfunctioning cells to maintain the normal development and homeostasis of multicellular organisms. Dysregulation of apoptosis can lead to a variety of diseases such as cancer, cardiovascular diseases and neurodegenerative disorders. Proapoptotic BAX plays a critical role as a gatekeeper of the apoptotic cell death pathway. BAX lies dormant in the cytosol until triggered by cellular stress to form a pore on the mitochondria and inflict irreversible damage. Apoptotic Repressor with CARD (ARC) is a cellular inhibitor of BAX-mediated cell death, however, the exact mechanism of how BAX is inhibited by ARC is not known. Here, we investigate the mechanism of BAX inhibition by ARC to understand how the critical step of BAX activation is regulated. Using biochemical and biophysical techniques, we have successfully produced functional recombinant ARC protein including full length-ARC and the monomer and dimer forms of the ARC-CARD domain. We show that ARC can directly inhibit BAX and the rate of functional pore formation in liposome assays. Our results provide the basis for future structural and functional studies to elucidate the detailed mechanism of the ARC-BAX interaction and potentially provide a pharmacologic strategy for modulating BAX-mediated cell death. We acknowledge support by National Institutes of Health (4R00HL095929) and Summer Undergraduate Research Program at Albert Einstein College of Medicine, and technical assistance by our colleagues Denis Reyna, Dat Mai, Diane Na, and Serena Schwechter.

Attention Effects on Auditory Processing of a Multiple Feature Pattern

T. Miller[a] S. Chen[b] and E. S. Sussman[b]

[a] Yeshiva University, Stern College for Women, NY [b] Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY

People are constantly inundated by a vast array of sensory input, including auditory input. As such, not only must the brain distinguish between different sounds, but it also must differentiate between properties of the sound features. Another study conducted by the Sussman laboratory analyzed the processing of multiple sound feature patterns in the absence of attention. Results indicated that the brain can track up to three feature patterns of auditory input. In this follow-up study, we asked whether attention would influence the brain's ability to process the unattended features. This study was conducted using electroencephalography (EEG) recordings of 11 healthy adults without hearing problems. Subjects were tested in three different conditions. Their task was to listen to the four-tone frequency pattern and respond whenever a pattern reversal was detected in all three conditions. Changes occurred *only* along the frequency dimension in the "frequency alone" (FA) condition and along duration and intensity dimensions in the "frequency duration" (FD) and "frequency intensity" (FI) conditions. Behavioral and ERP results demonstrated that unattended duration and intensity feature patterns were not tracked. In fact, the presence of more than one feature pattern hindered the subject's ability to track the frequency pattern. These results, when applied in a broader sense may suggest limitations in the extent to which people are able to process and accomplish multiple things at once.

Effects of Clade-specific Genetic Variation in the p6 Region of HIV's Gag Protein

§Eitan Novogrodsky, §Milka Rodriguez, §Arthur Ruiz, §Vasudev Rao, ¶Mario Pujato, ¶Andras Fiser, §Vinayaka R. Prasad
Departments of §Microbiology and Immunology, ¶Systems and Computational Biology, Albert Einstein School of Medicine, Bronx, NY

The p6 region of HIV's Gag protein plays a critical role in the assembly and budding of HIV-1 virus particles from the infected host cells. As budding progresses, the p6 region recruits host cell proteins from the ESCRT pathway – normally used to aid in exocytosis – to help detach the budding virus from the plasma membrane. A comparative sequence analysis from our laboratory showed a highly conserved leucine-tyrosine (LY) dipeptide in the p6 region of HIV-1 clade B but not in HIV-1 clade C. Dr. Prasad's laboratory created several clones of HIV with mutations in the p6 region in order to study the phenotypic manifestations of different portions of the p6 genome. The current study aims to insert six nucleotides corresponding to LY dipeptide into a representative HIV-1_{IndieC} to the effect of this change. Site-directed mutagenesis was performed to insert six nucleotides, *TGTACT*, into the p6 region of HIV-$1_{Indie\ C}$ *Gag*. The ligation with the remainder of the HIV-1_{IndieC} provirus created a complete HIV-1_{IndieC} molecular clone with the desired mutation. We hypothesize that HIV-1_{IndieC} containing the LY insertion will bud more effectively from the host cell membrane than wild-type HIV-1_{IndieC}. This research was sponsored by the Summer Undergraduate Research Program at Albert Einstein School of Medicine. Dr. Prasad, Arthur Ruiz, Milka Rodriquez, and Vasudev Rao graciously provided guidance and mentorship throughout the study.

