Joint Molecular Biosciences Graduate Student Association

8th Annual Graduate Student Research Symposium

Friday March 28th 2014 9:30am - 4:30 pm

Life Sciences Building Atrium
Letter from the Organizers

Welcome to the 8th Annual Graduate Student Symposium hosted by the Joint Molecular Biosciences Graduate Student Association (JMBGSA) of Rutgers University/RWJMS. We are delighted to have you join us today to support the outstanding work that graduate students here at Rutgers/RWJMS have been producing throughout their different stages of graduate learning.

As a student organization, the goal of JMBGSA is to facilitate the professional development of graduate students, and provide them with opportunities for social interaction with their peers. By allowing them to present their work to a critical audience, graduate students are able to hone their presentation skills, and receive input on their work from faculty from various departments. With these goals in mind, we organize the annual symposium, and each year we look to the university community to make it a success.

Through this symposium, we hope to not only showcase the graduate student research, but also to provide a platform for professional interaction between students, faculty and administration. We are proud to be able to provide the avenue for students from different labs to showcase their scientific endeavors that range from kinetic studies of polymerases in a bacterial system to disease knockout models in higher eukaryotes. Through both the oral and poster presentations, we sought to introduce some of the interesting basic and translational science conducted in Rutgers/RWJMS.

We have received tremendous help and support from our peers and faculty alike in putting this symposium together. We thank our advisor Dr. Janet Alder for her help and encouragement during the planning process. We also thank the graduate student office of both schools for their tremendous support of our program.

We gratefully acknowledge the faculty members who have taken time out from their busy schedules to participate as judges and give students feedback on their work. Since this event would not be possible without the participation of our fellow-graduate students, we applaud their efforts and thank them for their poster presentations and talks. We’d like to offer a very special thank you to all our generous sponsors as well for supporting graduate student research at Rutgers-RWJMS.

Thank you for joining us today. We sincerely hope that you will enjoy the 8th Annual Graduate Student Symposium and we hope to see you next year.

Sincerely,

JMBGSA Executive Board
PROGRAM

9:30 – 9:55 am  Registration and Breakfast
9:55 – 10:00 am  Opening Remarks
10:00 – 11:00 am  Oral Presentations -I
11:00 – 12:00 pm  Poster Presentations- I
12:00 – 1:00 pm  Lunch
1:00 – 2:00 pm  Keynote Speaker

Eddy Arnold, Ph.D

“Therapeutic targeting of polymerases for treatment of HIV and influenza virus infection”

2:00 – 3:00 pm  Poster Session- II
3:00 – 4:00 pm  Oral Presentations- II
4:00 – 4:30 pm  Awards Presentation
In vivo Evidence Suggesting a Role for Bacillithiol in Iron-Sulfur Cluster Metabolism in Staphylococcus aureus

Rosario-Cruz Z, Chalal HK, Mike LA, Skaar EP and Boyd JM

Staphylococcus aureus is a human pathogen and healthcare concern worldwide. S. aureus does not produce the nearly ubiquitous low-molecular-weight thiol glutathione, but instead produces bacillithiol (BSH). To have a better understanding of the role(s) of BSH in staphylococcal metabolism, we constructed and examined BSH-deficient strains. Phenotypic analysis found that BSH-minus strains were auxotrophic for the branched chain amino acids (BCAA) leucine (Leu) and isoleucine (Ile). Further analysis found that the activity of LeuCD and IlvD, the Fe-S cluster-dependent dehydratases required for the biosynthesis of Leu and Ile, were decreased in the cellular extracts of BSH-deficient strains. BSH-deficient cells also had decreased aconitase activity suggesting that the role of BSH in Fe-S cluster metabolism is not specific to the enzymes involved in BCAA biosynthesis. We found that overexpression of Fe-S cluster trafficking systems suppressed the Leu and Ile auxotrophies as well as the decreased activity of aconitase in cells lacking BSH. Similar to strains defective in Fe-S cluster metabolism, cells lacking BSH had increased pools of non-chelated iron. These data are consistent with the hypothesis that BSH may play a role in the trafficking of Fe-S clusters in S. aureus.
in vitro have enhanced myoblast development. A coupled electrical-mechanical response can be achieved with ionic polymer gels (IPGs), which actuate in an electric field. Our laboratory has used IPGs to fabricate actuating, coaxial, conductive, and nanofibrous scaffolds for skeletal muscle regeneration. These scaffolds bend under an electric field and then relax over time. The objective of this study was to determine a configuration of IPGs and nanofibers that would lead to sustained, reversible contraction during electrical stimulation. Electrospun scaffolds were fabricated using polycaprolactone and multi-walled carbon nanotubes to improve conductivity. A combination of poly(acrylic acid) (PAA) and poly(vinyl alcohol) (PVA) with a photo-induced cross-linking agent was added to the fibrous scaffolds in different configurations and cross-linked with UVA radiation, which cross-linked both the PAA and PVA components. A banding pattern of PAA/PVA was created using a photo-mask to selectively block UVA radiation. Actuation experiments were performed by submersing scaffold sections in a saline solution and applying 20 V. The scaffolds with bands of PAA/PVA achieved contractions in excess of 62%, which is a large improvement over the previous, coaxial architecture. We hypothesize that there exists an optimum aspect ratio of the IPG sections that will cause the greatest actuation/contraction. Future experiments will conduct in vitro studies to determine how the coupled electrical-mechanical stimulation will affect muscle cell proliferation and morphology.

10:45-11:00

Optimizing Design Of A Coated Neural Probe For Single Neuron Recordings In Brain-To-Computer Interface Devices

Singh S, Lo J, Damodaran V, Kaplan H, Shreiber D, Zahn J and Kohn J

Single-unit recording probes are used extensively for signal acquisition in Brain-to-Computer Interface devices. Current probes obtain accurate measurements in the short-term, but inflammation and encapsulation of the probe via glial scarring limits long-term signal acquisition. Probes that are small and flexible can minimize injury response, at the cost of increased likelihood of mechanical failure. Our group has fabricated flexible microelectrodes and coated these probes with an ultra-fast degrading polymer which confers temporary stiffness to improve the probe’s mechanical integrity. However, the vast number of possible geometries and stiffnesses for the probe and coating makes selecting an efficacious design a difficult process.

In this study, we designed and validated a computational model to simulate insertion of a coated neural probe of varying dimensions and material properties into brain tissue. Simulation results were validated by measuring force generated by the probe upon insertion into ex vivo chick embryonic brain tissue. Our model established a probability distribution of successful insertion based on probe and coating parameters, and suggests: 1) coating thickness has the biggest influence on insertion success, and 2) probes with a coating of 75 µm or larger will insert with minimal probe failure. This model provides a powerful design tool for predicting the full range of neural probes, which will insert successfully into tissue.
KENYOTE ADDRESS

Dr. Eddy Arnold, Ph.D

Distinguished Professor and Board of Governors Professor
Department of Chemistry and Chemical Biology
Rutgers University

Dr. Arnold received his B.S., M.S., and Ph.D. in Organic Chemistry from Cornell University under the guidance of Professor Jon Clardy. He continued his postdoctoral studies at Purdue University on virus crystallography and virus structure in the laboratory of Professor Michael G. Rossmann. In 1987, Dr. Arnold joined the Center for Advanced Biotechnology and Medicine, where he and his team began working with the late Dr. Paul Janssen to create drugs to fight HIV. Dr. Janssen was a legendary drug developer and founder of Johnson & Johnson subsidiary, Janssen Pharmaceutica. Dr. Arnold’s research was essential in bringing two anti-HIV drugs to market – Edurant in 2011 and Intelence in 2008.

Dr. Arnold’s current research program focuses on developing fundamental knowledge about and new therapeutics for diseases including HIV and influenza. The ultimate goal of the his group is to develop a new class of HIV therapeutics that will be effective against the increasingly prevalent viral strains that are resistant to current drug therapies. Dr. Arnold is one of the leading HIV research specialists in the world, who in 2013, won a $6.3 million NIH grant, in addition to his second consecutive NIH MERIT Award totaling $3.8 million, an honor bestowed on fewer than 5% of investigators. This past November, the Hyacinth AIDS Foundation honored Dr. Arnold for his significant accomplishments in HIV treatment research and development, and his ongoing work on the medical problems presented by resistant strains of HIV.
Poster Presentations Abstracts
Session I
11:00-12:00 pm

Genomic Instability and G2/M Checkpoint Defect in BRCA1 RING Domain-Mutant Mouse

Hamza A, Patel AN, Misenko DS, Li SM and Bunting SF

Individuals carrying germline mutations in the BRCA1 (breast cancer 1, early onset) tumor suppressor gene have a lifetime risk of developing breast cancer of about 80%. BRCA1 is an important factor in maintaining genomic stability by mediating DNA damage repair via the homologous recombination (HR) pathway. BRCA1 has a number of conserved protein domains, including a coiled coil domain, which aids in recruitment of Rad51 to break sites, a BRCT domain and an N terminal RING (Really Interesting New Gene) domain. The impact of RING domain mutations on HR is not clear. We characterized a BRCA1-mutant mouse model, BRCA1floxed/floxed, in which conditional deletion of exon 2 excises a large portion of the RING domain. BRCA1floxed/floxed cells have a defective G2/M cell cycle checkpoint after IR-induced DNA damage. In addition, we are testing the role of the RING domain in the maintenance of genomic stability and recruitment of 53BP1 (p53 Binding Protein 1), a key regulator of the choice between alternative DNA double-strand break repair pathways in mammalian cells. By clarifying the importance of the RING domain in mediating BRCA1 activity, we aim to better understand why patient mutations in the BRCA1 gene cause cancer predisposition and identify new targets for therapeutic intervention.

