August 21, 2010

ASBMB Graduate Student Research and Career Symposium

For Students, By Students

Northwestern University, Baldwin Auditorium
303 East Superior, Chicago, IL 60611

Meeting Organizers:
Darja Pollpeter, Northwestern University
David Courson, University of Chicago
David Taussig, University of Illinois - Chicago
AGENDA

Meeting Location: Baldwin Auditorium, Northwestern University

10:00 a.m. Introduction and Welcome

10:15 a.m. -11:00 a.m. Career panel Session #1
   Marueen Mullen, Hospira Inc. [Biotech]
   Emina Stojkovic, Northeastern Illinois University [Undergraduate Teacher]
   Sharon Housinger, U of C Laboratory School [K-12 Teacher]
   Jeffry Hoyer, Deerfield High School [K-12 Teacher]

11:15 a.m.-12:00 p.m.  Career panel Session #2
   Syed Rizvi, Husch Blackwell Sanders LLP [Patent law]
   Joy Ramos, Prescott Medical Communications [Science Writing]
   Sarah Kopecky-Bromberg, Abbot Laboratories [Science Writing]
   Rabia Mayas, MSI- Science Director [Administration]

12:00 p.m.-1:00 p.m. Lunch with presenters

1:00 p.m.-2:00 p.m. Student Research Talks Session 1
   Shihao Shen, University of Iowa
   Sam Nalle, University of Chicago
   Marina Pazin, Northwestern

2:00 p.m.-3:00 p.m. Poster Presentations

3:00 p.m.-4:00 p.m. Student Research Talks Session 2
   Laurie Risner, Loyola
   Dan Zheng, Northwestern
   Narjes Tavoosi, UIUC

4:00 p.m.-5:00 p.m. Special Topics Panel
   Ben Glick, University of Chicago
   Aixa Alfonso, UIC
   Vinzenz Unger, Northwestern
   Suzanne Pfeffer, Stanford


5:00 p.m. Closing remarks
# POSTER PRESENTATIONS

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maureen Richards</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Sam Light</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Shihao Shen</td>
<td>University of Iowa</td>
</tr>
<tr>
<td>Rakesh Mishra</td>
<td>University of Texas Medical Branch</td>
</tr>
<tr>
<td>Sankar Narayan Krishna</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Sam Nalle</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Mike VanGompel</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Kristen Mighty</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Cynthia Danielson</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Erin White</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Jaya Sastri</td>
<td>Loyola University Medical Center</td>
</tr>
<tr>
<td>Noah Birch</td>
<td>Loyola University Chicago</td>
</tr>
<tr>
<td>Kalpana Ramakrishnan</td>
<td>University of Illinois at Chicago</td>
</tr>
<tr>
<td>Lola Olufemi</td>
<td>Southern Illinois University</td>
</tr>
<tr>
<td>Marla Issac</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Guadalupe Navarro</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Jessica Bockhorn</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Laurie Risner</td>
<td>Loyola University Medical Center</td>
</tr>
<tr>
<td>Dan Zheng</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Andy Loria</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>David Courson</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Constance Markle</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Brigitte Ziervogel</td>
<td>University of Chicago</td>
</tr>
<tr>
<td>Darja Pollpeter</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Narjes Tavoosi</td>
<td>University of Illinois at Urbana-Champaign</td>
</tr>
<tr>
<td>David Taussig</td>
<td>University of Illinois at Chicago</td>
</tr>
<tr>
<td>Antonia Navarro</td>
<td>Northwestern</td>
</tr>
<tr>
<td>Diana Monsivais</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Marina Pazin</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>Danielle Glick</td>
<td>The University of Chicago</td>
</tr>
</tbody>
</table>
Inactivation of T regulatory cells Enhances Anti-Viral Responses and Delays Disease Progression in the TMEV Model of Multiple Sclerosis

Maureen Richards, Meghann Teague Getts, Stephen Miller.

Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

Abstract:

Theiler’s Murine Encephalomyelitis Virus is a naturally enteric mouse pathogen that results in an induced demyelinating disease (TMEV-IDD) in susceptible mouse strains. The disease develops secondary to persistent viral infection in the antigen presenting cells of the CNS. TMEV-IDD is a relevant model of MS as both disease states are characterized by demyelination and cellular infiltrates of activated CD4+ T cells. Previous work from our laboratory and others indicates that both resistant and susceptible strains mount a CD8+ T cell response specific to the virus. Our data shows that inactivation of Treg cells prior to infection results in delayed progression of TMEV-IDD and increased viral clearance from the CNS. Additionally, inactivation results in increased anti-viral response to TMEV by CD8+ T cells as shown by IFNg production and lysis of viral loaded target cells by in vivo CTL assay. Treg cells also regulate the CD4+ T cell antiviral response as depletion results in increased DTH responses to both the CD4+ and CD8+ dominant epitopes. Our data also show that SJL mice have a significantly higher population of Tregs post infection than B6 mice. SJL mice have a greater amount of CD4+CD25+Foxp3+ cells as well as CD4+CD25-Foxp3+ cells in both the CNS and periphery after infection. This increased population results in an unfavorable ratio of activated CD8+ T cells to Treg cells in the SJL strain of mice. SJL mice have approximately 1 activated effector CD8+ T cells for every 9 Treg cells in the CNS after infection; while B6 mice have 1 activated CD8+ T cell for every 1 Treg. This unfavorable ratio in SJL mice inhibits viral clearance mechanisms by CD8+ T cells. We hypothesize that Treg cells are activated to suppress viral clearance mechanisms and proliferate in an antigen specific manner due to a cross reactive epitope with TMEV that exists in the SJL repertoire of Treg cells but does not exist within the B6 repertoire of Treg cells.
Identification and Analysis of Functionally Dynamic Elements of the Type I Dehydroquinate Dehydratase

Sam Light\textsuperscript{1,2}, George Minasov\textsuperscript{1,2}, Arnon Lavie\textsuperscript{3}, Michael Caffrey\textsuperscript{3}, Wayne Anderson\textsuperscript{1,2}.

Center for Structural Genomics of Infectious Diseases\textsuperscript{1}, Department of Molecular Pharmacology and Biochemistry, Feinberg School of Medicine\textsuperscript{2}, University of Illinois at Chicago, Department of Biochemistry and Molecular Genetics, Chicago, IL\textsuperscript{3}

Abstract:

The biosynthetic shikimate pathway is a composed of 7 enzymes which catalyze sequential reactions to generate chorismate, a precursor the aromatic amino acids and a number of secondary aromatic metabolites. We present crystal structures of the third enzyme in the pathway, the type I dehydroquinate dehydratase, from intestinal pathogens Clostridium difficile and Salmonella typhimurium. In addition to an apo structure, structures complexed with substrate and covalent reaction intermediate before and after dehydration were determined. This series of apo and liganded structures demonstrates two conformations of histidine-143 which position the residue for a role in establishment and breakdown of the covalent intermediate and in proton shuttling between the ring and leaving group. A second structurally dynamic region of the protein is a loop region which is disordered in the protein’s apo state but is ordered and interacts with the reaction intermediate in the liganded structures. Site-directed mutagenesis shows that loop residues which hydrogen bond with reaction intermediate are important for substrate binding and catalysis. Comparison of wildtype and the glutamine-236 to alanine mutant reveals that upon loop closure the loop residue glutamine-236 is necessary for inducing arginine-214’s displacement to the conformation it assumes in the reaction intermediate bound structures. This observation defines an untold function of loop closure in coordinating active site residues and provides a basis for the surprisingly large effect of glutamine mutation on the enzyme’s kinetics.
Evolution of alternative splicing in primate brain transcriptomes

Shihao Shen², Lan Lin³, Peng Jiang¹, Beverly Davidson¹,³,⁴, Yi Xing¹,²,⁵.

Department of Internal Medicine, University of Iowa, Iowa City, IA 52242, USA¹, Department of Biostatistics, University of Iowa, Iowa City, IA 52242, USA², Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA 52242, USA³, Department of Neurology, University of Iowa, Iowa City, IA 52242, USA⁴, Department of Biomedical Engineering, University of Iowa, Iowa City, IA 52242, USA⁵

Abstract:

Alternative splicing is a predominant form of gene regulation in higher eukaryotes. The evolution of alternative splicing provides an important mechanism for the acquisition of novel gene functions. In this work, we carried out a genome-wide phylogenetic survey of lineage-specific splicing patterns in the primate brain, via high-density exon junction array profiling of brain transcriptomes of humans, chimpanzees and rhesus macaques. We identified 509 genes showing splicing differences among these species. RT–PCR analysis of 40 exons confirmed the predicted splicing evolution of 33 exons. Of these 33 exons, outgroup analysis using rhesus macaques confirmed 13 exons with human-specific increase or decrease in transcript inclusion levels after humans diverged from chimpanzees. Some of the human-specific brain splicing patterns disrupt domains critical for protein–protein interactions, and some modulate translational efficiency of their host genes. Strikingly, for exons showing splicing differences across species, we observed a significant increase in the rate of silent substitutions within exons, coupled with accelerated sequence divergence in flanking introns. This indicates that evolution of cis-regulatory signals is a major contributor to the emergence of human-specific splicing patterns. In one gene (MAGOH), using minigene reporter assays, we demonstrated that the combination of two human-specific cis-sequence changes created its human-specific splicing pattern. Together, our data reveal widespread human-specific changes of alternative splicing in the brain and suggest an important role of splicing in the evolution of neuronal gene regulation and functions.
Regulation of TNF mediated antiapoptotic signaling in human neutrophils: role of PKC and ERK1/2.

