THE 23rd ANNUAL GRADUATE STUDENT RESEARCH SYMPOSIUM
The Graduate School of Biomedical Sciences
Baylor College of Medicine
Houston, Texas

October 20, 2011

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Cover Legend:
Johnston’s Organ (JO) is Drosophila auditory organ in the second segment of the antennae. JO contains several hundreds of mechanosensory neurons with specialized sensilla. Here is a 3D projection of immunostained pupal JO. HRP (red), a neuronal membrane marker, shows the morphology of these specialized auditory neurons. Eyes shut (green) gives a two dots staining pattern along the sensilla. Olfactory neuronal axons from the third segment (bottom side) converge with axons from auditory neurons on the basal side of JO (top side). Drosophila’s ear-Johnston’s Organ were cultured and immunostained by Tongchao Li, Ph.D. candidate in Developmental Biology Program. Laboratory of Hugo Bellen, Ph.D. and Andy Groves Ph.D.

A publication of The Graduate School of Biomedical Sciences, Baylor College of Medicine, Houston, TX 77030
Oral Presentations – Cullen

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Awards Ceremony & Reception
Thursday, October 20, 2011
3:45 – 5:00 PM

8-Stranded Beta-Barrel Jelly Roll Awards
Professor John J. Trentin Scholarship Awards
Deborah K. Martin Achievement Award in Biomedical Sciences
Marc Dresden Excellence in Graduate Education Award
Milton Gregory Poster Awards
Beckman Poster Awards
Mavis P. Kelsey Student Speaker Awards
Beckman Platform Award
Welcome to Baylor College of Medicine’s 23rd Annual Graduate Student Symposium! Thank you for joining us as we take time to honor all graduate students at BCM and to showcase some of the exceptional biomedical research being performed at our institution.

This year’s symposium features student poster presentations and platform talks. Students chosen to give talks represent the best of their respective departments, having won awards at scientific retreats. These talks and poster presentations provide an invaluable opportunity for students to discuss their research with a diverse group of faculty and peers. Year after year, the Symposium showcases excellent student research; we think you’ll agree this year promises to be no exception. In addition to the outstanding student work, we are pleased to have Dr. Erich Jarvis from Duke University, culminate the day’s events by delivering the Joseph L. Melnick Distinguished Lecture.

The events of today would not have been possible without the help of many dedicated students and faculty. Members of the Graduate Student Council have graciously volunteered their time and efforts all year. For their hard work and their indispensable help with the symposium, we extend our sincerest thank you.

A special thanks is also owed to Melissa Houghton and Dr. Gayle Slaughter for their leadership roles in planning and organizing all aspects of today’s symposium. We also thank Dr. Hiram Gilbert for his work in organizing the Symposium and his continued mentorship of the GSC. Additionally, our sincere gratitude goes out to each and every faculty and staff member at BCM who work tirelessly day after day to ensure students receive the best educational experience possible. We would especially like to recognize Donna Otwell, Dr. Scott Basinger, Dr. William Brinkley and all of our faculty mentors and program administrators for their dedication. Finally, we thank our poster and presentation judges who volunteered their time to help make this a successful symposium, and of course we thank Dr. Erich Jarvis for joining us at BCM to deliver the Distinguished Lecture.

It has been our pleasure serving you over the last year and we hope you enjoy today’s Symposium.

Alison Obr, GSC President
William Choi, GSC Vice President
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I am honored to join you in celebrating the research achievements of the students in the Baylor College of Medicine Graduate School of Biomedical Sciences at the annual Graduate Student Research Program.

In the competitive research environment that we work in today, the ability to communicate research findings to colleagues and to the public is critical. The research findings presented by BCM graduate students are clearly of the highest quality and speak well to the efforts of our internationally recognized Graduate School faculty.

One of the highlights of my career has been mentoring students. There is no greater professional satisfaction than to see a trainee achieve success in their field of research. Graduate students at BCM have the determination and stamina needed to pursue and solve the scientific questions of the future and become leaders in research discovery.

On behalf of the Baylor College of Medicine faculty, congratulations on creating an outstanding Graduate Student Research Program for 2011. You have our best wishes for continued success and our commitment to support you as you complete your training in preparation for an exciting career in biomedical research.

Paul Klotman, M.D.
President & CEO
Baylor College of Medicine
The Graduate Student Research Symposium is a time where we acknowledge and celebrate the enormous research contributions from graduate students during their training to become independent researchers. Graduate students are authors on over 40% of the publications of our graduate faculty. Their enthusiasm and dedication means long hours in the lab or on the computer designing and conducting the experiments that bring new knowledge and insights about biology and human disease. Presenting that knowledge to others in the form of talks, posters, abstracts and publications is an essential part of being a scientist. This symposium showcases student research by providing them an opportunity to communicate their results and ideas to an audience of diverse scientific interests.

A symposium of this magnitude does not happen spontaneously. Graduate students themselves have taken the responsibility of planning this symposium in the style of a national scientific meeting. Last year’s Graduate Student Council began planning for the Symposium with the invitation of today’s speaker, and this year’s Graduate Student Council arranged the scientific program. Gayle Slaughter and John Rodgers helped by critiquing the platform presentations and Melissa Houghton assembled the abstract book. I would like to acknowledge them all for their effort.

I look forward to the Symposium every year as a special time where the research excellence of our students and their mentors is made so clear through the quality and quantity of the research that will be presented in talks, posters, and abstracts. I am glad that you could join us for this celebration of excellence, and I urge you all to participate actively in the platform and poster presentations.

H. F. Gilbert, Ph.D.
Dean
Graduate School of Biomedical Sciences
Erich Jarvis, Ph.D.
Associate Professor, Department of Neurobiology
Investigator, Howard Hughes Medical Institute
Duke University Medical Center

Dr. Erich Jarvis is a world-renowned neurobiologist at Duke University Medical Center studying vocal communications using songbirds as the main animal model. His laboratory uses an integrative approach that combines behavioral, anatomical, and molecular biological techniques to delineate the molecular pathways involved in the perception and production of learned vocalizations. The overall goal of his laboratory is to better understand the neural mechanisms for vocal learning and the basic mechanisms of brain functions in hopes to lead to treatment for speech related problems.

Besides making break-through discoveries in his laboratory, Dr. Jarvis has a passion for dancing while growing up in Harlem, NY. He attended the prestigious High School of the Performing Arts in New York City, and received scholarships to continue his dancing career. Instead, he decided to pursue a scientific career. Dr. Jarvis attended New York’s Hunter College with a double major in biology and mathematics and received a Bachelor of Arts in 1988. He continued his Ph.D. and post-doctoral studies in Dr. Fernando Nottebohm’s laboratory at The Rockefeller University studying the molecular behavioral mechanisms of song-associative learning in songbirds. In 1998, Dr. Jarvis moved to Duke University Medical Center (DUMC) where he started leading his own group of scientists. He is now a tenure-track Associate Professor and an Investigator of the Howard Hughes Medical Institutes (HHMI).

Throughout his scientific career, Dr. Jarvis has won many awards including NIH Director’s Pioneer Award given annually to the top 1.5% of applicants, the Whitehall Foundation Award, NSF Alan T. Waterman Award, and Human Frontiers in Science Program Young Investigators Award, to name a few. His research has been recognized by Discover Magazine as a top 100 science discoveries in 2005 and National Science Foundation top 10 science stories of 2005.

Dr. Jarvis’ dedication, perseverance, and enthusiasm for the field of science are truly inspiring. The Graduate Student Council is honored to have him for this year’s Distinguished Guest Lecturer.
SYMPOSIUM FUNDING ACKNOWLEDGMENTS

We are grateful to the following for their continued support of the Symposium:

- **JOSEPH L. MELNICK, Ph.D.** whose endowment supports The Joseph L. Melnick Distinguished Guest Lecturer and the Symposium Awards Reception. Dr. Melnick was the first Dean of the Graduate School of Baylor College of Medicine and served in that capacity from 1968 to 1991.

- **HARRIS BUSCH GRADUATE STUDENT SYMPOSIUM FUND**—Dr. Harris Busch was the Michael E. DeBakey Professor of the Department of Pharmacology. Dr. Busch was the Chairman of the Department of Pharmacology from July 1, 1960 to June 30, 1998.

- **MAVIS P. KELSEY AWARDS**—The Mavis P. Kelsey fund supports outstanding research by graduate students. This endowment will provide a monetary award for each graduate student who is selected to speak for his or her program.

- **MILTON GREGORY AWARDS**—The Milton Gregory endowment will support the twelve poster awards at the annual symposium. The funds are in honor of outstanding research by graduate students.

- **BECKMAN BEST POSTER AND BEST PLATFORM TALK AWARDS** – Sponsored by Beckman Instruments, Inc. Beckman Instruments of Fullerton, California, operates on a worldwide basis distributing products used in laboratories for biological analysis in all phases of the battle against disease, from pioneer medical research through drug discovery to clinical diagnostics.
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Leukemias derived from MLL-translocations account for 70% of infant leukemias and roughly 10% of adult leukemias. Patients suffering from these leukemias have poor prognoses in general and often relapse after treatment due to the aggressive nature of the disease. Thus, there is a pressing need for effective treatments against mixed lineage leukemias.

Leukemias derived from MLL-translocations all bear a chromosomal rearrangement of the MLL gene on chromosome 11q23. These rearrangements are sufficient to induce mixed lineage leukemias within hematopoietic stem cells. It has been shown that DOT1L is recruited by MLL-rearrangements. DOT1L is a non-SET domain histone methyltransferase that methylates lysine 79 of histone 3 (H3K79).

DOT1L is required for the maintenance of mixed lineage leukemias. Also, DOT1L uses the small molecule s-adenosyl methionine (SAM) as a cofactor for methylation of histones. In addition, there is only one published DOT1L inhibitor. For these reasons, DOT1L makes an attractive target for rational structure-based drug design.

Our goal is to synthesize and evaluate small molecules as potential DOT1L inhibitors. Design of these small molecules will be rationally guided by the crystal structure of human DOT1L(1-472), containing the methyltransferase domain of DOT1L bound to SAM. Synthesis of these compounds is accomplished through standard synthetic chemistry. Potential inhibitors of DOT1L are first evaluated for potency by a scintillation-based nucleosome activity assay. Sub-micromolar inhibitors are then tested on MLL-AF10 transformed leukemic cells for efficacy and on hematopoietic stem cells for general cytotoxicity. Additionally, crystal structures of DOT1L with inhibitor bound are obtained to develop structure-activity relationships (SAR) as part of the rational structure-based approach.

This research was funded by a training fellowship from the Keck Center of the Gulf Coast Consortia, on the Pharmacological Sciences Training Program, National Institute of General Medical Sciences (NIGMS) T32GM089657.

Contributors: Yao, Yuan; Chen, Pinhong; Diao, Jiasheng; Cheng, Gang; Deng, Lisheng; Song, Yongcheng
COMBINING T CELL THERAPY WITH ANGIogenic INHIBITION FOR THE IMMUNOTHERAPY OF MELANOMA

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Advisor: Gianpietro Dotti, M.D.-Department of Medicine
Teresa Hayes, M.D./Ph.D.-Department of Medicine

Melanoma has become an increasingly urgent public health concern worldwide. In the United States, 1 in 58 will be diagnosed with melanoma this year, making the incidence of developing melanoma the greatest among all cancers. Since conventional treatments including surgery, radiation and chemotherapy often fail in patients with metastatic melanoma, new therapeutic options are needed. Melanoma is highly susceptible to T cell immunotherapies based on infusion of tumor infiltrating T lymphocytes (TILs) or T cells engineered to express transgenic T cell receptors (TCRs). Unfortunately, TIL generation fails in more than 50% of patients and the generation of TCR transgenic T cells is restricted to HLA-A*0201 patients.

Targeting melanoma using T cells redirected with chimeric antigen receptors (CARs) circumvents the HLA restriction of transgenic TCRs and allows for the generation of cytotoxic T cells for the majority of patients. CARs specific for tumor associated antigens provide T cells with cytotoxic activity based on the expression of immunoreceptor tyrosine-based activation motifs (ITAMs) on CD3-ζ. The addition of a costimulatory endodomain to a CAR molecule, a second generation CAR, allows CAR-modified T cells to activate costimulatory signals that are generally not provided by tumor cells. There have been several dual signaling moieties established in the context of second generation CARs but a consensus has not been reached on which signaling domain pairs work best. Groups have also gone one step further to strengthen the co-stimulation of second generation CARs by considering stimulation of the PI3kinase/Akt pathway by adding a third stimulatory motif such as 4-1BB or OX40. These constructs have superior in vitro proliferation but more studies are needed in vivo.

Using available technology we aim to generate a CAR that targets High Molecular Weight Melanoma Associated Antigen (HMW) which is a cell surface proteoglycan expressed on 90% of melanoma lesions with restricted expression on normal tissues. HMW is thought to promote migration, invasion and metastasis of melanoma cells through its interaction with the extracellular matrix (ECM). Given its limited expressivity, HMW is an ideal antigen to use for CAR-based T cell therapy. Monotherapy, however may not suffice to combat the multitude of tumor evasion strategies therefore a combination of T cell therapy coupled with angiogenic inhibition may yield a more powerful response. Tumor angiogenesis, the formation of new vasculature from existing vessels, promotes tumor growth, persistence and metastasis. Thrombospondin-1 (TSP-1) is a 420kD glycoprotein implicated as a potent anti-angiogenic factor. TSP-1 is endogenously expressed at sites of tissue remodeling where it modulates extracellular matrix structure. Delivery of anti-angiogenic factors including TSP-1 has proven to be challenging due to their short circulating half-life and toxicity from systemic administration. By utilizing the specificity of CAR-based therapy, TSP-1 can be delivered locally; circumventing the disadvantages of current anti-angiogenic factor therapy. We hypothesize that adoptive T cell therapy targeting the HMW antigen coupled with the potent anti-angiogenic effects of TSP-1 expression will have markedly improved results in melanoma patients compared to conventional T cell therapy.

Contributors: Arambula, Claudia; Savoldo, Barbara; Yvon, Eric; Ferrone, Soldano; Dotti, Gianpietro.
SEVERE REDUCTION IN SYNTAXIN1 EXPRESSION LEVELS DECREASES SYNAPTIC EFFICIENCY

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The SNARE complex is widely accepted as the core of the membrane fusion machinery. However, priming and docking defects can be found in mammalian knockouts of Synaptobrevin and SNAP25, and acute deletions of Syntaxin1. This suggests that SNARE proteins also play important roles in distinct steps leading to neurotransmitter release. To test this idea, the effect of reduced Syntaxin1 levels on release properties was analyzed. A mouse line endogenously expressing the t-SNARE isoform Syntaxin1B (Stx1B), with GFP fused to the C-terminus, was generated. Originally intended for imaging studies, immunoblotting showed that expression of the Stx1B-GFP fusion protein in homozygotes is reduced compared to wildtype levels. Whole-cell patch clamp recordings from autaptic neurons derived from homozygotes show that synaptic transmission is similar to wild type neurons. To remove possible redundancy from the isoform Stx1A, Stx1B-GFP mice were crossed with Stx1A knockout mice. Whole-cell patch clamp recordings from these Stx1 double mutant neurons show reduced spontaneous and Ca2+-evoked release. Priming of the readily releasable pool is impaired and vesicular release probability is lower. Analysis of responses due to hypertonic stimulus also show that vesicles are less sensitive and have a reduced tendency to fuse, indicating decreased fusogenicity. These phenotypes suggest that the level of Stx1 expression is a critical factor for synaptic efficiency. In addition, knockdown of residual Stx1 in double mutants further reduced readily releasable pool size, but not vesicular release probability. This suggests that priming of vesicles having low release probability requires a threshold number of SNARE protein. Based on these results, we propose a model in which priming and normal release properties require threshold levels of a SNARE protein, to reach critical numbers of functional complexes per vesicle. Overall, this study shows that expression levels of a SNARE protein are a critical determinant of vesicular release efficiency.

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Acquisition and honing of motor skills depends on the iterative fine-tuning of neural circuitry throughout life. In vivo imaging studies have revealed one example of this fine-tuning: With learning of a novel motor skill, new synapses are formed onto the apical dendrites of corticospinal neurons in motor cortex, and if stabilized these synapses appear to represent a durable structural correlate of skill memory.

MECP2 duplication syndrome is a single-gene developmental disorder in which patients demonstrate impairments in motor skill learning, along with intellectual disability, autistic features, and epilepsy. The mouse model for MECP2 duplication recapitulates many of these features, but interestingly early on exhibits enhanced motor learning and memory on the rotarod task.

We hypothesize that the enhanced motor behavior in these mice could stem from abnormal biases in motor learning-associated structural plasticity. Pilot studies bear out an upregulation in dendritic spine formation and loss with motor learning in these mice which exceeds that seen in wild-type animals, and correlates with enhanced learning on the task.

Contributors: Ash, Ryan; Zoghbi, Huda; Smirnakis, Stelios
Background: Junctophilin 2 (JPH2) plays a critical role in junctional membrane complex formation in cardiomyocytes and loss of JPH2 results in spontaneous Ca2+ release via RyR2. Separately, defective sarcoplasmic reticulum (SR) Ca2+ handling including diastolic SR Ca2+ leak is known to contribute to both the induction and perpetuation of atrial fibrillation (AF). Recently, several JPH2 mutations have been identified in hypertrophic cardiomyopathy (HCM) patients. Thus, we screened 203 unrelated HCM patients and identified a novel missense mutation, E169K in the JPH2 protein in a patient with HCM and paroxysmal AF. Hypothesis: JPH2 interacts with RyR2 via E169 site and; E169K mutation disrupts this interaction leading to diastolic SR Ca2+ leak via RyR2, causing AF. Methods and Results: We generated 2 transgenic mice that overexpressed 1) JPH2 with E169K mutation (EK-Tg) and 2) WT JPH2 (WT-Tg) that served as control. On Western Blot, JPH2 expression was increased by 2-3 fold in both the mouse groups compared to non-transgenic controls. Echocardiography at 6 months of age revealed normal fractional shortening in all mice. At baseline, EK-Tg mice had a shorter RR interval (104±1 ms, n=10) vs. WT-Tg mice (123±5 ms, n=8, p=0.001). EK-Tg mice also had a shorter PR interval (36±1 ms, n=10) vs. WT-Tg mice (41±1 ms, n=8, p=0.01). On intracardiac electrophysiology studies, EK-Tg mice had shorter atrioventricular refractory period (AVERP) (39±2 ms, n=10) vs. WT-Tg (51±2 ms, n=8, p=0.005). On overdrive pacing of the atria in the presence of epinephrine and caffeine, EK-Tg mice had a 57% incidence of AF (n=10) vs. 21% in WT-Tg (n=8, p<0.05). EK-Tg mice pre-treated with flecainide, a class IC antiarrhythmic with RyR2 stabilizing properties, were completely rescued from inducible AF (0%, p<0.001 vs. EK-Tg). Isolated atrial cardiomyocytes from EK-Tg mice had a higher Ca2+ spark frequency (6.7±0.8 events/100(m/s, n=20) vs. cardiomyocytes from WT-Tg (1.8±0.5 events/100(m/s, n=10, p<0.001). JPH2 levels were significantly lower in RyR2 channels immunoprecipitated from EK-Tg mice vs. RyR2 from WT-Tg mice (0.31±0.08 vs. 1, p<0.001). In 3 separate mouse lines that had increased, normal and reduced expression of JPH2, level of JPH2 correlated with incidence of AF (p=0.04). Lastly, JPH2 levels were found to be reduced in atrial samples from patients with paroxysmal AF in comparison with patients in sinus rhythm (0.6 vs 1, respectively). Conclusion: JPH2 mutation, E169K, reduces JPH2-RyR2 co-immunoprecipitation, increases SR Ca2+ leak and promotes AF. Reduced JPH2 levels promote AF inducibility. RyR2 stabilizers like flecainide can prevent AF induction in mice with E169K mutation. Thus, JPH2 structural protein and especially the E169 site can serve as promising drug targets for future research.
The U.S. Centers for Disease Control and Prevention (CDC) has taken a special interest in Francisella tularensis (Ft), the causative agent of tularemia. Despite the fact that human cases are rare (approximately 100/year in the U.S.), as few as one to ten CFUs can be infectious, making it an ideal bioweapon and appropriately classified Category A Select Agent. Faced with this threat scientists are working to better understand the microorganism. Three subspecies of F. tularensis exist: Ssp. mediasiatic, holartica and tularensis. Subspecies holartica (Type B) and tularensis (Type A) are capable of causing disease in humans, although Type B is not as severe. In the 1950s a live-attenuated vaccine (designated Live Vaccine Strain, or LVS) was generated from Type B Ft. While it is administered to military and at-risk personnel, it is not FDA-approved and lacks many qualities of an effective vaccine, the most important of which is that the mechanism of attenuation is unknown. If improved, more effective vaccines are to be developed, a better understanding of F. tularensis pathogenesis is of critical importance.

Using comparative genomics we have previously identified genes that are present in the Type B strain but are either lacking or disrupted in LVS. The 18 resulting proteins are therefore most likely responsible for LVS attenuation. Along with several hypothetical proteins this list includes virulence factors involved in attachment, host immune evasion and replication. It is important to confirm that these proteins are indeed responsible for Type B attenuation, which, due to the previous lack of genetic tools and the required biohazard precautions has not been explored. Recently, a type II intron system (termed “TargeTron”) has been adapted for use in Francisella that enables the stable, permanent and specific disruption of genes. We therefore propose that the disruption of a subset of these 18 protein candidates in virulent Type B Ft will result in attenuation as shown by reduced intracellular replication in macrophages and decreased infectivity in BALB/c mice. Once the attenuating mechanism of LVS is known, a rationally designed attenuated vaccine can be tested in protection studies against virulent Francisella strains.

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The impacts of different substitutions on the function of a protein are not always similar. While some substitutions cause a large effect, some are neutral and harmless. It is important to be able to distinguish a deleterious substitution from a harmless one especially in a clinical setting. We hypothesize that the functional impact of a substitution depends on two main factors- the positional importance of the residue and the magnitude of the mutation. We test this hypothesis using bacterial RecA protein that plays a central role in homologous recombination and regulates DNA repair. It is also a key component of the bacterial SOS response where it controls the expression of many other DNA repair genes. The experimental assay quantitatively measures the impact of a mutation on RecA-RecA interface by looking for cell survival after DNA damage due to UV exposure and recombination efficiency by means of quantitative phage transduction on selective medium. About 26 different mutations have been made on the RecA-RecA dimer interface. Each of these is being tested for phenotype using the assays as mentioned above. We expect to see a direct correlation between the functional impact of these substitutions on the position of the residue and the magnitude of the mutation.

Contributors: Atri, Benu; Katsonis, Panagiotis; Adikesavan, Anbu K; Lichtarge, Olivier
Proximity to an explosion exposes one to rapid changes in air pressure, which can cause a mild traumatic brain injury (mTBI). Clinical data from recent military conflicts suggest that blast-induced mTBI may induce long-lasting changes in behavior, but the mechanism underlying this form of brain injury has not been elucidated. Recently, calpain-mediated disruption of the axon initial segment (AIS) was reported as a new mechanism of neuronal injury, but it is unknown if the AIS and other excitable domains of axons are susceptible to damage following blast-induced mTBI. Using a novel blast tube, we have exposed rodents to controlled blast overpressures. Two weeks following blast exposure, animals underwent behavioral testing and were sacrificed for examination of the AIS using immunofluorescence. We report here preliminary results from rodents exposed to a blast overpressure of 690 kPa, which lasted approximately 5 ms. Cognitive function of the animals was assessed using a novel object recognition task. Control animals spent significantly more time with the novel object while animals exposed to a blast spent equal amounts of time with the familiar and novel objects, indicating a disruption of learning and/or memory. Using immunofluorescence to stain for the AIS-specific cytoskeletal protein βIV spectrin and a novel counting technique, we do not see disruption of the AIS in the cortex, but we do see a significant decrease in the length of the AIS in injured animals. However, we do see a significant increase in staining for the injury markers BAPP and Iba-1 in the corpus callosum. Preliminary results indicate that exposure to the rapid changes in air pressure caused by an explosion induce changes in rodents at both a behavioral and molecular level.

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PROFILING EVOLUTIONARILY IMPORTANT PROTEIN RESIDUES IN THE PDB

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The PDB consists of over 70,000 known protein structures from sources such as NMR, X-ray crystallography, and CryoEM. However, the data consists mostly of raw atom coordinates and residue types. By combining this information with databases of other protein properties, such as solvent accessibility and residue conservation, this project aims to explore unknown relationships between these properties and the protein structure.

In addition to the raw structure data, our database includes information from PISA (Protein Interfaces, Surfaces and Assemblies), DSSP (Dictionary of Protein Secondary Structure), and our lab's database of Evolutionary Trace (ET) calculations for all proteins in the PDB.

This database provides a mechanism for making a wide variety of observations about the relationship between protein structure and residue importance, and here are presented a subset of observations. Cysteine, glycine, and tryptophans tend to be important, with any given residue of these types having about a 50% chance of being in the top third of importance. However, glycines, being much more common in general, are the most represented type of residue among all important residues. The database also recapitulates the known property that important residues tend to be physically close to one another. Also, it is observed that there is a positional bias in that the C terminal of proteins tend to be enriched for both highly important and highly unimportant residues. Lastly, the database was used to identify which residue types tend to interact at interfaces with what other residue types, showing, for example, that cysteines only tend to interact with other cysteines.

In this project, a computer database was used to confirm previous observations, such as the clustering of residues by importance and the overall elevated importance of certain residues, such as glycine. It was also used to make novel observations, such as the sequence bias for residue importance, raising questions for future research determining the cause of this bias.

In addition to finding and exploring new relationships, future work would include making this database public and incorporating additional sources of information into the database schema.

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Mutations in MECP2 cause Rett Syndrome (RTT), a devastating neurological disorder typified by a period of normal development followed by a slow and progressive decline of cognitive and psychomotor skills. The molecular mechanisms leading to this decline are completely unknown, but human data suggest that some mutations alter the rate at which symptoms develop. To gain insight into the factors that contribute to disease onset and progression we generated two new mouse models harboring either MeCP2-R270X or MeCP2-G273X expressing alleles. Despite differing by only three amino acids, mice expressing these mutant forms of MeCP2 progressively develop phenotypes at strikingly different rates. We interrogated these two models using a variety of molecular assays and discovered one key distinguishing factor: differential alteration of ATRX localization within the nervous system. Further investigation of ATRX mislocalization in various RTT models revealed that it mirrors the progression of phenotypes. This disruption is cell-autonomous, brain specific, and inversely related to changes observed in mice that overexpress MeCP2. These findings provide new insights into an in vivo molecular mechanism which can account for the pattern of disease onset that distinguishes RTT from other childhood neurological diseases.