Carcinoma Associated Fibroblasts in the tumor microenvironment affect growth, invasiveness, and drug response of human pancreatic cancer cells


Pancreatic cancer is one of the most aggressive malignancies, with a 5-year overall survival of less than 5%. Tumor drug resistance to conventional chemotherapy, such as gemcitabine, is often a significant contributor to poor overall survival. One of the common mechanisms of gemcitabine resistance is activation of cell signaling via increased phosphorylation of Mitogen-Activated kinase (MAP) kinases, leading to increased tumor survival and reduced sensitivity to chemotherapeutic agents. A growing body of evidence suggests that the CXCL12/CXCR4 signal transduction axis in the tumor microenvironment is an important mediator of tumor migration, growth, and drug resistance. We hypothesized that carcinoma-associated fibroblasts (CAFs), an important cellular component of the TME, play a contributory role in the growth, invasiveness, and drug response of pancreatic cancer cells (PCCs) by activating CXCL12/CXCR4-mediated signal transduction. To test this, we used an in vitro model system to study the growth, invasion, and drug response of human PCCs in the presence or absence of in vitro-generated CAFs. Functional studies demonstrated that co-culture of PCCs with CAFs led to significant increase in tumor cell invasion, which was abrogated by blockade of CXCR4 by AMD3100-plerixafor (AMD3100), a CXCR4 antagonist, and by siRNA-mediated knockdown of CXCR4 in CAFs. Increased invasion ofPCCs after co-culture with orthotopically derived CAFs was also abrogated by CXCR4 blockade, demonstrating a critical role for this receptor in regulating the tumor-promoting abilities of CAFs. Additionally, we investigated the effect of CAFs on the MAPK Signaling pathway (MEK1/2, p-MEK1/2, ERK1/2, p-ERK1/2, Akt, and p-Akt) via Western blot assays. Further, we
examined the effect of AMD3100 on the chemo-resistance of PCCs to gemcitabine. Our results indicated that AMD3100 reversed gemcitabine-mediated chemo-resistance of PCCs cells in the presence of CAFs or CAF-conditioned media. In co-culture with pancreatic cancer cells, CAFs induced activation of MAPK signaling pathways and enhanced transcription of Mn-SOD, G6PD, and catalase, genes involved in reactive oxygen species (ROS) pathways. Using phloretin, a ROS inhibitor, we observed attenuation of MAPK signaling, SOD activity, and reversal of drug resistance in CAF-exposed pancreatic cancer cells. These findings implicate CXCL12/CXCR4-dependent MAPK signaling and ROS pathways in CAF-mediated modulation of the growth, migration, and drug resistance of PCCs.

**Altering murine leukemia virus integration through disruption of BET protein interaction**


We report alterations to the murine leukemia virus (MLV) integrase (IN) protein that successfully result in decreasing its integration frequency at transcription start sites (TSS) and CpG islands, thereby reducing the potential for insertional activation. The host BET proteins Brd2, 3 and 4 interact with the MLV IN protein primarily through the BET protein ET domain. Using solution NMR, protein-interaction studies, and next generation sequencing, we show that the C-terminal tail peptide (TP) region of MLV IN is important for the interaction with BET proteins and that disruption of this interaction through truncation mutations affects the global targeting profile of MLV vectors. The use of the unstructured tails of gammaretroviral INs to direct association with complexes at active promoters parallels that used by histones and RNA polymerase II. Viruses bearing MLV IN C-terminal truncations can provide new avenues to improve the safety profile of gammaretroviral vectors for human gene therapy.

**Interaction between the TRPM7 ion channel and the signaling protein 14-3-3θ**

Cai N, Overton JD, and Runnels LW

TRPM7 is a unique channel-kinase that conducts Ca$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$. Recently, the channel has been implicated in stroke for causing Ca$^{2+}$ overload in neuron cells under ischemic conditions. The channel contains an α-kinase at the protein’s C-terminus, but the function of the kinase domain remains largely unknown. This study aims to probe the function of TRPM7 kinase domain and to determine its role in regulating the channel activity. A yeast two hybrid identified a small signaling protein 14-3-3θ as a binding partner of TRPM7. In the present study, we confirmed the binding between TRPM7 and 14-3-3θ by using in vitro biochemical analysis. We discovered that kinase-inactivated TRPM7 mutants failed to interact with 14-3-3θ, suggesting a requirement of a functional kinase domain in the interaction between TRPM7 and 14-3-3θ. In addition, we also showed that overexpression of 14-3-3θ in HEK-293 cells caused a significant reduction in TRPM7 protein levels, indicating an inhibitory role of 14-3-3θ in regulating TRPM7 expression. As 14-3-3θ is a protein that is actively involved in regulating apoptotic signals, its interaction with TRPM7 may give us clues on how the channel is involved in ischemia-induced neuronal cell death. This research will not only give critical insights into the regulation of this channel-kinase, but also shed light onto the possibility of targeting TRPM7’s kinase or its interaction with 14-3-3θ as a treatment for stroke.
Roundabout 2 acts non-cell autonomously during *Drosophila melanogaster* dorsal vessel formation

Canabal-Alvear JJ, Cheng YS and Kramer SG

Biological tubes are required for the development of complex organisms given that they distribute molecules to important tissues. In this study, we investigate an unknown function for the receptor Roundabout2 (Robo2) during lumen formation of the Drosophila dorsal vessel (DV), a simple linear tube required to pump hemolymph throughout the embryo. Two major steps are required for DV formation. First, specified cardioblasts (CBs) migrate toward the dorsal midline of the embryo and second, the CBs undergo cell shape changes to form a linear tube. The two rows of CBs are flanked on either side by two rows of non-muscle pericardial cells (PCs). CBs express a single Roundabout receptor (Robo1), while PCs express both Robo1 and Robo2. Our lab has shown that loss of Robo1 results in defects in lumen formation. This work investigates the role for Robo2 in lumen formation through loss-of-function (LOF) and gain-of-function (GOF) studies. In robo2 LOF embryos, we observe defects in lumen formation suggesting a non-cell autonomous role for Robo2 in this process. Furthermore, ectopic expression of robo2 at low levels in the CBs results in a robo LOF phenotype, while expression of Robo2 at high levels in the CBs produces a robo GOF phenotype. These results suggest that Robo2 can both antagonize as well as mimic Robo function in the DV. We are currently investigating Robo2s non-cell autonomous roles during cardiac migration and tube formation from both PCs and, unexpectedly, the overlying ectoderm by using a combination of genetic, structure function and live imaging analysis.

Notch1-Driven Sox9 Expression Mediates Motility and Invasion in NSCLC Adenocarcinoma


We sought to identify a novel target gene that mediates Notch1 signaling in non-small cell lung cancer (NSCLC). We analyzed three NSCLC microarray datasets for genes whose expression correlated significantly with HES1, a canonical Notch target gene. Transcription factor binding site analysis on the identified putative target genes demonstrated that SOX9 had multiple putative binding sites within -1000 bp upstream of the transcription start site (TSS). *In vitro* gain- and loss-of-function studies demonstrated that SOX9 responds specifically to Notch1 signaling in NSCLC adenocarcinoma cell lines. Chromatin ImmunoPrecipitation (ChIP) demonstrated that the Notch1-RBPJκ complex binds directly to the SOX9 promoter. Site-directed-mutagenesis identified the exact binding site for this complex -10 base pairs upstream of the SOX9 TSS. We demonstrated that Notch1 induces SOX9 expression independently of TGF-β signaling. Functional *in vitro* assays demonstrated that Notch1 signals through Sox9 to regulate cell morphology, motility, and invasion, and that Sox9 mediates Notch1 induced repression of E-cadherin expression. Immunohistochemistry of patient NSCLC samples revealed that Sox9 is overexpressed in the majority of NSCLCs and inversely correlated with E-cadherin expression. *In vivo* studies using the Notch1-induced lung cancer mouse model validated that Notch1 and Sox9 are coexpressed in the same cell population, and that Notch1 induces Sox9 expression *in vivo*. These data define Sox9 as a novel target of Notch1 in NSCLC which plays a key role in invasion and cell motility, in part through modulating E-cadherin levels. Sox9 inhibition may provide novel therapeutic options for NSCLC without the side effects of Notch inhibitors.
Sirtuins Mediate Cohesion of Silenced Domains in *Saccharomyces Cerevisiae*

Chen YF and Gartenberg MR

Assembly of yeast heterochromatin requires Sir2, the founding member of a conserved sirtuin family of NAD⁺-dependent protein deacetylases. Sirtuins serve as metabolic sensors involved in cellular lifespan, and they have been linked to cancer and numerous human diseases. Recently we found an unanticipated role for yeast Sir2: the protein is required for cohesion of yeast heterochromatin. Tethered Sir2 fragments mediate cohesion in the absence of other Sir proteins whereas synthetic heterochromatin domains that lack Sir2 do not mediate cohesion at all. We have mapped the domain of Sir2 that is responsible for cohesin enrichment at heterochromatic regions and we have identified point mutations in this domain that abolish heterochromatin cohesion. These Sir2 mutations bear little impact on heterochromatic repression in most standard phenotypic assays. However, de novo establishment of silencing is delayed. Whether cohesion and silencing of heterochromatin are truly coupled is discussed.