ACT: From the time blood is drawn until the test, how long can it be?
Is it necessary to perform ACTs in the Operating Room?

Michael Plietz, Vilma Padilla, Carole Pineda, Khrishan Naraine, Ari Leifer MD, Mark Greenberg MD, Jacob Rand MD, Amy Fox MD MS
Albert Einstein College of Medicine, Montefiore Medical Center- Department of Pathology, Cardiology, and Hematology

The levels of anti-coagulants needed during cardiac procedures are found indirectly by monitoring activated clotting time (ACT). The ACT test measures the time needed for the formation of fibrin (clot) in the blood. The ACT is regularly performed in the Operating Room directly after the blood sample is drawn from the patient. However, when the ACT cannot be performed immediately, it is imperative to see how long a sample of blood provides accurate measurement. In this study, blood samples were collected during cardiac catheterization and bypass procedures during their normal drawing for the ACT test. Twenty-two patients, accounting for thirty-six samples, had Kaolin-activated ACT tests done using the i-STAT at 0 min and then at five minute intervals until 20 minutes was reached. The ACT test results of the aged and time-zeroed samples were compared using a Paired Observation T-test and repeated measures of analyses of variance (ANOVA). When the results were split by medications, there was seen to be a significant change in the unheparinized values at the 10-minute mark and each time value afterwards (P-value = .0007). Patients who had only taken Angiomax had a significant difference within the first five minutes (P-value = .0094). Patients taking Angiomax and Plavix did not have a significant change in ACT values in the first 25 minutes. Early results for high heparin samples have shown instability in results within ten minutes, but no significant difference until 20 minutes in time (P-value = .0333). Based on the initial samples, it seems accurate for the ACT to be performed within 10 minutes, unless the patient is receiving only Angiomax.

Rab1a regulates endocytic vesicle processing through interaction with Kifc1

by: Jose Quiroz, Aparna Mukhopadhyay, and Allan W. Wolkoff

Receptor-mediated endocytosis is a mechanism by which cells take up and process macromolecules via a series of endocytic vesicles. We recently reported a novel pathway by which Rab1a regulates endocytic vesicle processing by recruiting the microtubule-based motor Kifc1. The mechanism by which this occurs is unknown and was the subject of the current investigation in which we examined whether Kifc1 and Rab1a interact with each other either directly or through a protein complex.

Human hepatoma-derived HuH7 and human embryonic kidney-derived HEK293 cell lines expressing super folder green fluorescent protein (SFGFP) or Rab1a-SFGFP fusion protein were prepared. Fluorescence microscopy of Rab1a-SFGFP expressing cells revealed punctate distribution of fluorescence throughout the cytoplasm. This distribution was similar to that of endogenous Raba1a in untransfected cells as determined by immunofluorescence using specific antibody. To test whether Kifc1 interacts with Rab1a, a plasmid encoding Kifc1-flag or empty vector (pFlag-LMV-5c) was transfected into Rab1a-SFGFP or SFGFP expressing HEK293 cells. Cell lysates were subjected to immunoprecipitation with GFP antibody linked to agarose beads. Following SDS-PAGE, immunoblot analysis revealed the presence of Kifc1 in immunoprecipitates from Rab1a-SFGFP expressing cells but not from cells expressing SFGFP alone.

In summary, immunofluorescence microscopy verified the successful preparation of stable cell lines expressing Rab1a-SFGFP in HEK293 and HuH7 cells. Immunoprecipitation of Rab1a-SFGFP cells transiently transfected with Kifc1-Flag showed that Rab1a and Kifc1 were in the immunoprecipitate. We conclude that Rab1a and Kifc1 are associated with each other in a protein complex and do not just independently bind to endocytic vesicles. Whether other proteins are in this complex remains to be discovered.

