Summer Undergraduate Research Program

August 7, 2008

Gerstner Sloan-Kettering
Graduate School of Biomedical Sciences
Preface

The mission of Gerstner Sloan-Kettering Graduate School of Biomedical Sciences is to advance the frontiers of knowledge by providing to gifted and creative students in an interactive, innovative, and collegial environment the education and training they need to make new discoveries in the biological sciences.

PhD Program

The goal of the Gerstner Sloan-Kettering graduate program is to train a cadre of outstanding scientists who will exploit new advances and developing fields in biomedical sciences and apply their training directly to advancing the understanding of human disease. The School offers the next generation of basic scientists a program to study the biological sciences through the lens of cancer -- while giving students the tools they will need to put them in the vanguard of research that can be applied in any area of human disease.

Summer Undergraduate Research Program

The Gerstner Sloan-Kettering Graduate School sponsors a ten-week research program for outstanding undergraduate students who are interested in pursuing a career in biomedically related sciences. The explosion in knowledge that has driven recent progress in the diagnosis and treatment of cancer reflects the vitality of laboratory science at Sloan-Kettering. Students learn from scientists who are conducting research in areas such as developmental biology, genetics, structural biology, computational biology, cellular and molecular sciences, immunology, molecular pharmacology and chemistry, among others. Students have the opportunity to:

- Obtain hands-on research experience in cutting-edge laboratories
- Interact with faculty, postdoctoral fellows, and graduate students
- Attend a weekly seminar series, with presentations by Sloan-Kettering faculty
- Attend workshops such as presentation skills, interview skills, and others
- Attend and present at works-in-progress sessions with the cohort of summer students
- Present their research at a special poster session at the end of the program

Further information about the school and its programs can be found at www.sloankettering.edu or by contacting us at gradstudies@sloankettering.edu.

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Expression of HIV-1 Gag and Production of Virus-like Particles in Mouse Cells
Camara Awkward-Rich, Nathaniel Martinez, and Marilyn Resh
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Mice provide an ideal model animal in which to study human disease given the deep understanding of their immune function, the large supply of animals with known genetic mutations, and the cell lines derived from them. However, due to multiple restrictions on viral entry, transcription, and assembly in murine cells, mice have not been available as a resource for studying HIV/AIDS. Even the expression of known cofactors in murine cells does not result in the release of infectious particles. Recently, a system has been developed that overcomes some of these barriers. Transfection of murine cells with codon optimized HIV-1 Gag-Pol resulted in the expression of Gag-Pol in these cells, and also in the release of virus like particles. Gag is the main structural protein of HIV that is responsible, among other things, for the virus’ ability to localize to and bud from the plasma membrane, and it alone is sufficient and necessary for the production of virus-like particles (VLPs). The purpose of this project was to explore the viability of this as the basis for a mouse model of HIV/AIDS. To do this, two murine fibroblast cell lines, along with a control cell line, were transfected with HIV-1 Gag in various vectors and then checked for Gag expression by means of Western Blot and immunofluorescence microscopy. The cell media was then purified and Western blotting was used to check for released VLPs. Both murine cell lines showed expression of Gag and, more importantly, efficient production and release of virus-like particles when compared to the COS-1 control cells. The results thus far indicate that this is a viable, useful model by which to study HIV assembly in murine cells and, potentially, in the animals themselves.

Temporal Mapping of Granule Cell Production in the Post-natal Mouse Cerebellum
Luis Barraza, Emilie Legue, and Alexandra Joyner
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The cerebellum (Cb) exhibits a complex morphology: along the medial-lateral axis it consists of two lateral hemispheres and a central vermis; along the anterior-posterior axis it is divided into folia. In the vermis, folia are grouped into zones: anterior, central, posterior, and nodular. Our goal is to understand how the Cb acquires its final shape. This work focuses on the timing of production of granule cells (GCs) in the different zones of the Cb since GC proliferation is critical for acquisition of foliation. Our approach is to temporally mark the precursors of GCs using genetic inducible fate mapping. This method is based on the Cre-lox system. Cre is a recombinase specific for loxP sites. By fusion with a modified estrogen receptor (ERT) that binds tamoxifen (Tm), the entry of Cre into the nucleus is temporally controlled by addition of Tm. To visualize Cre activity we use reporter genes whose transcription is blocked by a stop sequence flanked by loxP sites. Upon entry into the nucleus, Cre excises the stop sequence thereby genetically labeling the Cre-expressing cell and its descendants. To induce recombination in GC precursors we use a transgene expressing CreERT in GCs from a Math1 promoter sequence. We use the reporter line Tau_{lox-stop-lox-gfpIRESnlacZ}^lox after recombination, GFP (in the axons) and LacZ (in the nucleus) are expressed under the control of the Tau promoter in differentiated GCs. Tamoxifen induction at post-natal day 10 (P10) revealed higher production of GCs in the central zone (CZ) compared to the anterior zone (AZ). With later inductions we are determining the latest stage when GCs are produced in the CZ and the AZ. In parallel, examination of morphology and cell proliferation at the times of induction shows that GC precursors persist longer in the CZ (up to P16) than in the AZ. To test how differential timing of production of GCs is involved in the acquisition of Cb shape, we will repeat this study in mutants with foliation defects.
Regulation of Recombination by SUMO: Novel Interactions with Rad51 and Rad52 and the Effects of Sumoylation on Break-induced Recombination
Ellora Berthet, Milica Arneric, and Xiaolan Zhao
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Break induced recombination is an essential mechanism for repairing DNA lesions and must necessarily be closely regulated. Recent research has uncovered a role for SUMO (small-ubiquitin-like modifier) in this process. Additionally, the Slx5-Slx8 complex has been shown to affect the sumoylation of recombination proteins. By a two-hybrid screen, we have identified novel interactions in *Saccharomyces cerevisiae* between SUMO and two recombination proteins, Rad51 and Rad52. This allows for a potential role for SUMO in mediating the previously identified interaction of the Slx5-Slx8 complex with Rad51 and Rad52. In order to further elucidate the regulatory role of SUMO, we are currently examining the effects of sumoylation-site mutations in Rad52 and Rad59 on break-induced recombination through the use of a chromosome fragmentation vector assay. This could suggest a sumoylation-mediated model for the regulation of recombination in yeast.