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LABELING NONVIRAL VECTORS AT SPECIFIC NUCLEOTIDE SITES

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The covalent attachment of fluorescent dyes, peptides, and other chemical moieties to DNA vectors, siRNAs, and plasmids is a commonly implemented strategy in research into human gene delivery. However, no adequate method exists for the controlled modification of closed, circular DNA vectors at specific sites. We present a method, CLASS (Controlled Labeling at Specific Sites), that allows the controlled labeling of supercoiled DNA at specific nucleotide sites that does not require the gross disruption of DNA structure. We found that this process is most efficient with DNA circle lengths under 1000 bp. We therefore labeled 339 bp DNA minicircles with superhelical densities of $\sigma = -0.03$ and $-0.06$ with FRET dyes Alexa-488 and Alexa-568 at two different sites on opposing sides of the minicircle. Restriction digests in combination with fluorescence gel imaging confirmed that labeling was specific to these sites. Our synthesis utilizes common linking chemistry and thus allows for universal site-specific modification using most available dyes and functional groups. Towards that end, we demonstrated a single-site, covalent labeling of biotin on a supercoiled 333 bp DNA minicircle. Electrophoretic mobility shift assays (EMSAs) using streptavidin confirmed the presence of biotin at the single site.

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The characterization of Bas0520, a novel heme-uptake protein from Bacillus anthracis

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B. anthracis is a Gram-positive bacterium that causes anthrax disease. High concentrations of B. anthracis cells are found in the blood and tissues of hosts that succumb to anthrax infection, denoting an exceptional ability to acquire essential nutrients. Iron is a growth-limiting nutrient for bacterial pathogens, as in the host where extracellular iron levels are low. In addition, mammals compartmentalize the majority of iron within heme to counteract free iron toxicity, as well as acting as an innate resistance mechanism by providing a barrier to subvert iron accessibility. Since iron is essential for metabolism and growth, B. anthracis must possess efficient mechanisms to acquire host-iron. B. anthracis is able to sequester host-iron by directly binding iron with siderophores. While siderophores provide an iron source, these do not target the most abundant host iron-pool: hemoproteins. B. anthracis harbors an eight-gene isd locus that encodes a system enabling iron scavenging from hemoproteins during infection via the use of near-iron transporter domains (NEATs). A NEAT domain is a conserved motif attributed to heme and hemoglobin (Hb) binding, heme extraction from Hb, and heme transfer. The B. anthracis isd NEAT proteins consist of a cell wall-anchored protein that binds heme (IsdC) and two secreted hemophores, IsdX1 and IsdX2, both of which are proposed to scavenge heme from host Hb in the extracellular environment during infection. However, deletion of IsdX1, IsdX2, and IsdC did not lend to a reduction in virulence, suggesting other proteins may be more important or that there is functional redundancy. Interestingly, there are other NEAT proteins not encoded in the isd locus. The cell envelope protein, BslK, was found to bind and transfer heme to IsdC. Also, Carlson et al. (2009) compared gene expression of B. anthracis in iron-deplete media to iron-replete media, and identified bas0520 to be upregulated in low-iron conditions. The group generated a deletion mutant and showed that lack of bas0520 resulted in attenuated virulence of B. anthracis in an inhalational anthrax murine model of infection.

In silico analysis indicates bas0520 harbors a putative NEAT and several leucine-rich repeats (LRRs). Further analysis of the putative bas0520 NEAT domain revealed sequence similarity to the NEAT of isdC (38.3%), isdX1 (45.6%), bslK (63.7%), and the 5 NEATs of isdX2 (43.1%). Bas0520 NEAT domain was purified and specific heme binding and heme scavenging from Hb was assessed demonstrating this domain contains binding activities. Growth assays comparing B. anthracis Sterne (WT) and B. anthracis Sterne Δbas0520 in iron deficient media with heme and Hb as the iron sole source demonstrate WT has a higher growth rate than mutant strains. These results suggest that Bas0520 is a conduit of iron uptake during anthrax infection. The characterization of iron uptake mechanisms in pathogenic bacteria will aid in identification of virulence determinants and the development of therapeutics to treat bacterial infections.

Contributors: Balderas, Miriam; Maresso, Anthony
FREE CHOLESTEROL UNCOVERS DIFFERENTIAL rHDL FORMATION BY APO A-I AND A-II

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Cardiovascular disease (CVD) is a major cause of human mortality and identification of therapies addressing its underlying causes has a high public health priority. Dysregulated lipid metabolism leading to elevated LDL-cholesterol (C) and low plasma HDL-C are important CVD lipid risk factors. In the statin age, low HDL-C has emerged as the most important lipoprotein disorder for which current therapies are inadequate. HDL-C and CVD risk are mechanistically linked to reverse cholesterol transport (RCT) - the transfer of free cholesterol (FC), via HDL, from macrophages in the subendothelial space of atherosclerotic lesions to the liver for disposal. Although some therapies modestly increase HDL-C and reduce CVD events, new and more effective therapies producing robust increases in HDL-C and RCT are needed. Before this can occur, the determinants of RCT need to be more thoroughly understood.

To elucidate the structures, mechanisms, and molecular determinants associated with formation of HDL, we first determined the in vitro physical processes by which apos A-I and A-II (in both dimeric and monomeric forms) interact with model membranes (DMPC) and varying amounts of FC to form rHDL (reconstituted HDL). Employing kinetic turbidimetry and size exclusion chromatography, we established that rHDL formation from apo A-II and reduced/carboxymethylated (rcm) apo A-II is weakly dependent on FC content. rHDL formed from apo A-I and rcm A-II were smaller than those from apo A-I. FC (0 – 15 mol%) increased rates of rHDL formation from (1.7 ± 0.08) x 10-2 sec-2 to (2.1 ± 0.03) x 10-2 sec-2 for apo A-II and from (5.1 ± 0.02) x 10-2 sec-2 to (7.1 ± 0.02) x 10-2 sec-2 for rcm A-II. No rHDL formed above 20 mol% FC. Despite the greater lipophilicity of apo A-II vs. apo A-I, FC is a poorer inhibitor of rHDL formation from apo A-II up to 20 mol% FC above which rHDL no longer forms. We propose that at 20 mol% the sizes of FC-DMPC domains are too large to be circumscribed by apo A-II and rcm A-II. These data reveal FC as a potential intrahepatic determinant of HDL formation. Using these understandings, we are designing cell studies to investigate the determinants associated with formation of nascent HDL from macrophages with and without cholesterol loading. These studies will provide clues as to the importance of domain size on the efflux phenomena as a function of cholesterol concentration.

Contributors: Pownall, Henry J
STEM CELL ANTIGEN-1 (SCA-1) REGULATES MAMMARY TUMOR DEVELOPMENT AND CELL MIGRATION

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Background: Stem cell antigen-1 (Sca-1 or Ly6A) is a glycosyl phostidylinositol (GPI)-anchored cell surface protein associated with both stem and progenitor activity, as well as tumor initiating-potential. However, at present the functional role for Sca-1 is poorly defined.

Methodology/Principle Findings: To investigate the role of Sca-1 in mammary tumorigenesis we used a mammary cell line derived from a MMTV-Wnt1 mouse mammary tumor that express high levels of endogenous Sca-1. Using shRNA knockdown, we demonstrate that Sca-1 expression controls cell proliferation during early tumor progression in mice. Functional limiting dilution transplantations into recipient mice demonstrate that repression of Sca-1 increases the population of tumor propagating cells. In scratch monolayer assays Sca-1 enhances cell migration presumably by limiting cell adhesion. Microarray analysis indicates that repression of Sca-1 leads to changes in expression of genes involved in proliferation, cell migration, immune response and cell organization.

Conclusions/Significance: Sca-1 exerts marked effects on cellular activity and tumorigenicity both in vitro and in vivo. A better understanding of Sca-1 function may provide insight into the broader role of GPI-anchored cell surface proteins in cancer.

Contributors: Batts, Torey; Machado, Heather; Zhang, Yiqun; Creighton, Chad; Li, Yi; Rosen, Jeffrey
Tumors of the central nervous system are the second most common cancer in children. Deaths caused by CNS tumors are among the highest in pediatric cancers, and over the past three decades there have been few therapeutic advances. The optimal treatment of childhood brain tumors, particularly glioblastoma multiforme (GBM) and medulloblastoma (MB) requires a better understanding of tumor biology and the development of novel treatment strategies. Two of the most fundamental, though poorly understood questions regarding GBM and recurrent MB are: which invading cells have the ability to populate new tumors and what molecular mechanisms drive tumor progression? The emerging concept of the “tumor stem cell” argues that although either cancer stem cells or their more differentiated progeny may be capable of migrating into normal tissues, only stem cells have the self-renewal capacity to create clinically meaningful macroscopic disease. Therefore targeting genes essential for self-renewal of stem cells may result in a cell population with limited proliferation potential and a significantly reduced capability of developing recurrent tumors. BMI-1 is a known regulator of self-renewal and may directly participate in tumor invasion and metastasis. BMI-1 is a member of the polycomb group gene family of transcription repressors and is required for maintenance of self-renewal in neural stem cells. Over expression of BMI-1 has been reported in multiple tumors and we have detected high levels of BMI-1 mRNA transcripts in 34/48 human MB tumors using qRT-PCR. We have hypothesized that over expression of BMI-1 facilitates the development of metastatic foci in both cancer stem cells and non-stem cells, and that silencing BMI-1 expression will suppress tumorigenicity and eliminate tumor-forming capabilities of invading cells by blocking epithelial to mesenchymal transition. To accomplish this we have utilized a lenti-viral shRNA specific for BMI-1 to silence expression in GBM in vitro, we demonstrated a 50% reduction in GBM cell proliferation after exposure to the virus. To further examine the impact of silenced BMI-1 on tumor formation, we injected the lenti-virus transduced tumor cells from two GBM xenograft models into the brains of SCID mice. Significant suppression of tumor formation was observed in both models. Gene expression analysis of these tumor cells demonstrated decreased BMI-1 expression. More importantly, we identified a novel subset of downstream genes that were affected by silenced BMI-1 gene. Given the important role of BMI-1 in stem cell renewal, and its elevated levels in multiple cancers, our findings provided additional experimental evidence to support the prioritization of BMI-1 as a potential therapeutic target in the treatment of pediatric brain tumors.

Contributors: Baxter, Patricia; Zhao Xiumei, Liu Zhigang, Yu Litian, Gurusiddappa Sivashankarappa, Voicu Horatiu, Leung Eastwood, Adesina, Adekunle, Chintagumpala, Murali, Blaney, Susan, and Li, Xiao-Nan
STUDIES IN FRUIT FLIES HAVE PROVIDED SIGNIFICANT INSIGHTS INTO OUR UNDERSTANDING OF THE MECHANISMS BY WHICH NEURODEGENERATION OCCURS IN CERTAIN MUTANTS. IN A FORWARD GENETIC SCREEN IN OUR LAB, WE IDENTIFIED MISSENSE MUTATIONS IN THE aats-met GENE, CODING FOR THE MITOCHONDRIAL METHIONYL-tRNA SYNTHETASE, THE ORTHOLOG OF HUMAN MARS2. THESE PROTEINS ARE REQUIRED DURING PROTEIN SYNTHESIS IN MITOCHONDRIA. THE FLY MUTATIONS HAVE BEEN SUCCESSFULLY RESCUED BY EXPRESSION OF DROSOPHILA AND HUMAN cDNAs. WE ALSO CONFIRMED USING A FLAG TAG THAT THE PROTEIN IS MITOCHONDRIALLY LOCALIZED. HOMOZYGOUS MUTANT CLONES PRODUCED WITH THE eYFLP/FRT TECHNIQUE REVEAL A GRADUAL LOSS OF SYNAPTIC TRANSMISSION OVER THE COURSE OF FOUR WEEKS AS GAUGED BY THE PROGRESSIVE LOSS OF DEPOLARIZATION IN ELECTRORETINOGRAMS, SUGGESTING A PROGRESSIVE DEGENERATION. ADDITIONALLY, TRANSMISSION ELECTRON MICROSCOPY OF AGED aats-met MUTANT EYE TISSUE DISPLAY A DISORGANIZED AND SEVERELY ALTERED MORPHOLOGY OF PHOTORECEPTORS, WITH GREATER MITOCHONDRIAL MASS AND LIPID DROPLETS IN BOTH THE RETINAL AND LAMINAR LAYERS. INTERESTINGLY, THE MUTANT PHENOTYPES ARE Milder AT 18°C, ALLOWING FOR THE GENERATION OF TRANSHETEROZYGOUS ESCAPERS. THESE ESCAPERS EXHIBIT NEURODEGENERATIVE PHENOTYPES, REDUCED LIFESPAN, AND FLIGHT MUSCLE DEGENERATION. WE SUBSEQUENTLY FOUND THAT THE MITOCHONDRIA EXHIBIT DEFECTS IN COMPLEX I AND AN INCREASE IN REACTIVE OXYGEN SPECIES LEVELS. IN ADDITION, THESE MITOCHONDRIA EXHIBIT AN UNFOLDED PROTEIN RESPONSE, SUGGESTIVE OF PROTEIN MISFOLDING. WE ALSO NOTED THAT MUTANT TISSUES WERE SMALLER AND FOUND THIS TO BE DUE TO DECREASED CELL PROLIFERATION SECONDARY TO ROS. ADDITIONALLY, ANTIOXIDANTS IMPROVE SURVIVAL TO ADULTHOOD, THE DEGENERATION PHENOTYPE, AND OVERALL EYE APPEARANCE AND SIZE. FINALLY, WE FOUND THAT MUTATIONS IN THE HUMAN MARS2 LOcus ARE RESPONSIBLE FOR THE NEUROLOGICAL DISEASE ARSAL (AUTOSOMAL RECESSIVE SPASTIC ATAXIA WITH ASSOCIATED LEUKOENCEPHALOPATHY), WHICH HAD BEEN MAPPED TO THIS REGION. ANALYSIS OF 60 PATIENTS SHOW THAT THEY EXHIBIT DIFFERENT COMBINATIONS OF THREE REARRANGEMENTS IN THE MARS2 GENE, IDENTIFIED BY COPY NUMBER VARIATION ANALYSIS. IN ADDITION, THESE PATIENTS HAVE REDUCED LEVELS OF THE MARS2 PROTEIN AS WELL AS MITOCHONDRIAL TRANSLATION DEFECTS, AND PATIENT CELLS HAVE HIGHER ROS LEVELS AND REDUCED CELL PROLIFERATION RATES. Thus, our Drosophila study of aats-met will provide insight into the progression and pathology of this and related diseases.

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TARGETING DENDRITIC CELLS IN VIVO USING LYMPHOCYTES MODIFIED TO EXPRESS TUMOR ANTIGENS AND DENDRITIC CELL-ACTIVATING MOLECULES FOR CANCER VACCINATION

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Cancer vaccines have shown promise in small animal models of cancer, but have thus far been disappointing in clinical settings. Cancer vaccine success is dependent on the delivery of tumor-associated antigens (TAA) within lymphoid tissue in the context of costimulatory molecules and immune stimulatory cytokines. Lymphocytes expanded ex vivo readily migrate to lymphoid tissue following infusion. Lymphocytes may also be genetically modified to express both TAA and dendritic cell (DC)-activating molecules. These properties make lymphocytes ideal candidates as cellular vehicles for TAA delivery to lymph node-resident DC in vivo. We hypothesize that lymphocytes modified to express the DC-activating molecules flagellin and CD40 ligand (CD40L) may efficiently target TAA to lymphoid tissue and prime anti-tumor T cell responses via the induction of DC maturation.

Administration of Ova-pulsed lymphocytes primes peptide-specific CD8+ T cells, and leads to decreased tumor growth and increased survival in mice subsequently challenged with B16-Ova (p<0.05). Vaccination with Ova- or Trp2-pulsed lymphocytes in combination with LPS or anti-CD40 results in decreased tumor growth and increased survival in mice with pre-established B16-Ova or B16-F10 tumors (p<0.05). Vaccination with Ova- or Trp2-pulsed lymphocytes alone did not inhibit tumor growth. Lymphocytes genetically modified to express the toll-like receptor 5 agonist flagellin or CD40L induced the maturation of dendritic cells in vitro. Vaccination with Trp2-bearing lymphocytes genetically modified to express flagellin or CD40L inhibited tumor growth in mice with pre-established B16 melanoma tumors in comparison to unmodified lymphocyte vaccination (p<0.05). The efficient delivery of TAA to lymphoid tissues by lymphocytes-modified to express DC-activating molecules overcomes a major limitation of other vaccine strategies.

Contributors: Bear, Adham; Turnis, Meghan; Song, Xiao-Tong; Cruz, Conrad; Gottschalk, Stephen; Rooney, Cliona; Foster, Aaron
Heart failure affects more than 5 million Americans and has diverse etiologies; most commonly hypertension, coronary artery disease, and diabetes. While the molecular mechanisms remain poorly understood, research has shown that aberrant cardiac calcium handling plays a role in the progression of this disease. Junctophilins are a novel family of proteins that help approximate the plasma membrane and sarcoplasmic reticulum (SR) forming junctional membrane complexes. Specifically, junctophilin-2 (JPH2) is the predominant cardiac isoform that has been shown to approximate plasma membrane L-type calcium channels and SR ryanodine-receptor 2 (RyR2) facilitating calcium-induced calcium release.

Previous efforts in our lab have shown that sh-RNA-mediated knockdown of JPH2 leads to heart failure. In addition, JPH2 was found to be down-regulated in heart failure patients, and several mutations were identified in JPH2 in a cohort of patients with hypertrophic cardiomyopathy (HCM). Our lab has generated three transgenic mouse lines expressing these observed mutations. With this information we hypothesize that mutations in JPH2 lead to a heart failure phenotype due to aberrant calcium handling in the heart and provides a novel therapeutic target.

Initial long term echocardiography and survival studies have shown that the transgenic JPH2 mutant mice fail to develop spontaneous hypertrophy or heart failure. Current efforts have focused on subjecting these mice to exercise, pharmacological, and hypertensive stress to assess the ability of these mutant mice to compensate for physiologic stress compared to both nontransgenic mice and wild-type JPH2 transgenic mice. Preliminary exercise studies show mild physiological hypertrophy, but no significant differences from non-transgenic controls at the conclusion of exercise or after one month of rest. A more strenuous protocol using tail weights is now being tested. In addition, cohorts are being subjected to low dose isoproterenol stress using implantable pumps or transaortic constriction. Co-immunoprecipitation studies have been used to explore the binding site between RyR2 and JPH2, and its possible disruption by the disease-causing mutations. Finally, future studies will use calcium imaging to study calcium handling in the mutant transgenic cells, and electrophysiology to assess whether these mice are predisposed to arrhythmias.
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Congenital diaphragmatic hernia (CDH) is a life-threatening sporadic birth defect that affects approximately 1 in 4000 newborns. Terminal deletions of chromosome 9p have been identified in several patients with CDH. However, the gene(s) that contribute to the development of CDH in these patients has yet to be identified. In a recessive ENU mutagenesis screen we identified a mouse strain that was homozygous for a truncating mutation (L826X) in Frem1—a basement membrane encoding gene located on chromosome 9p22.3. A portion of Frem1-deficient mice develop retrosternal CDH. Penetrance of this phenotype is highly dependent on strain background, providing evidence for the existence of one or more genetic modifiers. Several other genes have been implicated in the development of retrosternal CDH in mice including Slit3—which encodes another extracellular matrix protein—and Gata4—which encodes a transcription factor that has also been implicated in the development of CDH associated with 8p23.1 deletions in humans. We have shown that both of these genes interact genetically with Frem1 in other developmental processes. Analyses of Slit3 and Gata4 mouse models of CDH suggest that a number of histopathologic changes in the anterior diaphragm may contribute to an increased risk of developing CDH. These changes include decreased cell proliferation, increased cell death, decreased diaphragm thickness and disorganized collagen fibrils in the central tendon. We are presently working to determine if similar changes are present in Frem1-deficient mice. Preliminary results of phospho-histone H3 immunostaining revealed a trend towards decreased cell proliferation in the anterior and central diaphragm of Frem1L826X/ L826X mice compared to littermate controls. A similar pattern has been reported in Slit3 mice, suggesting that the extracellular matrix plays an important role in regulating cell proliferation in the developing diaphragm. We are also working to determine the expression pattern of Frem1 and associated genes in the developing diaphragm. These studies will help us understand the mechanisms by which Frem1 deficiency causes Retrosternal CDH in mice and may provide insight into the causes of similar hernias in humans.

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B-VITAMIN DEFICIENCY IS PROTECTIVE IN EXPERIMENTAL COLITIS

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Background: Methionine (Met) cycle is disrupted in inflammatory bowel disease (IBD) as shown by elevated levels of homocysteine (Hcys). It is suspected that elevated Hcys increases inflammation in these patients. Some IBD patients with elevated Hcys have deficiencies in the B vitamins. Met cycle activity is critical for normal cell function and supplies the methyl donor, S-adenosylmethionine (SAM) for methylation reactions. SAM is converted to S-adenosylhomocysteine (SAH) when used as a methyl donor. SAH, an inhibitor of methyltransferases, is metabolized to Hcys. Hcys can be disposed of via 2 pathways: transsulfuration (TS) requiring vitamin B6 or remethylation (RM) requiring B12. The reaction of SAH to Hcys is reversible, so both metabolites can accumulate when Hcys disposal is impaired. However, the role of Met cycle disruption in the activation, progression, and remission of IBD is poorly understood. We hypothesize that Met cycle disruption due to vitamin deficiency exacerbates experimental colitis.

Methods: We tested the role of Met metabolism using mouse model of colitis. Mice received either a purified diet (Con) or a purified B6/B12-deficient (Def) diet to disrupt Met cycle for 2 weeks. Half of the mice in each diet group were then treated with 3-5% dextran sulfate sodium (DSS) for 3-5 days to induce colitis. We measured weight change and scored a disease activity index (DAI). Plasma and tissue were analyzed for Met metabolomics, myeloperoxidase (MPO) activity, and histopathology. A subgroup of mice in the same treatment groups also received a 4 hour continuous infusion of 13C-2H3-methionine to quantify Met kinetics. We measured enrichment of 13CO2 of expired air by IRMS and labeled Met and Hcys in tissues by GCMS.

Results: Unexpectedly, mortality was lower in Def mice (37.5%) after 5% DSS (3 days) when compared to Con mice (87.5%). Although no mortality was seen in 3% DSS (5 days), Def mice had lower DAI scores compared to Con mice and lost less body weight. Histological score was reduced; MPO activity and mRNA expression of TNF and iNOS were significantly reduced in Def mice. Methionine kinetics indicated that although TS was significantly reduced, RM was significantly higher in the Def diet fed mice. Metabolomic profiling showed that SAH, Hcys, and cystathionine were significantly elevated in Def diet mice compared to Con. Markers of B6 deficiency were significantly different, while B12 markers were minimally affected.

Conclusions: Met cycle disruption via B6 deficiency was protective during colitis. This is in contrast to studies in IBD patients which associate elevated Hcys with increased inflammation. We postulate that suppression of Hcys disposal via transsulfuration leads to conservation of methyl groups which alters epigenetic regulation of the inflammatory process.

Contributors: Stoll, Barbara; Puiman, Patrycja; Bauchart-Thevret, Caroline; Chacko, Shaji; Marini, Juan; Stabler, Sally; Burrin, Douglas
DOPAMINE IS REQUIRED FOR LEARNING AND FORGETTING IN DROSOPHILA

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After learning, it is unknown why animals begin to forget what they have learned. Using Drosophila, we sought to uncover molecular and neuronal circuits that may regulate forgetting. We found that blocking dopamine neuron output after learning increased memory retention of aversive olfactory memories. This suggests that, after learning, dopamine neurons are actively causing forgetting. Furthermore, stimulation of dopamine neurons after learning accelerated forgetting. Our data suggest that activity of a subset of dopamine neurons innervating the mushroom bodies is necessary and sufficient to regulate forgetting. In addition, blocking these dopamine neurons immediately after learning seems to specifically preserve early labile memory, whereas stimulation can cause forgetting of both labile and stable odor memories. Finally, we demonstrate that activity from this subset of dopamine neurons is also required for robust memories to form. Therefore, our data indicates that dopamine plays a dual role in learning and forgetting in Drosophila.

Contributors: Sandoval, Isaac C; Nicholas, Eric P; Davis, Ronald L
Multiple sclerosis (MS), an autoimmune disease of the central nervous system, affects more than a million people worldwide. Like most autoimmune diseases, the exact cause or trigger of MS is unidentified, pathophysiology poorly understood and treatment inadequately developed. Currently there exists no curative therapy for this disease. Autophagy influences many cellular behaviors including antimicrobial defense, maintenance of immune cell homeostasis, tolerance, and antigen presentation. Although involved in infection, inflammatory or neurodegenerative diseases, potential roles of autophagy in autoimmunity are inadequately investigated.

We hypothesized that autophagy in cells of myeloid origin might modulate development of MS, a demyelinating autoimmune disease of the central nervous system (CNS) where microglial cells, CNS-resident macrophages of myeloid origin, play an important role. To test this hypothesis, we induced experimental autoimmune encephalitis (EAE), widely regarded as an animal model of MS, in wild type or myeloid-specific autophagy knock-out (ATG7 KO) mice. We monitored disease severity by grading each animal in a scale of 0-5 (0: no disease, 5: death from EAE) and studied adaptive and immune functions potentially involved in autoimmunity.

We found that despite similar incidence and onset, severity of EAE is significantly lower in ATG7 KO mice in comparison to littermate controls. Currently we are investigating the underlying mechanisms.

Our data demonstrate for the first time that ablation of autophagy in myeloid-cells inhibits progression of EAE, a mouse model of multiple sclerosis. This might help in better understanding of the underlying pathophysiology and future development of novel therapeutic strategies for ameliorating these types of autoimmune disease.
THE CEREBROVASCULAR AND ANTIOXIDANT EFFECTS OF HYDROPHILIC CARBON CLUSTERS IN A MILD TRAUMATIC BRAIN INJURY MODEL COMPPLICATED BY HEMORRHAGIC SHOCK IN RATS

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Introduction: Traumatic brain injury (TBI) is associated with oxidative damage which may impair autoregulation of cerebral blood flow (CBF) and worsen outcome in the context of hypotension. Both superoxide radicals and hydroxyl radicals are increased in experimental TBI models and are rapidly released upon resuscitation (Fabian et al 1995). Polyethylene glycolated hydrophilic carbon clusters (PEG-HCCs), which are highly modified nano-structures derived from single walled carbon nanotubes, have potent antioxidant properties in vitro models of oxidative stress and have a more rapid onset of action than either superoxide dismutase or a small molecule antioxidant, PBN (Kent et al, 2011). The present study was to determine the potential beneficial effects of PEG-HCCs on resuscitation CBF and superoxide levels in a model of mild TBI complicated by hemorrhagic shock (HS). Methods: Long Evans rats were randomly assigned to 4 groups: 1. Sham + vehicle (n =9), 2.Sham + PEG-HCCs (n=4), 3. TBI + HS + vehicle (n= 12), 4. TBI + HS + PEG-HCCs (n = 10). For TBI + HS, an impact injury of 3m/sec and 2.5 mm deformation was induced followed by withdrawal of blood sufficient to reduce mean arterial blood pressure (MAP) to approximately 40 mmHg. After 50 min. an initial resuscitation phase (30 minutes) was started with administration of Lactated Ringer’s solution to achieve a MAP of 50mmHg. The third phase or “definitive hospital care” also lasted for 30 min. during which the room air was switched to 100% oxygen and the shed blood was re-infused. The drug PEG-HCCs (2mg/kg body weight) or vehicle was given immediately prior to the definitive hospital care phase and the animals were monitored for 6 hrs post-TBI by MAP, intracranial pressure and CBF. The CBF was assessed in the injured cortex and the contralateral cortex and expressed as % baseline. A superoxide dye was injected 6 hrs post-TBI. Results and Conclusions: We investigated whether the highly modified nanomaterial, PEG-HCC’s, that have shown rapid antioxidant properties in cultured cell lines, would improve CBF and reduce levels of superoxide in an animal model of mild TBI accompanied by hemorrhagic hypotension. Treatment with PEG-HCCs rapidly increased cerebral blood flow at the injury site, and CBF was restored to pre-injury levels in the perilesional cortex. The restoration of CBF was transient and lasted ~2 hours after a single injection which is the approximate half life of PEG-HCC’s in blood. Lack of effect in the contralateral hemisphere indicates that this improvement in CBF is not a non-specific CBF raising effect of the PEG-HCC’s. PEG-HCC treatment also significantly lowered levels of superoxide in the brain and more dramatically in the cerebral blood vessels 6 hrs post-TBI.