Rakesh K Mishra¹, Ritu Kulshrestha², S.K.Chhabra³, S.K.Bansal¹

Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas-077755¹, Department of Pathology², Department of Pulmonary Respiratory Physiology, 1Department of Biochemistry, V. P. Chest Institute, University of Delhi, Delhi-110007, India³

Abstract:

TNF is implicated in the suppression of neutrophil apoptosis during sepsis. Multiple signaling pathways are involved in TNF-mediated anti apoptotic signaling; a role for the MAP kinases (MAPK), ERK1/2, and p38 MAPK has been suggested. Anti apoptotic signaling is mediated principally through TNF receptor-1 (TNFR-1), and the PKC isotype-delta (PKC) is a critical regulator of TNFR-1 signaling. PKC associates with TNFR-1 in response to TNF and is required for NF_B activation and inhibition of caspase 3. The role of PKC in TNF-mediated activation of MAPK is not whether the MAPK, ERK1/2, and p38 MAPK are involved in TNF anti apoptotic signaling and whether PKC is a key regulator of MAPK activation by TNF. In human neutrophils, TNF activated both p38 MAPK and ERK1/2 principally via TNFR-1. The MEK1/2 inhibitors PD098059 and U0126, but not the p38 MAPK inhibitor SB203580, decreased TNF anti apoptotic signaling as measured by caspase 3 activity. A specific PKC antagonist, V1.1 PKC-Tat peptide, inhibited TNF-mediated ERK1/2 activation, but not p38 MAPK. ERK1/2 inhibition did not alter recruitment of PKC to TNFR-1, indicating PKC is acting upstream of ERK1/2. In HL-60 cells differentiated to a neutrophilic phenotype, PKC depletion by PKC siRNA resulted in inhibition of TNF mediated ERK1/2 activation but not p38 MAPK. Thus, ERK1/2, but not p38 MAPK, is an essential component of TNF-mediated antiapoptotic signaling. In human neutrophils, PKC is a positive regulator of ERK1/2 activation via TNFR-1 but has no role in p38 MAPK activation.
In-silico docking study of Homology Model of Mitogen-activated Protein Kinase Kinase 4 (MAP2K4/MEK4) with Genistein and its Analogs

Sankar Narayan Krishna¹, Li Xu¹, Rebecca Farmer², Xiao Ke Huang¹, Antoinette Nibbs², Karl Scheidt², Wayne Anderson³, Raymond Bergan¹.

Department of Medicine, Northwestern University, Chicago, IL 60611¹, Department of Chemistry, Northwestern University, Evanston, IL 60208², Department of Molecular Pharmacology & Biological Chemistry, Northwestern University³

Abstract:

Metastasis or spread of Prostate Cancer (PCa) to other parts of the body is the second highest cause of death due to cancer among men in the United States. Our lab has shown that 4,5,7-trihydroxyisoflavone (genistein), inhibits the initiating step of cell invasion, as well as the downstream formation of metastasis associated with PCa, by inhibiting Mitogen-activated protein kinase kinase 4 (MAP2K4/MEK4) activity. In particular, it has been shown that MEK4, a 399 amino acid protein, activates the established pro-invasion protein, p38 MAPK. MEK4 increases cell invasion and induces matrix metalloproteinase type 2 (MMP-2). Genistein inhibits MEK4 kinase activity in vitro, and MEK4-mediated signaling in intact PCa cells. In related studies, a series of genistein analogs have been synthesized and tested for their ability to inhibit prostate cell invasion, using a Boyden chamber assay system. In order to investigate the structure-activity relationship of MEK4 with genistein and synthetic analogs, an in silico model of MEK4 was constructed. Using this model, while blinded to the Boyden chamber assay results, activity prediction after virtual docking (Autodock 4.0) was done. In particular, the analogs and genistein were first segregated based on their optimal scoring binding sites on the protein target and then the Autodock estimated Inhibition constant (Ki) was determined. In silico results were then compared to Boyden chamber assay results. The similarities and differences between in silico and experimental findings and thus the potential of using this in silico modeling technique for predicting analog activity will be discussed.
The Role of Myosin Light Chain Kinase and Intestinal Barrier Dysfunction in Graft-versus-Host Disease

Sam Nalle1, Jerrold Turner1.

University of Chicago, Chicago, IL1

Abstract:

Graft-versus-host disease (GVHD) is a potentially fatal complication following bone marrow transplantation (BMT) that is characterized by destruction of host epithelial tissue. The intestine is a target organ of GVHD, and due to its role of establishing a mucosal barrier against luminal adjuvants, may also be a driving force of the disease. Conditioning with irradiation and/or chemotherapy prior to BMT, as well as inflammatory cytokines and alloreactive donor cells following BMT, can damage the intestinal epithelium. We have hypothesized that this epithelial damage leads to barrier dysfunction, which is critical for GVHD pathogenesis. A key mediator of this barrier dysfunction, measured as increased paracellular permeability, may be the tight junction-associated protein myosin light chain kinase (MLCK). Inflammatory cytokines can drive expression and activation of MLCK in intestinal epithelium, leading to actomyosin contraction and barrier loss. In a mouse model of GVHD, we show increased MLCK expression and activity in intestinal epithelial cells, which correlates with increased permeability. The increased permeability also parallels weight loss and clinical symptoms of disease, suggesting an active role in GVHD development. To test this, we evaluated GVHD in MLCK-/- mice after allogeneic BMT. These mice develop markedly less severe GVHD than wild-type controls, including decreased intestinal permeability, less weight loss, fewer clinical symptoms, less histological damage, and greater survival. We then evaluated GVHD in mice expressing a constitutively active MLCK in intestinal epithelial cells. These mice have accelerated GVHD compared to wild-type controls. Taken together, these data suggest that MLCK-mediated increases in intestinal permeability can regulate global GVHD severity and provide the foundation for investigating MLCK as a non-immunosuppressive pharmacological target for reducing GVHD.
Identification of RNA Targets of the Conserved Spermatogenesis Factor, Boule.

Michael VanGompel1, Eugene Xu1.

Northwestern University, Chicago, IL1

Abstract:

Though many RNA-binding proteins have been identified in the testis, validated mRNA targets of these proteins remain scarce, and how such protein-RNA interactions mediate RNA storage and translation activation remains unclear. Boule is one such RNA-binding protein. Boule is the ancestral member of the DAZ (Deleted in Azoospermia) family, with orthologs in nearly all metazoans, and gave rise to homologs Dazl (DAZ-Like) and DAZ in vertebrates and higher primates, respectively. Boule mutations lead to a pachytene arrest in Drosophila males and C. elegans females, and human BOULE can rescue meiosis in the fly testis, suggesting a conservation of Boule meiotic function. In Drosophila, boule is thought to regulate the translation of the Cdc25 homolog twine. We have shown that Boule is not required for meiosis in mice, but instead is necessary for differentiation beyond the round spermatid stage. To further determine Boule function, we sought to identify RNA targets of Boule and any associated functions. Boule was immunoprecipitated from whole testes, and co-precipitating RNA was purified and analyzed. We detected interactions between Boule and mRNAs important for spermatid differentiation, as well as with Cdc25a mRNA, and followed up on both observations. Our data is the first evidence for a direct interaction between Boule and Cdc25 homologs, so we pursued the possibility that Dazl compensates for Boule in our mutants. To further examine how Boule regulates its spermatid RNA targets, we performed microarrays on control and knockout 24-day old testes, when defects first appear in mutants, and also on control and Boule null purified adult round spermatids. Several mRNA targets that interact with Boule are absent specifically in mutant round spermatids, suggesting that Boule is involved in RNA stability. Our results have widened the possibilities of how Boule and the DAZ family control fertility.
Mutational Analysis of HPV31 E1^E4 in the Context of Complete Viral Genomes: Effect on Late Viral Functions

Kristen Mighty¹, Laimonis Laimins¹.

Northwestern University, Chicago, IL 60611¹

Abstract:

The most abundantly expressed protein in the productive phase of the human papillomavirus (HPV) life cycle is E1^E4, yet its function is poorly understood. Previous genetic analyses found that stable keratinocyte cell lines harboring genomes with truncated E1^E4 resulted in defective viral amplification and reduction in late gene expression. To better understand the role of E1^E4 in the life cycle, we carried out a detailed mutational analysis, focusing on conserved regions among high-risk and low-risk HPVs. Stable keratinocyte cell lines harboring wild-type (WT) and mutant E1^E4 genomes were established and examined for effects on the viral life cycle. Interestingly, although the RXL motif in the central region of E4 is a putative cyclin binding domain, mutagenesis of this region does not affect viral genome amplification or late gene expression. Other E1^E4 mutants were also tested, including mutation of the conserved LLXLL and PTTP motifs; these regions were found to have no effect on late viral functions or E1^E4 protein levels. Additional genetic analyses examining the effects of a series of C-terminal truncations of E1^E4 on the viral life cycle are in progress.
The role of the ubiquitin-proteasome pathway in rhTRIM5α restriction of HIV-1

Cindy Danielson1, Thomas Hope1.