The Generation of Monoclonal Antibodies Specific for the Phosphorylated Forms of the p15\textsuperscript{raf} Oncogene
Emily Casey, Gavin Porter, Jonathan Gill, Nicole Draghi, Kevin Chua, and Lisa K. Denzin
Gerstner Sloan-Kettering Graduate School, Memorial Sloan-Kettering Cancer Center, New York

The 15 kDa proliferating cell nuclear antigen (PCNA) associated factor (p15\textsuperscript{raf}) is an oncogenic protein that interacts with PCNA in the nucleus. p15\textsuperscript{raf} is ubiquitously expressed, and its overexpression has been linked to several cancers. Although association with PCNA, cellular localization, and overexpression in cancer suggests a possible role in cell proliferation, the specific functions of p15\textsuperscript{raf} have yet to be elucidated. Previous mass spectrometry analyses have determined that p15\textsuperscript{raf} is phosphorylated at two residues. We hypothesize that p15\textsuperscript{raf} phosphorylation controls p15\textsuperscript{raf}-PCNA interactions. In order to study the functional role of p15\textsuperscript{raf} phosphorylation, the development of monoclonal antibodies (mAbs) specific to the phosphorylated forms of this protein is crucial. In order to screen these antibodies for specificity, phosphorylated and non-phosphorylated versions of the protein must be generated. Mutations of p15\textsuperscript{raf} at the two sites identified as phosphorylated were generated by substituting the two Ser with Ala and subcloned along with the wild type protein into a His-tagged vector for protein expression in *E. coli*. Following purification, the wild type and mutant proteins will be phosphorylated in vitro in order to screen for phospho-p15\textsuperscript{raf} specific mAbs. These phospho-specific mAbs will be the key tools in unveiling the role of phosphorylation of p15\textsuperscript{raf} and should aid in understanding the function of this protein in cancer.
**Donor-Derived T Cell Number and GVHD are Significantly Increased Following Transplantation of CD27-/- T Cells**

Talia Chapman, Gabrielle Goldberg, Ali Krimmer, and Marcel van den Brink
Gerstner Sloan-Kettering Graduate School, Memorial Sloan-Kettering Cancer Center, New York

Allogeneic hematopoietic stem cell transplantation (Allo HSCT) is a potentially curative therapy for hematopoietic cancers such as leukemia, lymphoma, and myeloma. Graft versus host disease (GVHD) is a major complication of these transplants. A principal aim of HSCT research is to decrease the incidence or intensity of GVHD without decreasing the graft versus tumor (GVT) effect of the transplant. CD27, a costimulatory molecule expressed on T cells, binds to CD70, a receptor on antigen presenting cells. CD27-CD70 interactions enhance T cell proliferation. We tested the hypothesis that CD27-/- T cells have less allo-reactivity leading to decreased GVHD. Lethally irradiated BALB/c mice received T cell depleted bone marrow from C57BL/6 mice and T cells from either wild type (WT) (n=10) or CD27-/- (n=10) C57BL/6 mice. Surprisingly, mice that received CD27-/- T cells died significantly earlier from GVHD than those that received WT T cells. We therefore analyzed T cell numbers and phenotype by flow cytometry. Seven days after transplant, BALB/c mice that received CD27-/- T cells showed a significant increase in splenic CD4+ T cell number (WT: 8.38x10^4 ± 7.94x10^3 vs. CD27-/-: 1.85x10^5 ± 1.73x10^4). This was also the case for splenic CD8+ T cells (WT: 1.58E+05 ± 1.89x10^4 vs. CD27-/-: 3.00x10^5 ± 4.53x10^4). In the mesenteric lymph nodes, CD4+ T cells were significantly increased in number among the mice receiving CD27-/- T cells (WT: 5.33x10^4 ± 5.99x10^3 vs. CD27-/-: 1.21x10^5 ± 2.98x10^4), but CD8+ T cell number was not significantly different. This increase in T cell number is associated with increased GVHD. Contrary to expectations, CD27-/- T cells led to an increase in allo-reactivity and GVHD.