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The silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) coregulator promotes breast carcinogenesis through multiple cellular pathways

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Aberrant regulation of nuclear receptor coregulators is associated with a wide variety of human diseases. For instance, steroid receptor coactivator-3 (SRC-3) is an oncogene and increased SRC-3 expression is found for a significant number of human breast tumors. We recently determined that the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) coregulator could interact with SRC-3, increasing its intrinsic transcriptional activity and association with an estrogen receptor-(ER) target gene, cyclin D1 (Mol Endocrinology 24:1187, 2010). Expression of SRC-3 and SMRT were also positively correlated in a small cohort of breast tumors. We therefore wanted to examine the impact of SMRT in breast cancer using control versus SMRT-specific siRNA in ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast cancer cell lines. Depletion of SMRT reduced proliferation of MCF-7 cells grown as a monolayer on plastic under basal, estradiol (E2) or 4-hydroxytamoxifen (4HT) treatment conditions. The relative agonist activity of 4HT with respect to cell growth was not increased by SMRT depletion. In contrast, growth of MDA-MB-231 cells was not reduced by SMRT depletion. Flow cytometric analysis of cell cycle distribution revealed the E2-induced shift in synchronized populations from the G1 to S/G2/M phase was compromised in SMRT-depleted MCF-7 cells, consistent with a loss of estrogen induction of cyclin D1 expression in these cells. Moreover, a hormone independent increase in the percentage of SMRT-depleted MCF-7 cells in the subG0 population was detected suggesting an increase in apoptosis in SMRT depleted cells that was confirmed by Cell Death ELISA, annexin V staining, and PARP cleavage. Short hairpin RNA technology was used to generate MCF-7 cells with stable knockdown SMRT which produced fewer colonies in soft agar assays in comparison to control cells indicating that SMRT promotes anchorage-independent growth. The number of mammospheres formed by SMRT-depleted MCF-7 cells also was reduced compared with control cells and their structures were less well organized. Collectively, these data reveal that SMRT impacts breast tumorigenesis through multiple pathways that inhibit apoptosis, promote cell proliferation and promote anchorage-independent cell growth, and suggest SMRT is a potential therapeutic target for breast cancer.

Contributors: Chaubal, Vaishali; Karmakar, Sudipan
Estrogen receptor-alpha (ER) is a type I nuclear receptor essential for development and function of the female reproductive system. However, it is also involved in etiology and progression of diseases such as breast cancer and osteoporosis. Ligand-activated ER regulates gene transcription mostly through binding on DNA at estrogen response elements primarily located upstream of target genes. To directly visualize the mechanisms involved in ER mediated gene regulation, we have created and validated a microscopy-based approach to quantify coregulator recruitment to an ER-occupied promoter locus (Sharp et al, 2006, J Cell Sci; Berno et al, 2008, PloSOne; Ashcroft et al, 2011, Gene) as part of a platform that is amenable to high content analysis and screening (HCA/HCS). This system exploits an engineered dual stable HeLa cell line harboring a microscopically-visible, multicopy integration of the ER-regulated prolactin promoter array and GFP-ER (PRL-HeLa). The goal of the current project is to identify the full spectrum of ER coregulators in order to create a more complete picture of ER-regulated transcription in a cellular context. We have performed a screen of >1000 antibodies recognizing factors that have a role in gene transcription by HCA, and have identified members of the mediator complex and other basal transcription machinery that target the PRL array following treatment with estradiol (10-8M), tamoxifen (10-8M) or bisphenol A (10-6M). In parallel, we have also performed GFP-ER immunoprecipitation in estradiol-treated (10-8M) PRL-HeLa extracts followed by mass spectrometry (IP-MS) to identify ER interactors. IP-MS identified known (i.e. SRC-3) and novel (i.e. SIRT1). We also coupled these datasets with a siRNA screen to look for functional consequences of loss of coregulatory proteins on ER loading to the array or its stability/levels. In the RNAi screen we identified helicases, ubiquitination enzymes, and nuclear receptors as possible modulators of ER action on the PRL array. One of the more interesting factor identified was the glucocorticoid receptor (GR). In further exploring the cross-talk between ER and GR, we observed, after treatment with agonists for both receptors, an ‘assisted loading’ phenomenonas described recently (Voss et al, Cell, 2011), which suggests that ER can enhance GR targeting to the PRL array through chromatin modifications. Future studies will focus on defining how the factors identified in the HTS affect ER transcriptional activity and what are the consequences of ER-dependent GR “assisted loading” in PRL-HeLa and MCF-7 cells.
Hemophilia B is a hereditary disorder characterized by a potentially life-threatening bleeding diathesis caused by loss of adequate function of factor IX (F.IX), a key circulating coagulation factor secreted by the liver. Replacement of the defective F.IX gene in hepatocytes using adenovirus (Ad) vectors could lead to recovery of normal hemocoagulation function. Although preclinical efficacy using this strategy has been shown in murine and canine hemophilia models, therapeutic levels in humans has yet to be achieved. When higher doses of vector have been used, systemic immune responses have led to serious adverse effects that hinder clinical use. Evidence suggests that the severity of the immune response is dose-dependent; therefore, achieving therapeutic transgene levels with lower vector doses could mitigate immune response-associated complications. We have observed that inhibition of Src family kinases by the small molecule inhibitor PP2 increases first-generation Ad-delivered reporter gene expression in vitro in a promoter-independent manner. shRNA knockdown of c-Src, a principal Src family member, further increases transgene expression, specifically implicating the activity of c-Src in the enhancement. Internalization of the vector as measured by quantitative PCR is not increased after PP2 treatment, while mRNA levels as measured by quantitative RT-PCR increased. These observations suggest that enhancement of transgene expression is mediated by transcriptional activation and not increased transduction efficiency. Pharmacologic inhibition of the transcription factor STAT3 attenuates PP2-mediated enhancement, suggesting that PP2 inhibition leads to increased STAT3 activation. Hepatocellular carcinoma cells were transduced with a helper-dependent Ad delivering F.IX gene behind the liver-specific PEPCK promoter. Increased F.IX secretion as measured by ELISA was detected after PP2 treatment. These experiments demonstrate the efficacy of PP2 to increase expression of Ad-delivered therapeutic transgenes including F.IX in vitro and provide preliminary evidence that PP2 could be used to lower effective vector doses in gene therapy applications. Future work will focus on the efficacy of PP2 at increasing circulating F.IX levels in a murine model of hemophilia B.
We previously reported that exon-focused clinical aCGH, in which array probes are concentrated in exons of disease genes, enables the detection of small copy-number variations (CNVs) affecting single genes or even single exons. We now show that, in addition to detecting intragenic CNVs associated with specific dominant clinical phenotypes, exon-targeted aCGH unveils heterozygous single-gene mutations in recessive disease genes (i.e. establishing carrier status) and in disease genes for late onset dominant disorders (i.e. potentially predicting disease susceptibility). DNA from 9,005 anonymized clinical patients was analyzed by exon-targeted aCGH. Computational analyses revealed CNVs in 3,946 individuals, 1,295 of whom exhibited a single CNV affecting a single gene. Eighty-six of these CNVs encompassed or disrupted one of 242 genes implicated in severe, pediatric, recessive disease [Bell et al. Sci Transl Med 3:65ra4 (2011)] and for which at least one exon had enhanced probe coverage on our array. In total, 39 unique genes were affected, including AHI1 (Joubert syndrome, type 3), ATR (Seckel syndrome, type 1), BCKDHB (classic maple syrup urine disease, type 1B), and CRTAP (osteogenesis imperfecta, type IIB). For 77/86 individuals, a brief (1-10 words) clinical indication was available; 6 of these descriptions suggested the possibility of recessive disease corresponding to the affected gene [e.g. “dysmorphic features” and VPS13B (Cohen syndrome)], though no indications were pathognomonic for any disorder. Our cohort also includes examples of potentially damaging single-gene CNVs in genes associated with susceptibility to dominant late onset disease, for example SPAST (autosomal dominant hereditary spastic paraplegia, type 4) and EXT2 (multiple exostoses, type 2). Previous investigation of carrier status and genetic load, whether population-based or grounded in recently acquired personal genome sequences, has focused on simple nucleotide variation (SNV). Our findings demonstrate that assessing CNV using clinical exon-targeted aCGH is an effective means not only for diagnosing genetic illnesses, but also for identifying CNV resulting in recessive carrier states and potential predisposition to late onset dominant disease. Which of these many variants are likely medically actionable and reportable to clinicians is an evolving question, made particularly challenging when the mutation is novel and has not been functionally or epidemiologically assessed.

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ABNORMAL NEURONAL ACTIVITY IN AN INDUCIBLE TRANSGENIC APP MODEL

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Our studies are aimed at characterizing the abnormal neuronal electrical activity occurring in a mouse model of Alzheimer's disease (AD) and how neuronal excitability is affected by amyloid precursor protein (APP) expression. A high percentage of AD patients experience seizures, which may contribute to the cognitive dysfunction that characterizes disease progression. Our APP-overexpression model of AD exhibits aberrant electrical activity, providing us with a model of abnormal neural networks that are created by expression of the transgene. Unlike other mouse models for AD, transgenic APP expression in our mouse can be temporally controlled using the tet-off system, which allows us to shut down APP overexpression rapidly and reversibly. This system is ideally suited to examining the impact of APP overexpression on electrical activity. Our data using indicates that abnormal activity is prevalent among our APP-expressing mice, and multiple mice have exhibited seizures during EEG recordings. This data also suggests that shutting down APP expression can significantly decrease the amount of abnormal spiking. Pathological levels of Aβ have been proposed as a contributor to the excitability and disruption of normal negative feedback. We used 10 mg/kg of the gamma secretase inhibitor LY411575, which selectively decreases levels of Aβ and does not affect APP levels and can be used to separate out the effect of Aβ from APP. The results from these EEG recordings show no change in the amount of abnormal spiking and suggest that Aβ production is not contributing to generation of abnormal hypersynchronous electrical activity. Further work is necessary to determine the mechanism by which hypersynchronicity is initiated and can be acutely halted in this AD mouse model. These experiments will help to resolve how abnormal neural networks and seizures are linked to overexpressed and faulty APP production.

Contributors: Born, Heather; Bradley, Kathryn; Yoo, Jong; Noebels, Jeffrey; Jankowsky, Joanna
Acute myeloid leukemias (AML) represent a heterogeneous group of blood malignancies with poor prognosis. Development of AMLs is associated with a blockade in terminal differentiation of myeloid cells leading to accumulation of immature blasts and emergence of a transformed leukemic initiating cell (LIC) population with long term self renewal properties. Genetic and phenotypic properties LICs are highly variable among AML patients as a consequence of variable cytogenetics, stem/progenitor cell of origin and disease progression. Efficient targeting of LICs to eradicate AMLs requires a detailed understanding of the common molecular signatures that distinguish LICs from normal stem/progenitor cells.

The orphan nuclear receptors (ONRs), NR4A1 and NR4A3, are potent tumor suppressors of AML. They are silenced in all human AML LICs irrespective of patient cytogenetics. Deletions in mice lead to extremely rapid postnatal AML development due to disruption of hematopoietic stem cell (HSC) homeostasis and the emergence of a transformed radioresistant LIC. We now show that acute rescue of NR4A1 or NR4A3 in human AML cells inhibits their proliferation and reprograms a subset of gene signatures that distinguish all primary human LICs from normal HSCs regardless of cytogenetic background. Through integration of NR4A regulated gene signatures with chemical genomics we have identified novel chemical activators of NR4As that reduce AML leukemogenicity, reprogram a subset of common LIC gene signatures, and surprisingly, drive trans-lineage priming of human AML blasts.

Together, our results identify NR4A1/3 as novel therapeutic targets in AML and provide a general strategy for discovering chemical modulators of ONR activity and delineating mechanisms of tumor suppressor gene silencing.

Contributors: Boudreaux, Seth; Conneely, Orla
Background: Psychological or neurocognitive impairment is often seen in medulloblastoma survivors after craniospinal radiation, however, significant variability in outcomes exists. This study investigated the role of antioxidant enzyme polymorphisms in moderating this outcome, and hypothesized that patients who had polymorphisms associated with lower antioxidant enzyme function would have a higher occurrence of impairment.

Methods: From the CCSS cohort, 109 medulloblastoma survivors and 143 siblings were identified who completed the CCSS Neurocognitive Questionnaire (NCQ) and the Brief Symptom Inventory-18 (BSI-18), and who provided buccal DNA samples. Real-time PCR allelic discrimination was used for SOD2 (rs4880), GPX1 (rs1050450), and GSTP1 (rs1695 and rs1138272) genotyping and PCR for GSTM1 and GSTT1 gene deletions. Outcomes on NCQ and BSI-18 subscale scores were examined in association with genotypes and clinical factors, including age at diagnosis, gender, and radiation dose, using univariate and multivariable ANOVA.

Results: Patients < 7 years old at diagnosis displayed more problems with task efficiency (p<0.001) and fewer problems with somatic complaints (p=0.004) than patients ≥ 7 years old. Females reported more organization problems than males (p=0.02). Patients with homozygous GSTM1 gene deletion reported higher anxiety (mean null genotype=47.3±9.2, non-null=43.9±7.8; p=0.04), more depression (null=51.0±9.8, non-null=47.0±9.4; p=0.03), and more global distress (null=50.2±9.7, non-null=45.2±9.9; p=0.01). All associations for the GSTM1 polymorphism remained statistically significant in a multivariable model controlling for age, gender, and radiation dose.

Conclusions: Homozygous GSTM1 gene deletion was consistently associated with greater psychological distress in medulloblastoma survivors across multiple domains, suggesting that this genotype may predispose patients for increased emotional late effects. GSTM1 may be of greater importance in the protection of the brain against damage from free radicals compared to other enzymes due to its function in processing of both radiation- and chemotherapy-induced free radicals. If validated in future studies, GSTM1 genotype may have utility as a risk marker for late effects.

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Cocaine (COC) is one of many illicit drugs that are commonly abused worldwide. Dependence on such substances is a significant public health issue. Currently, there is no drug approved by the Food and Drug Administration to treat COC dependence; however, recent data indicates that modafinil (MOD) is effective in reducing the subjective and reinforcing effects produced by COC. It remains unclear how MOD produces these reductions, yet information about the actions of MOD may be garnered by co-administering medications that either exacerbate or attenuate MOD’s documented effects. Much work has focused on the monoamine neurotransmitter dopamine (DA), which has been widely speculated to mediate the effects of MOD. MOD was recently discovered to bind and inhibit the membrane DA transporter, which increases DA within the brain and is an effect similar to that observed after COC administration. In addition, DA has long been implicated as critical to the rewarding and reinforcing effects produced by stimulants such as COC. Based on the preceding information, I hypothesize that MOD reduces the subjective and reinforcing effects of COC by increasing DA concentration in the brains of COC-abusing individuals. This hypothesis will be tested by enrolling participants who are not currently seeking treatment into phase I clinical trials. Participants will be administered MOD plus either lisdexamfetamine, which will increase brain DA, haloperidol, which will decrease brain DA, or placebo, then challenged with COC. Changes in DA concentration will be measured several ways, both directly and indirectly. These include the participants’ subjective responses, and measures of plasma prolactin and DA.

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LOSS OF RHO GDI( LEADS TO A TAMOXIFEN-RESISTANT PHENOTYPE IN ER(-POSITIVE BREAST CANCER CELLS DUE TO ALTERED SENSITIVITY TO GROWTH FACTOR SIGNALING PATHWAYS

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We have found that tamoxifen-resistant, metastatic breast tumors have lower levels of Rho GDI( than their tamoxifen sensitive counterparts. Rho GDI( is a negative regulator of the Rho family of proteins, which play an important role in the regulation of the actin cytoskeleton. While the Rho pathway is known to influence metastasis in breast and other types of cancers, it is unclear how this pathway causes ER(-positive breast cancer cells to become tamoxifen-resistant. Since the loss of Rho GDI( causes an increase in the activity of the Rho GTPases (RhoA-C, Rac, and Cdc42) it is possible that increased activity of specific downstream effectors may lead to tamoxifen resistance through crosstalk with the ER( signaling pathway. In addition, breast cancer cells which become resistant due to long term culture in the presence of tamoxifen, also exhibited a decrease in endogenous Rho GDI(. We hypothesize that loss of Rho GDI( results in a metastatic and tamoxifen-resistant phenotype due to crosstalk between the Rho GDI( and ER( pathways. To investigate this I will: 1.) Determine the effects of decreased Rho GDI( and its downstream effectors on hormone resistance in ER(-positive breast cancer cells using both in vitro soft agar assays and in vivo tumor xenograft growth experiments. 2.) Determine the effects of Rho GDI( loss on distant metastasis of ER(-positive breast cancer cells using both in vivo animal models and in vitro invasion and migration assays with specific signaling inhibitors to block ER( activation. 3.) Determine if the effects of Rho GDI knockdown on the ER( signaling are due to enhanced sensitivity of growth factor signaling pathways and subsequent crosstalk through non-cannonical ER( signaling. We will compare ER( phosphorylation and acetylation status in response to treatments with tamoxifen, growth factors, and various inhibitor combinations. We propose that disruption of bi-directional crosstalk may be a useful strategy to prevent or reverse tamoxifen resistance.

Contributors: Brusco, Lauren; Barone, Ines; Gu, Guowei; Covington, Kyle; Fuqua, Suzanne AW
The progesterone receptor (PR) is a member of the nuclear receptor superfamily of transcription factors and is most commonly activated by binding its ligand, progesterone. Upon ligand binding, PR undergoes a conformational change and translocates into the nucleus where it binds as a dimer to the progesterone response element (PRE) of target genes. Such target genes are thought to be responsible for the anti-proliferative and proliferative effects of progesterone within the uterus and mammary gland, respectively. However, direct PR target genes that contribute to the anti-proliferation or proliferation within these reproductive tissues are yet to be fully characterized. To address this matter, we used PR knockout mice to distinguish the gene expression profiles within the uterus and mammary gland in response to in vivo treatment with estrogen and progesterone for 48 hours. Initial mining of our uterine Affymetrix microarray data has allowed us to identify a number of potential direct and indirect PR target genes in the uterus. One gene of particular interest is the homeodomain only protein Hopx. Recently, Yamaguchi et al. have shown that Hopx is epigenetically silenced in uterine endometrial cancer and suppresses estrogen-stimulated proliferation in cancer cells. Because of its role in suppression of proliferation, we hypothesized that Hopx may be involved in the normal anti-proliferative role of progesterone within the uterus.

PR-dependent induction of Hopx was validated by qPCR and localization determined by in situ hybridization. Hopx expression and localization within the mouse uterus were consistent with studies in the human. In order to determine the function of Hopx within the normal uterus, we compared the uterine physiology of our wild type mice with that of Hopx null mice. Quantitative BrdU immunohistochemistry detected no statistical difference in cellular proliferation between wild type and Hopx null mice under our treatment conditions, suggesting that Hopx is not sufficient to inhibit estrogen-stimulated proliferation within the uterus. These findings were further supported by uterine weight/body weight ratios as well as expression analysis of cell cycle genes such as c-fos and cyclin D1. These results suggest that even though Hopx can suppress estrogen-stimulated proliferation of cancer cells in vitro, Hopx is not sufficient to suppress estrogen-stimulated proliferation of normal cells in vivo. Our findings further suggest that there may be additional mutations necessary to drive transformation of normal endometrial cells to cancer cells and that Hopx silencing alone is not sufficient to increase cellular proliferation in response to hormone stimulation.

Contributors: Bucher, Ashlee R; Conneely, Orla M
Rett Syndrome is a severe, X-linked, neurological disorder that affects 1 in 10,000 girls and presents with developmental regression, including concomitant autistic features. Mutations in methyl CpG binding protein 2 (MECP2) cause more than eighty percent of cases. Mecp2 function has been eliminated in Rett Syndrome mouse models, recapitulating many aspects of the human disease. Studies of this model have shown that symptoms can be reversed by restoration of Mecp2 function in symptomatic mice, and partially rescued with other factors. This provides substantial evidence that therapeutic intervention in Rett Syndrome is possible. Unfortunately, as a widespread epigenetic factor, MECP2 levels are extremely dosage sensitive, making direct manipulation a poor treatment option. Therefore, we have employed a random mutagenesis dominant screen in the mouse model to identify secondary molecules and downstream pathways that are important for suppression or amelioration of symptoms and may lend themselves to the development of new pharmacological treatments for Rett Syndrome. By employing a combination of SNP linkage mapping and whole exome sequencing strategies, we have mapped five mutations that lead to increased longevity, better functioning, and improved health in the presence of Mecp2 mutation, though none suppress the disease entirely. The candidate genes identified are unlikely to have been found using the reverse genetics approach that is more common when working with mice. All have given us new insight into MECP2 function and disease pathology; one candidate in particular is a drug target that suggests a new approach to Rett Syndrome treatment from a systems biology, rather than a purely neurological, perspective.
Background: P-TEFb (Positive Elongation Factor b) is a crucial host co-factor required for productive HIV-1 replication. P-TEFb is composed of a regulatory subunit Cyclin T1 and a catalytic subunit CDK9. The kinase activity of P-TEFb is dependent on the phosphorylation of Thr-186 in the T-loop of CDK9, following which a conformational change in the catalytic core of the enzyme permits access of substrates and ATP. This activated form of P-TEFb is recruited to the viral LTR by HIV-1 transactivator protein Tat to stimulate processive elongation of the viral transcripts by RNAPII. In resting CD4+T cells, which are refractory to HIV-1 replication, levels of T-loop phosphorylated CDK9 are low, but upon cellular activation, there is a significant increase in phosphorylated CDK9 levels. This suggests that the final phosphorylation status of CDK9 or the equilibrium between phosphorylated CDK9 and dephosphorylated CDK9 is likely due to a balance between the concerted actions of kinases and Ser/Thr phosphatases. We therefore attempted to delineate the role of the kinase CDK7 and the phosphatase PPM1A in regulating CDK9 T-loop phosphorylation and its effect on HIV-1 transcription.

Principal Findings: Our results indicate that WT-CDK9 and the catalytically inactive CDK9 D167N mutant are phosphorylated to a similar extent, suggesting that CDK9 T-loop is likely phosphorylated by an upstream kinase rather than an autophosphorylation event. Inhibition of CDK7 kinase activity by inhibitor H8 in Jurkat cells led to a marked decrease in CDK9 T-loop phosphorylation. Analogous inhibition of CDK7 kinase activity in an in vitro model of HIV-1 latency accelerated the return of activated provirus to latency. In contrast to the positive role of CDK7 in phosphorylating the CDK9 T-loop, we show that PPM1A inhibits HIV-1 proviral transcription from the viral LTR by dephosphorylating the CDK9 T-loop, thus preventing its activation and recruitment by viral protein Tat. PPM1A did not have an effect on CDK8 mediated activation of HIV-1 transcription in an artificial P-TEFb tethering system, suggesting that PPM1A catalytic activity might be specific for CDK9. Additionally the protein levels of CDK7 and PPM1A did not vary in resting and activated CD4+T cells, the physiologically relevant cell type for HIV-1 replication.

Significance: In order to gain deeper insights into P-TEFb function and its central role in HIV-1 transcription, it is necessary to understand how association and interaction with various protein modulators regulates P-TEFb activity in the cell. Our study highlights the complex interplay between CDK7 and PPM1A in modulating the CDK9 phosphorylation status and its consequent effect on HIV-1 replication.

Contributors: Budhiraja, Sona; Ramakrishnan, Rajesh and Rice, Andrew P.
SODIUM CHANNEL AUXILIARY SUBUNIT $\text{Na}_v\beta 4$ IS A CELL TYPE-SPECIFIC COMPONENT OF THE AXON INITIAL SEGMENT

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Action potentials are initiated at the axon initial segment (AIS). The molecular composition of the AIS, characterized by high-densities of voltage-gated Na$^+$ and K$^+$ channels and supporting scaffold proteins, is similar among cell types; firing patterns, however, vary between neuron classes. Differences in voltage-gated Na$^+$ channel regulation could contribute to their divergent physiologies. The specific composition the multimeric Na$^+$ channel complex dramatically influences channel activity as each modulatory $\beta$ subunit differentially affects $\alpha$ subunit physiology. Sodium channel auxiliary subunit beta-4 ($\text{Na}_v\beta 4$) favors open-channel conformation at depolarized potentials by destabilizing the inactivation state. Recent evidence indicates that $\text{Na}_v\beta 4$ may facilitate the generation of resurgent Na$^+$ current. $\text{Na}_v\beta 4$ expression is limited to a specific subset of cells in the nervous system and is correlated with high-frequency firing capacity. To determine the subcellular localization of $\text{Na}_v\beta 4$, we generated antibodies targeting $\text{Na}_v\beta 4$-specific epitopes. $\text{Na}_v\beta 4$ is enriched at the AIS of cerebellar Purkinje neurons and spinal motor neurons. $\text{Na}_v\beta 4$ is also enriched at nodes of Ranvier, sites of action potential propagation in myelinated axons, in the central and peripheral nervous system. To determine the AIS targeting motif of $\text{Na}_v\beta 4$, we expressed GFP-tagged terminal truncation mutants in cultured hippocampal neurons, a cell-type devoid of endogenous $\text{Na}_v\beta 4$ expression. Like full-length $\text{Na}_v\beta 4$, $\text{Na}_v\beta 4\Delta C$, but not $\text{Na}_v\beta 4\Delta N$, localized to the AIS. Subsequent single point mutation analysis of the $\text{Na}_v\beta 4$-GFP construct revealed that residue C28 is required for $\text{Na}_v\beta 4$ AIS localization. To determine if $\text{Na}_v\beta 4$ is recruited to the AIS through interaction with $\text{Na}_v\alpha$ subunits, we silenced $\text{Na}_v\alpha$ expression in developing neurons. In the absence of $\text{Na}_v\alpha$, $\text{Na}_v\beta 4$ was not enriched at the AIS. Once properly targeted to the AIS and nodes, what is the functional role of $\text{Na}_v\beta 4$? Immunofluorescence studies in optic nerve during postnatal development showed that $\text{Na}_v\beta 4$ incorporation into nodes of Ranvier correlates with the onset of high-frequency firing in retinal ganglion cells. To further characterize the functional role of $\text{Na}_v\beta 4$, we are generating $\text{Na}_v\beta 4$ conditional knockout mice that will be subjected to a range of physiological and behavioral tests. Modulation of $\text{Na}_v\alpha$ subunits through interaction with $\text{Na}_v\beta 4$ may provide a mechanism to sustain high-frequency firing at the AIS. Our results suggest that the cell-type specific molecular composition of the AIS may contribute to the variability in firing patterns observed between neuron classes.