IMPROVED SAMPLING OF CONFORMATIONAL SPACE FOR ACCURATE CHEMICAL SHIFT PREDICTION OF INTRINSICALLY DISORDERED PROTEINS

Authors

Steven Reisman, Ertan Eryilmaz, David Cowburn

Department

Biochemistry Department, Albert Einstein College of Medicine, Bronx NY

Intrinsically disordered proteins (IDPs) lack rigid tertiary structures when isolated, but under physiological conditions, when interacting with their functional partners, attain well defined structures. Hence, IDPs challenge the notion of direct structure-function relationships. Due to the disordered nature of IDPs many conformational degrees of freedom exist. Understanding the conformational dynamics and disorder to order transitions of IDPs remains challenging. In response to such a difficult issue, various software tools and experimental procedures have been utilized in search of information on IDPs. Experimentally, nuclear magnetic resonance (NMR) spectroscopy has proven to be a useful tool in overcoming these challenges. NMR probes both local (in atomic resolution) and global properties of IDPs. Particularly, NMR chemical shifts report on the local environment of specific atoms. The chemical shift of an atom on a peptide residue is sensitive to the orientations of bonds and other nearby atoms. Although NMR is quite useful in understanding IDPs, this technique may suffer from spectral overlaps and ambiguous chemical shifts due to sequential repeats present in IDPs. Thus various computational approaches have been developed to accurately predict NMR chemical shifts of IDPs. In conjunction with other software which predict structure from chemical shifts, molecular dynamics (MD) tools and direct NMR experiments, we are closer in understanding IDPs. In this study, we have employed a flexible-meccano (FM) generated structure ensemble and all-atom MD simulations on C-intein of *DnaE Npu* to improve the accuracy of NMR chemical shifts predicted by a program called SPARTA+[1].

Acknowledgements

This work was supported by the Albert Einstein College of Medicine Summer Undergraduate Research Program. Special thanks to David Cowburn and Ertan Eryilmaz for their guidance and tutelage throughout the project.

Exploiting SIMPLE in the Secretion of Membrane Protein Cargos in Exosomes

Melanie Reschke, Hong Zhu, Wenjing Li, Chi-Wing Chow
Department of Molecular Pharmacology
Albert Einstein College of Medicine of Yeshiva University
Bronx, New York 10461 USA

Acknowledgements: Victoria Freedman, Rosa Garcia, and the Summer Undergraduate Research Program of the Graduate Division of Biomedical Sciences at Albert Einstein College of Medicine of Yeshiva University.
Funding: National Institutes of Health, Muscular Dystrophy Association

Intercellular communication within an organism is a complex and multifaceted process that, if disrupted, can lead to disease such as neurological disorders and tumor proliferation. Much of the current signaling process is carried out by small diffusible ligands binding to targeted receptors. Increasing evidence, however, has shown an important role of small vesicles. Exosomes are a type of secreted vesicle defined by their size (50-100nm) and their biogenesis in multivesicular endosomes (MVE).[1] Once secreted, signaling may occur via fusion of the exosomes with neighboring recipient cells. Cargos delivered by exosomes may alter cellular homeostasis of the recipients. Thus, understanding the exosomal pathway may provide insights to new therapeutic approaches for difficult to treat pathologies.

We have recently showed that overexpression of the protein SIMPLE (Small Integral Membrane Protein of the Lysosome) increases exosome secretion. Conversely, mutations in SIMPLE are associated with type 1C Charcot-Marie-Tooth (CMT1C) neuropathy. Mutation of SIMPLE causes reduced exosome production with disrupted MVE formation.[2]

Here we utilize a candidate approach to determine membrane proteins secreted via exosomes and the role of SIMPLE in this process. We found that proteins involved in ligand-receptor signaling pathways are more likely to be secreted via exosomes than membrane channels. In addition, secretion of a few proteins is potentiated by overexpression of SIMPLE.

In future studies we intend to test the ability of cells to take up exosomes and incorporate the functional cargo. Exosomes may provide a new therapeutic approach for pathologies of signaling pathways and immunological responses.

References:

1. Thery C., Ostrowski M., and Segura E. 2009. "Membrane vesicles as conveyors of immune responses". *Nature Reviews Immunology.* 9 (8): 581-593.
2. Zhu, H., Guariglia, S., Yu, R.Y.L., Li, W., Brancho, D., Peinado, H., Lyden, D., Salzer, J., Bennett, C., and Chow, C.W. (2012) Mutation of SIMPLE in Charcot-Marie-Tooth 1C Alters Production of Exosomes. Submitted.

Relating Neuronal Population Responses in Monkey V4 and V1 to Perceptual Judgements

Brian Sanders, Seiji Tanabe, Amin Zandvakili, Adam Kohn

Dept. Neuroscience, Albert Einstein College of Medicine, Bronx NY

Neurons in sensory cortex show weak but systematic correlations with perceptual decisions. The relationship between the activity of individual sensory neurons and the animal's behavior means that even neurons in early sensory cortex carry information about an upcoming decision. However, visual discrimination is more accurate than would be predicted from the responses of single neuron recordings. Correlation within neuronal populations can increase the amount of information encoded by a population of neurons. Therefore, perception should result from a pooling of individual signals into a summation of neuronal input.