The neurotherapeutic valproic acid stimulates cell cycle machinery during embryonic rat cerebral cortex development: Potential role of HDACs

Connacher RJ, Zhou XF, and DiCicco-Bloom E

Valproic acid (VPA) is a neurotherapeutic drug commonly used to treat epilepsy, migraine, bipolar disorder and depression. However, exposure to VPA during fetal development induces brain malformations. Using embryonic day 14.5 (E14.5) Rat cerebral cortical precursors *in vitro* and *in vivo*, our lab has shown that VPA stimulates DNA synthesis and cell division and increases cell numbers. This stimulation corresponds with rapid increases in G1 cyclin proteins D3 and E as well as levels of acetylated histone 3, suggesting that VPA may promote G1-S phase transition by histone deacetylase (HDAC) inhibition. To begin defining mechanisms, we are examining HDAC expression in the cerebral cortex by RT-PCR, western blot, and immunohistochemistry. Preliminary assessment of class 1-IV HDAC message was conducted on multiple brain regions during development showing wide expression during development and some region and time specific differences. Protein level was assessed for cell cycle regulating HDAC 1 and 2, revealing differing expressions in the cell. We also confirmed that VPA inhibits HDACs in primary cells by enzyme assay, revealing dose dependent inhibition. These studies suggest multiple HDACs are present in the developing cerebral cortex and can be inhibited by VPA, although GOF and LOF studies are needed see if specific HDAC inhibition elicits VPA’s mitogenic effects.

Doc is a Kinase that inactivates Elongation Factor Tu

Cruz JW, Rothenbacher FP, Maehigashi T, Lane WS, Dunham CM and Woychik NA

Bacterial toxin-antitoxin (TA) systems are small operons which consist of a labile antitoxin and a stable toxin. The Doc toxins, derived from phd-doc TA systems, are potent inhibitors of growth. They are widespread in Archaea and Eubacteria and are found in many pathogens. Despite their important role in growth control, the target of these toxins has remained elusive. The Doc toxin from bacteriophage P1 has served as a model for the family of Doc toxins. The presence of a Fic domain, though slightly
altered in length and sequence, provided a hint to the mechanism of P1 Doc action, as most proteins with this conserved domain inactivate GTPases by addition of an adenylyl group.

We incubated pure P1 Doc protein with an *in vitro* translation system in the presence of both \([\alpha-32P]\) ATP and \([\gamma-32P]\) ATP to check for adenylyl transferase and kinase activity, respectively. We discovered that P1 Doc toxin is a kinase, which specifically targets the threonine-382 residue of the essential elongation factor EF-Tu. This modification was prevented when EF-Tu was pretreated with kirromycin.

We determined that P1 Doc toxin, containing a degenerate Fic motif, is an EF-Tu kinase. As with other Fic domain proteins, Doc targets a pivotal GTPase for inactivation through a post-translational modification at a functionally critical acceptor site. Knowledge of the target and function of this representative Doc toxin suggests that all Doc family members are kinases and presents exciting possibilities for the development of novel antibacterial therapeutics for pathogens that contain phd-doc TA systems.

**From Stop to Go: the role of a specialized elongation factor eEFSec in recoding UGA for Selenocysteine incorporation**

Dubey A, Szwed AM and Copeland PR

The incorporation of the 21st amino acid selenocysteine (Sec) into proteins requires the recoding of in-frame UGA, usually a termination codon, to a Sec codon. While it is known that Sec incorporation utilizes a dedicated set of cis and trans acting factors to synthesize selenoproteins, how this recoding event takes place at the elongating ribosome is unknown. eEFSec, the selenocystyl-specific elongation factor, is a specialized elongation factor that bears structural resemblance to the canonical elongation factor eEF1A. However, eEFSec recognizes only one species of tRNA, Sec-tRNASec, and delivers it to the ribosome only in response to an in-frame UGA in the appropriate context. To elucidate eEFSec’s role in recoding UGA to Sec, we have conducted a systematic analysis of all four eEFSec domains using chimeric eEFSec/ eEF1A proteins in our recently developed eEFSec-dependent assay. In addition, through immunoprecipitation in cell and tissue extracts, we have identified eEFSec-specific interacting proteins that may act as regulatory elements of this specialized elongation cycle. Altogether, our results shed light on the molecular and cellular mechanism of recoding a Stop codon to selenocysteine.

**Expression of Aurora kinase C splice variants in human oocytes and cumulus cells**

Fellmeth J, Treff N, and Schindler K

Accurate chromosome segregation is a requirement for the successful formation of gametes. The Aurora family of kinases plays an integral role in maintaining proper ploidy during gametogenesis by correcting erroneous attachments between kinetochores and spindle microtubules. Aurora kinases are well conserved throughout evolution (Kimura et al., 1997; Niwa et al., 1996) and were identified in human diseases and disorders from cancer to infertility (Dieterich et al., 2007; Sen et al., 1997).

In mammals, there are three Aurora homologs in humans (A, B, and C). Aurora kinase A (AURKA) localizes to the spindle poles and aids in formation of a bipolar spindle during cell division (Farruggio et al., 1999). Aurora kinase B (AURKB) localizes to the centromeres where it helps to regulate spindle-
microtubule attachments and signaling for anaphase onset (Adams et al., 2000). Aurora kinase C (AURKC) is the most recently discovered and localizes and functions similarly to Aurora kinase B, but is found mainly in gametes (Tseng et al., 1998). There are three splice variants of Aurkc differing from alternative splicing in exon 1 (Figure 1A, B)(Bernard et al., 1998; Tseng et al., 1998; Yan et al., 2005). Aurkc_v1 is the longest transcript; it encodes a protein that is 309 amino acids and has the highest level of expression in the testis relative to other tissues (Figure 1B, C)(Yan et al., 2005). Aurkc_v3 is the shortest, encoding a 275 amino acid protein, and was identified next in a screen of human placental cDNA using probes for an Aurora homolog from Xenopus laevis (Figure 1B, C)(Bernard et al., 1998). Most recently discovered was Aurkc_v2 which was found in a screen to isolate and study Aurkc_v3 from human testis (Yan et al., 2005). It encodes a 290 residue protein (Figure 1B, C). The proteins encoded by these splice variants contain successively shorter N- termini while the rest of the protein (where the catalytic domain lies) remains identical (Figure 1C). While these variants are known to exist in testis tissue, it is not known whether they are expressed in oocytes or the relevance of these truncated isoforms.

The goal of our study was to determine which variants are expressed in human oocytes. We performed qPCR on cDNA prepared from single oocytes, sperm, and cumulus cells collected at a reproductive medicine clinic using variant specific Taqman assays. We found all three variants expressed in all three tissues at varying amounts.

Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2


Hepatitis C virus (HCV) is a significant public health concern with approximately 160 million people infected worldwide. HCV infection often results in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. No vaccine is available and current therapies are not effective against all genotypes. HCV is an enveloped virus with two surface glycoproteins, E1 and E2. E2 binds to the host cell through interactions with cell surface proteins and serves as a target for neutralizing antibodies. Little is known about the molecular mechanism that mediates cell entry and membrane fusion. Here we describe the structure of the E2 core domain in complex with an antigen-binding fragment (Fab) at 2.4 Å resolution. The E2 core has a compact, globular domain structure, consisting mostly of β-strands and random coil with two small α-helices. The strands are arranged in two, perpendicular sheets (A and B), which are stabilized by an extensive hydrophobic core and disulphide bonds. Sheet A has an IgG-like fold that is characteristic of viral and cellular proteins, whereas sheet B represents a novel fold. Solution-based studies demonstrate that the full-length E2 ectodomain has a similar globular architecture and does not undergo significant conformational or oligomeric rearrangements on exposure to low pH. Thus, the IgG-like fold is the only feature that E2 shares with class II membrane fusion proteins. These results provide unprecedented insights into HCV entry and will assist in developing an HCV vaccine and new inhibitors.
The Actin Cytoskeletal Regulator Ena/VASP is Required for Proper Cardiac Tube Formation in Drosophila melanogaster

King T and Kramer S

The Drosophila dorsal vessel (DV) is comprised of two rows of cardioblasts (CBs) that migrate toward the dorsal midline, align, and change their shape, allowing them to make dorsal and ventral attachments with their contralateral partners, creating a central lumen. Slit and its receptor Roundabout are required for lumen formation in the cardiac tube by negatively regulating CB cell adhesion. Furthermore, Netrin and its receptor Frazzled/DCC also play a role in CB outgrowth and alignment. While several receptors and ligands that are important for mediating changes in CB shape have been identified, we know very little about the downstream proteins that allow for remodeling of the cytoskeleton. In this study, we investigate the role of the actin regulator protein Enabled (Ena) in DV morphogenesis. Loss of Ena in the DV results in cell shape and alignment defects. Live imaging analysis of DV formation in ena mutants shows reduced CB leading edge protrusion length as compared to WT. Furthermore, in fixed embryos, we observe gaps in the DV between contralateral CBs. These gaps occur most often between a specific genetic subtype of CBs, which express the transcription factor seven-up and form the ostia or the valves of the heart. In WT embryos, these gaps between Svp+ CBs are observed, but only transiently during the final stages of DV closure, and resolve upon closure. Together our data suggests that Ena modulates the actin cytoskeleton in order to facilitate the final closing of the DV during cardiac tube formation.