**Identifying a Role for the Switch µ Transcript in Class Switch Recombination**

Katherine Helming, Bao Vuong, and Jayanta Chaudhuri
Gerstner Sloan-Kettering Graduate School, Memorial Sloan-Kettering Cancer Center, New York

In order to produce antibodies with different effector functions, B cells undergo class switch recombination (CSR), a process that changes the constant coding region of the gene encoding the immunoglobulin heavy chain via DNA recombination. Switch regions are important segments of the gene that lie upstream of constant coding regions and guide CSR. Activation-induced cytidine deaminase (AID) facilitates double stranded DNA breaks at switch regions by converting cytidine residues to uridine; the DNA between the switch regions is removed and the free ends of DNA are brought together through non-homologous end joining. Transcription through the switch region is required for CSR, and the intronic switch µ RNA is spliced out. The purpose of transcription in CSR is unclear.

The hypothesis of this experiment is that the switch µ transcript plays a role in CSR, either by permitting DNA recombination or recruiting proteins to the DNA. The role of the switch µ RNA transcript will be investigated by identifying the proteins that bind to it using an RNA affinity tag described by Srisawat and Engeleke (Methods, 2002). A streptavidin binding RNA biotin motif was inserted 5’ and 3’ of the switch µ transcript. Results from an in vitro transcription assay suggest that the switch µ transcript can be isolated by streptavidin agarose beads, which will allow for future studies of the switch µ transcript and its associated proteins in cells. It is possible that AID binds to the switch µ transcript because both transcription and AID are required for CSR. This hypothesis will be tested by identifying the RNAs that bind to AID through a RIP ChIP assay. Identification of proteins bound to the switch µ transcript will provide a better understanding of CSR and how B cells produce antibodies to protect the body from infection.
Characterizing the Role of Acvr1 in Mouse Gastrulation

Olga Herrera, Mary Madabhushi, Asma Amleh, Kat Hadjantonakis, and Elizabeth Lacy
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The formation of the primitive streak on the posterior side of the embryonic day (e)6.25-e6.5 mouse embryo marks the onset of gastrulation, a developmental process that generates the three definitive germ cell layers: ectoderm, mesoderm and endoderm. Bone morphogenetic proteins (BMPs), a distinct group of ligands within the TGF-β superfamily, play crucial roles in gastrulation by regulating a number of cellular processes, including proliferation, differentiation, and survival. BMPs activate signaling by binding to heteromorphic complexes comprised of type I and II serine/threonine kinase receptors. The type I BMP receptor Acvr1 -also known as Alk2- is expressed in the visceral endoderm (VE) at e6.5 and within the node and notochord at e8.0-e8.5, as well as in some mesodermal and endodermal tissues. Prior to, and during the initial stages of gastrulation, the VE surrounds the entire epiblast, the cell layer that will give rise to all cell types in the fetus; following gastrulation the VE forms the endodermal layer of the yolk sac. In addition to mediating nutrient-waste exchange, the VE provides important signals that organize and regulate the developing gastrula. Previous studies found that embryos homozygous for an Acvr1 null mutation arrest during early gastrulation with defects in formation of VE and mesoderm. We hypothesize that this early developmental arrest results from loss of Acvr1 signaling specifically in the VE. To explore this hypothesis and define the function of Acvr1 in mouse development, we analyzed homozygous Acvr1 mutant embryos at e7.5 for markers of endodermal differentiation via in situ hybridization and immunohistochemistry. Preliminary data indicate that the VE does not differentiate properly, suggesting that Acvr1 signaling within the VE is critical for correct formation and function of this endodermal tissue.

Characterization of Mouse Embryonic Expression of Pericentrin and Akap9 and Their Gene Trap Knock-out Phenotypes

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Centrosomes are the main microtubule organizing center of most vertebrate cells and act both as templates for primary cilia and as spindle poles during mitosis. They are composed of a pair of centrioles surrounded by an amorphous pericentriolar material (PCM). Pericentrin (Pcnt) and A-kinase anchoring protein 9 (Akap9) are pericentriolar proteins that are involved in regulating mitosis and ciliogenesis. In this study, we investigated the expression and function of Pcnt and Akap9 in mouse embryos utilizing two mouse lines that carry gene trap disruptions in either the Akap9 or the Pcnt gene. Mutant embryos for either Pcnt or Akap9 gene trap alleles fail to close the neural tube and die at e8.5. To determine the primary cause of the mutant phenotype, we are using in situ hybridization expression analysis of Brachyury and Cerl. The expression of Brachyury and Cerl mark the primitive streak and the anterior visceral endoderm respectively, which are the primary tissue organizing centers of the embryo. Majority of Pcnt mutants showed defects in defining the primitive streak. We used lacZ expression to follow gene expression in embryos that were heterozygous for the gene trap alleles. We found broad expression of Pcnt and Akap9 with high expression levels in the neural tube, somites and the heart. At e7.5, Akap9 showed polarized expression to the apical side of the cells in the epiblast. A striking phenotype seen at e10.5 is intense expression of Akap9 in differentiated cells, suggesting that Akap9 has important functions, in addition to mitosis. To further investigate the role of Akap9 in cell migration and mitosis, we are analyzing the location of Akap9 in explanted epiblast cells. Future studies will include further characterization of the mutant phenotype and comparison of cell migration between mutants and the wild type epiblast.
Cellular Localization, Kinesics, and Microstructure of Paxillin Recruitment Following T Cell Receptor Activation