To better understand this pooling, we implanted microelectrode arrays in areas V1 and V4 of macaque monkeys and recorded responses of populations of neurons while monkeys performed an orientation discrimination task. The animals were briefly presented with a sinusoidal grating and their task was to report whether the grating's orientation was closer to horizontal or to vertical than 45 deg.

We first confirmed that trial-to-trial fluctuations in single V1 and V4 neuron responses were predictive of the monkey's decisions (choice probability). We then related the simultaneously measured population (2-10 neurons) responses to those same perceptual judgments using binary classifiers. The performance of the classifiers was cross-validated by predicting decisions on a subset of trials. The classifiers we explored performed similarly well and not significantly better than the best individual cell within each population. Thus, we found that pooling a small random sample of the sensory representation is equivalent to sampling the single best neuron in the pool for generating the decision variable.

Acknowledgements: Carlyn Patterson and Stephanie Wissig for their insightful discussions and suggestions. This work was funded by grants from the Einstein SURP (YI) and by the Roth Institute Scholars Program.

Characterization of *Toxoplasma gondii* Jumonji C1 histone demethylase

Katelyn Scharf, Zoi Tampaki, Kami Kim
Department of Medicine and Department of Microbiology and Immunology
Albert Einstein College of Medicine, Bronx, NY 10461

Toxoplasma gondii is an opportunistic pathogen that affects approximately one third of the human population. Current drug therapies affect tachyzoites, the quickly replicating form of the parasite, but are unable to penetrate the cyst of the bradyzoites, Toxoplasma's latent phase. Consequently, researchers are investigating the effect of epigenetic modifications on the parasite's biology.

DNA inside the nucleus is wrapped around histones, which are proteins characterized by a globular head and terminal tail of amino acids. The tails of the histones are subject to posttranslational modifications (PTMs) such as acetylation and methylation by chromatin remodeling molecules. This project focuses on the histone demethylase Jumonji C1 (JmjC1).

Our prior studies have shown JmjC1 to be located on promoter regions of genes that are silenced in the tachyzoites. In such conditions, JmjC1 appears to involved in repression of genes that are likely to be activated in the bradyzoite stage. In order to study the function of JmjC1 with a specific interest in the gene's function under bradyzoite conditions, we used the HA-tagged JmjC1HA strain.

Staining and fractionation analysis of the JmjC1HA parasites showed that JmjC1 is localized in the nucleus. Fractionation also showed trace amounts of the protein the cytoplasm, suggesting that the molecule may also have non-histone substrates. Furthermore, we performed genome-wide chromatin immunoprecipitation analysis coupled to microarrays (ChIP-chip) of JmjC1HA in the bradyzoite state and these results are still being processed. Future research on the role of JmjC1 histone demethylases in bradyzoite conditions is necessary to understand the function of this gene.

Acknowledgments:
Dr. Kami Kim, Dr. Zoi Tampaki, Dr. Sheila Nardelli, Dr. Catherine de Beaumont, and the rest of the Kim Lab. Funding through SURP 2012.

Developing a Microarray CGH Database to Review Test Performance, Establish New Guidelines for Quality Assurance, and Identify Bronx Specific CNVs

Brandon Schein, Jianli Li and Rizwan Naeem
Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine

Array Comparative Genomic Hybridization (aCGH) is used to detect DNA copy number variations (CNV) in the genome with applications in both research and clinical diagnosis for personalized medicine. Montefiore Medical Center offers this test to a pediatric population. We developed a laboratory specific aCGH database in order to review test performance, identify Bronx specific CNVs, and establish new guidelines for quality assurance (QA). Multiple QA measurements are assessed during the testing process to ensure the generation of high quality and reliable data. We discovered a strong correlation between the ratio of reference DNA to test DNA labeling dyes and the value of the Derivative Log Ratio Spread (dLRsd), a known QA indicator of microarray noise levels. The correlation suggested that appropriate labeling of reference and test DNA generated microarray data with low noise levels. Additionally, a positive correlation was found between the dLRsd and the number of calls detected by analysis software. This observation was further validated by analyzing the differences in the results of repeated tests. This analysis showed that reductions in dLRsd values lead to fewer variations in detected calls. This data review led us to add a new QA measure, testing the ratio of dye labeling, prior to hybridization on microarray slides. In addition to other QA measures already in place, the appropriate ratio of labeling dyes can be used to predict the outcome quality of microarray tests. This is especially important for the dye labeling of control cases and confirmatory repeat tests, since a false positive or false negative of a control requires the entire batch of samples to be retested. We believe that this QA project will improve aCGH based genomic testing and reduce the costs associated with repeat testing.