Contributors: Buffington, Shelly; Ho, Tammy (Szu-Yu); Zhang, Chuansheng; Rasband, Matthew
PROINSULIN-EXPRESSING MACROPHAGES INFILTRATE OBESE ADIPOSE TISSUE AND CONTRIBUTE TO INSULIN RESISTANCE

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Background: Chronic inflammation of obese adipose tissue underlies insulin resistance in type 2 diabetes (T2D). While the heterogeneous adipose tissue macrophage (ATM) population constituting most of the immune infiltrate is known to locally compromise metabolic homeostasis and to be required for insulin resistance, specific ATM subsets and their respective contribution to the inflammatory milieu require further clarification. Studies in our laboratory identified a bone marrow-derived myeloid cell population present only in diabetic and insulin resistant animals. These cells express proinsulin and are therefore termed ectopic proinsulin-producing cells (EPPC). EPPC are present in liver, dorsal root ganglia (DRG) and peripheral nerves of ob/ob mice, high fat diet (HFD)-fed mice and streptozotocin-treated mice and rats. EPPC produce TNF-, a pro-inflammatory cytokine important for adipose inflammation and insulin resistance. In obese diabetic models, these cells are present in adipose tissue. We hypothesized EPPC comprise an ATM subset critical for promoting insulin resistance.

Results: To test the hypothesis, we analyzed EPPC distribution in visceral adipose tissue of mice fed HFD (60% kcal from fat) for 0, 8 and 16 weeks versus chow-fed littermates. Insulin gene expression and proinsulin immunopositive cells occur only in HFD mice and increase with duration of feeding. These EPPC co-express macrophage markers CD11b and F4/80 and preferentially localize to crown-like structures (CLS), collections of ATMs surrounding necrotic adipocytes whose number correlates with degree of insulin resistance. Overall, EPPC comprise the majority of the CLS macrophage population and uniformly co-express TNF-. When placed on HFD, MIP-GFP mice (which express GFP behind the mouse insulin promoter) have GFP(+)/proinsulin(+) CLS ATMs. To determine EPPC contribution to insulin resistance, we crossed inducible diphtheria toxin receptor (iDTR) mice with mice harboring insulin promoter-regulated Cre recombinase (RIPCre mice). Animals receiving bone marrow transplants from these iDTR/RIPCre mice undergo EPPC deletion upon systemic DT administration. An iDTR/RIPCre transplant recipient group was placed on 16 week HFD and treated with DT. Compared with an iDTR transplant negative control group, these mice had reduced adipose inflammation and improved insulin sensitivity.

Conclusions: We have identified a proinsulin-producing, proinflammatory macrophage population enriched in crown-like structures of obese adipose tissue that contributes to systemic insulin resistance. As EPPC appear in multiple diabetes models, we are investigating how the systemic diabetic environment influences adipose inflammation and insulin resistance.

Contributors: Buras, Eric Dale; Yang, Lina; Kaila, Vishal; Kusui, Shinnosuke; Kojima, Hideto; Chan, Lawrence
DEVELOPING PROGNOSTIC MARKERS FOR PEDIATRIC BRAIN TUMORS: TOWARDS PERSONALIZED THERAPY FOR EPENDYMOMA

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Background. Ependymomas (EPN) account for ~10% of intracranial tumors in children, typically presenting in the first five years of life. Despite recent advances in neurosurgical & radiotherapy techniques in the management of this disease, 5 year overall survival remains around 50%. Progression free survival (PFS) is worse, & late recurrences are not uncommon. These abysmal statistics stem from our profound lack of understanding about the biology of ependymoma. Currently the role of chemotherapy is not established & no compelling drug targets are known.

Materials & Methods. In an attempt to identify molecular markers that could predict clinical outcome, we analyzed 118 cases of pediatric intracranial ependymomas collected from Texas Children’s Hospital (n = 36) & Children’s Oncology Group (n = 82 from a single clinical protocol) using Affymetrix 250k Sty1 SNP arrays for copy number aberrations (CNAs) & loss of heterozygosity (LOH). Additionally, a subgroup was analyzed for risk of relapse against the expression of 367 miRNAs (TaqMan platform). Subjects ranged between 4 days & 19 years of age, with 44% under the age of four. Clinical features of this patient cohort are consistent with the disease epidemiology, with the ratio of infratentorial to supratentorial tumors being 2.11, & 44% of the cases were classified as anaplastic (WHO grade III). All but 8 patients received postoperative radiotherapy, & >76% received complete resection during their initial surgery. CNA calls were made based on a reference panel of matched blood samples from over half of these subjects, using the segmentation algorithm distributed by Partek, Inc.

Results. Visualization of these CNAs by unsupervised hierarchical clustering demonstrated three distinct groups. The first group is defined by multiple aberrations involving whole chromosomes or arms, with 6- & 9+ being the most common abnormalities. A second group dominated by 1q+ separated from the remaining cases with balanced karyotype or small focal CNAs. Patients with 1q+ ependymoma had significantly worse PFS, even after correction for all available clinical factors (Cox model HR = 3.940, p = 0.002). Membership in the 1q+ group was as predictive of outcome as tumor location & degree of surgical resection. Further investigation into gene expression within these 1q+ cases revealed gene transcripts (CHD1L – 1q21.11, PARP1 – 1q42.12) that could better identify high risk patients. This expression-based classifier was validated as prognostic using 23 independent EPN tumors, & immunohistochemistry against CHD1L proved prognostic on 170 independent FFPE cases. Additionally, immunoblot for PARP1 & CHD1L on a subset of the remaining frozen tissues (n = 21) suggested that observed expression intensities paralleled protein levels. Roughly 7% of the population not at risk via this signature had significant risk associated with overexpression of a miRNA cluster on chromosome 19q13.42. Inclusion of both signatures accounts for a majority of high risk cases.

Conclusions. We have developed a CNA-based molecular classification that has prognostic significance. Additionally, transcript over-expression of two genes within the CNA-based classifier was more predictive of PFS, & was further augmented by the addition of a classifier built upon miRNA expression in ch19q13.42. The two-gene expression signature was validated using array data in an independent sample, & immunohistochemically, both in a subset of cases & on an independent archive of EPNs. Both of these genes, PARP1 & CHD1L, are novel EPN related genes, & are currently targetable for chemotherapy. We are currently utilizing one such compound in combination with radiotherapy against a proprietary xenograft mouse model with known 1q+ and identical histolopathological features to the human disease.

Contributors: Burstein, Matthew; Chow Thomas; Shen, Jianhe; Wang, Hongmei; Su, Jack; Adesina, Adekunle; Dauser, Robert; Whitehead, William; Jea, Andrew; Curry, David; Chintagumpala, Muraili; Guerra, Rudy; Man, Chris; Lau, Ching
Detecting fast changes in the environment is important for an animal's survival. We presented observers with two brief flashes of variable duration—one following the other—and asked them to report whether they perceived both flashes or instead a single flash (a temporal fusion of the two). We found that certain combinations of durations engendered the double-flash perception more than others. With the behavioral data in hand, we then imaged participants on this task while we measured their neural activity with high temporal acuity using magnetoencephalography (MEG). We found several brain regions in which the neural activity correlated with the perception (one flash or two). A potential model for temporal separation of events is discussed.

Contributors: Cai, Mingbo; Bohuslav, Gregory; Rezaire, Roozbeh; Eagleman, David
The cochlea is the auditory part of the mammalian inner ear. The sensory epithelium of the cochlea is called the organ of Corti, which is comprised of sensory hair cells and supporting cells. Atoh1, the mouse homolog of Drosophila proneural gene atonal, is a bHLH transcription factor. Atoh1 is the earliest known gene expressed in hair cells, with its expression upregulated and maintained in hair cells until postnatal stages. There is a severe phenotype in the cochlea of Atoh1-null mice: the structure of the organ of Corti is disrupted and hair cells are completely absent from the sensory epithelia. In addition, over-expression of Atoh1 can also induce ectopic hair cells in the nonsensory epithelium of cochlea. These data provided strong evidence showing Atoh1 is both necessary and sufficient for hair cell differentiation.

To dissect Atoh1’s role during cochlear development, we established a conditional knockout (CKO) system that allows us to delete Atoh1 from hair cells at different stages using the inducible Cre-lox approach. Our preliminary data using this system showed that removal of Atoh1 in the newly differentiating hair cells resulted in the loss of most hair cells, with few hair cells remaining probably due to the inefficiency of Cre recombination. We also detected massive cell death in the sensory epithelia of these CKO cochleas. Interestingly, most of these apoptotic cells localized underneath the remaining hair cells, implying the removal of Atoh1 in hair cells might have a non-cell-autonomous effect on the surrounding supporting cells.

As a transcription factor, little is known about Atoh1 function on a cellular level. So far, very few genes are known as the direct targets of Atoh1 in hair cells, leaving Atoh1 function in hair cell development mysterious. Recently, two genome-wide studies have generated lists of Atoh1 targets in cerebellum and dorsal spinal cord, respectively, indicating Atoh1 might play diverse roles in different biological processes. From these two lists, we picked several candidate genes which show specific expression pattern in hair cells. We are currently examining whether these genes are Atoh1 targets in hair cells as well as their potential function in hair cell development. Meanwhile, in order to systematically identify Atoh1 targets in hair cells, we are also performing genome-wide screens in the mouse neonatal cochlea: RNA-seq to compare the transcription profiles in the Atoh1-expressing and Atoh1-non-expressing cells; Atoh1 ChIP-seq to identify genomic Atoh1-binding sites. These approaches combined might allow us to identify more Atoh1 targets in hair cells and to unravel the molecular role of Atoh1 during cochlear development.

Contributors: Cai, Tiantian; Klisch, Tiemo; Zoghbi, Huda; Groves, Andy
Obesigenic diets cause infiltration of leukocytes and inflammatory molecules expression in the adipose tissue (AT). Anti-inflammatory AT macrophages (ATM) maintain AT homeostasis through the secretion of anti-inflammatory molecules and the phagocytosis of apoptotic adipocytes. Macrophage polarization reflects the microenvironment and can depend on cytokines and fatty acids available. In-vitro, Toll-like receptor -2 and -4 are required for saturated fat-induced inflammatory activation of macrophages, while unsaturated fats are ligands for PPARy. The project goal is to analyze the very early inflammatory response to a diet high in milk fat that is rich in palmitic and oleic acid. I hypothesize that ATM initiate early changes and TLR-2, -4 are necessary for their inflammatory activities. We have examined AT molecular and cellular changes after 3 days of HF feeding, and utilized genetically-deficient mouse models to test this hypothesis. Expression of CCL2, F4/80, CXCL1 and Spp1 was significantly increased in the mesenteric AT. TLR2 was necessary for F4/80 expression, while CCL2 expression required TLR4. TLR4-/− mice, in contrast to wildtype and TLR2-/− mice, exhibited a significant increase in M2 macrophage markers. A macrophage cell line upregulated M1 macrophage markers (eg. TNFα) upon treatment with palmitic acid while oleic acid treatment upregulated M2 markers (eg. Arginase-1). These results suggest the early changes in response to a high milk fat diet are localized in the mesenteric tissue and involve receptors for fatty acids.

Contributors: Camell, Christina; Smith, C.Wayne
Orotidine 5'-monophosphate decarboxylase (ODCase) is essential for de novo synthesis of pyrimidine nucleotides. It is considered to be one of the most proficient enzymes known because it catalyses the decarboxylation of orotidine 5'-monophosphate to uridine 5'-monophosphate with a rate enhancement of ~1017 without the aid of cofactors or metal ions. The structural factors responsible for this extraordinary rate enhancement are an area of active study. This project is a systematic test of the effect of small insertions on ODCase function with an emphasis on identifying residue remote from the active site that are important for catalysis. For proteins in general, there are not many systematic studies of insertions reported.

To study the tolerance to insertions and determine remote residues important for enzyme function, an arginine-proline (RP) sequence (5-AGGCCT) was inserted every two amino acids, scanning the length of the entire protein. A set of 121 ODCase single insert mutants was constructed. Since a functional enzyme is needed for E. coli growth on M9 media, growth curves in M9 were obtained to identify insert mutants with slower growth times. Sixty-two insert mutants showed growth times similar to wild type, while the other fifty-nine exhibited slow or no growth. To determine if slower growth is due to effects on protein stability (i.e. aggregation, degradation etc.) or protein function, soluble and insoluble protein fractions were studied by western blot. Of interest are five insert mutants at positions away from the active site, which exhibit slow growth times but wild type protein expression levels. These insertions are postulated to impair catalytic function but not stability. These mutant enzymes were purified and kinetic analysis showed 103 - 105-fold decreases in enzyme catalytic activity in comparison to wild type. Taken together, these results provide a correlation between bacterial growth and ODCase enzyme function, where 1000-fold decrease in enzyme activity leads to a doubling of the growth time of E. coli cells harboring the mutant protein. Although the selected insert mutants are remote from the active site and do not interact directly with the substrate, x-ray crystallography and molecular modeling suggest they act at a distance to disrupt enzyme interactions with the substrate 5’-phosphate group.

Insert mutants that did not affect ODCase function were also analyzed. When considering the insert mutants showing wild type expression levels as well as wild type growth, these were primarily in the coils or alpha-helices located on the outside of the TIM beta-barrel structure, indicating low tolerance to small insertions in the internal eight beta-strands. The only exception was one insert mutant in the sixth beta-strand, located 11Å away from conserved active site residues.

This study provides new information on insertion tolerance of a highly proficient enzyme as well as key “remote” residue positions vital for enzyme function.

Contributors: Cardenas, Ana Maria; Palzkill, Timothy
NEW DRUG TO COMBAT MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

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Advisor: E. Zechiedrich, Ph.D.-Department of Molecular Virology & Microbiology

Background: Multidrug-resistant (MDR) bacterial infections cost ~99,000 American lives and over $3 billion annually. In addition, the drug development pipeline has few options to combat drug-resistant pathogens. Two strategies to reduce the time between drug development and clinical application are to derivatize current antibiotics and to re-profile older compounds already approved for human use.

Methods: Over ten years, we have collected >6,000 E. coli clinical isolates from Ben Taub General Hospital and the Michael E. DeBakey VA Medical Center in Houston, Texas. These isolates range from susceptible to all antibiotics to nearly pan drug-resistant. Based on the hospital-derived antibiotic susceptibility profiles and patient information, as well as their fluoroquinolone MICs determined in our laboratory, we chose representative isolates to test the efficacy of derivatized compounds of the natural antibiotic fosmidomycin.

Results: One successful drug derivative was ciclopirox, an FDA-approved antifungal drug. Regardless of fluoroquinolone MICs or how many antibiotics the E. coli clinical isolates resisted, ciclopirox blocked bacterial growth at 10 µg/ml in rich medium and 2.5 µg/ml in minimal medium for 48 hours and at least 72 hours, respectively. Ciclopirox MICs were not affected in laboratory strains lacking the clinically relevant MDR efflux pump AcrAB/TolC. Bacterial growth was rescued when either media was supplemented with iron, but not with magnesium, zinc or nickel. Additionally, our preliminary data show that ciclopirox blocks bacterial growth of MDR Klebsiella pneumoniae and Acinetobacter baumannii clinical isolates similar to E. coli isolates.

Conclusions: 1. Ciclopirox can inhibit E. coli bacterial growth in the presence of existing drug-resistance mechanisms. 2. The mechanism of how ciclopirox blocks bacterial growth represents a novel target for future drug exploitation and that it may involve iron acquisition or sequestration. 3. Revamping older drugs proven safe for humans may expand the drug arsenal to treat MDR bacterial infections.

Contributors:  
Genetically engineered mouse models represent powerful tools for studying breast cancer initiation and progression. The lack of mammary-specific promoters, however, has proved to be a major challenge in the field. Currently, transgenes are expressed from the mouse mammary tumor virus long terminal repeat (MMTV-LTR) and whey acidic protein (Wap) promoters. Individual lines vary in expression pattern and level depending on the site of integration, and none are entirely mammary specific. Also, MMTV and Wap promoter-driven expression is found throughout the ductal tree and is regulated by lactogenic hormones, not recapitulating human disease initiation, which is likely driven from a small number of cells.

We developed a novel mouse model to study breast cancer initiation, circumventing the problems associated with MMTV and Wap-driven transgenes. We generated a ROSA26-targeting vector containing a lox-STOP-lox cassette upstream of our chosen oncogene (Prdm14) and an IRES-EGFP marker. When knocked-in to the ROSA26 locus, this construct allows for spatial and temporal Cre-mediated excision of the STOP cassette and activation of oncogene expression. Utilizing an intraductal injection of a self-deleting lentiviral Cre recombinase will have several advantages: 1) self-deletion will reduce toxicities associated with sustained Cre expression, 2) injection will ensure mammary-specific expression and will reduce animal production time/costs, 3) timing/titer of lentiviral injection will control the type and number of cells expressing the oncogene and 4) ROSA26-driven oncogene expression is predictable, and not subject to position-effect variegation that leads to transgene silencing. We will use this system to determine if Prdm14 expression in a limited number of mammary cells is sufficient to drive tumorigenesis and will use the lentiviral footprint and EGFP marker to establish tumor clonogenicity and transplantability.

Contributors: Carofino, Brandi; Justice, Monica
The non-structural protein 1 (NS1) of influenza virus is a potent antagonist of the cellular antiviral interferon (IFN) response and consists of two domains, a dsRNA binding domain (RBD) and an effector domain (ED). Although, initially sequestration of dsRNA was considered the primary mechanism for countering IFN, subsequent studies have shown that the interactions of the ED with various cellular proteins are likely involved. Among various influenza virus strains, NS1 is relatively well conserved with major differences occurring in the linker region and the C-terminus, where several NS1 proteins contain truncations. The highly pathogenic H5N1 viruses that are currently a threat for another influenza pandemic, contain a five amino acid deletion in the linker region and have also been shown to contain a four amino acid virulence determinant at its carboxy terminus, known as the PDZ-binding motif (PBM). How these differences contribute to virulence remains unknown but these differences seem to have an effect on NS1 function that may be strain specific. To further understand these differences, we were interested in determining the crystal structure of the H6N6 NS1. The H6N6 NS1 contains a longer linker than the H5N1 NS1 and has the PBM. Currently we have successfully cloned, expressed and crystallized the H6N6 ED and the H6N6 full-length protein and will be able to determine the effect that a longer linker would have on NS1’s ability to interact with different cellular partners and/or modulate its affinity for dsRNA, which could ultimately affect Influenza A virus virulence.
Many key pathways associated with mammalian development have also been shown to be important in cellular transformation. Among these “dual-role” pathways are the fibroblast growth factor (FGF) and Wnt signaling pathways.

FGF and their receptors (FGFRs) are expressed in most tissues and play pivotal roles in development, wound healing and neovascularization, as well as being upregulated in many cancers, including prostate, mammary, renal, kidney, bladder, and testicular tumors. In order to study FGFR1 in prostate tumorigenesis, our lab has developed an inducible, prostate specific (probasin promoter) FGFR1 transgenic mouse model, JOCK1 (juxtaposition of CID and kinase). Using this mouse model we demonstrate that induced FGFR1 signaling in prostate progenitor cells (in vitro and ex vivo) and whole prostate in vivo is critical for tumor initiation and early maintenance and that prolonged exposure to FGFR1 (42 weeks) results in prostate cancer with distant metastasis.

The Wnt pathway is vital for proper embryogenesis and adult tissues homeostasis by regulating stem cell self-renewal and differentiation. The misregulation, namely uncontrolled activation, of this important “stem pathway” is associated with various cancers, including colon, breast and prostate cancer. Recently, using the MHC I promoter, our lab has developed a ubiquitously expressed iLRP5 (Wnt co-receptor) transgenic mouse model, Ubi-Cat (ubiquitously expressed inducible β-catenin). Although MHC I is expressed ubiquitously throughout the body we observe higher expression of our transgene in the prostate stroma compartment. We have demonstrated that inducing LRP5 crosslinking in vitro and in vivo results in prostate hyperplasia and thickened stroma eventually leading to high-grade Prostatic Intraepithelial Neoplasia (PIN) and adenocarcinoma (60 weeks). Implicating the importance of reactive stroma (RS) in tumorigenesis.

In order to delineate possible crosstalk and synergism between these two pathways, we crossed JOCK1 and Ubi-Cat transgenic lines. By 8-12 weeks of induction, the Ubi-Cat/JOCK1 mice had developed widespread hyperplasia, with focal areas of high-grade PIN. Adenocarcinoma along with an extensive RS was observed by 24 weeks, which is a significant acceleration of either of the single transgenic animals. We also observed increase expression of a fibroblastic RS and upregulation of the transcription factor Sox9, an upstream regulator of Snail1/2 (EMT regulators).

Contributors: Shahi, Payam; Seethammagari, Mamatha; Ittmann, Michael; Spencer, David M.
Abnormal neuronal activity during development has been shown to alter important aspects of neuronal maturation. Classic examples include tactile and visual sensory deprivation affecting the somatosensory cortex as well as the ocular dominance columns of the primary visual cortex, respectively. Our laboratory is interested in understanding the effects of hyperactivity upon the developing hippocampus. We have previously shown that under control conditions dendrites of CA1 pyramidal neurons increase in dendritic length and branching complexity. In addition, synaptic proteins such as NR2A and PSD95 concurrently increase in expression over time. When chronic, (4-7 days), spontaneous network bursting is induced in sister slices via the GABAa receptor antagonist bicuculline methiodide the expression of the postsynaptic proteins decreases and the CA1 basilar dendrites fail to grow. Stereological analysis has been preformed and no pyramidal cell loss is observed. To begin to explore the mechanisms involved in the alterations induced by hyperactivity a time course was preformed. We were surprised to find that by four hours there was a 40% and 70% reduction in the protein concentration of PSD95 and NR2A respectively. Antagonism of the NMDA receptors with APV prevented the epileptiform activity-induced reduction of protein expression. Additionally, when slices are exposed to epileptiform activity for one hour, there was a 20% reduction in burst duration compared to slices acutely treated with bicuculline. Prolonged network bursting further reduced the burst phenotype of slices grown in control media and acutely exposed to bicuculline. Surprisingly, only after four hours of epileptiform activity we observed a 26% and 16% decrease in the number of branch points and total dendritic length of the basilar dendrites of CA1 pyramidal neurons compared to control slices. An emerging mediator of neuronal properties is the protein phosphatase 2b, commonly known as calcineurin. When slices were pretreated with the calcineurin antagonist FK506, the mean burst duration was only reduced by 20% compared to slices not treated with FK506, 69%. In addition, FK506 and Cyclosporin-A completely suppressed the epileptiform induced effects on the glutamatergic synaptic markers NR2A, NR1 and the PSD95. Similarly, suppression of calcineurin activity blocked bicuculline-induced reductions in dendritic arborization both in total dendrite length and number of branch points. These results suggest that these apparent compensatory responses of the hippocampus to chronic network hyperexcitability are mediated by calcineurin-dependent signaling mechanisms.

Contributors: Casanova, J.R.; Nishimura, M.; Lam, T.T.; Le, J.T.; Swann, J.W.
Astrocytes are the most abundant cell types of the central nervous system (CNS). Yet, we are only recently starting to understand their functions and biology. Astrocytes are involved in blood brain barrier formation, synaptogenesis and metabolic regulation and their deregulation leads to several disorders such as malignancy, neurological and neurodegenerative diseases.

During spinal cord development, neurogenesis precedes gliogenesis and astrocytes emerge from neural stem cells (NSCs) following the preset pattern of the embryonic spinal cord. However, lineage progression from NSCs to astrocytes is unknown. Recently, the CCAAT box-element binding transcription factor NFIA was shown to be both necessary and sufficient for gliogenesis in the embryonic chick spinal cord. Particularly, NFIA remains expressed throughout astrocyte development, promotes astrocyte precursor (ASP) migration and regulates the expression of the terminal astrocyte differentiation marker GFAP, making NFIA a crucial factor for astrocyte lineage progression. Characterizing target genes of NFIA could therefore be a good starting point to better understand the different stages of astrocyte lineage progression.

Using a temporal gene expression profiling of NSCs from developing spinal cords, encompassing neurogenesis and gliogenesis, we identified 20 potential transcriptional target genes of NFIA, among which ARHGEF4 (GEF4). GEF4 is a guanine exchange factor of the small G-protein Rho family and has previously been involved in cell migration. GEF4 has four conserved NF1 DNA binding sites and its expression is correlated with NFIA since overexpression of NFIA in the chick embryonic spinal cord leads to ectopic expression of GEF4. In the spinal cord, GEF4 is first expressed in the ventricular zone and at later stages is found in both the ventricular zone and migrating cells into the mantle zone. Based on these previous observations, we hypothesized that GEF4 is involved in glial cells migration. Gain-of-function studies in the embryonic chick spinal cord reveal that GEF4 is sufficient to promote precocious migration of astrocyte precursor (ASP) only.

Current studies are focusing on better characterizing GEF4 expression in the spinal cord as well as define its expression in the brain. Loss-of-function studies are also underway both in the chick and mouse embryonic spinal cords using shRNA and knock-out, respectively. Future studies will set to confirm whether GEF4 is a transcriptional target of NFIA and functional studies will confirm the role of GEF4 in ASP migration and characterize the molecular mechanism involved.
Identification of new genes involved in aging and elucidation of their molecular function will provide insight into prevention of age-related functional decline. Neurosensory systems and neuroendocrine signaling transduce nutrient signals and modulate organismal function to regulate aging, but the underlying mechanisms are poorly understood. We hypothesize that neurosensory inputs signal through neuroendocrine systems to modulate organismal physiology and lifespan. Olfactory signals have been shown to play a key role in aging, but little is yet known about how taste signaling impacts aging. The development of new heterologous systems and gustatory receptor mutants has allowed us to study the effects of activating or silencing gustatory signals on longevity, physiology and behavior in Drosophila melanogaster.

We found that mutation of the Gr5a trehalose receptor results in decreased triglyceride levels, reduced fecundity, disrupted sleep homeostasis, and a significant reduction in mean lifespan. Gr5a mutants are unable to maintain trehalose homeostasis, and activation of Gr5a signaling resulted in sensitivity to starvation. While control flies increased sleep consolidation on high sugar diets, both Gr5a trehalose receptor mutants and Gr64 sugar receptor mutants were unable to shift sleep patterns in response to changes in dietary sugars that they cannot taste.