Jeong Min Kim, Emily Quann, and Morgan Huse
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The antigen-specific T cell immune response is initiated by the activation of the T cell receptor (TCR) via the binding of an antigenic peptide presented by major histocompatibility complex (pMHC). TCR activation is followed by the reorientation of the microtubule organizing center (MTOC) toward the immunological synapse (IS). This sets up an axis of polarity within the cell, enabling the directional secretion of cytokines and cytolytic factors toward the antigen-presenting cell (APC). This process of polarization depends on early TCR signaling, but the downstream events are unclear. Paxillin, a cytoskeletal adaptor protein that binds to both signaling and structural molecules, has been shown to localize to the MTOC of both unstimulated and activated T cells, as well as to the IS, and is phosphorylated downstream of TCR stimulation. This suggests that paxillin may be involved in MTOC reorientation. Our lab investigated the cellular localization, kinetics, and microstructure of paxillin recruitment after TCR activation by examining the recruitment behavior of green fluorescent protein (GFP) tagged paxillin in primary mouse T cells expressing the 5C.C7 TCR. Responses were examined both by epifluorescence imaging of conjugates between T cells and APCs, and also by total internal reflection fluorescence (TIRF) imaging of T cells in contact with glass surfaces presenting pMHC. The preliminary results show that paxillin localizes to the MTOC and the IS, as previously shown, and also that paxillin is recruited to the IS within 90 seconds after TCR activation. Paxillin does not form microclusters in the plasma membrane, but rather shows initial, prominent localization in the presumptive lamellipodia and later diffuses throughout the cell. We plan to expand the experiments with shRNA knock-down of paxillin, small molecule inhibitors of downstream events, and double infections with other fluorescently tagged proteins to observe various downstream events together.

Analysis of Blood and Lymphatic Vascular Development in Mutant Vezf1 Embryos using Immunostaining

Candice King, Pauline Ocaya, and Heidi Stuhlmann
Weill Medical College of Cornell University, New York

The vascular endothelial zinc finger 1 (Vezf1) gene is involved in the development of the blood vascular and lymphatic systems. The absence of Vezf1 causes decreased lymphatic and blood vascular integrity in mouse embryos. Embryos that are heterozygous for the Vezf1 gene exhibit edema and lymphatic hypervascularization associated with hemorrhaging in the jugular region. Homozygous embryos present an even greater degree of vascular defects, causing localized hemorrhaging and death.

Lymphatic vessel defects in the head and neck regions of Vezf1 +/- embryos at day 12.5 of gestation have been described previously by the Stuhlmann laboratory. However, a detailed analysis of lymphatic structures at other development stages and in other regions of the embryo has not been performed. To study the effects of Vezf1 loss of function in lymphatic and vascular systems I am undertaking a detailed analysis in vivo, in knockout embryos and in embryoid bodies that are derived from mutant embryonic stem (ES) cells using immunofluorescence and immunohistochemistry.
The Birth and Death of the Centrosome
Kristin Knouse, Won-Jing Wang, Rajesh Soni, and Meng-Fu Bryan Tsou
Gerstner Sloan-Kettering Graduate School, Memorial Sloan-Kettering Cancer Center, New York

The centrosome—centrioles surrounded by pericentriolar material—is the major microtubule-organizing center in animal cells and has profound effects on spindle bipolarity at cell division. Mono- or multipolar spindle formation can compromise sister chromatid segregation and therefore regulation of centrosome number is essential for genomic integrity. Here we characterize two of the mechanisms that contribute to this control: centriole disengagement, which licenses centrioles for duplication, and centriole degeneration, which leads to centrosome reduction.

Centriole disengagement, which gives birth to a new centrosome, requires the protease separase. The substrate of this activity remains unknown. Scc1, a subunit of the cohesin complex required for sister chromatid cohesion, is the only known separase substrate. To test if Scc1 also serves as the substrate of disengagement activity, we silenced Scc1 expression in human cells using RNA interference. Cells were examined with correlated time-lapse and fixed-cell microscopy. Intriguingly, defects in both centrosome and centriole cohesion were observed in some cells, suggesting a role for Scc1 in maintaining centriole engagement and centrosome integrity.

Centriole degeneration is necessary to reduce centrosome number during gametogenesis. The nature of such activity is not understood. We hypothesized that degeneration of axoneme, structurally similar to centrioles, might occur by a similar mechanism. Using extracts from the eggs of *Xenopus laevis*, we observed a novel activity that targets axoneme for destruction. Data revealed that degeneration occurs selectively in interphase by an energy-dependent process that strips the axoneme of its nine exterior microtubule doublets, resulting in complete destruction of axonemal microtubules. Characterization of this novel activity may shed light on the death of a centrosome, a missing puzzle piece required to explain the homeostatic control of this remarkable organelle.
ADP-Ribosylation Activity of Arr in Facilitating the DNA Damage Response
Lucy X. Li, Christina L. Stallings, and Michael S. Glickman
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DNA double-stranded breaks (DSBs) are potentially lethal injuries to the cell that can be generated by ionizing radiation and endonucleases but also by products of host immune systems. Inaccurate or incomplete repair of these breaks leads to larger genomic instability and, in many cases, cell death. To circumvent such a cell fate, mycobacteria are able to restore genomic integrity using homologous recombination and nonhomologous end joining.