Exploring Extra Pericentromeres and Telomeres

Naomi Schwartz[1], Yinghui Song[1], Jidong Shan[1], Tae Moon Kim[3], Paul Hasty[3], and Cristina Montagna[1, 2]

[1]Department of Genetics and [2]Pathology, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461. [3]Department of Molecular Medicine and Institute of Biotechnology, University of Texas Health Science Center, San Antonio, TX 78245.

Inefficient DNA repair is a major cause of genetic instability and chromosome abnormalities, common characteristics of cancer cells. Proper genome maintenance is critical to the cell's well-being, and thus, several repair pathways have evolved to cope with DNA damage. One of such pathways includes the homologous recombination protein RAD51, a RecA recombinase important for replication fork maintenance. Proper ATP binding is critical for RAD51 function: when RAD51's lysine K133 (an important ATP binding factor) is mutated into its defective K133A form, chromosomal rearrangements occur within the cell. Previous research identified a hitherto undescribed chromosomal abnormality, Extra Pericentromeres and Telomeres (EPTs), that appears within these mutated cells, yet there has been no further study of these novel rearrangements. This study focuses on EPTs specifically: how many occur, which chromosomes they affect, and whether they affect single chromosome duplication or multi-chromosome fusion. Mouse metaphases derived from K133A cells were visualized using Spectral Karyotyping. Of the 33 spreads analyzed, 63 EPTs were located, as well as a multitude of other structural rearrangements. The majority of the EPTs visualized affected three specific chromosomes: 1 and 11 (mostly duplicated), and 8 (mostly fused to chromosome 11). In order to ensure that these results are accurate, and to further explore the complexity of EPTs, additional experiments are necessary. These include using chromosome-painting probes to visualize chromosome 11 specifically, and specific probes to visualize the centromeres and telomeres. EPTs are formed by mutated RAD51 proteins, and are far more complex than originally thought, requiring much additional study.

Acknowledgements: I would like to thank the Molecular Cytogenetic Core at Albert Einstein College of Medicine for assisting me with the execution of the SKY hybridizations, image acquisition, and analysis. I would also like to thank the members of the Montagna and Hasty labs for providing support and sharing reagents. Additional thanks go to the Summer Undergraduate Research Program at Albert Einstein College of Medicine, for the funding allowing me the opportunity to carry out this research summer project, as well as to NIH CA013330-38 to I.D. Goldman and ACS 120025 to C. Montagna for additional funding.

Title: The Search for Downstream Effectors of TORC1 Signaling

Authors: Scott R Shuldiner (Department of Biology, Tufts University, Medford MA; Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY), Manuel Sanchez-Casalongue (Department of Biochemistry, Albert Einstein College of Medicine), and Ian M Willis (Department of Biochemistry, Albert Einstein College of Medicine; Department of Systems and Computational Biology, Albert Einstein College of Medicine)

Abstract: The target of rapamycin complex 1 (TORC1) signaling pathway controls diverse cellular processes including lifespan, cell growth, metabolism and autophagy. Deregulation of TORC1 signaling is correlated to disorders such as cancer, neurodegeneration, diabetes and cardiovascular disease. The protein kinases Kns1 and Mck1 have recently been found to play an integral role downstream of TORC1 in the transcriptional repression of tRNA and ribosome synthesis. Little is known of the mechanism by which Kns1 is activated. In response to cellular stress, Kns1 becomes hyperphosphorylated, accumulates in the nucleus and primes the RNA polymerase III subunit Rpc53 for phosphorylation by Mck1 to effect transcriptional repression. We used Synthetic Gene Array (SGA) methodology to generate strains to elucidate other roles for Kns1 and Mck1 downstream of TORC1. Viable gene deletion strains and their *mck1*Δ*kns1*Δ counterparts were screened for rapamycin hypersensitivity. Here, we present a set of synthetic negative interactions identified by our SGA. In addition, we mined high-throughput phosphoproteomic datasets and identified potential substrates of Kns1. These proteins were tagged and their phosphorylation status evaluated in response to inhibition of TORC1 signaling and dependence on Kns1. Preliminary data shows that one candidate protein, a regulatory subunit of a certain protein kinase, is differentially phosphorylated upon rapamycin treatment in a Kns1-dependent manner. This finding implicates regulation of the protein kinase as a downstream effector of the TOR signaling pathway.