Temporally-Specific Wnt3 Secretion From Thalamocortical Axons Induces Ribosomal Composition Changes In The Developing Neocortex

Kraushar ML, Wijeratne HRS, Thompson K, Viljetic B, Hart RP and Rasin MR.

The development of the six layers of the mammalian neocortex requires coordination of both extrinsic cues with intrinsic cellular processes. At mid-neurogenesis, axons originating from the thalamus grow towards the nascent neocortex and secrete the morphogen Wingless-related MMTV integration site 3 (WNT3). Our findings show that thalamic WNT3 released at mid-neurogenesis induces changes in the protein makeup of the ribosomes and thereby regulate polysome assembly and mRNA translation in the developing neocortex. These findings may illustrate a new role of WNT signaling as a mechanism of rapid spatiotemporal control of complex developmental systems such as the neocortex.

Metabolic and photosynthetic alterations in a starchless mutant of Chlamydomonas reinhardtii

Krishnan A, Vinyard DJ, Kumاراتwamy GK, Gu H, Ananyev G, Posewitz M, and Dismukes GC

The ADP-glucose pyrophosphorylase deficient (starchless) mutant of Chlamydomonas reinhardtii (Cr), sta6, is known to accumulate lipids under nitrogen deprivation conditions, but has a lower growth rate and biomass yield under photoautotrophic conditions that offset its biotechnological value. To investigate the cause, we have probed the flux of electrons through the photosynthetic electron transport chain, adenylate-, pyridine nucleotide-, and metabolite- pool sizes by LC-MS/MS. The PSII quantum yield of charge separation in sta6 was 100% of the control strain, cw15, while the oxygen evolution rate and whole chain electron transfer (PSII+PSI) are greatly reduced. The PSII flux was restored to the
control strain levels upon addition of artificial PSII or PSI electron acceptors (DCBQ and PNDA, respectively), demonstrating blockage further downstream of the photosynthetic electron transport chain. RuBisCO protein levels were normal, but the Calvin-Benson-Basham (CBB) metabolite levels particularly RuBP, were greatly reduced, indicating insufficient substrate for carboxylation as the primary origin of reduced growth by sta6. Consistent with this loss of CO2 fixation, neither protein nor lipid content changed appreciably under nutrient replete conditions, while the precursors to lipids (malonyl-CoA) and proteins (glutamate and glutamine) significantly accumulated. Sta6 cells attempted to compensate by producing more total adenylates and pyridine nucleotides, but NAD(P)H measurements revealed much slower kinetics of reoxidation following illumination. These data reveal that the large increase in production of lipids in wild type Cr strains under nitrogen deprivation requires intact CCB flux for efficacy in addition to overexpression of the lipid assembly genes.

Engineering TPP1 (Tri-Peptidyl Peptidase I) variants with longer intra-lysosomal half-lives for effective enzyme replacement therapy to treat LINCL (Late-Infantile Neuronal Ceroid Lipofuscinosis)

Kuber N and Lobel P

TPP1 is the enzyme deficient in the genetically inherited disorder LINCL. IV administration of recombinant TPP1 has shown to improve longevity in a LINCL mouse model. Overcoming the blood brain barrier is a major hurdle in IV therapy. There is a need for effective transport of the injected TPP1 across the blood brain barrier and a variant that has a longer half-life in the lysosome. Such a variant will be a good drug candidate to treat LINCL in humans. Half-life of TPP1 can be enhanced by mutating its amino acid sequence conferring overall stability to the folded protein. There are several ways that the sequence can be mutated to stabilize secondary structures and tertiary folds. A combination of strategies will help to engineer a variant that has higher conformational stability than the currently available recombinant TPP1. Mutations in the primary structure has yielded several interesting observations. Preliminary screening techniques indicate that the variant P554I is likely to increase the intra-lysosomal half-life of TPP1 by at least two-fold. Further confirmation of this finding is pending. The success in finding a suitable TPP1 variant to treat LINCL will be unique amongst lysosomal proteins. The techniques used can be applied to other disease-producing lysosomal proteins.

A cis-element of Notch1 Regulates Gene Expression in Neural Progenitors during Spinal Cord Development

Li Y, Tzatzalos E, and Cai L

Notch1 signaling is critical for neural development. Its activity is required for the maintenance of neural stem cell properties and neural cell fate determination. Although the downstream pathway regulated by Notch1 is extensively investigated, the molecular mechanism that regulates Notch1 expression is still not fully understood. Here we report the regulatory activity of a recently identified 399 bp cis-element in the second intron of Notch1 (Notch1CR2, or CR2) during embryonic and neonatal spinal cord development. In transgenic mice, the spatiotemporal activity of CR2 was detected from embryonic day 9.5 to postnatal day 7. CR2 is preferentially active in ependymal cells, which are the stem cells in spinal cord. It is also observed in the progenitors of interneurons, especially the GABAergic interneurons. We further explored the transcription factor binding sites on CR2 by in ovo electroporation and
Electrophoretic Mobility Shift Assay (EMSA). A 140 bp sub-region shows ability to drive reporter activity and its first 32bp has potential for binding with transcription factors. In summary, CR2 regulates gene expression in neural progenitors of spinal cord, which may provide new insights into the regulatory mechanism of Notch1 expression in neural development.

**Fusogenic anti-PSMA Liposomes for Antivascular Chemotherapy**

Locke T, Gomez A, and Sofou S

Advanced solid cancer currently has no cure. Effective interventions will likely require a combination of therapies, of which antivascular therapy may be a key element. Antivascular therapy aims to kill tumor cells by cutting off blood flow, depriving tumors of nutrients needed to survive. For maximum efficiency, a therapeutic agent needs to specifically target tumor endothelial cells, preferably using a uniquely, highly expressed targeted moiety, and avoid becoming entrapped within the delivery vehicle or in an intracellular compartment. The objective of this study is to design a new class of liposomes that can target tumor vasculature, and release therapeutic agents into the cytoplasm of tumor endothelial cells. The central hypothesis of this study proposes that by choosing the Prostate-Specific Membrane Antigen (PSMA) to specifically target the tumor vasculature, and the fusion peptide GALA conjugated to specific lipid moieties - to act as a pH-sensitive means of fusing drug carrying liposomes with intracellular endosomes - better tumor killing outcomes will be observed. Preliminary findings have established that lipid-conjugated GALA embedded in a liposome forms an alpha helix in acidic conditions, triggering pH-dependent release from endosome analogs. We plan to test our hypothesis through the following specific aims: 1) to engineer liposomes containing anti-PSMA ligands and GALA in order to investigate the conditions in which the corresponding functionalities operate effectively; 2) to show that anti-PSMA fusogenic liposomes loaded with doxorubicin exhibit a) selective targeting of tumor endothelium analogs, b) effective release of doxorubicin, and 3) enhanced killing of targeted cells.

**PI3K/Akt/mTOR signaling in in vivo and in vitro traumatic brain injury**

Nikolaeva IK, Baehr L, Valenziano J, Firestein BL, Meaney DF, and D'Arcangelo G

Traumatic brain injury (TBI) is a prevalent cause of persistent neurological damage in the population. Typically, the brain suffers initial physical damage, followed by secondary damage due to the release of glutamate and other amino acids into the extracellular matrix. Previous work suggests that the PI3K/mTOR pathway is upregulated within several hours of moderate to severe TBI. This upregulation may be beneficial in that it may contribute to both glutamate clearance and axonal regrowth, but it may also produce undesirable effects such as seizures and inflammation. In this study, we confirmed an increase in PI3K/Akt/mTOR signaling within the first several hours after cortical impact in mice in vivo. Specifically, we observed significant upregulation of phosphorylation in mTORC1 downstream targets S6 and S6 kinase. To better understand the role of mTOR in the initial phase of TBI, we used an in vitro model of neuronal stretch injury to examine PI3K/Akt/mTOR signaling at different time points after injury. We also exposed cortical neurons to high levels of Glutamate to simulate the excitotoxicity that occurs in the secondary phase of in vivo TBI. Finally, we manipulated the activity of the PI3K/mTOR pathway with pharmacological compounds or gene knockdown strategy in cultured neurons to determine whether it suppresses or augments cell death in response to stretch injury or excitotoxicity. We propose that there is interplay between PI3K/Akt/mTOR activity and neuronal cell death caused by physical
injury and excitotoxicity caused by TBI. The results of these studies will inform future work aimed at identifying molecular targets to improve recovery from brain injury.
Frazzled/DCC facilitates cardiac cell outgrowth and attachment during Drosophila dorsal vessel formation