Our data suggest that the Gr5a trehalose receptor may play a critical role in regulating organismal physiology, behavior, and longevity and that sugar receptors control the ability to increase sleep consolidation in response to dietary sugar. As the brain coordinates physiological processes across different tissues and Gr5a mutants exhibit alterations in energy balance, we have begun to test the hypothesis that Gr5a controls endocrine signals to centrally modulate nutrient homeostasis, sleep, and aging.

Contributors: Chan, Tammy; Waterson, Michael; Linford, Nancy; Pletcher Scott
It has been shown that cancer stem cells (CSCs) from human breast cancer have increased resistance to conventional therapies. However, it is still not known which mechanisms contribute to their therapeutic resistance. In order to study breast CSCs, our laboratory has employed a p53-null tumor model to mimic human breast cancer, and we previously identified that a Lin-CD29HCD24H subpopulation in the tumors has tumor-initiating properties. Hence, this subpopulation is considered as the CSCs in our model. Subsequent studies in our laboratory showed that Lin-CD29HCD24H cells are more resistant to ionizing radiation (IR) due to more efficient DNA repair. However, the mechanisms which contribute to this efficient repair are unclear. The DNA damage response (DDR) includes activation of cell cycle checkpoints, apoptosis, and DNA repair. To repair DNA double strand breaks caused by IR or chemotherapy, cells may undergo homologous recombination (HR) or non-homologous end joining (NHEJ). Studies have shown that adult stem cells, such as mouse hair follicle bulge stem cells and mouse hematopoietic stem cells are more radioresistant due to higher NHEJ activity. Furthermore, glioma stem cells are also more resistant to IR due to the preferential activation of the DNA damage checkpoint response. Accordingly, we speculate that CSCs of p53-null tumor model exhibit increased DDR possibly as a result of elevated NHEJ activity. To date, both higher protein expression and activity of Rad51, a key element of HR repair, were detected in non-CSCs from p53-null tumors. Furthermore, higher NHEJ activity was observed in the CSC population using a high-throughput NHEJ Assay.

Contributors: Chang, Chi-Hsuan; Zhang, Mei; Rosen, Jeffrey
NODE OF RANVIER FORMATION IN THE CNS: INTERACTIONS BETWEEN NODAL MEMBRANE PROTEINS AND EXTRACELLULAR MATRIX COMPONENTS

Kae-Jiun Chang  
Program in Developmental Biology  
Advisor: Matthew Rasband, Ph.D.-Department of Neuroscience

Action potentials are initiated at axon initial segments (AISs) and regenerated at gaps between myelin sheaths, called nodes of Ranvier. Myelin sheaths enwrap axons and actively cluster the voltage-gated sodium channels at nodes, which are crucial for saltatory nerve conduction. The nodes of Ranvier and their flanking paranodes and juxtaparanodes are highly specialized domains of myelinated axons, consisting of distinct protein complexes. In the peripheral nervous system (PNS), gliomedin, a nodal extracellular matrix (ECM) component, has been shown to play an instructive role in clustering axonal nodal membrane proteins and cytoskeletal scaffolds, which are followed and constrained by paranodal junction formation. In the central nervous system (CNS), gliomedin is absent from the nodes and in vitro studies suggested that unknown soluble glial factor(s) may play an equivalent role in the CNS to that played by gliomedin in the PNS. In adult rodents, several CNS nodal ECM components have been identified, including brevican (Bcan), versican V2 (VcanV2), brain link protein 1 (Bral1), tenascin-R, phosphacan, neurocan and oligodendrocyte myelin glycoprotein (OMgp). Whether any of these components plays a role in node formation is still unknown. In this study, we excluded OMgp from CNS nodal ECM and found Bcan and VcanV2 appear around nodes before robust node formation. Neuron glia-related cell adhesion molecule (NrCAM) was identified as another CNS nodal ECM molecule. Interactions between nodal ECM components and known axonal nodal membrane proteins were tested with COS-7 cell surface binding assays, hippocampal neuron AIS binding assays and in vitro pulldown assays. Whether the ECM components can play a role in directing clustering of nodal molecules was directly tested with clustering assays by using cultured dorsal root ganglion neurons in the absence of glial cells in vitro. So far, we have been able to show Bcan has the ability to induce node-like clusters and suggested its functional role in CNS node formation.

Contributors: Chang, Kae-Jiun; Susuki, Keiichiro; Rasband, Matthew
TEMPURA, A NOVEL PROTEIN PRENYLASE, REGULATES NOTCH SIGNALING DURING LATERAL INHIBITION AND CELL FATE DECISIONS

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To further unravel the molecular mechanisms required for proper Notch signaling we are trying to identify new players that affect Notch signaling. We therefore carried out a forward genetic screen and isolated 7 alleles of an uncharacterized Drosophila gene that we named tempura. tempura mutants exhibit bristle loss (balding) on the notum and wing notching, indicating altered Notch signaling activity. The balding defect in the mutant clones is caused by loss of Notch signaling as multiple neuron/sheath cells are found in the sensory clusters on the notum. The expression of Tramtrack, a Notch downstream target, is reduced in the precursors of the external cell lineages, further supporting Notch-dependent cell fate transformations. We also observe an accumulation of Delta in sensory organs, which is likely to lead to the cell fate determination problems that we observe in tempura mutants. In addition, the density of mutant sensory clusters is higher than in wild type tissues, indicating a defect in lateral inhibition, another function of Notch signaling. We also noted an accumulation of Scabrous, a secreted Notch modulator. This suggests a problem with the secretion of Scabrous in mutant clones, which in turn leads to an impairment of Notch signaling during lateral inhibition.

tempura is an evolutionarily conserved gene which encodes a 398 amino acid protein. Its function is unkown. It contains a domain that shows homology to subunit of geranylgeranyl transferase II (GGT II), also called RabGGT. The RabGGT complex contains an and a subunit and adds geranylgeranyl groups to Rabs with the assistance of Rab escort protein (REP). Rabs are small GTPases which regulate vesicle trafficking. Without geranylgeranylation, Rabs are not tethered to their target membranes and vesicle trafficking is impaired. CoIP experiments in S2 cells show that Tempura can interact with the RabGGT subunit and REP. Our working hypothesis is that Tempura function as a novel subunit of RabGGT and modulates the modification of certain Rabs that are involved in trafficking of Notch signaling components. Indeed, we found that overexpression of dominant negative Rab1 can cause strong Scabrous accumulation in sensory organs and that Tempura can interact with Rab5 and Rab11, which play important role in Notch signaling.


MUTL INHIBITS STRESS-INDUCED MUTAGENESIS INDEPENDENTLY OF MISMATCH REPAIR

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Under adverse conditions bacteria increase mutation rate by various mechanisms, controlled by stress responses. In an E. coli model system, stress-induced mutations (SIM) are of two types: stress-induced point mutations and amplifications. Point mutagenesis requires the proteins of homologous recombinational double-strand-break (DSB) repair, DinB error-prone DNA polymerase, and activation of the SOS DNA-damage response, the RpoS general/starvation stress response and the RpoE extracytoplasmic stress response. The mechanism of point mutation is a switch to error-prone DSB repair using DinB under stress, controlled by the RpoS response. Amplification results from replicational template switching causing microhomologous recombination under stress. Mismatch repair (MMR) is a highly conserved pathway that corrects DNA replication errors and inhibits partially homologous recombination. Previous work suggested that MMR became limiting transiently during SIM: (1) the SIM mutation spectrum is 1bp deletions in small mononucleotide repeats, a spectrum characteristic of MMR-defective cells; (2) Harris et al. 1997 Genes Dev found that overproduction of MutL MMR protein inhibits SIM and not generation-dependent mutation, as if MutL became limiting transiently for MMR during SIM. However, the number of MutL molecules per cell was not reduced in Western blots. We wondered: does MMR really become limiting during SIM because of limiting functional MutL? Using a chromosomal system to overproduce components of the mismatch repair system, we find that first, overproduction of MutL, but not MutS or MutH, reduces point mutation 10-15 fold. However, second, overproduced MutL still inhibits SIM in a ΔmutS background, in which no MMR can occur. We conclude that overproduced MutL inhibits SIM independently of MMR. Third, overproduced MutL, but not MutS, inhibits amplification 15 fold, and overproduced MutH inhibits amplification 4-fold. Whether MutS/MMR are required for this inhibition of amplification is not yet known. We are now testing the hypothesis that overproduced MutL activates UvrD, which may block SIM by its known anti-RecA activity.

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Chaperonins are barrel-like multi-subunit complexes that assist the de novo folding and refolding of misfolded proteins. Chaperonins could be characterized into two groups: the type I chaperonin such as GroEL, and type II chaperonin such as TRiC/CCT and Mm-Cpn. The mechanism of Type I chaperonin assisted protein folding is well characterized: the chaperonin employs a cofactor to encapsulate its central cavity upon ATP binding. The encapsulation process triggers the release of the substrate into the cis-ring central cavity for its refolding. However, unlike GroEL system, type II chaperonin does not have cofactor to assist in protein folding; instead, they have the built-in lid to encapsulate the central cavity upon ATP hydrolysis. Previously our studies showed that the ATP hydrolysis not only triggers the encapsulation of the chaperonin, but also the refolding of substrate. (Douglas, et al, cell, 2011) In this study, we take advantage of an Mm-Cpn mutant (Cpn-rls mutant) that uncouples the lid encapsulation from substrate release in the presence of ATP. The deficiency of substrate refolding activity could be recovered by inclusion of non-hydrolysable ATP analog (ATP•AlFx) into the reaction, presumably because ATP•AlFx could provide extra force to disrupt the interaction between the chaperonin and substrate, therefore trigger substrate refolding process. The substrate-trapped ATP-bound state of Cpn-rls mutant provides an excellent specimen to investigate how the chaperonin interacts with the substrate. From the cryo-EM study, about 25% of all the particles in this state have extra densities inside one of the two rings. As a comparison, the chaperonin-only control does not have extra densities inside any of the two rings, suggesting the extra density seen in the substrate-trapped ATP-bound state of Cpn-rls mutant corresponds to the substrate density. Moreover, the substrate density binds to the apical region of the chaperonin, indicating the apical region is involved in the recognition of the substrate. As a comparison, In the substrate refolding efficient chaperonin ATP•AlFx state, extra substrate density could be found among ~40% of the particles at the bottom of central cavity. It may indicate that the substrate has been released into the central cavity for its refolding process.

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DETERMINING THE ROLE OF ChREBP IN HEART METABOLISM

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Myocardial function depends on a fine equilibrium between the work the heart needs to perform and the energy that the heart is able to synthesize. To meet the high energy demands of the contracting muscle, the heart needs to produce a constant and plentiful supply of ATP. This energy is primarily produced by the oxidation of fatty acids, glucose, lactate, and other oxidizable substrates. Alterations in energy metabolism were found to cause contractile dysfunction, myocardial infarction, and heart failure. Despite of the intensive work, further studies are needed to establish the mechanisms of energy metabolism abnormalities causing these symptoms. Carbohydrate response element binding protein (ChREBP) has been recently identified as major mediator of glucose and lipid metabolism. ChREBP is a member of the basic helix-loop-helix/leucine zipper (bHLH/ZIP) family of transcription factors. It is activated by glucose and binds to the carbohydrate response element (ChoRE) in the promoter of certain lipogenic and glycolytic genes such as liver-type pyruvate kinase (L-PK) and fatty acid synthase (FAS). Though most of the functional and mechanistic studies on ChREBP are conducted in liver, recent publications as well as the studies in our lab suggest that the physiological roles of ChREBP might not be limited to liver. The expression of ChREBP in heart is comparable with in liver. Known ChREBP targets were reported to be induced in cardiomyocytes in response to glucose and were involved in cardiomyocyte survival. We believe that ChREBP is an important metabolic regulator in heart and that ChREBP might link the energy metabolism to heart diseases. In this study, we aim to characterize the role of ChREBP in heart. We first want to identify new ChREBP targets. Preliminary study done by our lab showed a couple of genes that are known to be involved in metabolic pathway might be regulated by ChREBP. I’m conducting the follow-up experiments to demonstrate the direct and functional binding of ChREBP to the gene promoters. Future studies using the cardiomyocyte-specific ChREBP knockout mice will provide further insight to the physiological roles of ChREBP in heart metabolism.

Contributors: Chen, Elaine; Chan, Lawrence
Reprogramming the Conformational Regulation of GPCR Signaling

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Advisor: Patrick Barth, Ph.D.-Department of Pharmacology

Signaling across biological membranes is critical to living cells and involves membrane-embedded receptors that transduce extracellular stimuli into cytoplasmic responses. G-protein coupled receptors (GPCRs) are one of the largest families of these receptors that serve this function and do so through long-range allosteric changes. My project is part of a general effort to uncover the sequence, structure, and energetic determinants governing GPCR signaling. I hypothesize that GPCR signaling is driven by conformational changes that allow them to toggle between functional states; computational designs that stabilize receptors in different states will allow us to reprogram their signaling properties and provide an atomic-level understanding of the underlying conformational regulation.

To address the relationship between protein stability and conformational plasticity in allosteric receptors, we have combined sequence bioinformatics techniques with the design mode of RosettaMembrane to probe the sequence space governing GPCR conformational stability. Sites predicted to be suboptimal for the stability of the receptor are targeted for design, and mutations that stabilize the receptor's inactive conformation are selected. The mutants are characterized via radioligand binding assays to measure apparent thermal stability. Our initial designs of the beta-1-adrenergic receptor (B1AR) have yielded mutants with increased apparent thermostability in comparison to a B1AR variant (M23) used in solving the B1AR structure (Warne et al. 2008).

Additionally we are working to modulate and switch the functional states of GPCRs. Using multi-state design with RosettaMembrane, we have been able to design mutants of rhodopsin as well as dopamine D2 receptor (D2DR) with increased constitutive activity over their respective wild types. Furthermore, we are able to design mutants of D2DR that display increased active-state stability. With over eight hundred GPCRs in the human genome, many of which have been implicated in disease studies, it is apparent that there is a critical need for atomic-resolution structural and mechanistic understanding of their signaling.

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Contributors: Chen, Kuang Yui Michael; Barth, Patrick
Mitochondria are highly dynamic organelles that form large interconnected intracellular networks. They can be actively transported along the cytoskeletons and continuously undergo fusion and fission, collectively termed mitochondrial dynamics. Disruption in mitochondrial dynamics may lead to dysfunctional mitochondria, and several diseases are associated with defects in the fusion and fission machinery. In mammals, mitofusin 1 and 2 (MFN1 and MFN2) have been shown to control mitochondrial fusion, and mutations in MFN2 causes Charcot-Marie-Tooth type 2A2 (CMT2A2), a heritable neuropathy that affects motor and sensory neurons with the longest axons. Mitofusins also play roles in other neurodegenerative disorders, including Amyotrophic Lateral Sclerosis, Parkinson’s disease, and Huntington’s disease. However, how mitofusins are involved in neuronal maintenance is not understood.

In an unbiased forward genetic screen designed to find mutations that cause neurodegenerative phenotypes, we identified 7 lethal mutations in Marf (mitochondrial associated regulatory factor). Marf encodes the Drosophila homolog of mammalian mitofusins. Our data show that homozygous Marf mutant clones in adult photoreceptors exhibit an age-dependent degeneration, suggesting that Drosophila Marf plays an evolutionarily conserved role in neuronal maintenance. In addition, motor neuron axons of the Marf mutants possess fragmented and fewer mitochondria, a phenotype that is more severe in longer axons and their associated terminals. This suggests that Marf is involved in axonal mitochondrial transport. Surprisingly, Marf mutants display a severe overgrowth of the neuromuscular junctions (NMJs) in the third instar larvae, and synaptic transmission is affected upon repetitive stimulation. We also found that loss of Marf causes reduced electron-transport chain (ETC) complex I-IV activities and an increase in reactive oxygen species (ROS) level. We hypothesize that increased ROS levels and impaired mitochondrial transport in Marf mutants cause abnormal NMJ morphology and photoreceptor degeneration, and that impaired synaptic transmission of Marf mutants is due to lack of ATP at the NMJs.

Notch signaling is critical during development directing binary cell fate between progenitors and differentiated cells. Aberrant Notch function causes several human skeletal diseases such as Spondylocostal dysostosis (SCDO) and Alagille syndrome. Recently, cartilage specific gain- or loss- of-function of Notch mouse models have been made in our and other laboratories. Although detailed phenotypic studies of these mice have been conducted, a full knowledge of the mechanisms of the pathway is still lacking. The canonical Notch pathway involves Rbpj as a co-factor to induce target gene expression. However several findings suggest Rbpj-independent mechanisms exist to promote Notch signaling transduction. Our goal is to unravel the molecular mechanisms by which Notch signaling controls cartilage development. We hypothesize that the function of Notch signaling is mediated by both the canonical and non-canonical pathways. To study the gain-of-function of Notch in cartilage, we crossed a Col2a1-cre line with a line having a conditionally activated allele of Notch1 intracellular domain (N1ICD). Resulting mutant mice have shortened limbs, undersized ribs, and virtual absence of the spine and tail. Decreased Alcian blue staining highlights the general defects of chondrogenesis. The decreased expression of Sox9, Col2, Col10 in the mutant suggests the inhibition of chondrocyte differentiation by Notch signaling. On the other hand, complete loss-of-function of Notch (achieved by deleting Presenilin 1/2 in cartilage) causes undersized and underdeveloped skeletal elements. The limbs and spine are about 40% shorter in the mutants at 3 weeks of age. Interestingly, cartilage specific deletion of Rbpj causes only a mild phenotype. The body weight and histology of cartilage appear to be normal at 3 weeks of age. To test whether Notch signal transduction depends on Rbpj, we made mice with deficient Rbpj on the Notch gain-of-function background. These mice have nearly normal limb structure but demonstrate irregular curvature of the spine with a shortened tail, suggestive of incomplete rescue. To completely map the Notch signaling pathway, we are identifying Notch targets by a CHIP-seq approach in the ATDC5 cells, a chondrocyte cell line. We have achieved high specificity and enrichment of the occupancy of the Notch targets using a tagged system. We hope to identify differential targets of canonical vs. noncanonical Notch signaling using this approach.

Contributors: Chen, Shan; Tao, Jianning; Jiang, Ming-Ming; Bertin, Terry; Lee, Brendan
TRIM63 encodes Muscle Ring Finger 1 (MuRF1) protein, which is a sarcomere M line protein with E3 ubiquitin (Ub) ligase function. MuRF1 regulates muscle trophic homeostasis by targeting sarcomere proteins myosin heavy chain (MyHC) and myosin binding protein C (MyBPC3) for degradation by the Ubiquitin-Proteasome System (UPS).

Hypertrophic cardiomyopathy (HCM) is primarily a disease of sarcomere proteins. Because MuRF1 is a sarcomere protein, we hypothesized that it may be a causal gene for HCM. We screened 307 probands with HCM and 320 control individuals by Sanger sequencing of all exons and intron-exon boundaries of TRIM63 and identified 3 mutations in 5 HCM probands. The p.A48V and p.Q247X occurred in 2 independent probands and p.I130M in 1 proband. The mutations were absent in the control individuals, affected totally conserved amino acids and predicted to be probably pathogenic in in silico analysis.

To determine the effects of the mutations on E3 Ub ligase activity, we generated recombinant lenti and adenoviruses and assessed autoubiquitination in specialized HeLa cells in which ubiquitinated proteins could be labeled with biotin. The p.Q247X mutation led to complete loss of autoubiquitination, while p.A48V and p.I130M had modest effects, as detected by immunofluorescence (IF) and co-immunoprecipitation (Co-IP). To determine the effects of the mutations on ubiquitinylation of known MuRF1 substrates MyHC and MyBPC3, we expressed the wild type (WT) and mutant MuRF1 proteins in adult cardiac myocytes using recombinant adenoviruses. Expression of WT protein led to reduced MyHC and MyBPC3 levels. In contrast levels of these proteins were normal in the mutant groups. Treatment with MG132 normalized MyHC and MyBPC3 protein levels. Co-IP studies showed reduced levels of precipitated MyHC and MyBPC3 in the mutant groups as compared to WT MuRF1.

To determine the effects of the mutations on localization of MuRF1 to M line, we expressed the proteins in adult cardiac myocytes and detected localization by IF. The p.I130M and p.Q247X mutations were diffusely expressed without specific localization to the M line. In contrast p.A48V maintained localization to sarcomere M line. Mislocalization of the mutations did not affect CK enzymatic activity.

To link the mutations to cardiac hypertrophy, we assessed levels of selected signaling molecules involved in cardiac hypertrophy. Levels of p-mTOR and its downstream target S6K were significantly reduced in myocytes expressing WT MuRF1 but not in cells expressing mutant MuRF1. Treatment with MG132 normalized reduced levels of p-mTOR and S6K.

Thus, genetic data in human patients with HCM and mechanistic studies in adult cardiac myocytes identified TRIM63 as a novel gene for human HCM and established p-mTOR as a novel substrate for MuRF1 in the heart.

Contributors: Chen, Suet Nee; Rodriguez, Gabriela; Grazyna Czernuszewicz; Jianping Jin; Marian, AJ
Actin is one of the most important proteins in many key biological processes. Despite extensive studies on the structure of actin in the past few decades, there is still a gap in understanding the structural basis of actin polymerization, especially the actin nucleation. There are several important actin nucleators been discovered recently, and one group of them shares a special feature as tandem WH2 domains (WH2, Wiskott-Aldrich syndrome homology region 2, a known actin-binding motif). These nucleators include Spire, Cordon-Bleu (Cobl), VopL, VopF, Lmod etc. Here we present the very first crystal structure of an actin dimer in its native state in complex with Cobl. This 2.9Å structure provides a novel orientation of two actin molecules that has never been observed. It is also the first time that we discovered a new binding interface between WH2 domain and actin that the second WH2 of Cobl crosses through the middle of two actins, holding them closely together. These exciting discoveries may lead to a new hypothesis of the actin nucleation mechanism by Cobl. Based on the structural information, 14 mutations on Cobl were designed to reveal how point mutations of only one or two amino acids could lead to defects on its ability of promoting actin polymerization. Not only will this structure be a key to understand actin nucleation by Cobl in the atomic level, but also it will facilitate future structure-based therapy designs especially in neurobiology since Cobl is mostly enriched in the brain and known to be involved in neural tube formation.

Contributors: Wang, Qinghua; Ni, Fengyun; Tian, Xia; Ma, Jianpeng
ANTIMÜLLERIAN HORMONE LEVEL (AMH) IN HEALTHY GIRLS FROM CHILDHOOD TO ADOLESCENCE

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Objective: To establish normative data for antimüllerian hormone (AMH) concentration in children with respect to chronological age and pubertal maturity.

Study design: One hundred eighty-nine healthy girls, ages 5.00 to 17.99 years, living in Houston proper were evaluated in the General Clinical Research Center for physical exam and venipuncture. Body mass index (BMI), Tanner staging, luteinizing hormone (LH), follicle stimulating hormone (FSH), and serum AMH concentration were assessed at a single visit.

Results: No significant relationship was found between age and serum AMH concentration (R=0.10, p=0.18). The lower limit of AMH concentration was distinctly above the detection threshold for each age group. AMH was also comparable between premenarchal (2.75; 1.88 -4.9 ng/mL) and postmenarchal healthy girls (2.93; 2.15-4.35 ng/mL) (median; 25-75th percentile). BMI, race, Tanner stage, and patient's age of menarche were not significantly associated with AMH concentration in our study population.

Conclusion: Serum AMH concentration is relatively stable throughout childhood and adolescence in healthy girls indicating similar preantral follicle activity between age groups regardless of pubertal maturity. Under this model, the exponential reduction in follicles during children is chiefly primordial or early primary follicle loss which do not secrete AMH. Subsequent studies comparing AMH levels between healthy controls and pediatric cancer patients are needed to determine the effects of oncology treatment on ovarian reserve in childhood.

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Atrial fibrillation (AF) is the most common sustained arrhythmia, affecting 1-1.5% of the population in the developed world. The prevalence of AF is projected to increase at least 3-fold by 2050, which amounts to considerable economic and social burden. AF is associated with significant morbidity and mortality, primarily due to arterial thromboembolism leading to stroke. Currently, pharmacologic strategies focus on controlling the heart rate and/or converting the irregular rhythm by targeting Ca2+, K+ and Na+ channels. While these drugs increase the functional capacity and quality of life of the patients, most of them are proarrhythmic and some actually increase mortality. This is due to an incomplete understanding of the underlying mechanism. Recent studies are pointing to impaired intracellular Ca2+ handling as the culprit of cardiac arrhythmia, which dysregulation is closely related to the generation of delayed afterdepolarization (DAD). In particular, patients with chronic AF have been shown to have elevated levels of hyperphosphorylated ryanodine receptor 2 (RyR2), which are not only hyperactive and leaky but may also give rise to DAD in different mouse models. While much work has been done examining the kinases responsible for the phosphorylation events on RyR2, little is known about the phosphatases that are also found in the RyR2 macromolecular complex. Namely, protein phosphatase 1 (PP1), and 2A (PP2A) are both tethered to RyR2 via adaptor proteins spinophilin and PR130, respectively. While global PP1 activity has been shown to be elevated in patients with chronic AF, preliminary data from our group shows that its local activity on RyR2 is decreased. This suggests that it is the local and not global regulation of PP1 on RyR2 that plays a key role in determining the overall phosphorylation state of RyR2. We therefore hypothesize that a decrease in the local activity of PP1 on RyR2 leads to its hyperphosphorylation, which contributes to the trigger and/or maintenance of AF. To test this hypothesis, we propose to: 1) Determine the regulation of RyR2-bound PP1 on the phosphorylation state and activity of RyR2 in atrial myocytes; 2) Determine the regulation of RyR2-bound PP1 on AF inducibility in mice; and 3) Define the molecular mechanism by which PP1 regulates RyR2 in AF patients.

Contributors: Chiang, David; Wehrens, Xander
Transcription of the integrated human immunodeficiency virus type 1 (HIV-1) is dependent on the recruitment of the cellular co-factor Cyclin T1, which mediates the function of viral protein Tat. Resting CD4+ T cells are refractory to virus replication and contain low amounts of Cyclin T1 protein, whereas activated CD4+ T cells have high Cyclin T1 levels and support efficient viral replication. We previously found that up-regulation of Cyclin T1 protein occurs in the absence of increased Cyclin T1 mRNA, suggesting translational repression of Cyclin T1 in resting CD4+ T cells. We hypothesized that microRNAs repress Cyclin T1 translation in resting CD4+ T cells, and that this inhibition is lifted upon cell activation. Using microarray expression analysis, we identified miR-27b, miR-29b, miR-150, and miR-223 as significantly down-regulated upon CD4+ T cell activation. Over-expression of these miRNAs decreases Cyclin T1 protein in cell lines, while treatment of primary resting CD4+ T cells with antagomiRs against the miRNAs increases Cyclin T1 protein. Of these four miRNAs, we have shown that miR-27b directly targets the Cyclin T1 3’UTR, as mutation of a putative miR-27b binding site abrogated repression of a luciferase reporter cloned upstream of the Cyclin T1 3’UTR. Association of Cyclin T1 mRNA with the RNA-induced Silencing Complex (RISC) was demonstrated via Ago2 immunoprecipitation, while the decreased levels of Cyclin T1 mRNA pulled down following transfection of miR-27b and miR-29b antagomiRs indicates that targeting by RISC is partly mediated by these miRNAs.