Acknowledgements: SURP, Robyn D Moir and Jaehoon Lee

Community Norms for Diagnosis and Treatment of Uveitis

Joseph Soucy; Jennifer Wu; Helen Zhou; Kim M. Lee; Moriah Rabin; Kevin Jwo; William Burton, PhD; David C. Gritz, MD

Department of Ophthalmology and Visual Sciences and Department of Epidemiology and Population Health, Montefiore Medical Center and Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York, USA

Uveitis is inflammation of the middle layer of the eye (uvea). Since there are more than 150 different causes of uveitis, it is difficult for physicians to accurately identify the necessary diagnostic tests for determining the specific origin of uveitis in any particular patient. Our study aimed to examine the community standard for evaluation of uveitis among general ophthalmologists and uveitis specialists in the USA. Randomly selected participants were contacted via email and invited to complete an anonymous survey. The survey involved the diagnosis of eight clinical uveitis cases and knowledge of the Standardization of Uveitis Nomenclature (SUN) recommendations, which determine a classification system for uveitis. Responses were tallied and analyzed. The two most frequently cited work-ups for each of the eight cases were recorded, and significance tests were performed to compare the responses of general ophthalmologists to those of uveitis specialists. Preliminary results indicate that FTA-ABS and MHA-TP are the most frequently ordered work-ups for four of the eight uveitis cases; serum angiotensis converting enzyme (ACE) was the most frequently cited test for two cases. No significant difference exists between the responses of general ophthalmologists and those of uveitis specialists. Additionally, less than 25 percent of participants were aware of the SUN recommendations. Efforts must therefore be made to increase awareness of the SUN classification system; standardization of diagnostic uveitis testing would prevent the administration of many unnecessary tests. Future studies might attempt to promote this awareness and to examine the frequencies of additional uveitis treatment procedures.

Acknowledgements: Albert Einstein College of Medicine SURP; Research to Prevent Blindness Foundation

B7X: A Potential Target Molecule for Tumor Immunotherapy

Adina Sperling, Hyungjun Jeon, Xingxing Zang

Department of Microbiology and Immunology, Cancer Center, Diabetes Center, Albert Einstein College of Medicine, Bronx, New York

Monoclonal antibodies designed to target specific molecules on cancer cells has proven to be an effective immunotherapeutic approach in a variety of human cancers. The overexpression of T cell co-inhibitory B7x is linked to a variety of human cancers and in many cases correlated with poor clinical outcome. The objective of this study was to validate the use of monoclonal antibodies against B7x using a murine tumor model. We induced cancer in Balb/c mice using the 4T1/B7x cell line, a highly aggressive metastatic breast cancer line. Monoclonal antibodies were injected on D-1,3,7,11,14. The mice were sacrificed; tumors were excised, weighed, and measured at D-17. The number of pulmonary metastases were also counted. Our results demonstrated a significant decreased tumor burden in the 1H3 study group compared to the IgG control. In order to elucidate whether the mechanism of tumor suppression was via antibody-dependent cellular cytotoxicity (ADCC), we studied the effect of monoclonal antibodies against B7x in an *in vitro* model as well. We obtained splenocytes from Balb/c mice, and combined them with our 4T1/B7x target cells in the presence of 1H3 or control IgG. Target cells were then stained with PKH-26 and CFSE, and viability was determined by FACS analysis. Our *in vitro* model demonstrated that monoclonal antibodies against B7x induce tumor cell death via ADCC. These results suggest that monoclonal antibodies against B7x can be a promising immunotherapeutic method.