Macabenta FD, Jensen AG, Cheng Y, Kramer JJ, and Kramer SG

Drosophila embryonic dorsal vessel (DV) morphogenesis is a highly stereotyped process that involves the migration and morphogenesis of 52 pairs of cardioblasts (CBs) in order to form a linear tube. This process requires spatiotemporally-regulated localization of signaling and adhesive proteins in order to coordinate the formation of a central lumen while maintaining simultaneous adhesion between CBs. Previous studies have shown that the Slit/Roundabout and Netrin/Unc5 repulsive signaling pathways facilitate site-specific loss of adhesion between contralateral CBs in order to form a luminal space. However, the concomitant mechanism by which attraction initiates CB outgrowth and discrete localization of adhesive proteins remains poorly understood. Here we provide genetic evidence that Netrin signals through DCC (Deleted in Colorectal Carcinoma)/UNC-40/Frazzled (Fra) to mediate CB outgrowth and attachment and that this function occurs prior to and independently of Netrin/UNC-5 signaling. fra mRNA is expressed in the CBs prior to and during DV morphogenesis. Loss-of-fra-function results in significant defects in cell shape and alignment between contralateral CB rows. In addition, CB outgrowth and attachment is impaired in both fra loss- and gain-of-function mutants. Deletion of both Netrin genes (NetA and NetB) results in CB attachment phenotypes similar to fra mutants. Similar defects are also seen when both fra and unc5 are deleted. Finally we show that Fra accumulates at dorsal and ventral leading edges of paired CBs, and this localization is dependent upon Netrin. We propose that while repulsive guidance mechanisms contribute to lumen formation by preventing luminal domains from coming together, site-specific Netrin/Frazzled signaling mediates CB attachment.

A Label-free, Optical Scatter Imaging Technique to Detect Bax/Bak Mediated Apoptosis

Naser M, Graham MT, Pierre K, Hacihaliloglu I and Boustany NN

In this work we propose image processing based textural features for light scattering signals to facilitate the label-free monitoring of apoptosis. These features quantify the morphological changes cells go through during mitochondria-mediated apoptosis. Our primary investigation shows that these features can differentiate between isogenic apoptosis-competent cells expressing Bax and Bak, and Bax/Bak null apoptosis-resistant immortalized baby mouse kidney (iBMK) cells subjected to an apoptosis stimulus. Unlike biomolecular marker based TUNEL and annexin V assays or caspase activity based immunofluorescence techniques, label-free monitoring such as this can greatly facilitate and increase the throughput of apoptosis assays.
Translational Regulation Of Trpm7 Channel-Kinase Expression By Mg2+

Nikonorova I, and Ryazanov A

TRPM7 (Transient Receptor Potential Melastatin 7) is an essential and ubiquitous protein, comprising an ion channel and an alpha-kinase domain within one molecule. It plays an important role in maintaining magnesium homeostasis in the cell as well as in the organism. However, the amounts of TRPM7 protein are negligible, whereas TRPM7 mRNA levels are comparable to most abundant cellular proteins.

Results: We discovered that expression of TRPM7 is regulated at the level of translation by two upstream open reading frames (uORFs) within its 5’UTR. We employed a mammalian in vitro translation system to analyze the role of these two frames in TRPM7 translational regulation. The frames have 2 major effects: (1) they drastically inhibit its translation in general and (2) they promote its optimal translation at reduced magnesium concentrations. Based on our results, we propose a mechanism of how these two uORFs work synergistically to ensure TRPM7 protein expression under conditions of decreased magnesium concentration. To our knowledge this is the first reported instance where translation of a eukaryotic ion channel is shown to be regulated by magnesium level.

Developmental vulnerability of rat hippocampal neural stem cells to the neurotoxicant methylmercury (MeHg)

Obiorah M, Sokolowski K, and DiCicco-Bloom E.

The developing brain is sensitive to environmental neurotoxicants such as methyl-mercury(MeHg), to which humans are exposed via tainted seafood. Studies implicate association of perinatal exposure with learning, memory and IQ deficits in children, suggesting hippocampal dysfunction. Using single cell analysis, we showed that after 24 hrs, a single, low exposure (0.6μg/g) adversely affect neurogenesis in postnatal day 7 (P7) rat hippocampus (comparable to 3rd trimester in humans), a period of major neural stem cell (NSC) proliferation. NSC proliferation continues but wanes during prepubescence(P14) and adolescence (P21). However, the period of developmental vulnerability and lasting consequences of exposure on future neurogenesis remain undefined.

To define developmental vulnerability, we injected s.c P14 and P21 rats once with a low or high (5μg/g) MeHg dose and sacrificed 24h later, pulsing with S-phase marker EdU 2hbefore sacrifice. S-phase cells at P14 were not vulnerable to the low dose, but the high dose reduced mitotic cells in the dentate gyrus hilus by 29% without inducing cell death (cleaved caspase3) or altering the size of the intermediate neural progenitor pool (Tbr2+cells). These results indicate that P14 rats retain some vulnerability to MeHg. In contrast, P21 exposure (24 or 48 h) had no effect on S-phase cells or apoptosis at either time point. To determine long-term effects of early exposure, P7 rats were injected once with MeHg and assessed with BrdU immunolabeling at P21. Both low and high exposures reduced S-phase cells by 24% and 33% (respectively) at P21, suggesting a lasting deficit in the NSC pool. Consistent with reduced mitosis, preliminary studies suggest immature Dcx+ neurons are reduced by 20%, suggesting decreased neurogenesis. These studies will provide insights into vulnerable periods in hippocampal neurogenesis that may inform guidelines for seafood consumption in humans.
The role of nAChR N398 genetic variant in nicotine addiction


Addictive drugs such as nicotine mediate reward /reinforcing mechanisms within the mesolimbic pathway involving midbrain dopaminergic (DA) neurons via nicotinic acetylcholine receptors (nAChRs). Previously, genome-wide association analyses (GWAs) identified nucleotide polymorphisms (SNP) associated with increased risk for addictive phenotypes including the N398 SNP variation in the CHRNA5 gene (Beirut et al., 2008). Expression studies in mice and Xenopus showed increased nicotine consumption and a reduction in Ca2+ permeability suggesting a role for the a5 subunit of nAChR in addictive behavior (Changeux, 2010; Fowler et al., 2011). Given the evolutionary distance between mice and humans, identifying extracellular factors that affect addiction may correlate poorly, limiting the conclusiveness of these studies. We hypothesize that the N398 SNP causes modifications in nicotine-stimulated Ca2+ influx altering cellular events including neurotransmitter release. In our study, induced pluripotent stem cell (iPSC) lines were derived from cryopreserved lymphocytes from subjects with N398 SNP together with age and gender matched unaffected controls. Functional DA neurons were induced from these iPSC lines via neuronal induction protocol adapted from Kriks et al., 2012. Gene ontology (GO) analysis from total RNAsequencing and calcium imaging analysis revealed increased enrichment of genes associated with neuroactive ligand-receptor interactions, calcium signaling pathways and an increased network activity in N398 neurons. Interestingly, with the addition of 3mM of nicotine, neurons derived from control subjects exhibited less potentiation than N398 genotype. Together, these results suggest that the N398 SNP affects Ca2+-signaling in neurons, which may explain the predisposition of subjects carrying this mutation for addictive behavior.

Discovery of Novel Triazole-based Inhibitors of the Proliferation of Human Multiple Myeloma Cells

Peng Y, Avrutik JF, Mundra JJ, Howells RD, and Welsh WJ

Multiple myeloma (MM) is an invasive neoplasm derived from B-lymphocytes that proliferates in the bone marrow, accounting for 10% of all hematological malignancies and 1% of all cancers. In 2011, the American Cancer Society estimated that 21,700 people were diagnosed with MM, and 10,710 died of the disease. With treatment, the median survival of patients with MM is 3-5 years and the 5-year survival estimate is 40%. Obviously there is a compelling need for new, effective treatment options.

Naltrindole is a non-peptide delta opioid receptor-selective antagonist derived from naltrexone. Naltrindole also acts as a potent immunosuppressant in the allogeneic mixed lymphocyte reaction and in inhibiting renal graft rejection, and it was demonstrated that a non-opioid receptor target was involved in the immunosuppressant activity of naltrindole. In a recent report (Mundra et al., JPET, 342: 273-287, 2012), naltrindole inhibited human MM cell proliferation in vitro and in a murine xenograft model in vivo, by interaction with a non-opioid receptor target.

Molecular modeling of the naltrindole pharmacophore led to the synthesis of a series of tri-substituted triazoles that are novel, small molecule delta opioid receptor ligands (DOPs, see Peng et al., Bioorg. Med. Chem., 17: 6442-6450, 2009). In the present study, it was discovered that DOPs competitively
displace specific $^3$H-naltrindole binding to a non-opioid receptor binding site expressed in human U266 MM cells, and the DOPs also inhibit MM cell growth, like naltrindole. Of the DOP ligands tested thus far, the rank order of affinity for displacement of specific $^3$H-naltrindole binding was DOP 126>DOP 402=naltrindole>DOP 108>DOP 121. To assess their efficacy toward inhibition of cell growth, MM cells were incubated at 37°C for 72 h in the presence and absence of various concentrations ranging from 0.01 to 100 mM of naltrindole and the DOP ligands. The rank order for inhibition of MM cell growth was DOP 900>DOP 132>DOP 126>DOP 402=naltrindole>DOP 121>DOP 108. The EC$_{50}$ of DOP 900 was 360 nM, which was 61-fold lower than the EC$_{50}$ of naltrindole. This study provides a rationale basis for further evaluation of DOP compounds as anti-MM therapeutic agents.