However, the actual events leading to downstream repair remain unclear. DNA microarray analysis of *Mycobacterium smegmatis* upon induction of DSBs identified several genes potentially involved in regulating DNA repair. One of the most highly expressed genes was *arr*, a monoADP-ribosyltransferase previously known to confer rifampin resistance. Deletion mutants of *arr* were more sensitive to killing by oxidative stress than wild-type *M. smegmatis* and showed deregulation of translation related transcripts during DNA damage. The altered transcriptional profile was markedly similar to that of the stringent response, a general stress response where the cell metabolism diverts resources from protein synthesis. Subsequently, Arr was shown to interact with ribosomal subunit L11, a protein necessary for the stringent response. This suggests the stress created by DSBs acts as stimulus for the stringent response, perhaps, through Arr.

This work examines the significance of Arr’s catalytic activity during DNA damage. Three single amino acid mutations of Arr’s catalytic residues were made, and the mutants were expressed episomally and intrachromosomally to assess novel phenotypic differences. All three mutations abolished ADP-ribosylation activity. We are assaying to determine if the mutations phenocopy the null strain in terms of sensitivity to oxidative stress, deregulation of transcription, and inability to bind the L11 protein in yeast assays. The findings will demonstrate if Arr’s ADP-ribosylation activity is essential to these processes.
Diversity-Oriented Synthesis of Spiroketalts
Emily Lim, Jackie Wurst, and Derek S. Tan
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Commonly found in natural products, spiroketals have become a popular target of diversity-oriented synthesis. Their wide range of stereochemical diversity and ability to bind to a variety of different biological targets have made spiroketals an interesting scaffold for library synthesis. Here, kinetically controlled stereoselective spirocyclization using methanol was used to produce two diastereomeric spiroketals on a stereochemically diverse glycal epoxide core. This level of stereochemical diversity in spiroketal synthesis has proven useful in the formation of the complex libraries that we seek to create.

Mast Cells in Pancreatic Fibrosis
Sade McKenzie, Rosalia Mora, Arul Veerappan, Nathan O’Connor, Haitham Al-Salama, Daniel Rhoades, Karl Migally, Racha Estephan, Alicia Reid, and Randi B. Silver
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Rationale: Pancreatic fibrosis is observed in diabetes mellitus. Angiotensin (ANG) is known as a pro-fibrotic mediator. Based on our prior observations that mast cells (MC) synthesize renin, the rate-limiting factor in ANG formation, we hypothesized that MC are necessary for the development of pancreatic fibrosis in type I diabetes.

Methods: To induce type-I diabetes, rats were injected with a single dose of streptozotocin (STZ) with confirmed hyperglycemia for up to 1 month. Histochemical, immunochemical, and trichrome staining were performed on fixed sections of pancreas. Pancreas pepsin soluble collagen was quantified by Sircol assay. Pancreatic MC were isolated by magnetic immuno-selection, targeting the MC-specific FcεRI receptor. Renin activity was performed by RIA on MC lysates.

Results: Immuno-histochemical staining of MC shows their proliferation and migration from the pancreatic connective tissue to the parenchyma after 1 month of STZ-treatment. This was verified by counting the number of isolated pancreatic MC (14,405,941/g (STZ) vs 59,334/g (control)). Collagen staining is more abundant in the STZ-pancreas compared to controls. Total collagen content is upregulated in STZ-pancreas (143 µg/g) compared to controls (48 µg/g). The pancreatic MC are immuno-positive for renin and it is active as determined by RIA.

Conclusion: MC may play a pivotal role in collagen deposition in diabetic pancreatic fibrosis. We propose pancreatic MC as a therapeutic target in diabetes.
Regulation of Estrogen Related Receptor Alpha Localization and Signaling by the Kinesin KIF-17
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Kinesins are motor proteins that transport a variety of cargos such as organelles, vesicles, RNA, protein complexes and even viruses, to specific destinations in the cell in a microtubule and ATP dependent manner. KIF17 is part of the kinesin family and it was found - by the methods of yeast two-hybrid assay and immunoprecipitation - that one of the cargos that interacts with is Estrogen related receptor alpha (ESRRA). ESRRA is an orphan nuclear receptor structurally and functionally similar to the classic estrogen receptors (ER) but its activity is independent of any known ligands, like estrogen or estradiol. However, phosphorylation of ESRRA by EGF signaling pathway can make ESRRA to change its conformation and enhance DNA-binding. ESRRA activity is regulated in part by direct competition with ERs and it must go to the nucleus in order to activate transcription of its target genes. It has been previously reported that KIF17 is involved in regulating CREM mediated transcription by interacting with and controlling the intracellular localization of the transcriptional activator ACT in murine male germ cells. Under the light of this precedent, we hypothesize that KIF17 might be involved in regulating nuclear transport of ESRRA, and hence, its activity. Our goal is to reveal the functional significance of KIF17-ESRRA interaction in breast cancer cells and we will do this by first, determining the mechanisms by which KIF17 controls the intracellular distribution of ESRRA (nuclear vs cytoplasmic) in presence of EGF. We will measure alterations of ESRRA localization and determine the nuclear:cytoplasmic ratio by immunocytochemistry and fluorescence microscopy. This is important because ESRRA activity is related to aggressive tumor behavior and poor prognoses in breast cancer patients.