This project was funded by the Summer Undergraduate Research Program at Albert Einstein College of Medicine

Chorioamnionitis: Clinical Pathological Correlations and Neonatal Outcome

*Tauqeer, S., Abadi, J., Sansary, J., Abadi, M.
Departments of Pathology and Pediatrics
Jacobi Medical Center, Bronx, New York, USA
*DSSROP, Einstein Medical College, Bronx, New York, USA

BACKGROUND. Chorioamnionitis is the inflammation of the amniotic fluid, membranes, placenta and the decidua, often associated with *E. coli* and *Group-B Streptococcus* (GBS) infections among others. Chorioamnionitis is linked to preterm deliveries and premature rupture of membranes. The presence of GBS in pregnant women might be a risk factor for more severe placental infections (funisitis and vasculitis). **HYPOTHESIS.** We hypothesized that the presence of funisitis, vasculitis and GBS in women with chorioamnionitis, will lead to worse outcomes in the extremely premature neonates compared to the more mature ones. **METHODS.** Maternal demographics, placental pathology and neonatal outcome data was collected form 162 patients at Jacobi Medical Center, an inner city hospital, with great diversity. The patients were divided into three groups based on gestational age (GA). The data was statistically analyzed and correlations were made between various factors present with chorioamnionitis. **RESULTS and CONCLUSIONS.** The results showed an increased prevalence of funisitis, vasculitis and GBS in early gestation, which decreased as the pregnancy approached full term. The outcome and survival rates of neonates were directly related to the increasing GA. Maternal age did not have an effect on the prevalence of chorioamnionitis in this study. Future research can be directed to improve early detection and management of chorioamnionitis to avoid fatal or complicated neonatal outcome.

Identification of DNA Repair Genes Required During Denucleation of Lens Fiber Cells

Bella Wolf[1], Jian Sun[2], Tiphaine Chevalier[2], and Ales Cvekl[2,3]
[1]Stern College for Women, Yeshiva university, 245 Lexington Avenue, New York, NY 10016
[2]Genetics, [3]Ophthalmology & Visual Science, Albert Einstein College of Medicine, Bronx, NY 10461

The mammalian ocular lens is a structure that refracts light on to the retina. In order to achieve this it is of crucial importance that lens transparency is maintained. Subcellular organelles such as the nucleus reduce transparency of the lens fiber cells and hinder refraction of light. Therefore, one critical step in ocular lens development is the degradation of the nucleus in lens fiber cells in a process called denucleation. Failure to execute denucleation leads to abnormal lens fiber cell differentiation and cataract formation.

As the denucleation process occurs, double stranded DNA breaks form and chromatin degrades. Therefore previous research in our laboratory has indicated that in order to counteract this and ensure that apoptosis, which would produce optical irregularities and scattering of light, does not occur DNA repair enzymes are mobilized.

The purpose of this experiment is to identify the functional DNA repair enzymes participating in the denucleation process. The mRNA levels of embryonic stage 15.5 mice lens, which have not yet undergone denucleation in the lens fiber cells, is compared through the use of real time PCR to that of embryonic stage 17.5 mice lens, the approximate stage in which denucleation occurs.

Results show that most DNA repair enzymes do not show a significant change from E15.5 to E17.5; however, several DNA repair pathway genes (eg. Nbn (Nbs1), Mlh1, Xpa, Mpg) show a considerable up-regualtion. These results encourage the current hypothesis that there is a significant participation of certain DNA repair enzymes during the denucleation process.

Acknowledgements: This work is supported by NIH grants, 95261482, University Undergraduate Summer Research Scholars Program, Summer Undergraduate Research Program, and an unrestricted grant from RPB to the Department of Ophthalmology and Visual Sciences. I would like to thank Dr. Ales Cvekl, Jian Sun, and Tiphaine Chevalier for all of their help, guidance, and encouragement during this program.

Identification and functional characterization of an Aif-1 isoform in 3T3-L1 adipocytes

Sara Wong, Prameladevi Chinnasamy, Nicholas E. S. Sibinga
Department of Developmental and Molecular Biology and Department of Medicine/Cardiology, Albert Einstein College of Medicine, Bronx, NY

Obesity is a worldwide problem that leads to many other diseases including diabetes and metabolic syndrome. Work in our lab has shown that Allograft inflammatory factor-1 (Aif-1) knockout mice are resistant to obesity upon high fat diet feeding. Aif-1 is a conserved protein with EF hand motifs and eight known transcripts. It is expressed in monocytes/macrophages and various cells and tissues. This protein has been shown to play an important role in breast cancer cell migration, neointima formation, atherosclerosis, and type 2 diabetes. However, the role of Aif-1 in the pathogenesis of diet-induced obesity is still unclear. To evaluate the function of Aif-1 in adipocyte biology, we differentiated 3T3-L1, a cell line that can differentiate into adipocytes. Western blot analysis of 3T3-L1 showed a novel Aif-1 isoform approximately 14 kDa, which is different from the 17 kDa isoform present in mice. We hypothesize that the novel Aif-1 isoform might play a distinct role in adipocyte differentiation, lipid uptake, and cell survival. To test our hypothesis, we will first identify the mRNA transcript and protein sequence by RACE-PCR and mass spectroscopy, respectively. Our RACE-PCR has identified a full-length transcript from exon 4 through 8. Furthermore, our western blot analysis shows expression of this isoform is induced on day 4, as compared to day 8 through 15, during which expression gradually decreases. Our result suggests that the novel Aif-1 isoform might be a therapeutic target for metabolic diseases in regulating adipocyte function.