Quantifying The Structure-Function Relationship Of Astrocytes Under Induced Pathophysiological Conditions Of The Central Nervous System (CNS).

Kamau P, Ahmed KI, and Boustany NN

In response to CNS injury or disease, astrocytes undergo a unique transformation known as astrogliosis which is characterized by functional, molecular, and structural changes. While this transition of astrocytes to a “reactive” form is the hallmark of CNS pathophysiology, various aspects of this transformation are still not entirely understood. Our work is premised upon the utilization of an Optical Scatter Imaging (OSI) modality (based upon Fourier filtering) to detect and quantify the structural changes of reactive astrocytes and to correlate these changes with their functionality in the post-injury CNS environment. The morphological changes associated with astrogliosis were induced in vitro by treating astrocytes with dbcAMP and structure encoded images of the cells were acquired via the implementation of an optical Gabor filtering scheme within our imaging setup. Morphometric measures such as the size and degree of orientation of sub-cellular structures were extracted from these images and utilized to quantify the morphological changes of these reactive cells.

Structural Characterization of the Extracellular Domain of CASPR2 and its Association with TAG-1


Autism spectrum disorder (ASD) is a neurodevelopmental disorder that affects brain development, social and communication skills. Genetic studies point to the role of synaptic adhesion proteins in the pathogenesis of ASD. Most of these genes are important in controlling synaptic function, neuronal migration and connectivity, and synaptic recognition patterns in brain development. Genetic variations in the CANTNAP2 gene, encoding for contactin-associated protein-like 2 (CASPR2), have been implicated in a variety of neurodevelopmental disorders. We compared the cellular localization and glycosylation processing of WT CASPR2 to twelve point mutations identified in individuals with ASD. Using immunofluorescence confocal microscopy, we found that CASPR2 mutants showed various degree of endoplasmic reticulum retention and activation of the unfolded protein response. Using immunoprecipitation techniques we investigated the role of the mutations in the association with its known binding partner, contactin-2(TAG-1). It was possible to measure their interaction when both proteins are co-transfected but not when transfected independently, suggesting that the interaction happens co-translationally or that another protein mediates this interaction. Finally, we studied the overall architecture of the extracellular domain of CASPR2 using a combination of biophysical techniques. We determined that the extracellular domain of CASPR2 is monomeric in solution and that
has a characteristic conformation that differs substantially from the structure of the homologous $\alpha$-neurexin that we have solved previously. Taken together, these results give us insights into the subcellular localization of the full length CASPR2, the structure of its extracellular domain and its binding properties with TAG-1.

**Optimizing Design of a Coated Neural Probe for Single Neuron Recordings in Brain-to-computer Interface Devices**

Singh S, Lo J, Damodaran V, Kaplan H, Shreiber D, Zahn J, and Kohn J

Single-unit recording probes are used extensively for signal acquisition in Brain-to-Computer Interface devices. Current probes obtain accurate measurements in the short-term, but inflammation and encapsulation of the probe via glial scarring limits long-term signal acquisition. Probes that are small and flexible can minimize injury response, at the cost of increased likelihood of mechanical failure. Our group has fabricated flexible microelectrodes and coated these probes with an ultra-fast degrading polymer, which confers temporary stiffness to improve the probe’s mechanical integrity. However, the vast number of possible geometries and stiffnesses for the probe and coating makes selecting an efficacious design a difficult process. In this study, we designed and validated a computational model to simulate insertion of a coated neural probe of varying dimensions and material properties into brain tissue. Simulation results were validated by measuring force generated by the probe upon insertion into ex vivo chick embryonic brain tissue. Our model established a probability distribution of successful insertion based on probe and coating parameters, and suggests: 1) coating thickness has the biggest influence on insertion success, and 2) probes with a coating of 75 $\mu$m or larger will insert with minimal probe failure. This model provides a powerful design tool for predicting the full range of neural probes, which will insert successfully into tissue.

**Tunable HER2-targeted pH-responsive Liposomes: Potential for Selective Targeting and Killing of Cancer Cells with Low or Too Low HER2 Expression**

Sempkowski M and Sofou S

HER2-targeted carriers of chemotherapeutics have the potential to reduce the severity of side effects associated with traditional chemotherapeutics, yet they are still limited by the level of HER2 expression on the surface of cancer cells. HER2 expression is inherently variable between cancer cells comprising the same solid tumor, and cancer cells from metastases of the same patient, effectively decreasing the efficacy of HER2-targeted therapies. Here we utilize pH-responsive liposomes to induce local multivalency ('very sticky patches') on the surface of liposomes containing antiHER2-lipopeptides. This is enabled by inducing preferential partitioning of lipopeptides into lipid raft-like domains that are triggered to form on the liposome membrane at acidic pH values (6.7 ph).
Tunable Liposomal Cisplatin for Improved Interstitial Transport Within Solid Tumor Analogues

Sempkowski M and Sofou S

Triple negative breast cancer (TNBC) is a subgroup of breast cancer that lacks gene expression for estrogen receptor (ER), progesterone receptor (PR) and Her2/neu. In terms of treatment, this is a challenge in that therapies used in other types of breast cancer that express these receptors would be relatively ineffective in TNBC. To enable selective and effective treatment of TNBC solid tumors, we study a drug delivery carrier of cisplatin (CDDP) - a clinically accepted major line of therapy for TNBC - that combines enhanced uptake as well as potentially deep penetration and homogeneous distribution of the drug within tumors. The carrier we study is a tunable (pH-sensitive) liposome encapsulating cisplatin. These tunable liposomes are expected to be injected into the blood stream and enter the tumor exploiting the enhanced permeability and retention (EPR) effect characteristic of tumors caused by their abnormal and defective vasculature. Once these tunable liposomes are within the tumor interstitium, due to the inherent drop in pH (~6.0-6.7) from the accelerated metabolism of cancer cells, and the pH sensitivity of the liposomal membrane, the liposome membrane will phase separate becoming 'leaky' and the encapsulated drug will be released from liposomes into the tumor interstitium. The smaller size of cisplatin is expected to result in significantly greater diffusivities within the tumor interstitium - relative to the nanometer sized carrier- which should result in improved penetration and distribution profiles of cisplatin within solid tumors. Cisplatin is able to enter cells either via passive diffusion or via the copper transport protein CTR1. Cisplatin’s major molecular mechanism of action is damage of DNA caused by adduct formation, which leads to apoptosis. Tunable liposomal cisplatin circumvents high toxicities associated with administration of free cisplatin, by selectively releasing the drug within the tumor interstitium. Our studies include evaluation of the pH-responsive liposomal cisplatin in model conditions and in 3D multicellular spheroids which develop interstitial pH gradients.

Tunable Liposomal Cisplatin for Improved Interstitial Transport Within Solid Tumor Analogues

Stras S and Sofou S

Triple negative breast cancer (TNBC) is a subgroup of breast cancer that lacks gene expression for estrogen receptor (ER), progesterone receptor (PR) and Her2/neu. In terms of treatment, this is a challenge in that therapies used in other types of breast cancer that express these receptors would be relatively ineffective in TNBC. To enable selective and effective treatment of TNBC solid tumors, we study a drug delivery carrier of cisplatin (CDDP) - a clinically accepted major line of therapy for TNBC - that combines enhanced uptake as well as potentially deep penetration and homogeneous distribution of the drug within tumors. The carrier we study is a tunable (pH-sensitive) liposome encapsulating cisplatin. These tunable liposomes are expected to be injected into the blood stream and enter the tumor exploiting the enhanced permeability and retention (EPR) effect characteristic of tumors caused by their abnormal and defective vasculature. Once these tunable liposomes are within the tumor interstitium, due to the inherent drop in pH (~6.0-6.7) from the accelerated metabolism of cancer cells, and the pH sensitivity of the liposomal membrane, the liposome membrane will phase separate becoming 'leaky' and the encapsulated drug will be released from liposomes into the tumor interstitium. The smaller size of cisplatin is expected to result in significantly greater diffusivities within the tumor interstitium - relative to the nanometer sized carrier- which should result in improved penetration and distribution profiles of cisplatin within solid tumors. Cisplatin is able to enter cells either via passive diffusion or via the copper
transport protein CTR1. Cisplatin’s major molecular mechanism of action is damage of DNA caused by adduct formation, which leads to apoptosis. Tunable liposomal cisplatin circumvents high toxicities associated with administration of free cisplatin, by selectively releasing the drug within the tumor interstitium. Our studies include evaluation of the pH-responsive liposomal cisplatin in model conditions and in 3D multicellular spheroids which develop interstitial pH gradients.