BDNF Polymorphism & its Effects on Amygdala & Hippocampus Volumes & Behavioral Phenotypes
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No genetic association had been identified linking neurotrophin genes to deficits in human learning and susceptibility to psychiatric disorders until the recent discovery of a common genetic variant in the human brain derived neurotrophic factor gene (BDNF). This single nucleotide polymorphism (SNP) in the BDNF gene encodes in a valine (Val) to methionine (Met) substitution at codon 66 in the prodomain (Val66Met), and results in decreased trafficking of BDNF into the regulated secretory pathway important for learning and development. Recent human studies have shown that carriers of the Met allele have decreased hippocampal volume and impairment on hippocampal-dependent memory tasks. In parallel, a transgenic knock-in mouse that recapitulates the human phenotype has been created, in which a novel phenotype of increased anxiety is observed. The goal of this work is to examine the effects of BDNF genotype on levels of anxiety and underlying neural correlates (amygdala volume) in humans. We hypothesize that Met allele carriers will have higher anxiety levels in humans that will be correlated with amygdala volume. The results from this work may provide more insight on how carriers of the Met allele may have increased susceptibility to psychiatric disorders.
The hypothalamic paraventricular nucleus [PVN] may contribute to an increase tendency for hypertension in perimenopausal females (age 45 – 54) compared to young females. PVN neurons that express corticotrophin-releasing factor [CRF] are regulated by glutamate through activation of NMDA receptors. In other brain regions, estrogen levels can impact the subcellular distribution of NMDA receptors subunits, changing excitatory responses. Therefore, this study used quantitative electron microscopic immunocytochemistry to examine potential differences in levels and subcellular distribution of the phosphorylated NR2B subunit relative to CRF in the PVN of young (2 mo old) and perimenopausal (11 mo old) mice following slow developing hypertensive conditions, induced by angiotensin (600ng/kg/min for 2weeks). Polyclonal rabbit antibody to pNR2B-Tyr 1472 subunit and polyclonal guinea pig antibody to CRF were detected by immunogold and immunoperoxidase labeling, respectively. pNR2B immunoreactivity (-ir) is localized primarily on glial processes that surround dendrites and terminals, while CRF-ir is primarily in terminals. Some CRF in terminals contacted dendrites surrounded by pNR2B-ir glia indicating that CRF and glutamate can regulate common targets. Preliminary quantitative analysis revealed a trend for increased pNR2B gold particles in glia surrounding larger (> 800nm diameter) dendrites in young mice, regardless of treatment. However, following angiotensin administration, pNR2B gold particles in glia surrounding smaller (< 800nm diameter) dendrites tended to increase in the 11-month-old mice only. These findings suggest that pNR2B activation in glia can differentially effect excitation and inhibition in the PVN of young and perimenopausal mice following hypertensive conditions.

Thrombopoietin/MPL Modulates Regeneration of the Hematopoietic Stem Cell Pool Through Hemangiogenic Recovery of the Bone Marrow Microenvironment
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Hematopoietic stem cells (HSC) are capable of self-renewal and can give rise to all types of mature blood cells. The bone marrow (BM) microenvironment produces many different factors that regulate the equilibrium between stem cell self-renewal and differentiation. Thrombopoietin (TPO) has been characterized as a cell-extrinsic factor that is necessary for normal HSC maintenance and megakaryopoiesis. The TPO receptor, MPL, has been suggested to be expressed on long term (LT)-HSCs that reside within the bone marrow, close to the endosteum (osteoblastic niche) and/or sinusoidal blood vessels (vascular niche). Previous studies have shown that the BM vascular niche provides permissive conditions that support stem cell proliferation and differentiation. In this study, we started by investigating the differences between the numbers of HSCs in WT, TPO−/−, and MPL−/− steady state mice using the recently discovered SLAM code. Even though TPO/MPL deficient mice had reduced numbers of phenotypically marked HSCs, we found that HSCs lacking TPO or MPL were able to sustain long-term engraftment, but developed thrombocytopenia. We identified a potential role for TPO/MPL signaling in the regeneration of the HSC pool after myeloablation. Histological analysis of the BM vascular niche suggests that TPO and MPL are required for re-establishment of the bone marrow vascular niche following myeloablation. Lack of TPO and MPL following myeloablation results in a decrease in normal BM vasculature, lack of hematopoietic recovery, and ultimately the demise of TPO−/− and MPL−/− mice. These data support the hypothesis that TPO and MPL play a role in replenishing the HSC pool following myeloablation by supporting hemangiogenic recovery of the BM microenvironment. Studies focusing on the mechanism for stem cell self-renewal are important as it may prove to have many clinical implications.
Accurate segregation of chromosomes during meiosis is required for the production of viable gametes in eukaryotes. In order to properly segregate, each pair of homologs must undergo crossing over. This process is tightly controlled to prevent aneuploidy. As a consequence of crossover control, each pair of homologs receives at least one crossover (obligate crossover). There are two additional manifestations of crossover control. First, the distribution of crossovers along a chromosome is nonrandom and crossover formation in one region decreases the probability that a crossover will form in an adjacent region (crossover interference). Second, when the number of double strand breaks induced during meiosis is decreased, the number of crossovers is maintained at the expense of noncrossovers (crossover homeostasis). Previous research suggests that crossover homeostasis and crossover interference are linked under a single crossover control mechanism. If this is true, yeast mutants that show decreased levels of crossover interference should also exhibit a corresponding decrease in crossover homeostasis. Preliminary studies of msh5Δ and zip1Δ strains with return-to-growth and random-spore analysis confirm this hypothesis. My goal was to test whether crossover homeostasis can be detected in mutants that exhibit interference. csm4Δ mutants exhibit increased numbers of crossovers, but crossover interference is maintained (Alani et al., pers. comm. to S. Keeney). If crossover homeostasis can be detected in csm4Δ mutants, this will support the conclusion that crossover homeostasis is mechanistically linked to crossover interference. If crossover homeostasis cannot be detected, there may be mechanistic differences between the two processes. If crossover homeostasis is detected, the crossover homeostasis assay could be used as a surrogate for current interference assays and replace laborious and time consuming tetrad dissection methods to analyze interference.
**Effects of Simultaneous Loss of Cathepsin Protease B and S on Tumor Angiogenesis.**