Mapping the Surface of the TFIID Complex via Random Peptide Screening and SBED Label Transfer Assays

Lucille Woodley, Lihua Song, and Dr.Wei-Li Liu
The Liu Laboratory
Albert Einstein College of Medicine
Department of Anatomy and Structural Biology
Bronx, NY

Transcription is the process by which cells are able to create an RNA copy of a certain section of DNA. TFIID is a core promoter recognition complex that binds to promoter DNA of certain regions of DNA (1). TFIID is responsible for drawing other essential factors involved in transcription to the promoter region of the DNA. It is known that human TFIID is composed of fifteen different subunits.

There are many proteins in the nucleus that could potentially interact with TFIID. However, many of these are currently unidentified leaving our understanding of the overall process of transcription very limited. Our research goal is to identify novel factors capable of binding to TFIID.

To achieve this goal, we utilized the FliTrx Random Peptide Display Library screening system. This is a system for identifying protein-protein interactions. After sequencing, the results revealed sixty-six of the randomly generated peptides were able to bind to TFIID.

My project is to identify which subunits of TFIID are able to bind these novel peptide sequences. This process will identify which peptides are able to bind to TFIID and where. This was accomplished via SBED-biotin label transfer assays.

These experiments revealed that some peptides including 2-11, 2-17, 2-21, 4FT2, 9FT2, and 16FT2 possibly bind to TAF1. Peptide 2-17 is potentially able to bind TAF5. 4FT2 potentially targets TAF10.

There remain more peptides to be tested to reveal more protein-protein interactions. This knowledge will further our understanding of the way cells induce transcription and allow for many further investigations.

Reference

1. Liu WL, Coleman RA, Ma E, Grob P, Yang JL, Zhang Y, Daily G, Nogalws E, Tijan R. 2009. Structures of Three Distinct Activator-TFIID Complexes. *Genes & Development* **23**:1510-1521.

Special thanks to Albert Einstein SURP for funding this project.

The Role of Eicosanoids in Chagas Disease

Michael A. Zingman[1,2], Herbert B. Tanowitz[1], Shankar Mukherjee[1], Louis M. Weiss[1]

Affiliations:
[1]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY; [2]Northwestern University, Evanston, IL

Abstract:

American trypanosomiasis or Chagas disease (CD) is caused by infection with the protozoan parasite *Trypanosoma cruzi (Tc)*. Currently 18 million people are infected resulting in 21,000 deaths annually. Acute infection leads to myocarditis, focal ischemia, enhanced platelet activation and thrombus formation. Up to 30% of infected individuals that survive the acute stage progress to the indeterminate and chronic stages, associated with cardiomyopathy, vasculopathy, and sustained inflammation in the nervous system, digestive system, adipose tissue and heart. As our understanding of CD increases, it is becoming increasingly important to explore the role of eicosanoids or inflammatory mediators derived from arachidonic acid (AA) metabolism in the disease development. Parasite-derived thromboxane (TXA_2) has been shown to interact with host TXA_2 receptors (TP) to control parasitemia, mortality, and cardiac pathology in chagasic mouse models (CMM).

Recently, we found evidence that parasites express a protein similar to the TP receptor of mammals. We investigated the physiological significance of *Tc*TP with IBOP, a TXA_2-mimetic, on the binding of parasite receptors by measuring calcium mobilization. Furthermore, we evaluated the extent of AA metabolic pathways in the parasites by immunoblotting and observing immunofluorescence with antibodies directed against specific enzymes. We screened three parasite stage-specific libraries for cloning of *Tc*COX, *Tc*TP, and *Tc*PLA_2. Finally, we measured resolvin D1 (novel DHA-derived anti-inflammatory or pro-resolution mediator) levels in the plasma of CMM as well as in humans with chronic infection, in order to evaluate whether RvD1 could be used as a predictive marker to therapeutic windows and outcomes of the disease.