Department of Cell and Molecular Biology, Northwestern University, Chicago IL 60611

Abstract:

Background: rhTRIM5α blocks HIV-1 infection by interacting with the capsid core of the virus early after entry into the host cell. rhTRIM5α normally blocks the infection prior to reverse transcription, but proteasome inhibitors rescue reverse transcription while maintaining a block to infection. When expressed in cells, rhTRIM5α localizes to accumulations in the cytoplasm known as cytoplasmic bodies, which have been shown to stably associate with virus in the presence of proteasome inhibitors. While the proteasome thus appears to be involved in restriction, the details regarding its role remain unclear. Results: Immunofluorescent analysis of rhTRIM5α using specific ubiquitin antibodies revealed that rhTRIM5α cytoplasmic bodies contain polyubiquitinated proteins, but proteasome inhibition alters this localization. Mutant versions of rhTRIM5α were constructed to prevent involvement in the ubiquitin-proteasome pathway, and immunofluorescent analysis revealed different patterns of ubiquitination in these cytoplasmic bodies. To examine the role of the proteasome in restriction, expression of a fluorescently tagged subunit of the proteasome (LMP2-GFP) in cells expressing rhTRIM5α demonstrated relocalization of proteasomes to rhTRIM5α cytoplasmic bodies in the presence of virus or proteasome inhibitors, as well as revealed associations of proteosomes with virus in living cells. Conclusions: The ubiquitin antibody results demonstrate that ubiquitination within rhTRIM5α cytoplasmic bodies is dynamic, and analysis of rhTRIM5α mutants revealed that ubiquitination in these structures is complex, with rhTRIM5α likely playing a role in both conjugating and receiving ubiquitination. Analysis of proteasome localization showed that proteasomes are recruited to rhTRIM5α cytoplasmic bodies in the presence of virus, and that proteasomes associate with virus in living cells, which may lead to proteasomal destruction of rhTRIM5α-virus complexes.
Structural Transitions in the Centralspindlin Complex
Required for Microtubule Bundling During Cytokinesis

Erin White¹, H Raghuraman², Eduardo Perozo², Michael Glotzer¹.

Department of Molecular Genetics and Cell Biology, University of Chicago¹, Department of Biochemistry and Molecular Biology, University of Chicago²

Abstract:

At anaphase, a set of overlapping, antiparallel microtubules become bundled to form the central spindle. This structure is essential for completion of cytokinesis and can dictate the position of the division plane. Central spindle assembly is controlled by microtubule associated proteins and kinesin motors, most notably centralspindlin. Centralspindlin is comprised of two molecules each of a kinesin-6 motor protein, ZEN-4, and a Rho GTPase activating protein CYK-4. Through its targeted localization to a narrow region of microtubule overlap immediately following chromosome segregation, centralspindlin initiates central spindle assembly. Although it is known that microtubule bundling by centralspindlin requires the interaction between ZEN-4 and CYK-4, the mechanism of action of CYK-4 is not clear. CYK-4 binds ZEN-4 in the linker between the motor domain and the coiled-coil, a key region for kinesin motility. This raises the possibility that CYK-4 binding allosterically modifies the structural and/or biochemical properties of ZEN-4. Consistent with this model, CYK-4 binding decreases the rate of motility of ZEN-4 in microtubule gliding assays. To address whether CYK-4 binding induces conformational changes in ZEN-4, we used EPR spectroscopy. ZEN-4 molecules comprised of the minimal CYK-4 binding region and the coiled-coil were spin-labeled at specific positions and the distances between sites determined in the presence and absence of CYK-4. At several positions, CYK-4 did not significantly alter the distance distributions between probes on ZEN-4. However, CYK-4 binding induced dramatic conformational changes that resulted in fixed distances at several other positions in ZEN-4 that are highly variable when unbound. Combining these data with other structural considerations, we have developed a working model for the architecture of the centralspindlin complex and are examining the functional implications of these structural changes in ZEN-4 at the single-molecule level.
Identification of residues within the Linker2 region of rhesus TRIM5α that are required for retroviral restriction and cytoplasmic body localization

Jaya Sastri1, Christopher O’Connor1, Cindy Danielson2, Michael McRaven2, Edward Campbell1.

Stritch school of Medicine, Loyola University, Maywood, IL 601531, Fienberg school of Medicine, Northwestern University, Chicago, IL 606112

Abstract:

The TRIM5α protein from the rhesus monkeys (rhTRIM5α) is known to restrict HIV-1 infection following binding to specific determinants in the HIV-1 capsid. rhTRIM5α is also known to form cytoplasmic bodies in cells, though the role of these bodies in restriction remains controversial. We have identified a stretch of amino acids within the Linker 2 (L2) region which is required for the localization of a GFP-TRIM5α protein into cytoplasmic bodies. Scanning alanine mutagenesis of this region has revealed two discrete regions within L2 that are required for cytoplasmic body formation. Critically, mutations that perturb the localization of TRIM5α to cytoplasmic bodies also abrogate the ability of these proteins to restrict HIV-1 infection. This suggests that the ability of TRIM5α to localize to cytoplasmic bodies is relevant to the process of retroviral restriction. Cytoplasmic bodies may reflect an ability to multimerize that is critical for restriction. Alternatively, these bodies may allow recruitment of cofactors that are important in this process. This work was supported by K22 AI078757-01 to EC.
Determining the role of cysteine modification in regulating the binding of the MLL CXXC domain to non-methylated CpG DNA

Noah Birch¹, Laurie Risner¹, Nancy Zeleznik-Le¹.

Loyola University Chicago - Stritch School of Medicine¹

Abstract:

The Mixed Lineage Leukemia (MLL) protein can participate in leukemic fusions that necessarily retain the N-terminal CXXC domain which binds non-methylated CpG DNA. There is one cysteine, Cys1188, in this domain which does not coordinate zinc and is critically positioned on the DNA-binding surface though it does not directly interact with CpG DNA. Previous work in our lab has shown that mutation of Cys1188 to aspartate in the context of an MLL-AF9 fusion protein disrupts the ability of the CXXC domain to bind to DNA and prevents transformation in vitro and in vivo. In contrast, mutation of Cys1188 to alanine retains the CXXC domain’s ability to bind to DNA resulting in enhanced leukemogenesis in vivo. With a critical location on the DNA-binding surface and a thiol group susceptible to redox regulation, we hypothesized that the side chain of Cys1188 may be physiologically modulated to regulate DNA-binding affinity, allowing this residue to function as a molecular switch. As a preliminary test of this hypothesis, bone marrow progenitor cells were transformed with MLL-AF9 retaining the w.t. Cys1188 or a mutant C1188A which is not susceptible to post-translational modification. Several modifying agents were tested in a colony formation assay to observe the effects on transformation ability. Treatments with hydrogen peroxide or spermine nonoate showed dose-dependent cellular toxicities for w.t. and C1188A MLL-AF9 with no significant differences in colony forming ability between the two constructs. A modest dose-dependent decrease in proliferative capacity, however, was observed in w.t. MLL-AF9 cells treated with parthenolide compared to the C1188A control. This observation suggests that thiol modification within the CXXC domain may alter DNA binding affinity resulting in decreased transformation ability. We hope that our understanding of Cys1188’s role as a molecular switch will eventually allow for therapeutic targeting of the CXXC domain in MLL-associated leukemias.
CEH-28 regulates dbl-1 expression and neuroendocrine function of the M4 neuron

Kalpana Ramakrishnan¹, Paramita Ray¹, Peter Okkema¹.

Department of Biological Sciences, University of Illinois at Chicago¹

Abstract:

CEH-28 is an NK-2 family homeodomain transcription factor expressed in the M4 pharyngeal motor neuron in C. elegans. ceh-28 mutants exhibit synaptic defects in M4 that result in hyperstimulation of the target pharyngeal muscles, indicating that CEH-28 plays a role in regulating synapse assembly and function. Here we describe evidence that CEH-28 also regulates a neuroendocrine function of M4. dbl-1 encodes a BMP/TGF-β family ligand that functions in the Sma/Mab signaling pathway to regulate body size and male tail patterning. dbl-1::gfp is strongly expressed in the M4 neuron and a subset of additional pharyngeal and non-pharyngeal neurons. In comparison, dbl-1::gfp expression is specifically lost in the M4 cells of ceh-28 mutants. Further, we find that ceh-28 mutants and mutants in the Sma/Mab pathway show common morphological defects in the pharyngeal g1 gland cells, which are located adjacent to M4. Based on these findings, we hypothesize that CEH-28 is an upstream regulator of dbl-1 in M4, and that DBL-1 secreted from M4 regulates gland cell activity or morphology. The dbl-1 promoter contains several potential CEH-28 binding sites and we have begun characterizing this promoter to determine if it is directly activated by CEH-28. Our preliminary results suggest that the dbl-1 promoter contains separate regulatory sequences for expression in the pharyngeal and non pharyngeal neurons. Five-prime deletions that remove potential CEH-28 binding sites progressively reduce dbl-1::gfp expression in M4, and we are currently mutating candidate CEH-28 binding sites to test whether these sites are necessary for M4 expression.
Determining the Domain Organization of the Ioc2 subunit of the ISW1b Chromatin Remodeling Complex: The Identification of Novel Domains within Ioc2 That Distinguishes ISW1b

Lola Olufemi¹, Blaine Bartholomew¹.