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Cysteine cathepsins are a family of proteases which primarily function in terminal protein degradation, though they are also capable of processing other proteins. In cancers, cathepsins are often upregulated, and are thought to play important roles in tumor development and progression. Our lab has studied the roles of several members of the cathepsin family by analyzing individual cathepsin knockout mice, which were crossed to the RIP1-Tag2 (RT2) model of pancreatic islet cancer. The analysis revealed that certain cathepsins are functionally implicated in tumorigenesis. In particular, mice deficient in cathepsin B or S have a distinct angiogenic phenotype characterized by a marked decrease in angiogenic switching, microvascular density and tumor burden, and an increase in apoptosis.

Here, we study the effects of a simultaneous loss of cathepsins B and S in greater detail. B^-/-S^-/- RT2 mice were found to have reduced angiogenic switching compared to the individual B or S knockouts, and the overall tumor number and burden is also significantly decreased. Our aim is to examine the microvascular characteristics, including vessel branching, length, number, and density, functionality, and leakiness, of tumors and non tumor sites in B^-/-S^-/- mice. Immunofluorescence staining for cell type specific markers was done to quantify branch points, vessel length, and vessel number in RT2 tumors. Matrigel plug assays were conducted in B^-/-S^-/- mice to study angiogenesis outside the context of the tumor microenvironment, and vessel functionality and leakiness was assessed by lectin or dextran injection in these mice and the RT2 mice.

Preliminary data indicates that there is little change in vessel branching, length, or density in the context of tumor angiogenesis in the double knockouts. However, additional microvascular characteristics, including vessel functionality, leakiness, and angiogenesis outside the context of the tumor, remain to be quantified.

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**Glucocorticoid Sensitive IFN- Stimulated Genes**

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Glucocorticoids (GCs) are steroid hormones produced in the adrenal glands and are upregulated in the presence of stress. For this reason, they are also known as stress hormones. GCs bind the glucocorticoid receptor (GR), a ligand-dependent transcription factor that leads to changes in gene expression. Specifically, a wealth of data demonstrates GRs ability to repress the transcription of pro-inflammatory cytokines. Previous work in the lab has shown that direct treatment of macrophages with type I interferon (IFN) and Dexamethasone (Dex), a synthetic GC, inhibits the expression of IFN-stimulated genes (ISGs), including IP10, ISG56, and ISG15 (unpublished results). This finding suggested that GR may interfere with the Type I IFN-Jak/STAT signaling pathway; making IFN the only cytokine sensitive to GCs, both at the level of production and signaling. However, a thorough evaluation of the ISG expression profile affected by GCs in this context is lacking. Thus, we will be examining the nature and kinetics of genes stimulated by the IFN-Jak/STAT pathway that are GC sensitive. A complete understanding of the mechanism by which liganded GR affects type I- IFN signaling is essential and can potentially foster the development of treatments for a variety of inflammatory diseases.
Motor Function Behaviors of Mir Mutants in Drosophila
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MicroRNAs are a class of short noncoding RNAs that serve as posttranscriptional regulators of gene expression. In Drosophila, numerous miRs have been isolated; however the biological functions of many are unknown. We have screened a UAS-miR library (provided by E. Lai) for those miRs that, when overexpressed in myoblasts, lead to muscle defects. Using this strategy, we have uncovered several miRs that cause muscle phenotypes, which range from severe (large numbers of missing/mispatterned muscles) to mild (few muscles missing/duplicated).