**Sequence-specific RNAP-DNA interactions in transcription initiation and elongation: core recognition element (CRE)**

Vahedian-Movahed H, Zhang Y, and Ebright RH

The crystal structure of RNA polymerase open complex (RPo) indicates that RNAP core interacts with the transcription-bubble nontemplate-strand segment corresponding to positions -4 to +2 in which we designated it as core recognition element, CRE. In this study, we sought to investigate the sequence determinants, the recognition mechanism and the functional roles of CRE.

To determine whether RNAP-CRE interactions are sequence-specific, we constructed all possible nucleotide substitutions at each CRE position, and assessed effects on RNAP-DNA interaction inequilibrium binding experiments and high-salt-induced-dissociation off-rate experiments. We conclude that RNAP-CRE interactions are specific and that the consensus CRE is t/g-n-n-n-T-G.

To identify individual RNAP amino acids that mediate specificity at CRE positions +2 and +1, we constructed single Ala substitutions of RNAP residues that contact CRE positions +2 and +1, and assessed effects on RNAP-DNA interactions with promoter derivatives containing all possible nucleotide substitutions at CRE positions +2 and +1. We conclude that bR151, bD446, or bR451 mediate specificity at CRE position +2, and bW183 mediates specificity at CRE position +1.

To define the structural basis of specificity at CRE positions +1 and +2, we determined crystal structures of RPo derivatives containing all possible nucleotide substitutions at CRE positions +2 and +1. We conclude that specificity at CRE positions +2 and +1 manifests itself not only in quantitative differences in binding thermodynamics and kinetics, but also in qualitative differences in structure.

Our results establish that RNAP-CRE interactions are sequence-specific. We propose that RNAP-CRE interactions contribute to the sequence-specificity of promoter binding, promoter unwinding, promoter scrunching, and promoter escape during transcription initiation and also contribute to the sequence-specificity of pausing during transcription elongation.

**Accumulation Of Distinct Macrophage (Mp) Subpopulations In The Lung Following Nitrogen Mustard (Nm) Exposure; Contribution Of Splenic Monocytes**

Venesa A, Malaviya R, Laskin JD, and Laskin DL

NM (mechloretamine) causes lung injury and fibrosis. This is associated with MP accumulation in the tissue and the release of mediators implicated in toxicity. Herein, we assessed the phenotype and origin of MP accumulating in the lung after NM. Cells were collected by bronchoalveolar lavage (BAL) 1-28 d after i.t. saline or NM (0.125 mg/kg) administration to Wistar rats and stained with antibodies to Mac-1
(inflammatory cells) and CD43 (monocyte-derived MP). The majority (>97%) of cells from control animals were Mac-1-/CD43-, consistent with a resident alveolar MP phenotype. NM caused an increase in Mac-1+/CD43+ and Mac-1+/CD43- cells in the lung, which peaked at 3 and 7 d, respectively. By 28 d post NM, cells more closely resembled resident alveolar MP. To characterize these subpopulations, cells were analyzed for expression of pro- (CCR2, iNOS) and anti-inflammatory (IL-10) genes. NM caused a persistent increase (4-6 fold) in CCR2 expression by resident alveolar MP (Mac-1-/CD43-), with no change in iNOS or IL-10. Increased CCR2 was also observed in infiltrating Mac-1+/CD43- and Mac-1+/CD43+ cells after NM; at 3 d, expression was 2-3 greater than resident alveolar MP. CCR2 remained elevated for at least 7 d in Mac-1+/CD43+ cells. These cells also expressed increased iNOS, and to a lesser extent, IL-10, suggesting that within this population there are phenotypically distinct subsets. Splenectomized (SPX) rats were used to assess the role of the spleen as an extramedullary source of lung MP. Increased tissue damage was noted in histologic sections of SPX rats 3 d post NM; this correlated with increased Mac-1+/CD43+ cells in the lung. These studies show that NM treatment leads to infiltration of multiple inflammatory subpopulations into the lung some of which originate in the spleen. Moreover, it appears that spleen derived cells contribute to down regulating inflammation and initiating wound repair.

**Tet, the 5-methylcytosine Oxidase, is Essential in Drosophila**

Wang F, Banerji B, Minakhina S and Steward R

DNA methylation in Drosophila is controversial and recent genome wide bisulfide sequencing did not uncover any methylated cytosine. However, Drosophila has one well-conserved Tet gene, the homolog of the three vertebrate genes responsible for catalyzing 5-methylcytosine into 5-hydroxymethylcytosine. We identified Tet as an interactor of Zfrp8/PDCD2 in a yeast two-hybrid screen and confirmed the interaction of the vertebrate and Drosophila proteins by co-immunoprecipitation. We found that Tet is essential in Drosophila and that loss of one copy of Zfrp8 partially suppresses Tet lethality. Similarly, Tet dominantly suppressed position effect variegation, while PEV is not affected in the double-heterozygote Zfrp8+/+, Tet/+, suggesting that both genes have an effect on chromatin organization, but in opposite ways. Preliminary dot blot experiments using DNA extracted from larval tissues and anti-5hmC antibody suggest the existence of 5hmC in Drosophila. These results argue for the existence of DNA methylation and suggest that Tet functions together with Zfrp8 in epigenetic regulation.

**Characterization of Patient-Derived induced Pluripotent Stem Cell Conversion to Neural Stem Cells**

Williams ME and DiCicco-Bloom E.

The objective of this study was to generate neural progenitor cells (NPCs) from patient-derived induced pluripotent stem cells using a novel serum-free media as well as to characterize the cellular identity and proliferative phenotype of the induced cells. Furthermore, I also set out to study the effects of plating density on cell identity and proliferative capacity. Cellular identity was examined by using antibodies to stain for biomarkers specific to NPCs, such as Sox2, Nestin, and Pax6 and immature neurons including TuJ1 and Tau. Preliminary results suggest that I am successful in inducing the formation of NPCs from iPSCs with currently available methods; however there is a small subpopulation of cells with immature neuronal characteristics. This subpopulation of maturing NPCs appears to decrease as cell plating
density increases as evidenced by a decrease in the percentage of Tuj1+ cells decreasing from 15% to 4%. Interestingly, cell density has no effect on percentage of proliferating cells regardless of cell passage number as seen by a relatively consistent 35% EdU labeling index.

**The Polarity Protein PAR-1 Regulates Dendritic Spine Morphogenesis through Phosphorylating PSD-95**

Wu Q, DiBona VL, Bernard LP and Zhang H

The polarity protein PAR-1 plays an essential role in many cellular contexts including embryogenesis, asymmetric cell division, directional migration, and epithelial morphogenesis. Despite its known importance in different cellular processes, the role of PAR-1 in neuronal morphogenesis is less well understood. In particular, its role in the morphogenesis of dendritic spines, which are sites of excitatory synaptic inputs, has been unclear. We recently showed that PAR-1 is required for normal spine morphogenesis in hippocampal neurons. Moreover, we found that PAR-1 functions through phosphorylating the synaptic scaffolding protein PSD-95 at Ser561 in this process. Here we show that phosphorylation at this conserved Ser561 regulates an intramolecular interaction between SH3 and GK domain in PSD-95. In addition, we show that phosphorylation at Ser561 regulates the dynamics of PSD-95 synaptic targeting in live hippocampal neurons. Finally, we show that phosphorylation at Ser561 regulates the interactions between the GK domain in PSD-95 and other synaptic proteins, such as GKAP/SAPAP (Guanylate kinase-associated protein), LGN (the mammalian homologue of Pins) and SPAR (Spine-associated Rap-specific GTPase-activating protein). Together, our findings uncover a role of PAR-1 in spine morphogenesis in hippocampal neurons, through phosphorylating PSD-95 at Ser561.

**Structural and Functional Insights into Alphavirus Polyprotein Processing and Pathogenesis**

Yost SA, Shin G, Miller M., Elrod E., Grakoui AM and Marcotrigiano J

Alphaviruses, a group of positive-sense RNA viruses, are globally distributed arboviruses capable of causing rash, arthritis, encephalitis, and death in humans. The viral replication machinery consists of four nonstructural proteins (nsP1-4) produced as a single polyprotein. Processing of the polyprotein occurs in a highly regulated manner, with P2/3 cleavage influencing RNA template use during genome replication. The structure of precleavage P23 shows an extensive interface due to a ring formed by nsP3 which encircles nsP2. The P2/3 cleavage site is located at the base of a narrow cleft that is 40 Å away from the protease active site and is not readily accessible, suggesting a highly regulated cleavage. nsP3 contains a previously uncharacterized protein fold with a zinc-coordination site. Known mutations in nsP2 that result in formation of noncytopathic viruses or a temperature sensitive phenotype cluster at the nsP2/nsP3 interface. Structure-based mutations in nsP3 opposite the location of the nsP2 noncytopathic mutations prevent efficient cleavage of P23, affect RNA infectivity, and alter viral RNA production levels, highlighting the importance of the nsP2/nsP3 interaction in pathogenesis. A potential RNA-binding surface based on the location of ion-binding sites and adaptive mutations spans both nsP2 and nsP3. These results offer unexpected insights into viral protein processing and pathogenesis that may be applicable to other polyprotein-encoding, positive stranded RNA viruses such as HIV, hepatitis C virus (HCV), and Dengue virus.
Strong T Cell Receptor Mediated Signaling is not Sufficient to Induce PLZF Expression in Conventional T Cells

Zhang S and Sant’Angelo D

The transcription factor promyelocytic leukemia zinc finger (PLZF) is highly expressed in and essential for the function and development of innate T cells, including natural killer T (NKT) cells, a subset of γδ T cells (Vγ1.1Vδ6.3) and human mucosal-associated innate T (MAIT) cells. Upon activation, innate T cells respond rapidly and secrete large amounts cytokines. Studies have shown that in the absence of PLZF, NKT cells fail to release cytokines upon primary stimulation and need secondary stimulation to fully elaborate their effector functions, whereas ectopic expression of PLZF in conventional T cells results a spontaneous acquisition of an activated phenotype and rapid cytokine secretion. The activated/memory-like phenotype of innate T cells is also entirely dependent on PLZF. Therefore, it is important that PLZF expression is highly regulated and restricted to specific T cell subsets because aberrant expression of PLZF in random immune cells could lead to “hyperactivated” T cells that might cause autoimmune diseases.