Southern Illinois University, School of Medicine¹

Abstract:

ISW1 forms two distinct chromatin remodeling complexes in S. cerevisiae, ISW1a and ISW1b. ISW1a is composed of Isw1 and Ioc3 while, ISW1b is comprised of Isw1, Ioc2, and Ioc4. Isw1, functions as the catalytic subunit of the complex. Similar to Drosophila ISWI, Isw1 contains HAND, SANT, and SLIDE domains. Ioc2 has an atypical PHD motif that is often found in chromatin associated proteins and Ioc4 has a PWWP motif, a putative DNA binding domain, which is often found in enzymes that function in transcription regulation. Ioc3 has no conserved domains that have been previously characterized in S. cerevisiae; neither does it bear any similarity to proteins which their function has been defined. The objective of this project was to determine the domain organization of Ioc2 subunit of the ISW1b chromatin remodeling complex. Using mutational analysis, this work identifies regions of Ioc2 that contribute specific functions to the overall activity of the ISW1b complex. Our data identifies regions of Ioc2 that maintain ISW1b complex integrity, modulate nucleosome interaction, directionality during remodeling and spacing. When this domain of Ioc2 was mutated, it resulted in a complex that exhibited catalytic activity reminiscent of ISW1a, suggesting that this region of Ioc2 acts as the distinguishing feature between the ISW1a and ISW1b complexes. This work was supported by Public Health Service grant GM 70864 from the National Institute of Health.
βArrestin2/βArrestin1 Ratio (βA2/βA1) as Potential Molecular Risk Factor for Platelet-Activating Factor (PAF)-induced Enterocyte Apoptosis and Onset of Necrotizing Enterocolitis (NEC)

Marla Issac1, 2, Michael Caplan2, Tamas Jilling2.

Northwestern University1, Northshore University HealthSystems Research Institute2

Abstract:

Platelet-activating factor (PAF) and accelerated enterocyte apoptosis are key mediators in the onset and progression of Necrotizing Enterocolitis (NEC). In rat small intestinal epithelial cells (IEC6) PAF activates PAF receptor (PAFR), leading to an increase in apoptotic signaling. However, PAF does not induce apoptosis in other cell types including, macrophages and fibroblasts. PAFR can signal, in addition to G proteins, via the βarrestin family of proteins. We hypothesize that the βA2/βA1 mRNA and protein ratio are important factors determining PAF-induced cell death or survival and may underlie NEC susceptibility in neonatal rats. Objective: To determine if βA2/βA1 ratio determines the outcome of PAF-induced cell death or survival in vitro, and whether βA2/βA1 ratio correlates with NEC susceptibility in vivo. Methods: RNA was isolated from intestinal villi of adult female rats, mother-fed (MF), formula-fed (FF), and formula-fed with cold and asphyxia stress (FFCAS) i.e. NEC protocol, neonatal rats. RNA was also isolated from cultured IEC-6 and human embryonic kidney cells (Ad293). PAFR, βArrestin1, βArrestin2, and GAPDH mRNA levels were quantified using quantitative real time PCR. Caspase activity was assayed in cultured IEC6, Ad293, and IEC6 cells stably transfected with βArrestin2 fused to red fluorescent protein (IEC6-βArrb2RFP) Results: βA2/βA1 ratio was significantly higher in adult 0.0016±0.00015 and MF 0.00268±.0005 compared to FF 0.0003±0.00008. (p<.05,p
Androgen Excess in Female Mice Predisposes to Insulin Deficiency via AR in β-Cells

Guadalupe Navarro1, Liu Suhuan1, Franck Mauvais-Jarvis1.
Northwestern University1, Northwestern University2, Northwestern University3

Abstract:

In women, excess production of the male hormone testosterone is associated with insulin resistance and pancreatic β-cell dysfunction. Here, we hypothesized that excess testosterone exerts detrimental effects on β-cell function via acting on the androgen receptor (AR) in β-cells. We observe expression of AR in pancreatic β-cells. In order to address the role of the AR in β-cells in vivo, we generated a β-cell-specific AR deficient mouse (βARKO -/-) using the Cre-loxP strategy. βARKO -/- mice are born with the expected mendelian frequency and show no overt abnormality of energy homeostasis. Metabolic characterization was performed to ensure that AR action has not been altered in non β-cell tissues involved in energy homeostasis especially in the hypothalamus. Our first in vivo study consisted of determining the effects of excess androgen exposure on beta cell function. These mice were exposed to excess androgen using the AR selective ligand dihydrotestosterone (DHT) and β-cell stress induced by streptozotocin (STZ), respectively. Following STZ challenge, female control and βARKO -/- mice were relatively protected from STZ-induced insulin-deficient diabetes and showed minimal alteration in islet mass and pancreatic insulin concentration. Conversely, control female mice exposed to DHT became vulnerable to STZ and showed a severe predisposition to insulin-deficient diabetes with dramatic loss of β-cell mass and pancreatic insulin concentrations. We observed that the DHT-induced exacerbation of STZ-induced diabetes was significantly reduced in βARKO -/- mice. Female βARKO -/- mice exposed to DHT showed a less severe disruption in islet architecture and a more moderate decrease in β-cell mass and pancreatic insulin concentration.
Development of SRC AD2 peptides to reverse tamoxifen resistance in ER+ breast cancers

Jessica Bockhorn1, Geoffrey Greene1,2.

Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 606371, The Ben May Department for Cancer Research, University of Chicago, Chicago, IL 606372

Abstract:

Steroid receptor coactivators (SRCs) are part of a large family of coregulators that have demonstrated roles in breast cancer genesis and progression as well as tamoxifen resistance. Evidence suggests that these coactivators interact with both the ligand-binding (LBD) and aminoterminal (NTD) domains of ERα. Recent data indicate that the ERα NTD and SRCs have a prolonged interaction in tamoxifen resistant cells. It is hypothesized that the interaction between SRC and ER can overcome tamoxifen inhibition of AF-2/LBD and cause treatment resistance. Given that tamoxifen is only effective in approximately 50% of ERα positive breast cancers, these coactivators are not only a good marker for disease and treatment response, but also a possible target for drug therapy. We aim to identify the minimal region of SRC AD2 that binds ERα and develop small peptides to block SRC/ER interaction, thereby reversing tamoxifen resistance. We have used in vitro binding assays to narrow down the ER-interacting region of all three SRCs to the glutamine rich AD2 region. Mammalian two hybrid assays subsequently identified a minimal ERα NTD interaction domain. Finally, ERE-luciferase reporter assays were used to determine the effect of this minimal interacting region on ER transcriptional activity. Preliminary data show that this minimal SRC interacting region inhibits ER transcriptional activity. This work demonstrates the possibility of identifying drugs that could be used to disrupt the interaction between SRCs and ERα.
Structural basis for MLL CXXC domain protection against CpG DNA methylation and the essential role of this function in MLL-AF9 leukemia

Laurie Risner¹, Tomasz Cierpicki², Jolanta Grembecka², Stephen Lukasik², Relja Popovic¹, Monika Omonkowska², David Shultis², Nancy Zeleznik-Le¹, John Bushweller².

Loyola University Chicago, Maywood, IL 60153¹, University of Virginia, Charlottesville, VA 22904²

Abstract:

MLL is the target of chromosomal translocations which cause leukemias with poor prognosis. All leukemogenic MLL fusion proteins retain the CXXC domain which binds to nonmethylated CpG DNA. We present the solution structure of the MLL CXXC domain in complex with DNA, showing for the first time how the CXXC domain distinguishes nonmethylated from methylated CpG DNA. Based on the structure, we designed point mutations which disrupt DNA binding. Introduction of these mutations into MLL-AF9 results in increased DNA methylation of specific CpG nucleotides in Hoxa9, increased H3K9 methylation, decreased expression of Hoxa9 locus transcripts, loss of immortalization potential, and inability to induce leukemia in mice. These results establish that DNA binding by the CXXC domain and protection against DNA methylation is essential for MLL fusion leukemia. They also validate this interaction as a potential target for therapeutic intervention.
Nano-Flares for Gene and Molecular Detection in Living Cells

Dan Zheng¹, Dwight Seferos², David Giljohann¹, Chad Mirkin¹.