Pre-treatment with miR-27b decreases HIV reporter virus gene expression in HeLa cells; this effect is not observed when Cyclin T1 is knocked down with siRNA. Infection with NL4.3 virus also results in decreased virus production when miR-27b is overexpressed. Moreover, inhibiting miR-27b in primary resting CD4+ T cells leads to an increase in HIV transcription in an environment which is normally virus-restrictive. We have additionally shown that miR-27b and miR-150 can affect HIV latency in an in vitro model consisting of Jurkat cells infected with GFP reporter virus. After viruses are transcriptionally activated by TNF-α treatment, the rate of virus re-entry into latency, as measured by loss of GFP expression, increases when miR-27b and miR-150 are over-expressed. This result suggests that miR-27b and miR-150 can contribute to establishment of HIV-1 latency in CD4+ T cells through repression of Cyclin T1.
Norwalk virus is the prototype virus of the Norovirus genus in the Caliciviridae family. Noroviruses are highly contagious human pathogens that cause acute, epidemic non-bacterial gastroenteritis. The open reading frame 1 (ORF1) in these viruses encodes a non-structural protein p41 that exhibits all the signature motifs of SF3-2 helicase analogous to picornavirus 2C protein. In contrast to p41, 2C protein has been subjected to extensive mutational and biochemical studies. These studies have shown that SF3 motifs in the 2C protein are critical for NTPase activity and viral RNA replication. In addition, 2C protein is implicated in membrane reorganization to support efficient synthesis of progeny virions. It is possible that p41 in noroviruses has a similar function as the 2C protein. However as with crystallization attempts, most biochemical assays of these NTPase has been greatly hampered by insolubility and aggregation.

To facilitate crystallization, N-terminal deletions construct ((p41) lacking the predicted membrane anchoring region, was expressed in E.coli and purified to homogeneity. (p41 crystallized in two different crystal forms. In the first crystal form with P21221 space group, the asymmetric unit consists of one molecule. The (p41 structure consists of two domains. The N-terminal domain (NTD) composed of a three (-helix bundle is connected through a long hinge to the NTPase domain. Although the NTPase domain exhibits Walker A and B motifs, it significantly differs from typical AAA+ fold by having an insertion of two short (-sheets with a 310-helix after the B motif. In addition, the C-terminal five (-sheets and one (-helix are folded together with AAA+ motif as a part of the NTPase domain. As expected, the NTP binds at the P-loop. The (p41 structure is significantly different from the typical SF3 DNA helicase structures in both the domains. Positively charged patch for nucleic acid binding, a common feature in the SF3 helicase structures, is conspicuously absent in the (p41 structure.

In the second crystal form with P6322 space group the asymmetric unit consists of three molecules. In this crystal form, (p41 is engaged in extensive intermolecular interactions forming a tubular structure with a large hole having a diameter of 107Å. Even in such a higher-ordered oligomeric state, there are no extended patches of positively charged residues that could be implicated in binding to RNA. This observation provided an explanation for previous biochemical studies which failed to demonstrate helicase activity in p41. Similarly, no helicase activity has been demonstrated for 2C protein of the picornavirus.

In picoraviruses, however, the 2C protein has been implicated in the membrane reorganization activity. To examine the possibility that p41 may also be involved in such an activity, we transfected C-terminal GFP tagged full length or N-terminally truncated (p41 into 293T cells and observed their localization using confocal microscope. The full length protein forms perinuclear punctates in contrast to (p41 without the membrane anchoring region which is observed throughout the cytoplasm. These observations are similar to those made with 2C protein suggesting p41 also is involved in membrane organizing activity. It is likely, that the high-order oligomeric interactions observed in the formation of the tubular structure together with the NTPase activity may facilitate membrane reorganization, which is critical for replication of RNA viruses.

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Background: Glioblastoma multiforme (GBM) is the most common and most aggressive primary brain tumor in humans. Standard therapies are ineffective and result in significant morbidity and mortality. Thus, new targeted treatments are needed to improve outcomes, and adoptive immunotherapies have the potential to fulfill this need. Genetic modification of T cells with chimeric antigen receptors (CARs) enables the rapid generation of T cells that can recognize and kill tumors with specific tumor antigens. Erythropoietin-producing hepatocellular A2 (EphA2) receptor has been identified as an antigen that is overexpressed in many solid tumors including GBM. The aim of this study was to 1) generate a CAR specific for EphA2 and express it in T cells, and 2) determine if EphA2-specific T cells can selectively recognize and kill EphA2-positive GBM ex vivo and in vivo.

Methods: EphA2.CAR was generated by fusing the antigen binding domain of an EphA2 monoclonal antibody (4H5) to the signaling domain of a T-cell receptor. T cells were retrovirally transduced to express the EphA2.CAR (EphA2-specific T cells). Ex vivo function was determined by the ability of the EphA2-specific T cells to 1) proliferate and secrete cytokines in response to stimulation with EphA2-positive GBM cell lines, and 2) to kill EphA2-positive GBM and glioma-initiating cells in cytotoxicity and neurosphere coculture assays. The in vivo efficacy of EphA2-specific T cells was tested in an orthotopic glioma xenograft SCID mouse model.

Results: Expression of EphA2 receptor in GBM cell lines and primary GBM was confirmed by Western blot. EphA2.CAR was successfully expressed on T cells as determined by FACS analysis. These EphA2-specific T cells were able to recognize EphA2-positive GBM as evidenced by their production of high levels of IFN-γ and IL-2 compared to nonspecific T cells. EphA2-specific T cells were also able to kill EphA2-positive GBM in cytotoxicity assays and had potent activity against glioma-initiating cells as judged by their ability to prevent neurosphere formation and destroy intact neurospheres in coculture assays. Finally, testing the EphA2-specific T cells in an orthotopic xenograft SCID mouse model demonstrated the ability of these T cells to induce regression of established GBM tumors in vivo and increase overall survival.

Conclusions: We successfully generated an EphA2.CAR that is able to direct T cells to tumors that overexpress the EphA2 receptor. EphA2-specific T cells were able to recognize and target EphA2-positive GBM, secreting immunostimulatory cytokines in response and killing glioma and glioma-initiating cells ex vivo. In vivo, EphA2-specific T cells were able to cause regression of establish GBM xenografts in an orthotopic SCID mouse model. EphA2 has emerged as an important tumor antigen for immunotherapy, and targeting this receptor using EphA2.CAR-modified T cells shows promise for the treatment of GBM.

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HDAC3 REGULATES NUDC ACETYLATION IN MITOSIS

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Mitosis is a highly regulated process in which errors can lead to genomic instability, a hallmark of cancer. During this phase of the cell cycle, transcription is silent and RNA translation is globally inhibited. Thus, mitosis is largely driven by post-translational modification of proteins, including phosphorylation, methylation, ubiquitination, and sumoylation. Recent proteomics studies suggest that protein acetylation is as prevalent as protein phosphorylation. However, very little is known about the role of acetylation in mitotic progression.

Using anti-acetyl-lysine immunoprecipitation of mitotic HeLa cell lysates followed by mass spectrometry, we identified 51 unique non-histone proteins, including proteins involved in RNA binding and processing, and cell cycle regulation (1). One cell cycle protein identified is NudC, a highly-conserved dynein/dynactin associated factor that plays a role in mitosis and cytokinesis. I confirmed that NudC is acetylated in mitosis by immunoprecipitation followed by immunoblot. Moreover, NudC acetylation in mitosis increased upon treatment with the HDAC inhibitor apicidin that shows specificity towards HDAC3, suggesting that NudC acetylation is regulated by HDAC3.

Interestingly, comparing unperturbed HeLa cells to mitotically-enriched HeLa cells, NudC is more acetylated outside of mitosis. Utilizing a double thymidine block and release protocol, I enriched for HeLa cells in S, G2, early mitosis (prometaphase-like), late mitosis (anaphase), and G1. I found that NudC associates with HDAC3 throughout mitosis, which may explain its lower level of acetylation during mitosis. Immunofluorescence staining confirmed that NudC and HDAC3 co-localize during early mitosis.

Mass spectrometry analysis of NudC immunoprecipitated from unperturbed HeLa lysate identified one acetylation site on NudC at K39. I generated NudC K39 acetyl-deficient (K39R) and acetyl-mimetic (K39Q) mutants and will decipher the role of NudC K39 acetylation in mitotic progression. Additionally, how NudC acetylation/deacetylation regulates its association with various mitotic regulators to promote mitotic progression are under analysis.

These studies provide a framework from which to address how dynamic acetylation/deacetylation regulates the function and/or localization of proteins that drive mitotic progression and cell division. Understanding acetylation of cell cycle protein networks in cell cycle regulation is likely to reveal new paradigms for anti-cancer therapy.


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ROLE OF ANDROGEN RECEPTOR IN METASTASIS AND HORMONE RESISTANCE OF ESTROGEN RECEPTOR ALPHA-POSITIVE BREAST CANCER

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Background: We have previously developed a novel model of breast cancer metastasis involving shRNA knockdown of Rho GDI(, an inhibitor of the GTP hydrolyzing enzymes of the Rho family. Knockdown results in tamoxifen (Tam) resistant lung metastases in athymic nude mice. Also although it is known that androgen receptor (AR) is frequently expressed along with estrogen receptor (ER) ( in breast tumors, AR’s role in resistance or metastasis is unknown. Here we explore the role of AR in these processes in the Rho GDI( knockdown model.

Materials and Methods: shRNA knock down was used to block expression of Rho-GDI in the ER(-positive MCF-7 breast cancer cell line. Metastasis of the cells was analyzed by injection into athymic mice followed by histological analysis of isolated tissues. Growth of these cells was increased following injection into mice that were treated with tamoxifen, suggesting a tamoxifen resistant phenotype. To determine growth factor pathway activation western blot analysis was used for components of the AR and ER( signaling pathways. Reverse phase protein arrays (RPPA) were also used to analyze global changes in growth factor pathway expression. Growth assays were utilized to examine for effects of AR inhibitors.

Results: The MCF-7 Rho GDI( knockdown cells exhibited increased lung metastases, and were resistant to the growth inhibitory effects of Tam when grown as xenografts in vivo. We discovered that AR was overexpressed in the Rho GDI( knockdown cells as well, as confirmed by both western blot and RPPA. ER( expression was higher in knockdown cells with elevated AR expression, however these results need to be confirmed in additional clones. Inhibition of AR function using the antagonist bicalutimide, known as CasodexTM, resulted in significant reduction in the basal growth of Rho GDI( knock down cells. Experiments to determine the effects of Casodex on other properties of tumor cell invasion are underway.

Discussion: Given our data showing that AR was significantly overexpressed with the metastatic and Tam-resistant phenotype, we hypothesize that AR overexpression plays a role in these processes. We are focused on understanding whether the resistance conferred by AR overexpression is pivotal for the multistep process of metastasis. Our results suggest that AR may represent a new target for hormone therapy resistance of ER( positive breast cancer.

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THE BMP RECEPTORS, ALK2 AND ALK3, ARE ESSENTIAL FOR FERTILITY OF FEMALE MICE

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To achieve a healthy pregnancy, the embryo and the uterus must establish a cross talk that will be essential throughout gestation. The implantation of the embryo takes place in a limited window of time when the uterus is receptive and the embryo has reached the proper stage of development. This crucial synchrony is controlled by the sex hormones through the regulation of the expression of many downstream factors; intriguingly, these factors are connected by mutual regulation and feedback loops that make this regulatory network challenging to decipher.

BMP2 is one of these factors. Mice lacking this TGFβ superfamily member in the uterus cannot undergo the morphological and functional transformation of the endometrium that normally follows the attachment of the embryo(1). This transformation, called decidualization, is a fundamental step in the implantation process and is essential for preparing the uterus for the sheltering of the embryo. BMP2 signals through heterodimers of BMP type 1 and type 2 serine/threonine kinase receptors. A BMP ligand can bind different receptors in different contexts: the physiological association with a specific receptor depends on both the binding affinity and the actual availability of the ligand and the receptor in a specific environment. While the type 2 receptors are essential for recognizing the ligands, the type 1 receptors determine the specificity of the intracellular response. Three type 1 receptors, ALK2, ALK3, and ALK6, are known to mediate BMP signaling, and all expressed in the pregnant uterus.

To study the implantation process, we investigated the effects of deleting each of these receptors in the uterus. Although ALK6 null mice are sterile, this receptor is not required for the decidualization. We now demonstrate that ALK2 and ALK3 are essential for mouse fertility and are required during the peri-implantation period. Moreover, the differences presented by the ALK2 and ALK3 cKO mice suggest that the involvement of the TGFβ signaling in the regulation of the process goes beyond what is known for BMP2 and affects the early pregnancy at different steps. In particular, we discovered that ALK3 plays a role in the pre-implantation period, whereas absence of ALK2 affects the system at a later stage.

Considering the broad involvement of the TGFβ superfamily in many processes, it is crucial to further dissect these pathways in uterine biology to identify possible targets for future therapeutic manipulation.

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DEVELOPING A NOVEL ELECTROPORATION-BASED DNA VACCINE UTILIZING A SMALL MOLECULE REGULATED IMMUNE SWITCH

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Dendritic cell (DC) vaccines are a rapidly progressing area of research for developing new cancer treatments. Although ex vivo DC-based vaccines have been extensively studied for the treatment of cancer, they are limited by short DC lifespan after activation, labor-intensive production (requiring ex vivo manipulation of patient cells), high cost and consequent limited accessibility to the patient. Development of an inducible variant of CD40 (iCD40) and a composite inducible MyD88/CD40 (iMC) by our lab have been shown to increase the potency and lifespan of DC vaccines. These inducible receptors act as in vivo DC “switches” that lead to the priming and robust expansion of antigen (Ag)-specific T-cells capable of eliminating pre-established tumors in mice.

Despite the success of these DC “switch” systems, practicality and scalability of patient-tailored ex vivo DC vaccines remains a major hindrance in their widespread application. Therefore, the future of DC vaccines lies in the development of “off-the-shelf” methodologies. DNA vaccines are attractive for this purpose owing to their elegant simplicity, easy of production, and lack of anti-vector immune responses. In order to successfully vaccinate patients with DNA one must be able to deliver plasmid encoding therapeutic genes to target cells efficiently. In vivo electroporation is a relatively new technology just starting to be tested in clinical trials, and provides a safe, simple, and effective means by which to administer DNA vaccines. Data from our preliminary studies of the electroporative delivery of plasmids encoding the model antigen β-galactosidase (LacZ) in mice suggests that LacZ-specific T-cell responses are induced and that upon tumor challenge mice receiving prophylaxis via electroporation had slower tumor growth kinetics when compared to controls. Together this preliminary data supports the notion that DNA vaccination with tumor Ag by electroporation may be a simple and effective “off-the-shelf” cancer vaccine strategy. Additionally, this vaccination strategy may be enhanced by the addition of the iMC adjuvant. Therefore, further investigation of intradermal electroporation is warranted by these findings, to better characterize the induced immune responses and better optimize vaccination parameters.

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Dictyostelium discoideum, also known as a cellular slime mold or social amoeba, serves as an excellent developmental model. During starvation, the unicellular microbe aggregates with 50,000 to 100,000 conspecific amoebae to produce a multicellular migrating slug and then a fruiting body of various tissues. One of the challenges that developing amoebae face is the prevention of the exploitation by cheaters that avoid the non-"germ-line" cell fate. D. discoideum might prevent such exploitation by cooperating only with self/kin through allorecognition proteins. Recently, two polymorphic cell adhesion proteins, tgrB and tgrC, have been shown to be responsible for allorecognition in developing amoebae (Hirose et al. 2001. Science333(6041):467-70). A closely related pair of proteins, tgrE and tgrD, are also developmentally expressed and predicted to be cell adhesion proteins, but are not polymorphic (Benabentos et al. Curr Biol. 2009 Apr 14;19(7):567-72). Both tgrE and tgrD null mutants have severe developmental phenotypes, but do not segregate from wildtype cells during development, unlike the tgrB and tgrC null mutants. We hypothesize that the tgrE and tgrD cell adhesion system operates similarly to that of tgrB and tgrC and that these two systems have a common phylogenetic origin. Specifically, we hypothesize that tgrE and tgrD bind heterotypically in trans and in cis, and that proper binding is required for activation of developmental signaling pathways. Currently we are attempting to express tagged tgrE and tgrD proteins in D. discoideum to follow expression and perform CoIP, FRET studies, and other experiments for the functional characterization of these important mediators of D. discoideum development.

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Optogenetic approaches allow researchers to investigate causal links between neural activity and behavior. Light-activated proteins, such as channelrhodopsin-2 (ChR2), are genetically targeted to specific populations and types of neurons. Light, at specific wavelengths, is then used to elicit activity in the modified cells. Typically, wide-field illumination is used, limiting the effective depth in light-scattering brain tissue and preventing individual cells being targeted.

Utilizing two-photon stimulation to activate ChR2 would give precise control of intact brain tissue with single-cell and single-spike resolution. Our goal is to study the functional organization and plasticity of cortical microcircuits using optogenetic tools combined with random-access multi-photon (RAMP) microscopy. RAMP microscopes employ acousto-optic deflectors (AODs) for inertia-free steering of a laser beam and are well-suited to generating precise spatio-temporal patterns of stimulation. After identifying ChR2-positive cells with the RAMP microscope, user-defined arbitrary patterns of optical stimulation will permit experimental protocols where individual cells or sub-compartments receive unique temporal stimulation sequences.

We expanded the features of an existing RAMP microscope to activate ChR2 channels in Thy1-ChR2-YFP line 18 transgenic mice. Since ChR2 channels are expressed at a low density and have small conductance, the two-photon (920nm) beam was scanned rapidly across a user-drawn outline of the fluorescent soma. Initial results indicate that these “filling” scans reliably induce membrane currents large enough to elicit action potentials. Existing transgenic mice are however impractical for our experiments since it is difficult to identify ChR2 positive neurons based solely on membrane bound fluorescent proteins (i.e. YFP fused to the ChR2). Furthermore, we would ideally want feedback on the activation of ChR2 so that precise firing times can be determined. We are therefore working on an optogenetic approach that would insert ChR2 combined with cytosolic fluorescent proteins or genetically encoded Ca2+ sensors.
Introduction: Estrogen receptor (ER)-negative breast cancer has a particularly poor prognosis, and there are limited targeted agents for this subtype.

Aims and Methods: To identify nuclear receptor (NR) targets, we did microarray analysis of 227 ER-negative tumors, and performed hierarchical clustering using 41 NRs, and prediction analysis of microarrays (PAM) across the clustered groups. Pathway analysis was performed on PAM genes in these subtypes. We then compared each of these groups for associated clinical phenotypes such as the DNA damage repair signature.

Results and Conclusion: The 41 NRs clustered the tumors into 5 distinct groups. We found that the androgen receptor (AR) was the highest ranked discriminator of these groups. The AR group (group 5, 24% of the tumors) also showed higher expression of ERa and ERRg. This group probably corresponds to the previously described molecular apocrine group. Group 3 (37%) was characterized by neither high nor low expression of any specific NR, but demonstrated enrichment for genes involved in purine/pyrimidine metabolism, and the Notch/Wnt pathway. Group 4 (16%) expressed higher levels of PPARg and thyroid hormone receptor b. We have previously shown that a PPARg ligand can inhibit proliferation of breast cancer cells, thus PPARg may represent a new clinical target in ER-negative tumors. This group also expressed many immune regulatory genes. Group 2 (9%) was characterized by higher expression of the orphan receptor NURR1, suggesting that retinoic acid ligands might provide a potential therapy in this group. This group also showed enrichment for cell signaling molecules, and the MAPK/Jak/Stat pathways. Group 1 (14%) was characterized by elevated ERRa and RXR expression, and also displayed similarity in pathways with group 2. Vitamin D receptor was expressed in both groups 2 and 4. HER2 positive tumors were distributed between groups 1 and 3. We found that there was high concordance between the nuclear receptor signature that we have identified and a previously reported DNA damage repair signature. This may indicate that certain nuclear receptor profiles drive cancer cells to resist DNA damage. In conclusion, clinical targeting of NRs in selected ER-negative breast cancers may be promising since there are a number of agonists and antagonists currently available.

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**Purpose:** This project utilizes the DBA2/J mouse strain, which is considered a glaucoma model because it has an intraocular pressure (IOP) increase and retinal ganglion cell (RGC) death starting at 7 months. RGC death is a focal point of glaucoma, however the loss of function in RGCs prior to cell death is poorly characterized. Since understanding that dysfunction could have diagnostic utility, this project attempts to characterize it by recording RGC light responses. That data is combined with behavioral and anatomical information, and compared to IOP histories on an eye by eye basis. This approach addresses important concerns regarding the connection between RGC death and IOP in these mice.  

**Methods:** IOP was measured with a rebound tonometer, with at least 3 measurements taken at ~10 day intervals after mice reached 3 months of age. Groups of mice were then sacrificed at three month intervals, starting at 4 months. Before sacrifice, their optokinetic reflex threshold, a behavioral metric of visual acuity, was determined for both eyes. To characterize RGC function, retinas either had their RGC light responses recorded with a multielectrode array or, to assay degeneration, RGC cell bodies were back-filled with dye via the optic nerve.  

**Results:** The mouse population showed a consistent IOP elevation to an average of 25mmHg by 8 months, compared to an average of 13mmHg in age matched wild type mice. However there was significant variation in IOP history between mice and, within each mouse, between eyes. Females showed a more acute elevation than males, but right and left eyes were statistically identical. No statistically significant correlations could be detected between mouse behavior and IOP elevation. This effect is probably due to a considerable deficit in DBA/2J pre-IOP elevation behavioral contrast thresholds (mean of 73% contrast threshold) compared to age matched wild type mice (%12). A moderate positive correlation was observed between IOP elevation and retinal ganglion cell sensitivity, suggesting that DBA/2J mice with elevated pressure do have dysfunctional ganglion cells. Additional measurements to verify this finding statistically are underway. Anatomical results confirmed previous findings that showed RGC death starting at 8 months and progressively growing worse with age.  

**Conclusions:** By performing the first longitudinal study of pressure in these animals, we were able to correlate IOP history to the severity of mouse disease. Given the observed variation in pressure history between eyes, we believe this is an important approach. IOP history showed a moderate correlation to ganglion cell physiology, and little correlation to morphology and behavioral measures of contrast sensitivity.  

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NEUROONTAL AGGREGATES ARE ASSOCIATED WITH PHENOTYPIC ONSET IN THE R6/2 HUNTINGTON'S DISEASE TRANSGENIC MOUSE

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Huntington’s Disease is caused by the expansion of the polyglutamine tract expressed in the huntingtin protein. Data from patients show a strong negative correlation between CAG repeat size and age of disease onset. Recent studies in mixed background C57xCBA R6/2 mice suggest the inverse correlation observed in the human disease may not be replicated in some animal models of HD. To further clarify the relationship between repeat length and age of onset, congenic C57BL6/J R6/2 transgenic mice expressing 110, 260 or 310 CAG were tested in a comprehensive behavioral battery at multiple ages. Data confirmed the findings of earlier studies and indicate that on a pure C57BL6/J genetic background, R6/2 mice with larger repeats exhibit a delay in phenotypic onset with increasing polyglutamine size (6 weeks in 110 CAG and 17 weeks in 310 CAG mice). Further analysis confirmed a decrease in transgene transcript expression in 310 CAG mice as well as differential aggregated protein localization in association with repeat length. Mice expressing 110 CAG developed aggregates that localized almost exclusively to the nucleus of neuronal cells in the striatum and cortex. In contrast, tissue from 310 CAG mice exhibited predominantly extranuclear inclusions. Novel mutant protein analysis obtained using time-resolved FRET revealed that soluble protein levels decreased with disease onset in R6/2 mice while aggregated protein levels increased. We believe that these data suggest a role for aggregation and inclusion localization in HD pathogenesis and propose a mechanism for the age of onset delay observed in R6/2 mice.

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Autophagy is a cellular catabolic process utilized for degradation of cytoplasmic components through the lysosomal machinery. Autophagy is negatively regulated by the nutrient sensor mammalian target of rapamycin complex 1 (mTOR) and occurs at a low basal level in nutrient-rich conditions. A number of viruses induce autophagy and exploit the autophagy machinery for their replication but the signaling pathways through which these viruses activate autophagy are unknown.

Rotavirus (RV) is the leading cause of severe diarrhea among infants and young children. The RV nonstructural protein 4 (NSP4) was reported to colocalize with LC3, a marker of autophagy, associated with viroplasms, which are sites of nascent viral RNA replication and immature particle assembly. This interaction suggested a possible new involvement of NSP4 with autophagy in virus replication. In this study I determined (1) whether RV induces autophagy; (2) whether the autophagy machinery is required for viral replication; and (3) a mechanism of autophagy induction.

I found (1) RV infection induced the formation of LC3 II and LC3 puncta, characteristics of autophagy induction. (2) RV requires the autophagy machinery for replication. In infectivity assays, the yield of virus was reduced >90% in cells treated with the autophagy inhibitor 3-methyladenine or cells deficient in Atg 3 or Atg 5, proteins required for autophagy membrane formation, as compared to nontreated or parental cells. (3) Induction of autophagy was mediated by NSP4 functioning as a viroporin to increase cytoplasmic calcium. The increased cytoplasmic calcium activated the calcium-dependent calmodulin kinase kinase-β (CaMKK-β) to phosphorylate the adenosine monophosphate-activated protein kinase (AMPK), which in turn negatively regulated mTOR leading to the induction of autophagy. Inhibition of CaMKK-β by STO-609 abrogated NSP4/LC3 puncta formation and significantly reduced the yield of infectious RV. These are the first studies to define a role for autophagy in RV replication and to characterize a mechanism of viral-mediated Ca2+-activated signaling for the induction of autophagy.
Obstructive sleep apnea (OSA), a highly prevalent sleeping disorder, is characterized by repetitive closure of the pharyngeal airway with ongoing ventilatory effort during sleep. Clinical studies suggest that OSA is an independent risk factor for cardiovascular diseases (i.e. hypertension and stroke). Although OSA is a predictor for stroke, very little is known about how it affects the cerebral circulation.

Animal models have been frequently used in an attempt to better understand the pathological consequences of OSA. However, current animal models utilize intermittent hypoxia alone during the sleep cycle to mimic OSA. Thus, animal models are limited to the hypoxia component of OSA. True apnea involves hypoxia, hypercapnia, excessive negative intrathoracic pressures, sympathetic activation, arousal, and altered chemoreceptor and baroreceptor responses. In this study, we have 1) developed an improved model of OSA and 2) begun to examine the effects of OSA on cerebral artery function.