Cell specific factors controlling PLZF expression have yet to be defined. Recent studies, however, suggest that a strong T cell receptor (TCR) signal can induce PLZF expression in conventional T cells. This idea appears to be consistent with the generally accepted idea that NKT cell precursors receive a strong TCR signal during their development in the thymus. In this study, we used various reporter systems in combination with different means of TCR stimulation to test if PLZF can be induced in conventional T cells. Our results showed that a strong TCR signal alone is insufficient to induce PLZF expression in conventional T cells both in vitro and in vivo. Interestingly, by using reporter mice that estimate the strength of the TCR signal, we actually find that NKT cells do not receive stronger signals than conventional T cells in the thymus. Our results show that a strong TCR signal is insufficient to induce PLZF expression in conventional T cells. This is an important finding because it reveals that PLZF expression is a highly regulated process, which requires factors beyond those directly associated with TCR signaling events, to ensure the precise expression of PLZF in innate T cell lineages.

Effect of intracellular localization of Actinium-225-loaded nanocarriers on the therapeutic efficacy of alpha-particle emitters

Zhu C and Sofou S

Due to alpha particles’ high Linear Energy Transfer (LET) of 80-120 keV/µm and short range of 40-100 µm in tissues, alpha-particle emitters are potential drug candidates for targeted anti-cancer therapies. Upon cell internalization of the radiolabeled nanocarrier, intracellular localization is an important factor that determines therapeutic efficacy. Alpha-particles are most lethal when their trajectories carry them through a cell nucleus, and the probability of this occurrence increases dramatically for an alpha-emitter that decays while directly adjacent to the nucleus. We demonstrate that while Ac225-labeled antibodies result in high delivered radioactivities, lower activities delivered via targeted liposomes can achieve similar killing efficacies. Confocal imaging of fluorescent antibodies and liposomes suggest that the intracellular trafficking routes of Ac225- loaded liposomes will carry encapsulated alpha-emitters closer to the nucleus and thus, increase the chances of an alpha particle track that crosses the organelle.
Replication fork barriers guide the integration of LTR retrotransposons in fission yeast

Jacobs JZ and Zaratiegui M

Long Terminal Repeat retrotransposable elements (LTR-RTs) are a large group of eukaryotic Transposable Elements characterized by flaking repeats in tandem orientation—the LTRs. The LTRs of these elements contains the promoter and other sequences that recruit proteins involved in the expression, replication, silencing, organization, and stability of these elements. A successful transposable element must maximize its reproductive amplification without jeopardizing its host, and several characterized LTR-RTs appear to accomplish this through the selection of integration sites away from protein coding sequences. However, despite the high relatedness of all studied LTR-RTs, a universal mechanism that explains how these parasitic elements avoid coding sequences has not been established. Through alignment of previously published de novo integration sites of the LTR-RT Tf1 from the fission yeast Schizosaccharomyces pombe, we find a strong integration preference for integration events near the binding site of Sap1, a DNA-binding protein that controls the directionality of DNA replication by causing polar fork arrest. Interestingly, we further find that Sap1 binding alone is not sufficient for efficient Tf1 targeting, and that the fork blockage, caused by Sap1 or other barrier proteins, is an important secondary requirement. We further demonstrate this effect by showing that mutations in proteins responsible for the Sap1 polar fork arrest reduce transposition rates. We propose a model where Sap1 binding sites in proximity to blocked replication forks enhance the targeting and integration efficiency of retrotransposon Tf1, and that transposition likely occurs during S phase of the cell cycle.

An RNA-seq method for defining endoribonuclease cleavage specificity identifies dual rRNA substrates for toxin MazF-mt3

Schifano JM, Vvedenskaya IO, Knoblauch JG, Ouyang M, Nickels BE and Woychik NA

Toxin-antitoxin (TA) systems are widespread in prokaryotes. Among these, the mazEF TA system encodes an endoribonucleolytic toxin, MazF, that inhibits growth by sequence-specific cleavage of single-stranded RNA. Defining the physiological targets of a MazF toxin first requires the identification of its cleavage specificity, yet the current toolkit is antiquated and limited. We describe a rapid genome-scale approach, MORE (Mapping by Overexpression of an RNase in Escherichia coli) RNA-seq, for defining the cleavage specificity of endoribonucleolytic toxins. Application of MORE RNA-seq to MazF-mt3 from Mycobacterium tuberculosis reveals two critical ribosomal targets—the essential, evolutionarily conserved helix/loop 70 of 23S rRNA and the anti-Shine-Dalgarno (aSD) sequence of 16S rRNA. Our findings support an emerging model where both rRNA and mRNA are principal targets of MazF toxins and suggest that, as in E. coli, removal of the aSD sequence by a MazF toxin modifies ribosomes to selectively translate leaderless mRNAs in M. tuberculosis.
3:30-3:45pm

Exome sequencing to identify the genetic bases for lysosomal storage diseases of unknown etiology

Wang N, Gedvilaite E, Kumar D, Donnelly R, Xing J, Sleat D and Lobel P

Lysosomes are membrane-bound, acidic eukaryotic cellular organelles. As an enzyme container, they play important roles in the degradation of macromolecules. Monogenic mutations resulting in the loss of enzyme activities in the lysosome may lead to severe health problems, such as neurodegeneration and early death. These conditions are categorized as lysosome storage diseases (LSDs). The diagnosis of LSDs is typically straightforward. However, in some cases the underlying genetic defects are unknown. Here, we performed whole exome sequencing on 14 suspected LSD cases, with the goal of finding the causal mutations in each case. From the raw sequence data, we first identified DNA variants in each individual using three variant discovery pipelines: the Genome Analysis Toolkit, LifeScope and CLC Genomics Workbench. We then used the Variant Annotation Analysis Selection Tool (VAAST) to prioritize disease-causing mutations in 848 candidate LSD genes. As a probabilistic disease gene finder, VAAST integrates allele frequency, amino acid substitution severity and conservation information into a composite likelihood framework. Different from hard filtering methods, VAAST preserves all the candidates by listing them according to their disease-causing potential. So far, a number of candidate variants have been identified, and we are performing downstream mutation validation and proteomic analyses to investigate the potential connection between candidate variants and LSDs. Our project uses various bioinformatics analyses tools to decode enormous exome sequencing data, narrowing down candidate lists and largely increasing the efficiency of downstream proteomic studies. The study results will provide valuable insights into the genetic basis of LSDs.

3:45-4:00 pm

Investigating the role of the orphan GPCR, Gpr161, in adult neural stem cells

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Brain damage from injury, stroke, and neurodegenerative diseases is irreversible, with no known treatment. Transplantation of exogenous stem cells can replace lost neurons; however, to translate these procedures to human therapeutics would require surgery and immunosuppression. Stimulating the endogenous central nervous system (CNS) stem cells is an alternate route that could circumvent these difficulties. Understanding how CNS stem cell proliferation is regulated could provide molecular targets for therapeutic strategies. G protein coupled receptors (GPCRs) is a large superfamily of integral membrane receptors whose members are targeted by 50-60% of currently available drugs, making them the most important family of pharmaceutical targets. Gpr161 is an orphan GPCR expressed in adult CNS stem cell populations. Using the vacuolated lens (vl) mouse mutant, which carries a hypomorphic allele of Gpr161, we tested the hypothesis that Gpr161 regulates adult CNS stem cells under normal conditions and post-injury. The following results support our hypothesis: (1) immunohistochemistry (IHC) demonstrated decreased stem cell marker expression in adult Gpr161vl/vlCNS stem cell regions compared to Gpr161+/+; (2) decreased proliferation was observed in neurospheres from Gpr161vl/vl compared to those isolated from Gpr161+/+; (3) Gpr161vl/vlneurospheres also displayed reduced ability to self-renew and generate more stem cells; (4) Gpr161vl/vlreduced the proliferative response of CNS
stem cells in response to traumatic brain injury (TBI) \textit{in vivo}; and lastly, (5) Gpr161 overexpression increases proliferation in neurosphere cultures. This study identifies Gpr161 as a potential therapeutic target in recruiting the endogenous CNS stem cells to treat brain damage from injury and disease.
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