Northwestern University, Evanston, IL 60201¹, University of Tronto, Tronto, Canada²

Abstract:

The detection and imaging of biomolecules in living cells and organisms are of great importance for expanding the knowledge base of disease phenotypes at the molecular level as well as allowing early and accurate disease detection for a better patient outcome. High resolution temporal and spatial data on the expression of targeted genes or the level of small molecules in vitro will allow a far greater degree of understanding of the dynamic biophysical processes that occur within cells and organisms. Molecular beacons and aptamer-based probes are attractive detection and diagnostic tools, however, these probes are often difficult to transfect, require additional agents for cellular internalization, and can be unstable in cellular environments. Nanomaterials hold great promise for biology and medicine due to their unique size, properties, and ability to be functionalized with biological recognition elements. Previous research has identified several unexpected properties of high density DNA nanomaterials for cellular application, including enhanced enzymatic stability and the ability to readily enter living cells. By combining the merits of both DNA-AuNPs conjugates and the molecular beacons, we have demonstrated that a novel nano-material, termed nano-flare, can be fabricated for in vitro and in vivo visualization, detection, and quantification of biological targets. Nano-flares consist of a gold nanoparticle core functionalized with a dense monolayer of DNA with a high affinity for various molecular targets. The probes bind selectively to targets and release fluorescent reporters which indicate the presence of the analyte. Additionally, these nanoconjugates are readily taken up by cells to quantify intracellular analyte concentration. These nanoconjugates are a promising approach for the intracellular quantification of other small molecules or proteins, or as agents that use aptamer binding to elicit a biological response in living systems.
The GAP domain of CYK-4, a component of centralspindlin, contributes to RhoA activation during cytokinesis

Andy Loria1, Katrina Longhini1, Michael Glotzer1.

Department of Molecular Genetics and Cell Biology, University of Chicago1

Abstract:

Cytokinesis furrow formation is directed by two parallel pathways. The central spindle and astral microtubules serve as independent spatial cues that collaborate to ensure rapid and robust cell division. The central spindle-directed pathway involves recruitment of the critical GEF for cytokinesis, ECT-2, to the central spindle via direct binding to the phosphorylated N-terminus of the RhoGAP, CYK-4. In conjunction with the kinesin-6 motor ZEN-4, CYK-4 has a second critical role in microtubule bundling. The target of the CYK-4 RhoGAP domain has been the subject of debate. Two alleles of cyk-4 containing substitution mutations in the GAP domain of CYK-4 were recently isolated. cyk-4(or749) and (or570) embryos fail to complete cytokinesis. Canman et. al. demonstrated that depletion of CED-10/Rac1 or ARX-2/Arp2 could rescue cytokinesis in cyk-4(or749) embryos and concluded that CED-10 is the critical GTPase that is inactivated by the CYK-4 GAP domain. We have studied these alleles and found that they are defective in RhoA activation. Although depletion of CED-10 can rescue the extent of cytokinesis in cyk-4(or749) embryos, it neither rescues the reduced rate of furrow ingression nor the reduction in cortical NMY-2. These data suggest that CED-10 rescues via a bypass mechanism. Consistent with cyk-4(or749) embryos being deficient in RhoA activation, this allele is phenocopied by partial depletion of RhoA. Biochemical interaction studies support a model in which the GAP domain of CYK-4 has a direct role in activating ECT-2. We have generated integrated transgenes containing CYK-4 with an alanine substituted for the catalytic arginine residue. Both this transgene and its wild-type counterpart support central spindle assembly. However, embryos expressing the mutant form of CYK-4 and depleted of endogenous CYK-4, fail at a late stage of cytokinesis. We conclude that the GAP domain of CYK-4 has several functions including being directly involved in RhoA activation via ECT-2.
Actin Crosslinking Proteins Recognize Distinct Arrangements of Actin Filaments

David Courson¹, Ronald Rock¹.

University of Chicago, Chicago IL 60637¹

Abstract:

Self-assembly of complex structures is commonplace in biology but often poorly understood. In the case of the actin cytoskeleton, a great deal is known about the components that comprise higher order structures, such as lamellar meshes, filopodial bundles and stress fibers. Each of these cytoskeletal structures contains actin filaments and crosslinking proteins, but the role of crosslinking proteins in the initial steps of structure formation has not been clearly elucidated.

We employ an optical trapping assay to investigate the behaviors of two actin crosslinking proteins, fascin and alpha-actinin, during the first steps of structure assembly. Here we show that these proteins have distinct binding characteristics that cause them to recognize and crosslink filaments that are arranged with specific geometries. Alpha-actinin is a promiscuous crosslinker, linking filaments over all angles. It retains this flexibility after crosslinks are formed, maintaining connection even when the link is rotated. Conversely, fascin is extremely selective, only crosslinking filaments in a parallel orientation. Surprisingly, bundles formed by either protein are extremely stable, persisting for over 0.5 h in a continuous wash. However, using fluorescence recovery after photobleaching (FRAP) and fluorescence decay experiments we find that free fascin can readily displace fascin in the bundle. We present a simple avidity model for this crosslink dissociation behavior. Together, the binding and dynamics results presented here place constraints on how cytoskeletal structures assemble, organize, and disassemble in vivo.
Genetic and biochemical analysis of bacteriophage N4 gp2 and its role in N4 RNAPII transcription activation

Abby Markle1, Lucia Rothman-Denes1.

University of Chicago, Chicago IL 606371

Abstract:

Transcription of coliphage N4 middle genes is carried out by a phage-coded, heterodimeric (gp15/gp16) RNA polymerase (N4 RNAPII), belonging to the T7-like RNA polymerase family. Gp15 contains the DxxGR motif and is 145 residues shorter at its N-terminus than T7 RNAP; gp16 contains the conserved A, B, and C motifs. In contrast to T7 RNAP, N4 RNAPII displays no activity on double-stranded templates and non-specific low-level activity on single-stranded templates. Under limiting RNAPII concentrations, addition of the N4 early gene product gp2, a 128 aa sequence-independent, ssDNA-binding protein required for N4 middle transcription, is sufficient to recruit RNAPII to ssDNA. Alanine substitution mutagenesis of gp2 identified 21 single amino-acid substitutions that yielded proteins that failed to complement N4ORF2am infection in vivo. The mutant proteins were purified and assayed in vitro for their ability to bind ssDNA, interact with N4 RNAPII and activate transcription. The data revealed that the functions of gp2 are genetically separable. N-terminal substitutions abrogated RNAPII interaction and affected transcription activation but did not affect binding to ssDNA. Conversely, substitutions near W30 and F70 severely reduced ssDNA binding without affecting RNAPII interaction or transcription activation. Finally, C-terminal substitutions variably affected all three activities. To further investigate the interaction of gp2 N-terminus with RNAPII, a label-transfer strategy was employed. Gp2 mutants containing a single, surface-accessible cysteine residue were derivatized with a photoreactive, biotin-labeled crosslinker; upon incubation with and crosslinking to RNAPII, the label transferred to the gp15 subunit. Future experiments will investigate the role of gp2 in promoter recognition and in elongation. These results may inform research into the factor-dependent members of the T7 RNAP family, such as the nuclear-encoded mitochondrial RNAPs.
The Role of Microscopic Interactions for Effective Antibiotic Delivery across the Bacterial Outer Membrane.

BREGITTE ZIERVOGEL1, BALASUNDARESAN DHAKSHNAMOORTHY1, LYDIA BLACHOWICZ1, BENOIT ROUX1.

University of Chicago1

Abstract:

Outer membrane protein F (OmpF), of Gram-negative bacteria, allows diffusion of β-lactam antibiotics across the lipid barrier. The diffusion limit for translocating molecules is provided by the constriction zone, which defines both the channel diameter at the narrowest region, as well as electrostatic properties due to a unique arrangement of charged residues. Since reduced outer membrane permeability contributes to antimicrobial resistance, it is necessary to identify the role of drug-protein molecular interactions in antibiotic transfer in order to design antibiotics with improved diffusional characteristics. We have co-crystallized E. coli OmpF with various antibiotic molecules and observe the density corresponding to the antibiotic inside the OmpF pore. Results of this work give insights into how the charge distribution of the translocating molecule affects binding interactions within the OmpF constriction zone. Furthermore, functional assays and mutational analysis provide evidence that alteration of some key charged OmpF residues has an effect on bacterial cell survival. We are also using computational methods to model the pathways of diffusing antibiotics and measure their residence time in the OmpF pore. Here we are employing a new approach called the String Method with swarms-of-trajectories to study transition pathways for various zwitterionic and anionic molecules across both WT and mutant OmpF porins. Results of this work will therefore assist in the design of new antibiotics that are more effective in the treatment of bacterial infections. [Supported by NIH grant GM062342].
Paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2

Darja Bamming Pollpeter1, J.-Patrick Parisien1, Akihiko Komuro1, Aparna Ramachandran1, Jason Rodriguez1, Curt Horvath1.