An occlusion device (OD) has been developed, which is chronically implanted into the trachea of rats. When remotely activated by a computer, a balloon inflates for 10 seconds to obstruct the airway. Rats are exposed to 30 apneas/hr for 8 hrs a day during the sleep cycle (moderate OSA) for up to one month. The new model incorporates aspects of OSA (described above) which are not involved in “intermittent hypoxia” models. Airway pressure studies demonstrate that implantation of the device had no acute effect on normal pressure waveforms. During apnea, rats exhibit excessive negative intrathoracic pressures (~ -20mmHg; n=7). Furthermore, apnea causes a drop in pO2 from 98 to 51 mmHg, an increase in pCO2 from 28 to 35 mmHg, and a decrease in oxygen saturation from 98% to 87%. During an apnea, heart rate decreases by 43% (330 to 143 bpm). The effect of apnea on mean arterial blood pressure (MABP) varies with individual rats as it does in humans with OSA. After one week of apnea, MABP is not different in apnea rats when compared to sham controls (instrumented with OD but not inflated) [109 and 110 mm Hg (n=3 each), respectively]. Despite the lack of a difference in MABP, cerebral vessels from apnea animals exhibit signs of vascular dysfunction with attenuated nitric oxide-mediated dilations to ATP and enhanced endothelin-1 constrictions.

In summary, we have developed a rat model of OSA that better mimics the human OSA condition. Furthermore, we provide results showing vascular dysfunction in cerebral arteries. While there are no overt changes in MABP, we propose that endothelial dysfunction precedes and is responsible for hypertension that takes longer to develop.

Contributors: Durgan, David; Phillips, Sharon; Lloyd, Eric; Marrelli, Sean; Bryan, Robert
Rationale: Patients with Hodgkin’s Lymphoma (HL) who relapse after hematopoietic stem cell transplant (HSCT) have limited options for long-term cure. We have shown that infusion of cytotoxic T cells (CTL) targeting Epstein Barr virus (EBV)-derived proteins induced complete remissions in EBV+ HL patients. A limitation of this approach is that up to 70% of relapsed HL tumors are EBV-negative. For these patients an alternative is to target the cancer/testis antigen MAGE-A4 which is present in EBV antigen-negative HL tumors. Furthermore, epigenetic modification by clinically available demethylating agents can enhance MAGE-A4 expression in previously MAGE-negative tumors.

Experimental Design: We explored the feasibility of combining adoptive T cell therapy with epigenetic modification of tumor antigen expression. We further characterized MAGE-A4-specific T cell phenotype and function, and examined the effects of the epigenetic-modifying drug decitabine on these T cells.

Results: Cytotoxic T cells were generated specifically recognizing MAGE-A4 expressed by autologous HL targets and tumor cell lines. We found that the epigenetic-modifying drug decitabine – previously shown to increase tumor antigen expression in Hodgkin’s Lymphoma – did not compromise MAGE-A4 specific T cell phenotype and function. In patients treated with decitabine, we found an expansion of MAGE-A4 specific T cells and a broadened anti tumor T cell repertoire, consistent with increased antigen stimulation in vivo.

Conclusions: Adoptive transfer of MAGE-A4 specific T cells combined with the administration of epigenetic-modifying drugs to further increase expression of the protein may improve treatment of relapsed Hodgkin’s Lymphoma.
Miltefosine (hexadecylphosphocholine) is a synthesized membrane-active alkylphospholipid originally developed as an anticancer drug. This drug has broad-spectrum antiprotozoal and antifungal activities. In particular, miltefosine has been approved in India, Columbia, and Germany for the treatment of leishmaniases and is the only currently available effective oral treatment for this group of diseases.

To investigate these mechanisms, we used a functional genomic approach in the yeast Saccharomyces cerevisiae and identified about 150 genetic alterations that confer miltefosine-hypersensitivity or -resistance. By analyzing the screen results and some further experiments, we found that miltefosine can inhibit PIP2 synthesis and then further PIP2 dependent actin morphology and exocytosis.

Contributors: Dandan Cui1, Zhiwei Huang1 and Xuewen Pan1,2
Affective experiences and responses to cigarette-related cues have been frequently implicated in smoking relapse induction. A better understanding of smokers’ affective and cigarette-related responses may promote the development of more efficacious interventions for tobacco dependence. The analysis of EEG frequencies provides the opportunity to investigate brain processes involved in attentional capture: power in the alpha band (8-12 Hz) is inversely related to attentional demand in the environment. We hypothesized that, similarly to intrinsically emotional stimuli, cigarette-related cues would significantly reduce power in the alpha band in comparison to neutral cues. To study this question, we recorded the brain electrocortical activity (i.e., electroencephalogram) of 179 treatment-seeking smokers in response to neutral, pleasant, unpleasant, and cigarette-related pictures. We applied wavelet analysis to specifically isolate alpha band, a method that provides joint time-frequency resolution. We found that affective and cigarette-related stimuli induced greater alpha desynchronization than neutral stimuli. In addition, cigarette cues-related alpha desynchronization was similar to the level modulated by pictures of high arousal levels, such as erotica and mutilations. In conclusion, for smokers, cigarette-related cues capture attentional resources to the degree that is normally allocated to natural stimuli (e.g., erotica) that are critical for survival. Future studies may investigate if these cue-related alpha activities are related to smoking relapse.

Contributors: Cui, Yong; Versace, Francesco; Minnix, Jennifer; Robinson Jason; Lam, Cho; Engelmann, Jeffrey; Wetter, David; Brown, Victoria; Dani, John; Kosten, Thomas; Cinciripini, Paul.
Image-Guided Patterning of Hydrogel Scaffolds to Mimic Neurovascular Structural Relationships in the Neural Stem Cell Niche

James Curtis Culver
Department of Molecular Physiology & Biophysics
Advisor: Mary Dickinson, Ph.D.-Department of Molecular Physiology & Biophysics

Neural stem cells are maintained throughout adult life within localized microenvironments in the mammalian brain (1,2). The largest of these regions, the subependymal zone (SEZ), is located along the lateral walls of the two lateral ventricles. Previous reports have demonstrated that neural progenitors in the SEZ are closely apposed to blood vessels (1,2), and evidence suggests that this physical association is important for regulating neurogenesis (1). Our objective in this study was to elucidate the defining features of these vessels, and to engineer biomaterials that recapitulate the essential structural relationships between neural progenitors and vascular endothelial cells observed in this microenvironment. Our results show that there are structural and physiological differences between these vessels in the SEZ and vessels in non-neurogenic regions of the brain, and that image-guided two-photon photolithography can be used to fabricate hydrogel scaffolds that recapitulate these neurovascular structural relationships at subcellular resolution. This work could be applied as a strategy for maintaining neural stem cells in engineered tissue constructs, with potential applications in regenerative medicine.


Contributors: Culver, James C.; Hoffmann, Joseph C.; Poché, Ross A.; West, Jennifer L.; Dickinson, Mary E.
Background: Noroviruses (NoVs) are the major etiological agents of epidemic, acute nonbacterial gastroenteritis in humans. The replication of these viruses and host determinants of susceptibility remain poorly characterized and few therapeutic and prophylactic strategies exist. The absence of tissue culture or small animal models for cultivation of human NoV’s presents a technical challenge leading to a rare marriage of “bedside” and “bench” approaches for the study of this pathogen. Studies of human volunteers experimentally infected with a human NoV have provided the first clues to host determinants of susceptibility.

Specifically, lack of expression of histo-blood group antigen (HBGA), a group of glycans that are displayed on the surface of mucosal epithelia and in their secretions, is correlated with resistance to infection. High levels of antibody that block viral binding to HBGAs in vitro are correlated with protection from illness in experimentally infected individuals. The so-called “blocking assay” for measuring these antibodies requires expensive and difficult to obtain reagents. Blocking assay reproducibility is compromised by sensitivity to a multitude of reaction conditions. NoV virus-like particles (VLPs), which structurally and antigenically recapitulate native virions, hemagglutinate human erythrocytes. A shorter, simpler, and less expensive method such as a hemagglutination inhibition (HAI) assay could allow more rapid and reliable characterization of protective serum antibody.

Objective: To determine the correlation between HAI activity and protection from gastroenteritis in human volunteers experimentally infected with Norwalk virus, the prototypical NoV.

Results and Future Work: HAI activity increased significantly in individuals experimentally infected with Norwalk virus (n=18). The geometric mean titer of the HAI antibodies peaked at 28 days post-challenge. Pre-challenge HAI titer was a strong predictor of protection from the development of gastroenteritis among infected individuals (Mann-Whitney U, p=0.02; logistic regression; p=0.02). HAI titer strongly correlated with titer acquired when the same set of sera were analyzed using the blocking assay, at a pre-exposure time point (r=0.76) as well as at 28 days post-challenge (r=0.85, p < 0.01). Future work will determine whether the protective antibodies measured by these assays demonstrate any cross-protection against related human NoV’s.

The results of this work will facilitate NoV vaccine development and evaluation.

Contributors: Czakó, Rita; Atmar, Robert; Estes, Mary
BAYLOR COLLEGE OF MEDICINE

CROSS-SPECIES RNA-SEQ IDENTIFIES NOVEL GENES FUNCTIONING IN RETINAL DEVELOPMENT

Bryce Reid Daines
Department of Molecular & Human Genetics
Advisor: Rui Chen, Ph.D.-Department of Molecular & Human Genetics

The central goal of functional genomics is to elucidate the relationship between an organism’s genome and phenotypes. Advances in DNA sequencing technology are enabling the development of novel high-throughput functional genomics techniques. Importantly, these approaches are applicable to a broad range of organisms including established and emerging model organisms, non-model organisms, and humans.

Drosophila retinal development is a well established model for human eye disease and development for two primary reasons. First, the major regulators of vertebrate eye development are conserved in Drosophila. Second, many genetic tools are available for functional analysis in this species. To identify novel functional genes involved in eye development, we have developed a novel methodology: cross-species RNA-sequencing.

We performed tissue-specific transcriptome sequencing in five Drosophila species to discover conserved patterns of gene expression within third instar larva eye imaginal discs. Comparative analysis of these data identifies patterns of gene expression conserved across the Drosophila lineage. The conserved expression data were integrated with genome-wide epistasis experiments in which we perturbed members of the well-understood retinal determination network. The integrated dataset was used to make functional predictions on previously uncharacterized genes. We validated these functional predictions by screening for eye-specific phenotypes with RNAi and observed success rates as high as 56%. Development of this technique has led to the identification of many candidate genes for future functional analysis. It is likely that this methodology will have similar success when applied to other developmental processes and organisms including vertebrates and humans.

Contributors: Daines, Bryce; Li Yumei; Wang, Hui; Lopez, Irene; Hernandez, Eddie; Salvo, J. Scott; Mardon, Graeme; Chen, Rui
PREVALENCE OF XMRV IN THE BLOOD CELLS OF HIV-1 INFECTED PATIENTS

Bryan Patrick Danielson
Department of Molecular Virology & Microbiology
Advisor: Jason Kimata, Ph.D.-Department of Molecular Virology & Microbiology

Background: Xenotropic murine leukemia virus-related virus (XMRV) is a gammaretrovirus that was discovered in the prostatic tissue of prostate cancer patients in 2006, and was found in the blood of chronic fatigue syndrome patients in 2009. The prevalence, distribution, and pathogenic potential of the XMRV in the human population remain to be established. Since discovery of the virus, numerous studies have found little to no trace of XMRV in different cohorts of prostate cancer and chronic fatigue syndrome patients among others. The ability of XMRV to infect lymphocytes and cells of the reproductive tract suggest that the virus may share common transmission routes with human immunodeficiency virus (HIV). This, in addition to the immunological destruction caused by HIV type 1 (HIV-1) infection, suggests that HIV-1 infected individuals may represent a high-risk group for XMRV infection and spread. The primary objective for this study was to evaluate the prevalence of XMRV in the peripheral blood mononuclear cells (PBMCs) of HIV-1 infected patients and healthy blood donors from Houston, TX, and to determine whether presence of XMRV correlates with HIV-1 infection. Methodology/Principal Findings: DNA was isolated from the PBMCs of 169 HIV-1 infected treatment naïve patients, 81 of which were co-infected with HCV, and 54 healthy blood donors. DNA was screened for XMRV provirus with two sensitive, published PCR assays targeting XMRV gag and env genes and one sensitive, published, nested PCR assay targeting env. Detection of XMRV was confirmed by DNA sequencing. One of the 169 HIV-1 infected patients tested positive for gag by non-nested PCR whereas the two other assays did not detect XMRV in any specimen. All HIV-1 uninfected blood donors were negative for XMRV proviral sequences. Sera from 23 of the HIV-1 infected patients, including 15 coinfected with HCV, were screened for the presence of XMRV-reactive antibodies by Western blot. Thirteen of the 23 serum samples showed reactivity to XMRV infected cell lysate. Lastly, in cultured cells, HIV-1 coinfection did not enhance XMRV replication. Conclusions/Significance: The virtual absence of XMRV in PBMCs suggests that XMRV is not associated with HIV-1 infected or HCV-coinfected patients, or blood donors. While positive serum reactivity to XMRV suggests possible latent infection, we are unable to verify the antibodies are XMRV-specific.

Contributors: Danielson, Bryan; Gingaras, Cosmina; Vigil, Karen; Arduino, Roberto; Kimata, Jason
The medial habenula (MHb) has emerged as an important neural locus for nicotine addiction. Nicotinic receptors (nAChRs) in the MHb are important for somatic nicotine withdrawal, and the nAChRs in the MHb containing the alpha5 subunit modulate the aversive character of high nicotine intake. Furthermore, null mutation of the nAChR subunits found in high density within the MHb alters performance in various anxiety-related tasks, further corroborating the MHb’s role in modulating negative affect and the aversive aspects of nicotine addiction. We propose that nicotine acts through nicotinic receptors in the MHb to modulate the firing of MHb neurons as a neural mechanism for nicotine dependence. Primarily, we utilize patch clamp electrophysiology to investigate our hypotheses. Our data show that this occurs via a plasticity of intrinsic excitability, involving multiple ionic conductances. The results from our work may have implications for therapeutic interventions against nicotine abuse.

Contributors: Dao, Dang Q.; Dani, John A.; De Biasi, Mariella
ABSENCE OF WIP1 PARTIALLY RESCUES ATM DEFICIENCY PHENOTYPES IN MICE

Yolanda Darlington
Program in Cell & Molecular Biology
Advisor: Lawrence Donehower, Ph.D.-Department of Molecular Virology & Microbiology

Wildtype p53-Induced Phosphatase 1 (WIP1) is a serine/threonine phosphatase that dephosphorylates proteins in the ataxia telangiectasia mutated (ATM)-initiated DNA damage response pathway. WIP1 may play a homeostatic role in ATM signaling by returning the cell to a normal pre-stress state following completion of DNA repair. To better understand the effects of WIP1 on ATM signaling, we crossed Atm-deficient mice to Wip1-deficient mice and characterized phenotypes of the double knockout progeny. We hypothesized that the absence of Wip1 might rescue Atm deficiency phenotypes. Atm null mice, like ATM-deficient humans with the inherited syndrome ataxia telangiectasia, exhibit radiation sensitivity, fertility defects, and are T-cell lymphoma prone. Most double knockout mice were largely protected from lymphoma development and had a greatly extended lifespan compared to Atm null mice. Double knockout mice had increased p53 and H2AX phosphorylation and p21 expression compared to their Atm null counterparts, indicating enhanced p53 and DNA damage responses. Additionally, double knockout splenocytes displayed reduced chromosomal instability compared to Atm null mice. Finally, doubly null mice were partially rescued from infertility defects observed in Atm null mice. These results indicate that inhibition of WIP1 may represent a useful strategy for cancer treatment in general and A-T patients in particular.

Contributors: Darlington, Yolanda; Nguyen, Thuy-Ai; Moon, Sung-Hwan; Herron, Alan; Rao, Pulivarthi; Zhu, Chengming; Lu, Xiongbin; Donehower, Lawrence
CHARACTERIZATION OF THE ROLE OF WAC IN NEURODEGENERATION AND AUTOPHAGY

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Program in Developmental Biology  
Advisor: Hugo Bellen, Ph.D./M.B.A.-Department of Molecular & Human Genetics

Our lab has conducted a forward genetic screen to identify essential genes on the Drosophila X chromosome that affect the maintenance of the nervous system. Homozygous mutant clones in the eye were generated through the FLP/FRT system and electroretinogram recordings were used as a read-out of neuronal function in aging flies.

From this screen, we isolated six alleles of the Drosophila homolog of mammalian WW domain-containing adapter with coiled-coil region (WAC). Mutant clones in the eye manifest degenerative electroretinogram profiles. Transmission electron microscopy images show abnormal morphology and accumulation of mitochondria and autophagosome-like structures in the retina and the lamina. Acidified vesicles marked by lysotracker are strikingly absent in the fat body mutant clones of starved larvae indicating a defect in the autophagy-lysosome pathway.

Previously published studies in mammalian cell culture implicate WAC in a variety of processes. WAC has been shown to co-localize with the splicing factor, SNRP70, indicating a possible role in splicing. WAC has also been shown to be a functional partner of RNF20/40, regulating histone H2B ubiquitination and gene transcription. In addition, WAC has been shown to be involved in golgi membrane fusion through its interaction with VCIP135 and p97.

We are currently trying to determine the mechanism by which the Drosophila WAC mutations lead to our observed phenotypes. We are very interested in deciphering how a gene implicated in splicing, histone ubiquitination, gene transcription, and golgi biogenesis plays a role in neurotransmission and the autophagy-lysosome pathway.

Contributors: David, Gabriela; Tong, Chao; Yamamoto S; Jaiswal M; Bayat V; Xiong, B.; Bellen, Hugo
ALBUMIN BINDING PEPTIDE CONJUGATE FOR NEAR INFRARED FLUORESCENCE IMAGING OF LYMPHATIC DISEASE AND RESPONSE TO THERAPY

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Program in Translational Biology & Molecular Medicine
Advisor: Eva Sevick-Muraca, -Department of Radiology
Caroline Fife, M.D.-Department of Radiology

There is an urgent need for improved diagnostic imaging techniques to assess the architecture and function of lymphatic vasculature in vascular disease. We have developed and characterized a novel peptide imaging agent for non-invasive near-infrared fluorescence imaging of the lymphatic system. Our imaging agent consists of a cyclic albumin binding domain (cABD) peptide, with sequence, (RLIEDICLPRWGCLWEDDK), conjugated to a bright near-infrared fluorophore, IRDye800CW, allowing for enhanced vascular retention and signal. Synthesis of cABD-IRDye800 was conducted using solution conjugation followed by HPLC purification. Characterization of the peptide conjugate was performed using mass spectrometry to confirm the molecular weight, fluorescence spectroscopy to assess optical properties, and serum binding assays and Biacore surface plasmon resonance to determine binding affinity. SDS-Page studies were conducted to determine binding specificity, while chemical stability was monitored by HPLC. Subsequently, cABD-IRDye800 was used for fluorescence imaging to monitor lymphatic uptake and retention in normal mice and a VEGFR3 +/- (Chy) mouse model of lymphedema. Finally, AAV-vegfc was prepared from viral plasmid components by transfection of 293T cells, and harvested using density gradient centrifugation, for use in gene therapy. Our results showed that cABD-IRDye800 had a greater fluorescent yield than commonly used indocyanine green (ICG) as determined by extinction coefficient and quantum yield, a high affinity for albumin with IC50 and Kd in the nanomolar range, binding specificity for albumin as compared to the structurally distinct protein ovalbumin, and superior retention characteristics within mouse lymphatics when compared to IRDye800. The conjugate was also found to be stable in a 14 day stability study. Subsequently, cABD-IRDye800 was successfully used to show a lack of initial inguinal lymphatics in VEGFR3 +/- mice. These findings were confirmed by intravital imaging using Evans Blue, which showed a lack of dye uptake into the lymphatic vessels and inguinal lymph node of these mice. Finally, AAV-vegfc was produced, quantified and assessed for vegfc production for longitudinal imaging of response to viral gene therapy in Chy mice. C3H and Chy mice were treated with AAV-vegfc or soluble vegfc and imaged weekly using cABD-IRDye800. Initial results in the ear of the Chy mice have shown that AAV caused vessel dilation after six weeks, while soluble vegfc showed an increase in vessel density after two weeks. Similar changes were also seen in the inguinal region of the mice. cABD-IRDye800 has utility for assessing lymphatic function in mouse models of human lymphatic disease and potential for use in clinical diagnostic and therapeutic imaging of lymph and possibly blood vasculature as well.

Contributors: Davies-Venn, C.A., Wilganowski, N., Harvey, Barrett R., Aldrich, M.B., Kwon, S., Sevick-Muraca, E.M.
Balanced translocations are hallmarks of many human cancers and some are also used as prognostic markers. In pediatric acute lymphoblastic leukemia (ALL), the t(12;21), t(1;19), t(4;11) and t(9;22) translocations are used as part of a prognostication algorithm to stratify patients to risk-based therapy. These translocations are detected clinically by a combination of G-banding and fluorescent in-situ hybridization (FISH) but neither technique can identify the precise location of the breakpoints, which may have further prognostic significance. In addition, more than 10% of the cases fail to have a cytogenetic diagnosis due to various technical limitations, which may lead to under- or over-treatment. We therefore seek to develop a more robust, high-resolution alternative to classical ALL clinical cytogenetic diagnostic tests, to characterize patient samples on the developed platform, and test the hypothesis that the locations of the translocation breakpoints provide additional prognostic value. Previous studies in our lab revealed that cytogenetically balanced translocations are detectable by a custom high-density microarray due to micro-insertions and deletions observed only at high resolution. However, molecularly balanced translocations and complex rearrangements impeded translocation detection in roughly half the cases. Therefore we re-used the microarray design for sequence capture to detect translocations in two cryptic samples, 96C and 4. Each sample was known by FISH to harbor a t(4;11) translocation. Previous microarray results indicated the 3' end of the MLL gene on chromosome 11 in 96C was deleted, whereas sample 4 was diploid at high resolution across the entire 1.3 Mb target. Each sample was hybridized to a NimbleGen capture array, and the captured fragments were sequenced by 454 instruments. Coverage variability between samples was highly correlated, allowing coverage-based copy number analysis. Circular binary segmentation of a normalized coverage log ratio revealed a breakpoint in 96C within 50 bp of the 3' MLL deletion. Furthermore, chimeric sequences were identified and validated by PCR, providing base-pair resolved breakpoints of both derivative chromosomes in sample 4, and a novel MLL fusion partner, USP2, in sample 96C. In sample 4 we found a translocation event despite one of the partner breakpoints occurring within a gap in the microarray design. Robustness to design gaps is important given that structural variation is commonly associated with repetitive sequence. Our results show that 454 sequence capture determines copy number and base-pair sequence resolution of clinically relevant structural variations in ALL.
Aggression is a complex social behavior found widely throughout the animal kingdom. Drosophila males display distinct aggressive acts when defending a food territory, such as wing threats, charging or lunging behavior, and escalated fighting. Only a limited number of genes and even fewer neuronal circuits within the brain have been shown to modulate aggression. Our lab has identified tailless (tll) as a transcription factor that regulates aggression. Expression of tll during development is required to establish the posterior segments consisting of the eighth abdominal segment, the telson, and the posterior gut, as well as the anterior stripe which later gives rise to brain neuroblasts. In the adult fly, tll expression is restricted to the pars intercerebralis (PI) and the optic lobe. Knockdown of tll in the PI neurons leads to an increased aggression phenotype, which suggests an adult-specific role for tll in addition to its developmental role. We are in the process of identifying downstream targets of tll through mRNA sequencing from the PI neurons. In addition, we will identify the interacting proteins with Tll by co-IP using a tandem-affinity purification (TAP)-Tag method followed by mass spectrometry. Previous studies suggest possible interactions between Tll and histone-modifying proteins, such as Rpd3, dG9a and Lsd1. We will confirm these functional interactions by knockdown of the candidate genes and assaying the flies for aggressive behavior. Furthermore, we will examine PI function by measuring neuronal excitability in a WT and tll knockdown background by using 2 methods; measuring fluorescence in the PI by expressing UAS-GCaMP3, a calcium indicator of activated neurons, and by measuring voltage changes in the PI by electrophysiology. These results will help elucidate the mechanisms underlying aggressive behavior in Drosophila.

Contributors: Nomie, Krystle
TWO-STIMULUS FUNCTIONAL MAGNETIC RESONANCE IMAGING REVEALS
DIMINISHED MIDDLE CINGULATE SELF-RESPONSE IN CHILDREN DIAGNOSED
WITH AUTISM SPECTRUM DISORDERS

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Department of Neuroscience
Advisor: P Montague, Ph.D.-Department of Neuroscience
Terry Lohrenz, Ph.D.-Department of Neuroscience

Rapid and reliable functional magnetic resonance imaging (fMRI) paradigms will greatly enhance the efficacy of using fMRI to assess brain responses in individuals prone to motion artifacts and fatigue during typical fMRI experiments. Here we demonstrate robust visual responses in human middle cingulate cortex (MCC) during passive viewing of a single image of one's own face in control adults and typically developing children. We also show that these responses are diminished in individuals diagnosed with ASD. MCC response to age- and gender-matched “other” faces in the ASD group is comparable to the control cohorts. Responses in the fusiform face area also remain unperturbed in the ASD subjects suggesting that relatively low-level visual processing of faces remains intact. Together, these results are consistent with previous work demonstrating diminished perspective taking responses in the MCC during hyper-scanned social interaction in adolescents diagnosed with ASD. Furthermore, these results suggest the feasibility of using rapid single-stimulus fMRI to measure brain responses in children with and without the diagnosis of ASD.

Contributors: Kishida, Kenneth T.; Treadwell-Deering, Diane; Montague, P. Read
Airway epithelial cells (AECs) are found in the respiratory tract and provide initial protection against airborne pathogens. AECs prevent microbial colonization by generating antimicrobial agents and mounting an inflammatory response. In addition, studies suggested that beyond being a barrier, AECs also function as pathogen-sensors and clearance initiators. AECs are a common target of Pseudomonas aeruginosa (P. aeruginosa), a gram-negative bacterium which can cause pneumonia mainly in patients that are immunocompromised. Interestingly, in healthy individuals colonization of AECs by these pathogens rarely occurs. It has been shown that AECs are able to internalize P. aeruginosa and induce it elimination. However, the mechanisms responsible for pathogen elimination inside AECs still need to be identified. Autophagy plays a protective role in the cell via the degradation of damage organelles and proteins that otherwise could be harmful to the cell. It is also a key process in innate immunity and pathogen clearance in different cell types including macrophages. However, the role of autophagy during pathogen elimination in AECs is not completely understood.

Hypothesis: We hypothesize that AECs utilize autophagy as a strategy to fight infection in the respiratory tract; thereby preventing bacteria, including P. aeruginosa, from spreading to other tissues.

Objective: The purpose of this project is to evaluate whether P. aeruginosa is eliminated via autophagy in AECs. I will use a human airway epithelial cell line (A549), primary mouse tracheal epithelial cells and a conditional mouse model that is autophagy deficient in ciliated epithelial cells only. Understanding whether autophagy contributes to P. aeruginosa elimination in AECs may lead to new therapeutic approaches to better control infections in patients.