To assess the effect of these different miRs on muscle function, I have developed two behavioral assays. These assays are used to establish normal motor function in both the larval and adult stage. To screen for larvae motor behavior, I have completed a path length assay, which measures crawling motor ability in the 3rd instar larvae by quantifying the distance crawled in a set time. My data indicates that the control larvae exhibit a path length of 600 – 800 pixels. Motor function in adult flies is determined by completing a negative geotaxis assay. Drosophila exhibits a natural movement against gravity; a bang assay is used to quantify this behavior. My data indicates that control flies show a decrease in geotaxis behavior as the flies age.

These two assays are being used on miR mutants to determine whether there is a difference in larval and adult stage motor behavior when compared to controls. We will determine the degree to which a muscle developmental defect results in impaired motor function. In addition, the stage at which there is a direct difference in motor ability will also be determined. Taken together, these assays provide a novel way of assessing the behavioral output of muscle function and the contribution of miRs to this behavior.
### Investigating the Stem Biology of eXtraembryonic ENdoderm Cells: Generation of Reporters for Live Imaging

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Stem cells representing each of the three first lineages (trophectoderm, primitive endoderm (PrE) and epiblast) present in blastocyst stage mammalian embryos can be isolated, propagated and differentiated in culture. While embryonic stem cells (from epiblast lineage) have been extensively studied, few studies have focused on the biology of trophoblast stem cells and extraembryonic endoderm (XEN) cells (from PrE lineage). The goal of my SURP project was to develop live imaging tools to investigate various aspects of the stem biology of XEN cells including cell behavior, and differentiation. I exploited two complementary approaches: (1) the isolation of stable expressing cell lines upon transfection of established XEN cell lines, and (2) the derivation of new XEN cell lines from genetically-modified mouse strains.

The **first goal** focused on characterizing the dynamic cell behaviors, morphology, and movement of XEN cells. To visualize XEN cells using fluorescent microscopy, I generated lines expressing lipid modified fluorescent protein fusions, including a glycosyl phosphatidylinositol tagged green fluorescent protein (GFP) or a myristoyl- tagged Venus yellow fluorescent protein. 3D time-lapse imaging using laser scanning confocal microscopy allowed me to visualize the dynamic processes of these highly motile cells and provided quantitative information which could be correlated to determine the relation between shape, migration and vesicular trafficking.

The **second goal** focused on characterizing the differentiation properties of XEN cells. The PrE differentiates into two distinct cell types: the parietal and visceral (VE) endoderm. As a first step towards determining which signaling pathways are required to drive XEN cells into the VE lineage, I am generating XEN cells in which a GFP reporter is activated once cells are committed to VE fate. To do this, I am generating a XEN cell line carrying GFP under the cis-regulatory sequences of the VE-specific α-fetoprotein gene.

### Over-expression of the Human Replication Protein Mcm10

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Mcm10 is essential to DNA replication and is one of the factors recruited to the pre-initiation complex. It physically and/or genetically interacts with a number of other proteins critical to DNA replication, including Cdc45 (which induces origin unwinding), Mcm2-7 complex (replicative fork helicase) and DNA polymerase α/ε. These interactions suggest that Mcm10 plays an important role in origin initiation and in elongation of DNA chains during replication. Nevertheless, its exact functions are not known. The goal of this study is to isolate and to purify HsMcm10 for biochemical characterization.

Mcm10 was first identified in *S. cerevisiae* and is highly conserved across all species, including humans (HsMcm10). HsMcm10 shows 21% overall similarity with its *S. cerevisiae* homolog. It has conserved GKT (Walker A box) and DEXX (Walker B box) motifs, suggesting it might have an ATPase activity, and a zinc finger domain which is known to be important for single-strand DNA binding. A function of Mcm10 in DNA replication might be to bind to replication forks and to recruit replication proteins required for the initiation and elongation of nascent DNA chains.

In this study, *HsMcm10* was subcloned into various baculoexpression vectors for over-expression in insect cell lines. Also, we would like to determine the optimal conditions for Mcm10 over-expression in bacteria.
Adenomatosis polyposis coli, better known as the APC tumor suppressor, has been shown to play a critical role in regulating cell proliferation through the WNT/β-catenin signaling pathway. In addition, recent experiments have shown APC to associate with both (+)-ends of microtubules and with the lateral membranes of polarized epithelial cells. This suggests that APC may play a role in determining cell polarity. The dual functionality of APC has interesting implications for the development of the mammalian neocortex, which is derived from highly polarize neural progenitors in the ventricular zone and requires proper WNT/β-catenin signaling for normal development. Here we found that mice with their APC gene conditionally removed in the forebrain at an early stage of brain development exhibit a severe disruption in the organization and morphology of their cerebral cortex that is visible as early as E12. Qualitative observation of brains sectioned at E14 reveal a significant lack of a proper cortex as well as an abnormal development of the skull and ventral portions of the brain. Immunofluorescent staining of the transgenic brain sections demonstrate a complete disorganization of the cells and an apparent lack of neuronal progenitor cells. While the lack of organization may be attributed to the loss of the cells’ ability to maintain cell polarity, the early differentiation of neuronal progenitor cells contradicts the suggested role of APC as a tumor suppressor in the WNT signaling pathway. These results either suggests an alternative role of APC in the WNT signaling pathway in the context of brain development, or it may suggest an entirely new signaling pathway altogether.