Northwestern University, Evanston, IL 602081

Abstract:

Diverse members of the Paramyxovirus family of negative-strand RNA viruses effectively suppress host innate immune responses through the actions of their V proteins. The V protein mediates interference with the interferon regulatory RNA helicase MDA5 to avoid cellular antiviral responses. Analysis of the interaction interface revealed the MDA5 helicase C domain as necessary and sufficient for association with V proteins from human parainfluenza virus type 2, parainfluenza virus type 5, measles virus, mumps virus, Hendra virus, and Nipah virus. The identified approximately 130-residue region is highly homologous between MDA5 and the related antiviral helicase LGP2, but not RIG-I. Results indicate that the paramyxovirus V proteins can also associate with LGP2. The V protein interaction was found to disrupt ATP hydrolysis mediated by both MDA5 and LGP2. These findings provide a potential mechanistic basis for V protein-mediated helicase interference and identify LGP2 as a second cellular RNA helicase targeted by paramyxovirus V proteins.
Protein-phospholipid interactions in blood coagulation

Narjes Tavoosi1, Rebecca Davis-Harrison1, James Morrissey1.

Department of Biochemistry, University of Illinois at Urbana-Champaign1

Abstract:

Most steps in the blood coagulation cascade take place on membrane surfaces. Many clotting proteins bind to phosphatidylserine (PS)-containing membranes via GLA domains but it is not clear how GLA domains bind to membranes. Phosphatidylethanolamine (PE), strongly synergizes with PS to enhance factor X activation by the tissue factor-factor VIIa complex (TF:fVIIa). The mechanism of this synergy is poorly understood -- although a number of hypotheses have been put forward. Based on the results of our experiments, we now propose a new hypothesis to explain GLA domain binding to membranes which we term the ABC (Anything But Choline) hypothesis; it invokes two main types of protein-phospholipid interactions: a single L-serine-specific binding site in each GLA domain; and multiple “phosphate-specific” interactions in which the phosphate groups of non-phosphatidylcholine (PC) phospholipids interact with the tightly bound calcium ions in GLA domains. Any non-PC phospholipid that was tested, including PS with D-serine, supported much lower factor X activation rates than did PS with L-serine, but on the other hand, PS with D-serine synergized very well with L-PS to enhance factor X activation on TF-liposomes. Phosphatidylglycerol (PG) as a non-PC phospholipid synergizes with L-PS on TF-Nanodiscs as well. Biacore studies showed that factor X binds tighter to Nanodiscs when PG is present in the membrane along with L-PS. Structurally, PC is tri-N-methyl PE. To further test our hypothesis, N-Methyl PE derivatives’ ability to synergize with PS were compared. Mono- and dimethyl PE showed progressively weaker synergy with PS to enhance factor X activation by TF:fVIIa. Phosphatidic acid (PA) and diacylglycerol are only different by the presence of/absence of the phosphate group; as another test of our hypothesis, their abilities to synergize with PS were compared. PA synergized very well but diacylglycerol synergized very weakly with PS to enhance factor X activation by TF:fVIIa.
Exchanger-Ypt -Effector Module Required for Autophagy

Daivd Taussig¹, Zhanna Lipatova¹, Veena Suresh¹, XiuQi Zhang¹, Natalia Belogortseva¹, Shu Chen¹, Jane Kim¹, Yongheng Liang², Nava Segev¹.

University of Illinois at Chicago¹, Nanjing Agricultural University, Nanjing, China²

Abstract:

Autophagy, the process by which cells discard cytoplasmic proteins and organelles under stress conditions, has been implicated in multiple human diseases ranging from cancer to Alzheimer’s. This process is conserved from yeast to man and in yeast the multiple Atg proteins are required for this process. Autophagy starts with the formation of the preautophagosomal structure (PAS). However, it is not clear how PAS is formed. Ypt/Rab GTPases regulate the different steps of the intra-cellular trafficking pathways. These GTPases are activated by GEFs and when in the active form, interact with their downstream effectors. In yeast, Ypt1 GTPase regulates ER-to-Golgi transport and the TRAPPI complex acts as its GEF. We have originally shown that ypt1-1 mutant cells are defective in growth under nitrogen starvation, a hallmark of an autophagy defect. A non-essential subunit of the TRAPP complex, Trrs85, is required for autophagy. Here, we show that a module that includes a Trrs85-containing TRAPP GEF, the Ypt1 GTPase, and a Ypt1 effector Atg, functions in the assembly of PAS. First, Trrs85 interacts with TRAPPI, but not TRAPPI-specific subunits. Second, Trrs85-containing TRAPP acts as a Ypt1 GEF but not for a GTPase required for trans-Golgi-to-plasma membrane transport, Ypt32. Third, an Atg that acts as a PAS organizer under nutrient rich conditions, acts as a Ypt1 effector who’s localization to PAS depends on Ypt1. Together, these results define the first Ypt/Rab GTPase autophagy module that functions in PAS formation. Because Ypt1 and Trrs85 are conserved from yeast to humans, we propose that the Ypt/Rab-dependent autophagy module is also conserved.
DNA Methylation Analysis of Human Uterine Leiomyomas

Antonia Navarro

Northwestern University, Chicago, IL 60611

Abstract:

DNA Methylation Analysis of Human Uterine Leiomyomas

Antonia Navarro, Ping Ying, Jianjun Wei, Pan Du, Simon Lin, Serdar E. Bulun

Department of Reproductive Biology Research, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

ABSTRACT Uterine leiomyomas are the most common benign tumors of the female genital tract and affect 30% of women and up to 70% of African American women of reproductive age. The incidence of epigenetic marks such as DNA methylation in human uterine leiomyoma remains largely unknown, and to our knowledge, a genome-wide profile of DNA methylation in these benign tumors has not yet been performed. In this analysis to determine genome-wide analysis of differential DNA methylation patterns, we have quantified the DNA methylation levels of 27,578 CpG dinucleotides spanning 14,587 genes in 18 pairs of human uterine leiomyoma (ULM) and matched normal myometrium (NMM) samples using bisulfite-modified gDNA screening beadarrays. Supervised cluster analysis demonstrated significant differential DNA methylation in ULM compared to NMM. Approximately 1300 genes were identified: 734 being hypermethylated and 560 hypomethylated in uterine leiomyoma (ULM) compared to matched adjacent normal myometrium (NMM). The most differentially hypermethylated genes were DLEC1, KRT19, ALDH1A3, CDKN2B and THBS2 while the most differentially hypomethylated were KRTAP17-1, TRIM63, TPM3, HOXB3 and PCP4.
17-ß-Estradiol Induces COX-2 Expression in Endometriosis via ERβ Activity

Diana Monsivais¹, Serdar Bulun¹.

Northwestern University, Chicago, IL¹

Abstract:

Endometriosis is an estrogen-dependent disease affecting 5-10% of women of reproductive age; it is characterized by the presence of endometrial tissue outside of the uterine cavity and is associated with chronic pelvic pain, dysmenorrhea and infertility (1). It is well established that estrogen fuels the growth and survival of endometriosis; in endometriosis, ERβ mRNA expression is increased by 40-fold relative to the normal endometrium (2), thus transcriptional regulation by ERβ must play a pivotal role in this disease. Using endometriotic stromal cells isolated from patients undergoing surgical removal of endometriotic tissue, we confirmed that mRNA and protein levels of ERβ are increased relative to the normal endometrium. We performed a microarray analysis in the patient samples and identified that several genes are differentially regulated. Our microarray analysis and others (unpublished data, 3) show that genes in the prostaglandin synthesis pathway are differentially regulated in endometriosis relative to the normal endometrium. In various tissues and cell types 17-ß-Estradiol (E2) stimulates COX-2 expression, however it is unclear whether it is a genomic or non-genomic E2-mediated effect. Treatment of endometriotic stromal cells with various concentrations of TNFα and E2 elicited an increase in COX-2 expression. Since ERβ is the dominant ER subtype in endometriosis, we will determine whether it positively regulates COX-2 expression. Future experiments will identify whether COX-2 regulation by ERβ occurs by direct promoter binding and stimulation or via an indirect recruitment of transcription factors.
Platelet Activating Factor (PAF) Receptor Inhibits Intestinal Epithelial Cell Linear Migration Through its C-terminus

Marina Pazin¹, Tamas Jilling¹, Michael Caplan¹.

NorthShore University Health System, Evanston IL 60201¹

Abstract:

Although cell migration is critical for many biological processes, irregularities in this cellular function are associated with disease. Necrotizing enterocolitis, NEC, is a disease characterized by inflammation and necrosis of the intestine. Once damaged, the organ attempts self-repair through proliferation of intestinal epithelial cells at the crypt of the intestine and their migration into the denuded area. We have previously seen that activation of PAF receptor, a G-protein coupled receptor, inhibits the PI3K-AKT signaling cascade, and stops migration of rat intestinal epithelial cells (IEC6) into a wound created in a confluent monolayer. We have also observed that in a serum (FBS)-deprived medium in which migration is severely compromised, supplementation with TGFβ (1ng/ml) is able to restore migration in a focal adhesion kinase (FAK)-dependent manner. Consequently, we decided to explore the mechanisms by which PAFR activation regulated focal adhesion dynamics as well as the therapeutic potential of TGFβ in restitution of intestinal epithelium following injury. We now show that migration deficit caused by PI3K inhibition (20μM LY29004 or c-PAF) is restored by co-supplementation with TGFβ. The ability of TGFβ to support migration is dependent on calmodulin-dependent protein kinase (CaM) II. Moreover, we show that activation of PAFR leads to displacement of FAK from paxillin-positive focal adhesions. The region of PAFR responsible for FAK regulation is located within its C-terminus. Both PAFR activation and FAK inhibition lead to deactivation of AKT. We speculate that TGFβ has therapeutic potential in situations when epithelial injury is caused via mechanisms involving PI3K inhibition, such as PAF-dependent injury in intestinal inflammatory conditions.
Functions of BNIP3 in liver metabolism.

Danielle Glick1,3, Wenshuo Zhang2, Matthew Brady2, Kay Macleod1,3.

Committee on Cancer Biology, The University of Chicago1, Committee on Molecular Metabolism and Nutrition, The University of Chicago2, The Ben May Department for Cancer Research3

Abstract:

Regulation of autophagy is coordinated with metabolism in the liver and defects in autophagy lead to accumulation of damaged mitochondria, poly-ubiquitinated aggregates and lipid in the liver. The molecular mechanisms by which hepatocytes regulate autophagy in response to metabolic stress are not understood. Our work has identified BNIP3 (a hypoxia-inducible member of the Bcl-2 super-family) as a modulator of mitochondrial integrity and lipid metabolism in the liver. In contrast to most other adult mouse tissues, we have shown that BNIP3 is constitutively expressed in healthy adult liver and is induced to even higher levels by overnight fasting of mice. Furthermore, we show that targeted deletion of BNip3 in the mouse induces aberrant mitochondrial morphology, accumulation of liver triglycerides, increased cell death and infiltration of the liver by immune cells. Again, these effects were exacerbated by overnight fasting consistent with a role for BNip3 in the liver in responses to starvation. Given that autophagy, fatty acid oxidation, and gluconeogenesis are activated in response to starvation, our work assesses whether the effect of BNIP3 loss on metabolism is an indirect consequence of defective autophagy or a novel direct role for BNIP3 in metabolism.
SYMPOSIUM ATTENDEES

Chaitanya Aggarwal  
Pharmaceutical Biotech  
University of Illinois, Chicago  
1926 W. Harrison street  
Apt 1317  
Chicago, IL 60612  
Email: caggar2@uic.edu

Miranda Bernhardt  
ObGyn  
Northwestern University  
303 E. Superior St.  
Lurie 10-250  
Chicago, IL 60611  
Email: m-bernhardt@northwestern.edu

Axia Alfonso  
UIC

Mashal Almutairi  
center for pharmaceutical biotechnology  
UIC  
2310 N. harlem ave, apt # 504  
elmwood park, IL 60707  
Email: malmut3@uic.edu

Yao Bian  
Microbiology-Immunology  
Northwestern University  
320 E. Superior  
Searle 3-430  
Chicago, IL 60611  
Email: ybian@u.northwestern.edu

Irene Aninye  
Molecular & Integrative Physiology  
University of Illinois at Urbana-Champaign  
1310 Mitchem Drive  
Urbana, IL 61801  
Email: ianinye2@illinois.edu

Noah Birch  
Molecular & Cellular Biochemistry  
Loyola University Chicago - Stritch  
School of Medicine  
2160 S. First Avenue  
Bldg 112, Rm 328  
Maywood, IL 60153  
Email: nbirch@lumc.edu

Yaw Bediako  
Microbiology-Immunology  
Northwestern University  
303 E Chicago Ave  
Chicago, IL 60611  
Email: yaw@northwestern.edu

Jessica Bockhorn  
Biochemistry and Molecular Biology  
University of Chicago  
3550 N. Lake shore drive  
Apt. 2714  
Chicago, IL 60657  
Email: jbockhorn@gmail.com
Darcy Gordon  
Biology  
Illinois State University  
1513 Fell Avenue  
Bloomington, IL 61701  
Email: darcy.gordon@gmail.com

Jennifer Haick  
Biology  
Benedictine University  
2945 Wellington Ave  
Apt 206  
Lisle, IL 60532  
Email: jennifer_haick@ben.edu

Bin He  
Dept of Ecology and Evolution  
University of Chicago  
1101 E 57th St  
Chicago, IL 60637  
Email: hebin@uchicago.edu

Elizabeth Hjort  
Medicine  
Northwestern University  
303 E. Superior Ave. Rm 5-220  
Chicago, IL 60611  
Email: e-hjort@northwestern.edu

Carolyn Hollands  
Developmental Biology  
The University of Chicago  
5118 S Dorchester Ave Apt 308  
Chicago, IL 60615  
Email: chollands@uchicago.edu

Shiyuan Hong  
Microbiology  
northwestern University  
303 E Chicago Ave. Morton 6-693  
Chicago, IL 60611  
Email: shiyuan.hong@northwestern.edu

Sharon Housinger  
U of C Laboratory School

Jeffry Hoyer  
Deerfield High School

Heather Howell  
Microbiology-Immunology  
Northwestern University  
753 W. Bittersweet PL  
APT 3  
Chicago, IL 60613  
Email: HeatherHowell2010@u.northwestern.edu

Onyekachi Iroanya  
Dept of Cell Biology & Genetics  
University of Lagos  
Akoka-yaba  
Lagos, NO 115201  
Email: onyiiog@yahoo.com

Marla Issac  
IBiS  
Northwestern University  
2650 Ridge ave  
SB513  
Evanston, IL 60201  
Email: marlaissac2011@u.northwestern.edu
Amin Jahromi  
Biophysics  
506 E. Michigan St., APT# 24, Urbana, IL 61801  
Urbana, IL 61801  
Email: amin_h_j@yahoo.com  

Daniela Janevska  
Biochemistry/Molecular Biology  
Benedictine University  
9806 W 55th St.  
Countryside, IL 60525  
Email: daniela_janevska@ben.edu  

Samuel Jensen  
Neurology  
Northwestern University  
1735 W. Crystal St. Apt. 2F  
Chicago, IL 60622  
Email: samjensen@u.northwestern.edu  

Audrey Jerde  
Development, Regeneration, and Stem Cell Biology  
University of Chicago  
5841 S. Maryland Ave MC6088  
c/o Svensson lab  
Chicago, IL 60615  
Email: ajerde@uchicago.edu  

Amber Jolly  
Cell and Molecular Biology  
Northwestern University  
710 Oakton Street  
Evanston, IL 60202  
Email: amber-jolly@northwestern.edu  

Sonali Joshi  
Hematology/Oncology  
Northwestern University  
25 E Delaware Place  
Apt 1509  
Chicago, IL 60611  
Email: sonali-joshi@northwestern.edu  

Fred Kohlhapp  
Immunology  
University of Chicago  
1725 N. Honore St. apt 2R  
Chicago, IL 60622  
Email: kohlhapp@uchicago.edu  

Sarah Kopecky-Bromberg  
Abbot Laboratories  

Sankar Narayan Krishna  
Medicine  
Northwestern University  
303 E Chicago Ave, Ward 8-264  
Northwestern University  
Chicago, IL 60611  
Email: sankar-n@northwestern.edu  

Veena Krishnamoorthy  
Pathology  
University of Chicago  
5468 S Harper Avenue  
Apt # 3A  
Chicago, IL 60615  
Email: vkrishna@medicine.bsd.uchicago.edu
Breah LaSarre  
Microbiology and Immunology  
University of Illinois at Chicago  
835 S. Wolcott  
Chicago, IL 60612  
Email: blasar2@uic.edu

Kristen Lauing  
Cell Biology, Neurobiology, and Anatomy  
Loyola University Medical Center  
2160 S 1st Ave  
Building 110, Room 4244  
Maywood, IL 60546  
Email: klauing@lumc.edu

Mindy Leelawong  
Microbiology-Immunology  
Northwestern University  
303 E Chicago Ave  
Morton 3-681  
Chicago, IL 60611  
Email: m-leelawong@northwestern.edu

Sam Light  
Molecular Pharmacology and Biological Chemistry  
Northwestern University  
303 E.chicago  
Molecular Pharmacology and Biological Chemistry  
Chicago, IL 60657  
Email: samuellight2013@u.northwestern.edu

Chengyi Lin  
BMBCB  
Northwestern University  
2205 Tech Drive, Hogan 2-100  
Evanston, IL 60208  
Email: cylin@u.northwestern.edu

Kevin Little  
Medical Physics  
University of Chicago  
5339 S Harper Ave Unit 1  
Chicago, IL 60615  
Email: little@uchicago.edu

Andy Loria  
Molecular Genetics and Cell Biology  
University of Chicago  
920 East 58th Street  
Chicago, IL 60637  
Email: aloria@uchicago.edu

Jessica Lowry  
Pharmacology  
University of Illinois at Chicago  
835 S. Wolcott Ave. E403 MSB  
Chicago, IL 60612  
Email: jlowry3@uic.edu

Julia MacKenzie  
Microbiology and Immunology  
Northwestern University  
303 E. Chicago Ave. Ward 6-260  
Chicago, IL 60611  
Email: j-jackson@northwestern.edu
Rohit Malik
Molecular Pharmacology and therapeutics
Loyola University Chicago
2160 S. First Avenue
Maywood, IL 60153
Email: rmalik@lumc.edu

Joanna Mandecki
Organismal Biology and Anatomy
University of Chicago
5528 S. Hyde Park Blvd
Apt. 1202
Chicago, IL 637
Email: jmandecki@uchicago.edu

Constance Markle
Committee on Genetics
University of Chicago
6056 S Ingleside Ave
Chicago, IL 60637
Email: camarkle@uchicago.edu

Rabia Mayas
MSI- Science Director

Christine McCary
Medicine - Allergy/Immunology
Northwestern University
240 E Huron
McGaw M530
Chicago, IL 60611
Email: c-mccary@northwestern.edu

Erin McMurray
Biological Sciences
University of Illinois at Chicago
900 S Ashland
Chicago, IL 60607
Email: enhobbs@uic.edu

Kristen Mighty
Microbiology-Immunology
Northwestern University
303 E Chicago Ave
Morton 6-693, Laimins Lab
Chicago, IL 60611
Email: k-mighty@northwestern.edu

Rakesh Mishra
biochemistry and molecular biology
University of texas medical branch
515 1street,Apt#230
casa caribe
Galveston, TX 77550
Email: rakeshadams@gmail.com

Luciana Molinero
Medicine
University of Chicago
924 E. 57th St.
JFK-R302
Chicago, IL 60637
Email:
lmoliner@medicine.bsd.uchicago.edu

Diana Monsivais
Reproductive Biology
Northwestern University
303 E Superior Ave
Lurie 4-250
Chicago, IL 60611
Email: d-monsivais@northwestern.edu
Janitza Montalvo Ortiz  
Neuroscience  
Northwestern University  
1234 Elmwood Avenue  
Apt 4A  
Evanston, IL 60202  
Email: janitzaortiz2014@u.northwestern.edu  

Marueen Mullen  
Hospira Inc.  

Sam Nalle  
Pathology  
University of Chicago  
5841 S. Maryland Ave.  
AMB P513  
Chicago, IL 60637  
Email: snalle@uchicago.edu  

Antonia Navarro  
OB/GYN  
Northwestern  
1339 N. Dearborn st  
Chicago, IL 60610  
Email: antonianavarro2013@u.northwestern.edu  

Guadalupe Navarro  
Endocrinology  
Northwestern University  
300 E Superior  
Chicago, IL 60610  
Email: gnavarro@northwestern.edu  

Sonia Olikara  
Molecular Biology  
Loyola University Chicago  
2015 N Leavitt St  
Unit 3  
Chicago, IL 60647  
Email: solikara@lumc.edu  

Lola Olufemi  
School of Medicine  
Southern Illinios University  
3007 B Sunset Dr  
Carbondale, IL 62901  
Email: lolufemi@siu.edu  

Pinal Patel  
Chemistry  
Northwestern University  
2145 Sheridan Road  
Evanston, IL 60208  
Email: pinal-patel@northwestern.edu  

Marina Pazin  
Pediatrics  
Northwestern University  
2650 Ridge Ave Sb 632  
Evanston, IL 60201  
Email: marinapazin@gmail.com  

Suzanne Pfeffer  
Dept of Biochemistry, B-400  
Stanford University School of Medicine  
279 Campus Dr, MC 5307  
Stanford, CA 94305-5307  
Email: pfeffer@stanford.edu
Aaron Place
Pharmacology
835 S. Wolcott Ave. Rm. E403 MSB
Chicago, IL 60612
Email: atplace1@gmail.com

Darja Pollpeter
BMBCB
Northwestern University
2200 Campus Drive, Pancoe 4301
Evanston, IL 60208
Email: d-bamming@northwestern.edu

Sarah Prins
Cell Biology, Neurobiology and Anatomy
Loyola University Chicago
618 S Racine Ave
Chicago, IL 60607
Email: sprins@lumc.edu

Igor Rafalovich
Physiology
Northwestern
303 E Chicago Ave
Chicago, IL 60611
Email: igorrafalovich2013@u.northwestern.edu

Kalpana Ramakrishnan
Department of Biological Sciences
University of Illinois at Chicago
900 S Ashaland Avenue, MBRB 4060
Chicago, IL 60607
Email: kramak2@uic.edu

Joy Ramos
Prescott Medical Communications

Ravi Ranjan
Department of Pharmacology and Cancer Center
University of Illinois College of Medicine
909 South Wolcott Avenue Room 5100
MC 704
Chicago, IL 60612
Email: ranjan@uic.edu

Nathanael Reynolds
Pathology
3054 N. Sheffield Ave
Chicago, IL 60657
Email: n-reynolds@northwestern.edu

Maureen Richards
Microbiology-Immunology
Northwestern University
303 E Superior St
Tarry 6-718
Chicago, IL 60611
Email: maureenrichards2010@u.northwestern.edu

Laurie Risner
Molecular Biology
Loyola University Medical Center
2160 S. First Avenue, Bld 112
Cancer Center Room 328
Maywood, IL 60153
Email: irisner@lumc.edu

Syed Rizvi
Husch Blackwell Sanders LLP
Ronadel Ronquillo
BMBCB
Northwestern University
2205 Tech Drive, Hogan 2-100
Evanston, IL 60208
Email: r-ronquillo@u.northwestern.edu

Jaya Sastri
Biochemistry
Loyola University Medical Center
2160 S 1st avenue
Maywood, IL 60153
Email: jsastri@lumc.edu

Amy Sebeson
BMBCB
Northwestern University
2-100 Hogan Hall
2205 Tech Dr
Evanston, IL 60208
Email: acsebeson@gmail.com

Elizabeth Sefton
Obstetrics and Gynecology Labs
Northwestern University
303 E. Superior St
Luire 4-250
Chicago, IL 60611
Email: elizabethsefton2008@u.northwestern.edu

Shihao Shen
Biostatistics
University of Iowa
544 Hawkeye Dr
Iowa City, IA 52246
Email: shihaoshen@gmail.com

Ann Shim
MPBC
Northwestern University
745 N Fairbanks Ct.
Searle 8-417
Chicago, IL 60611
Email: a-shim@northwestern.edu

Bryan Singer
Committee on Neurobiology
University of Chicago
2651 W Thomas St
Apt 1F
Chicago, IL 60622
Email: bfsinger@uchicago.edu

Emina Stojkovic
Biology
Northeastern Illinois University
5500 N. St. Louis Ave
S-352-G
Chicago, IL 60625
Email: e-stojkovic@neiu.edu

Madhushalini Sukumar
Biological sciences
University of Illinois at Chicago
900 S Ashland Ave Rm4010
Chicago, IL 60607
Email: msukum2@uic.edu

Frances Szeto
Medicine
University of Chicago
1305 S. Michigan Avenue #904
Chicago, IL 60605
Email: fszeto@uchicago.edu
David Taussig  
Biological Sciences  
University of Illinois at Chicago  
2354 S Whipple #2F  
Chicago, IL 60623  
Email: dtauss2@uic.edu

Narjes Tavoosi  
Biochemistry  
University of Illinois at Urbana-Champaign  
415 MSB, MC-714, 506 S. Mathews  
Urbana, IL 61801  
Email: tavoosi2@illinois.edu

Joseph Tiano  
Medicine  
Northwestern University  
303 E. Chicago  
Tarry 15-762  
Chicago, IL 60611  
Email: j-tiano@northwestern.edu

Vinzenz Unger  
Northwestern

Eliza Vakana  
Cancer Center and  
Hematology/Oncology  
Northwestern University  
303 E. Superior St.  
Rm 3-220  
Chicago, IL 60610  
Email: elizavakana2013@u.northwestern.edu

Emily Vandenbroucke  
Pharmacology  
University of Illinois at Chicago  
835 S. Wolcott rm.E403 m/c868  
Chicago, IL 60612  
Email: evande3@uic.edu

Mike VanGompel  
Ob/Gyn  
Northwestern University  
303 E Superior St  
Lurie 7-250  
Chicago, IL 60611  
Email: g-van@northwestern.edu

Eric Voll  
Hematology/Oncology  
Northwestern University  
303 E Superior  
Chicago, IL 60610  
Email: e-voll@northwestern.edu

Maggie Walker  
MI  
Northwestern University  
303 E Superior ST  
Chicago, IL 60611  
Email: maggie.walker21@gmail.com

Zebin Wang  
BCMG  
university of illinois at chicago  
MBRB 2218  
chicago, IL 60607  
Email: zwang28@uic.edu
Erin White
Molecular Genetics and Cell Biology
University of Chicago
920 E. 58th St. CLSC 901
Chicago, IL 60637
Email: eawhite@uchicago.edu

Vipin Yadav
Molecular Biology
Loyola University Medical Center
2160 S. 1st Ave
Bldg 112/ Rm 310
Maywood, IL 60153
Email: vipin.yadav@gmail.com

Peng Yuan
Neuroscience
Northwestern University
2153 N Campur Dr
Evanston, IL 60208
Email: p-yuan@northwestern.edu

Dan Zheng
Chemistry
Northwestern University
2205 Campus Drive IBiS Program
Evanston, IL 60208
Email: danzheng2008@u.northwestern.edu

Brigitte Ziervogel
Biochemistry and Molecular Biology
University of Chicago
929 E 57th St
Chicago, IL 60637
Email: bkziervogel@uchicago.edu