Contributors: Yi, Xu; Eissa, Tony
Nutrient limitation has varying effects on DNA replication in different bacterial species. The stringent response to amino acid starvation inhibits replication elongation in the Gram-positive bacterium Bacillus subtilis, but not in the Gram-negative E. coli. Interestingly, we and other groups have found that E. coli DNA primase, which in B. subtilis is a target of (p)ppGpp, is also inhibited by (p)ppGpp in vitro. This led us to question why no in vivo effects are seen on primase in E. coli during amino acid starvation. A difference in the stringent response between these two organisms is that B. subtilis preferentially accumulates guanosine pentaphosphate (pppGpp) whereas E. coli preferentially accumulates guanosine tetraphosphate (ppGpp). Additionally, total (p)ppGpp levels may differ between the two organisms. We propose that these differences in pppGpp levels underlie the difference in replication control.

The E. coli gene gppA encodes a phosphatase responsible for the conversion of pppGpp to ppGpp. Deletion of gppA leads to increased pppGpp levels and increased total (p)ppGpp. We then analyzed replication elongation in these cells. We found that loss of gppA decreases the rate of replication elongation during amino acid starvation, and this effect is (p)ppGpp-dependent. Induction of (p)ppGpp in the absence of starvation is sufficient to attenuate the replication elongation rate, indicating that the effect we observe is directly attributable to (p)ppGpp induction and not to a secondary effect of starvation. Conversely, expression of gppA in B. subtilis, which does not have a gppA homolog, leads to decreased pppGpp levels and diminished replication arrest in response to amino acid starvation. This work suggests that high levels of pppGpp are important for replication control in B. subtilis while high total levels may be required for attenuation of replication elongation in E. coli. In addition to providing in vivo evidence for starvation-induced attenuation of replication elongation in E. coli, this work also demonstrates the potential for separate physiological roles of the two forms of (p)ppGpp.

Contributors: DeNapoli, Jessica; Tehranchi, Ashley, Wang, Jue D.
Bacterial cells outnumber somatic cells by 10:1 in the human body, and bacterial genes outnumber human genes by 100:1. This fact underscores the importance of studying the role of bacteria in relation to human health and disease. Previous studies demonstrate the role of the intestinal microbiome in the metabolism of complex polysaccharides, maintaining gut homeostasis, and development of the immune system. The composition of microbial communities colonizing the human body and their relationship to health and disease allows for the potential diagnosis, treatment, and prevention of diseases.

Lung cancer treatment causes a wide variety of detrimental side effects, but the administration of naturally derived treatments could potentially decrease the severity of side effects, if not lung cancer itself. Previous studies using lung cancer mouse models demonstrated the anti-tumorigenic activity of red ginseng extract (RGE), which has been used for centuries in Chinese medicine. The active components of RGE are ginsenosides produced by the hydrolysis of glycoside molecules. Ginsenoside hydrolysis is known to be catalyzed by glycosidases not expressed by mammalian cells suggesting that glycosidases from a non-mammalian source catalyzes the hydrolysis of RGE. We hypothesize that bacteria present in the intestinal microbiome possess glycosidases capable of ginsenoside hydrolysis. Identification of bacterial species capable of metabolizing RGE presents an opportunity for administration of identified bacterial species as a probiotic to increase the efficacy of RGE for the prevention and treatment of lung cancer.

To determine changes in the intestinal microbiome associated with RGE administration, 17 A/J mice were treated with RGE by oral gavage for one week. We collected stool samples on days 0, 3, and 7 and performed 16S rRNA pyrosequencing to identify changes in membership and abundance of the mouse intestinal microbiome. Preliminary results show distinct population shifts associated with RGE treatment. Shannon diversity indices increase temporally with RGE treatment. We see significant changes in relative abundance of four genera previously shown to hydrolyze RGE. The overall changes in the intestinal microbiome from treatment with RGE demonstrate the potential for development of a probiotic for co-administration with RGE.

Contributors: Smith, Diane; Niu, Tao; Hu, Ming; Petrosino, Joseph
Recurrent chromosomal aberrations are a hallmark feature of the human cancer genome. Copy number alterations result in causal events that can either activate oncogenes or inactivate tumor suppressor genes. Identification of such driver events remains a foundation for the development of effective, targeted therapeutic agents against cancer. We discovered recurrent 5p13 amplification in several solid tumors including melanoma, colon adenocarcinoma and non-small-cell lung cancer (NSCLC) by genome-wide array-based comparative genome hybridization method (Scott et al., 2009, Nature). Further integrative genomic and functional analyses identified GOLPH3 as one oncogene targeted by 5p13 gain. While its full biological activity remains to be fully elucidated, GOLPH3 influences signaling through the Akt-mTOR pathway that plays a critical role in cell growth and cancer. Interestingly, our data also shows that in vivo GOLPH3 growth advantage is abrogated by treatment with rapamycin, an mTOR inhibitor used for treatment of certain cancer cases. Currently we are using yeast and human systems to examine GOLPH3’s potential role in regulating the trafficking of signaling molecules upstream of the Akt-mTOR axis.

Contributors: Dogruluk, Turgut; Scott, Kenneth L.
The piggyBac transposon system, derived from the cabbage looper moth, is an emerging technology for genetic modification of mammalian and human cells. We compared a recently developed hyperactive piggyBac transposase enzyme (containing seven mutations – 7pB) to the most active transposase of Sleeping Beauty, SB100X, for gene transfer in human cells in vitro and to somatic cells in mice in vivo. Despite similar protein level expression to native pB, 7pB significantly increased gene transfer efficiency of a neomycin resistance cassette transposon in both HEK293 and HeLa human cells. SB100X exhibited transposition efficiency similar to native pB. The activity of hyperactive 7pB transposase was not affected by the addition of a 24kDa N-terminal tag, while SB100X manifested a 50% reduction in transposition. Following nucleofection in human primary T cells, 7pB increased long-term gene expression by 2-3 fold as compared to native piggyBac and SB100X. Hyperactive 7pB was compared to native pB and SB100X in vivo in mice using hydrodynamic tail vein injection of a limiting dose of transposase DNA combined with luciferase reporter transposons. We followed transgene expression for up to six months and observed approximately 10-fold greater long-term gene expression in mice injected with a codon-optimized version of 7pB compared to those injected with native pB or SB100X. We conclude that hyperactive piggyBac elements can increase gene transfer in human cells and in vivo and should be useful for a wide variety of cell and gene therapy applications.
Metazoan cells form cytoplasmic mRNA granules such as stress granules (SG) and processing bodies (P bodies) that are proposed to be sites of aggregated, translationally silenced mRNAs and mRNA degradation. Poliovirus (PV) is a plus-strand RNA virus containing a genome that is a functional mRNA; thus, we investigated if PV antagonizes the processes that lead to formation of these structures. We have previously shown that PV infection inhibits the ability of cells to form stress granules by cleaving RasGAP-SH3-binding protein (G3BP). Here, we show that P bodies are also disrupted during PV infection in cells by 4 h postinfection. The disruption of P bodies is more rapid and more complete than disruption of stress granules. The kinetics of P body disruption correlated with production of viral proteinases and required substantial viral gene product expression. The organizing mechanism that forms P body foci in cells is unknown; however, potential scaffolding, aggregating, or other regulatory proteins found in P bodies were investigated for degradation. Two factors involved in 5’-end mRNA decapping and degradation, Xrn1 and Dcp1a, and the 3’ deadenylase complex component Pan3 underwent accelerated degradation during infection, and Dcp1a may be a direct substrate of PV 3C proteinase. Several other key factors proposed to be essential for P body formation, GW182, Edc3, and Edc4, were unaffected by poliovirus infection. Since deadenylation has been reported to be required for P body formation, viral inhibition of deadenylation, through Pan3 degradation, is a potential mechanism of P body disruption.

Contributors: Dougherty, Jonathan; White, James, Lloyd, Richard.
Acute myeloid leukemia (AML) is associated with lack of differentiation within the myeloid lineage, leading to accumulation of transformed leukemic stem cells. Knockout of the Nr4a1 and Nr4a3 orphan nuclear receptors in mice leads to the rapid development of an AML like disease and death within 3-4 weeks after birth. Compared to normal human stem cells (HSCs), both NR4A1 and NR4A3 are also severely downregulated in leukemia initiating cells (LICs) from AML patients regardless of cytogenetic background. Recent studies in our lab have also shown that acute restoration of NR4A function in AML cells inhibits their proliferation and results in transcriptional resetting of a subset of gene signatures that distinguishes human LICs from normal HSCs. Therefore, we hypothesize that silencing of the NR4A tumor suppressor genes may be an obligate event in AML development, and re-expression of these genes may have important implications in the therapeutic treatment of human AML which is plagued with poor prognoses and few avenues of successful treatment. The overall goal of my project is to disclose the molecular mechanisms by which NR4As function as tumor suppressors of AML. The specific aims are:

1) To identify direct transcriptional targets of NR4As that mediate their tumor suppressor function in AML cells. We used two complimentary approaches to address this aim. First, by bioinformatics analysis of NR4A regulated gene signatures, we have identified a subset of genes whose promoters contain canonical NR4A response elements. Using ChIP assays in human AML cells (Kasumi-1, carrying the AML-ETO oncogene representative of >12% of AML patients), we have confirmed that NR4As are recruited to the promoters of the first tested of these genes and activate their expression in AML cells. Second, using genome wide ChIP-sequencing, we have used a non-biased approach to identify NR4A interacting cistromes and established the relationship between sites of NR4A transcription factor recruitment and gene regulation. This approach has identified the ETS family of transcription factors as cooperating factors necessary for NR4A-mediated gene regulation.

2) To identify coregulator proteins that mediate the transcriptional regulatory activity of NR4As in AML cells. By using NR4A immunoprecipitation followed by mass spectrometry, we have objectively identified 9 coregulators that interact with NR4A1. Several of these are implicated in regulation of HSC homeostasis, although their mechanisms remain poorly understood. Using reciprocal IPs, we will validate their interactions with NR4A1 in AML cells. In addition, we will examine their functional involvement in NR4A dependent transcriptional control of the endogenous targets identified in aim 1 using siRNA mediated knock-down or overexpression of individual coregulators, and we will examine their co-recruitment to NR4A target genes by ChIP-re-ChIP assays.
Greater than 90% of human multi-exon pre-mRNAs are alternatively spliced, contributing to proteome diversity and to regulation of tissue and developmentally-specific gene expression programs. RNA binding proteins regulate alternative splicing via sequence motifs within and surrounding alternative exons. However, the mechanisms by which regulatory proteins communicate with the basal splicing machinery to regulate splicing are not well understood. The spliceosome assembles on pre-mRNAs through a series of dynamic interactions between protein and RNA components. It is through regulation of these contacts that splicing regulatory proteins are thought to function. Muscleblind-like 1 (MBNL1) regulates several splicing transitions during normal vertebrate development. MBNL1 binds pre-mRNAs both upstream and downstream of alternative exons through four CCCH-type zinc finger domains. Our goal is to elucidate the mechanism(s) used by MBNL1 to activate splicing of alternative exons. Insulin receptor (IR) exon 11 and sarcoplasmic reticulum calcium ATPase 1 (SERCA1) exon 22 are two notable exon inclusion events known to be regulated by MBNL1 during vertebrate development. We have characterized the in vitro splicing of IR exon 11 using a three-exon substrate containing exon 11 and intronic regions previously shown to be required for MBNL1 activation between two flanking heterologous exons. The activation of IR exon 11 inclusion by MBNL1 is dose-dependent and is diminished by mutation of the previously-identified MBNL1 binding motif in the intron downstream of exon 11. Using two-exon substrates containing either the upstream or the downstream intron, we determined that MBNL1 promotes removal of the upstream intron, not the downstream intron. We therefore utilized a two-exon substrate containing the upstream intron to monitor the effect of MBNL1 on spliceosome assembly. Preliminary evidence indicates that MBNL1 promotes spliceosome complex A assembly. We next tested whether the 5’ splice site of exon 11 is required for MBNL1-mediated splicing activation of the upstream intron. The results indicated that mutation of the 5’ splice site of exon 11 did not abolish the ability of MBNL1 to activate splicing. This indicates that MBNL1 promotes communication across the exon to promote splicing, perhaps by recruiting splicing complexes to the 3’ splice site of exon 11. To determine whether different MBNL1 targets use the same or different mechanisms of splicing activation, we generated a SERCA1 splicing substrate containing exons 21, 22, and 23. Preliminary evidence indicates that MBNL1 activates inclusion of alternative exon 22 in vitro. Future studies will reveal whether mechanistic features of MBNL1-mediated regulation of IR exon 11 splicing are shared with SERCA1 exon 22.
Foxi3 is critical for pharyngeal arch development. Bones and cartilage of the jaw, as well as the middle and outer ear derive from the most anterior of transient embryonic structures called pharyngeal arches. Ventral outpocketings arising at the level of the midbrain, pharyngeal arches develop into these elaborate structures through a complex interplay of signals and interactions between ectoderm, endoderm, mesoderm, and cranial neural crest cells. Of particular importance on this process are regions of ectoderm between the arches in direct contact with underlying endoderm: pharyngeal pouches. We have identified a forkhead transcription factor, Foxi3, which is expressed broadly in the pharyngeal region prior to arch outgrowth, and which is rapidly refined to ectoderm and endoderm in the pouches. It is not expressed in mesoderm or neural crest. Foxi3 is critically important for development of anterior arch-derived structures; Foxi3 mutant mice completely lack mandibles, bones of the middle ear, outer ears, and tympanic rings. Although maxillas are present, these mice have dramatically malformed palates. The most proximal cause of these deformities appears to be massive apoptosis of post-migratory neural crest cells in the arches around E9.5, as evidenced by positive immunostaining for activated caspase-3. Since Foxi3 is a transcription factor that is not expressed in the arch mesenchyme, we hypothesize that it plays a vital role in regulating a signaling pathway or coordinating multiple signaling pathways that promote neural crest survival and differentiation in the pharyngeal arches. Neural crest cells define the proximal-distal axis in the arches by expressing Dlx transcription factors in a nested pattern. Prior to onset of neural crest apoptosis in Foxi3 mutants, populations of cell express the proper nested Dlx pattern, suggesting that Foxi3 is not required for the initial steps of post-migratory neural crest differentiation, nor is it required for immediate survival of neural crest cells. Thus far, we have determined that loss of Foxi3 affects Fgf8, BMP4, and Endothelin-1 (Edn1) signals in the pharyngeal epithelium. Fgf8 is expressed both in arch ectoderm, and in a Tbx1-dependent manner in endoderm. Loss of Foxi3 does not disrupt Tbx1 or endodermal Fgf8, but it delays onset of ectodermal Fgf8 expression up to a day of development. BMP4 and Fgf8 are expressed in a complementary pattern in the first arch, with BMP4 defining the more distal region of the mandibular process and the presumptive maxillary domain of the ectoderm. In Foxi3 mutants, BMP4 expression is expanded through the first arch ectoderm into the cleft between the mandibular process and premaxillary domain, overlapping the Fgf8 expression domain. Not surprisingly, a mesenchymal downstream target of BMP4, the transcription factor Msx1, is also expanded through the first arch. It seems possible that Foxi3 acts in a genetic pathway that mediates Fgf8-BMP4 compartmentalization in pharyngeal arches. Ongoing experiments to further define gene expression changes resulting from loss of Foxi3 in the mouse and overexpression in chick will more specifically pinpoint the place of Foxi3 in arch development and neural crest cell survival and differentiation.

Contributors: Edlund, Renee
Over a decade ago, functional magnetic resonance imaging (fMRI) studies demonstrated that a rate effect exists between finger tapping rate and neural response (Rao et al., 1996). This study investigates the use of supervised learning analyses to decode finger-tapping rate from fMRI data on an image-by-image basis. In this study, we used a unimanual motor task in which subjects were guided by an audiovisual computer interface to press a fiber optic button at different rates with their right index finger. Fifteen subjects participated in three runs. Each run was 650 s (325 TRs). Their finger-tapping rate ranged between 1 and 5 Hz equally distributed over 48 blocks. The data were analyzed with the support vector machine using the 3dsvm command in AFNI (Cox, 1996). Specifically, Support Vector Regression (SVR) was applied to investigate decoding of continuous values of finger tapping frequencies ranging 1 to 5 Hz. Support Vector Classification (SVC) was used to build pairwise classifiers of each of the five frequencies. In addition, we obtained functional maps with the general linear model (GLM) (Friston, 1995) to compare to those obtained from SVC and SVR (LaConte, 2005).

SVR prediction was highly correlated with the task frequencies ($r = 0.79$, STD = 0.09) and had a root mean square error (RMSE) of 0.83 Hz (STD = 0.17), indicating that fMRI data can support predictions of finger tapping frequencies. SVC predicted 44% of the rates correctly (chance was 20%). Second level group analysis was performed separately for SVR, SVC, and GLM results. Multiple comparisons corrected maps (Genovese et al., 2002) resulted in SVR: right sensorimotor cortex and bilateral superior temporal gyri (STG); SVC: bilateral sensorimotor cortex at 4 foci, bilateral STG, bilateral middle occipital gyri, left thalamus, left putamen, right culmen, right insula and right inferior temporal gyrus; and GLM: all previous regions with addition of the left supplementary motor area. Finally, the mean responses in these regions were plotted across the 15 TR task block. The response curves were clearly separable by rate. We conclude that fMRI can be decoded into tapping rates. Furthermore, the SVC and SVR neural maps are consistent with the GLM and previous literature.

Contributors: Eierud, Cyrus; Lisinski, Jonathan; Craddock, Cameron R.; LaConte, Stephen M.
Drug-resistant bacterial infections cost many lives and billions of dollars each year yet the fundamental molecular mechanisms underlying antibiotic resistance remain unknown. To determine bacterial genotypes underlying antibiotic resistance we used next generation sequencing to identify single nucleotide polymorphisms (SNPs) common in all (n= 144) fluoroquinolone-resistant Escherichia coli clinical isolates. Accompanying the well-known gyrA S83L mutation associated with fluoroquinolone resistance, SNPs corresponding to amino acid changes were identified in three genes (ligB, mutM and recG). These genes fall within a 15 kb region in the E. coli chromosome.

We investigated the conservation of these SNPs across evolution to determine if our results could apply to other drug resistant bacteria found in the clinic. I examined the linkage throughout bacteria by looking at the distances between the three genes. Retaining close positions on the chromosome was more likely to indicate conservation of amino acid variants. Using 220 bacterial genomes where the three genes were annotated I found this linkage is highly conserved within the order enterobacteriales. A local protein sequence alignment was performed on 86 sequences collected from 14 unique species of enterobacter for each of the three genes as well as gyrA, where a S83L mutation is indicative of fluoroquinolone resistance. Analysis of amino acid variants showed the majority (54%) of strains had all three variants and the S83L gyrA mutation was only seen in strains that had all three variants. This suggests the variants of ligB, mutM, and recG are necessary before the S83L mutation can occur in gyrA. Lindgren et al. surveyed the presence of mutations that cause fluoroquinolone resistant clinical isolates and saw elevated mutation rates in most isolates, including susceptible isolates. We hypothesize that bacteria with all three variants have slightly elevated mutation rates as opposed to isolates and laboratory strains that lack these three variants. Identifying unique SNPs that are correlated to antibiotics resistance may uncover how bacteria are able to evolve to cope with environments they are exposed to in an efficient manner.

Contributors: Evangelista, Michael; Bodine, Truston; Swick, Michelle; Hamill, Richard; Carlson, Kimberly; Sucgang, Richard; Zechiedrich, Lynn
POST-TRANSCRIPTIONAL REGULATION OF THE KRAS ONCOGENE

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Alternative cleavage and polyadenylation (APA) has recently been identified to play a key role in the post-transcriptional regulation of gene expression in eukaryotic cells. This together with regulation of messenger RNAs by small RNA molecules and RNA binding proteins (RBPs) forms an intricate network by which gene expression is controlled through the intrinsic stability, cellular localization and efficiency of ribosomal binding and translation of mRNAs into their encoded protein product. APA events can alter the length of the 3' UTR of a given transcript, which in turn determines the presence or absence of cis-regulatory elements. By eliminating some or all of these regulatory elements, it is thought that the usage of upstream (proximal) polyadenylation sites (PASs) can result in increased protein expression by rendering mRNA transcripts invisible to trans-factors such as micro RNAs and RBPs. Previous work has correlated the increased usage of proximal PASs with cellular proliferation and transformation events. In order to map PAS usage across the genome, we resorted to 3' Rapid Amplification of cDNA Ends (RACE)-based deep sequencing. One of the genes we found to undergo consistent and robust PAS switching is Kras. Kras GTPase is an oncogene known to be mutated and amplified in approximately 30% of all human tumors. It functions as an intermediate signaling protein from upstream cell surface receptors in the activation of a number of signaling pathways controlling cellular proliferation, differentiation and apoptosis. Analysis of Kras 3' UTR identity after murine T lymphocyte stimulation revealed a 3-fold increase in proximal PAS usage compared to the distal PAS. Subsequent experiments show that the isoform switching event results in a significant induction in Kras protein expression. We aim to show to that that alternative cleavage and polyadenylation events regulate Kras protein expression and function. We will examine the relative stabilities of the short and long Kras mRNA isoforms and identify the cis-elements and trans-factors responsible for the post-transcriptional regulation by Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation followed by high-throughput sequencing and miRNA profiling. We will also investigate the effect of Kras 3' UTR identity on cancer development and progression in vivo through a mouse model of human cancer.

Contributors: Fachini, Joseph; Chaudhury, Arindam; Ruch, Alexander; Chandler, Anita; Kongchan, Natee; Neilson, Joel
Abnormalities in chromosome number, or aneuploidy, is characteristic of many solid tumors. It leads to altered gene dosages and disruptions in the stoichiometry of protein complexes, and therefore imposes a potential stress on the cells. Aneuploidy is most likely generated by mistakes in mitosis when sister chromatids are separated and segregated. Spindle assembly checkpoint (SAC) is a critical monitoring mechanism that prevents chromosome missegregation. We and others have generated a number of mouse strains with various degrees of disruption of SAC. A common phenotype of these mice is the development of spontaneous tumors, suggesting that aneuploidy can be tumorigenic. The SAC mutant mouse strain created in our lab carries point mutations in Cdc20 (Cdc20AAA) that renders Cdc20 refractory to the inhibition by SAC. Our preliminary studies show that Cdc20AAA/AAA mouse embryonic fibroblasts (MEFs) have a higher percentage of aneuploidy compared to wildtype MEFs, leading to changes in metabolism, elevated levels of reactive oxygen species (ROS). As a result, the ATM-p53 pathway is activated and causes apoptosis and cessation of growth of Cdc20AAA/AAA MEFs. Loss of either ATM or p53 dramatically enhances the tumor phenotype of Cdc20+/AAA mice. Therefore, we propose that the ATM-p53 pathway serves as an aneuploidy checkpoint to prevent the expansion and transformation of aneuploid cells.

To further characterize the aneuploidy checkpoint, we next look into the molecular mechanism that leads to apoptosis in the aneuploid Cdc20 mutant MEFs. Our preliminary data showed that there was an increase in p53 acetylation in Cdc20AAA/AAA MEF cells. Acetylation is critical for the activation of p53 and Tip60 is the known acetyl transferase of p53 that regulates its function in apoptosis. Taken together, here I hypothesize that acetylation of p53 by Tip60 leads to apoptosis in Cdc20AAA/AAA MEFs. Further experiments will be performed to determine the regulating mechanism of Tip60 in Cdc20 mutant cells, as well as the downstream target protein of p53 that causes apoptosis.
Exposure of homeothermic animals to cold temperature leads to a number of adaptive physiological responses, which however can be excessive and adverse, contributing to disease. One of the main responses that act to prevent heat loss is vasoconstriction in cutaneous circulation. However, in patients with Raynaud’s phenomenon, cold induces excessive vasoconstriction, which can lead to pain and ischemia-reperfusion injury. Substantial progress was made in elucidating efferent mechanisms of cold-induced vasoconstriction, which include a reflex increase in local sympathetic output and augmentation of \( \alpha_2 \)-adrenoceptor reactivity on vascular smooth muscle cells (SMCs). However, the initial temperature-sensitive mechanisms are still poorly defined. In addition, why the vasculature is over-reactive to cold in Raynaud’s phenomenon is not fully understood. Recently, the transient receptor potential ankyrin repeat domain 1 (TRPA1) ion channel was established as a molecular sensor of cold. TRPA1 is primarily expressed in sensory neurons that relay the information about cold exposure to the central nervous system. At the same time, TRPA1 is expressed in vascular endothelium and contributes to arterial vasodilation by the well-described endothelium-dependent hyperpolarizing factor (EDHF) mechanism. This raises an intriguing possibility that cold-sensing TRPA1 is involved in the intrinsic vascular response to cooling and its activation by cold limits vascular SMC contraction via EDHF. We hypothesize that TRPA1 channel expressed in the endothelium of skin vasculature is activated by cold, leading to vasodilation by the EDHF mechanism. We further hypothesize that the loss of compensatory vasodilation due to gene mutations and resulting disruption of endothelial TRPA1 function and EDHF response leads to excessive vasoconstriction seen in Raynaud’s phenomenon. Specifically, we propose to (1) define the effects of cold on membrane potential and calcium signaling of vascular endothelial cells mediated by TRPA1 ion channel; (2) determine the involvement of the EDHF mechanism in the responses of mouse cutaneous blood vessels to cold; (3) evaluate the role of TRPA1 functional and genetic variation in the pathogenesis of Raynaud’s disease in humans.

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The role of estrogen hormone dependence in the metastatic process

Natalie Michelle Fernandez

Program in Cell & Molecular Biology
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The antiestrogen tamoxifen (Tam) and aromatase inhibitors are the most frequently prescribed hormonal agents targeting the estrogen receptor (ER) in breast cancer patients worldwide. However despite improvements in the efficacy of targeted hormonal therapies the development of hormonal resistance remains a clinical problem and the major cause of recurrence and mortality in ERα-positive, early advanced stage disease. Complications of tumor metastases are the most common cause of death in breast cancer patients. It has proven difficult to identify key regulators of metastasis, and there are only a handful of genes which have been shown to be essential for metastatic behavior. In order to increase survival of breast cancer patients a better understanding of the key regulators and molecular mechanisms of the metastatic process will help to develop new treatment options for metastatic disease. A frequent somatic mutation at nucleotide 908 of ERα has been identified in premalignant and invasive breast cancers1. This mutation induces a lysine to arginine change in amino acid 303 in the hinge domain that confers the ability of enhanced cell growth under low levels of estradiol and it's resistant to aromatase inhibitors and tamoxifen (Tam)2,3. The mutation resides at a region that can be posttranslationally modified and is adjacent to the ER( S5305 phosphorylation site. Several studies have demonstrated that this phosphorylation can modify the response of breast tumors to Tam2. It has also been demonstrated that an interplay between the IGF-1R signaling pathway, the K303R ER( and S305 phosphorylation, and this interplay results in enhanced cell growth and resistance to aromatase inhibitors3. When analyzing tumor samples for the presence of the K303R mutation, the mutant and the wild type alleles of ER( are both present in the primary tumor, but the wild type allele can be lost in the metastatic site. This has led us to hypothesize that the K303R mutation may confer a distinct advantage for cancer cells and the metastatic process. Using two groups of 35 athymic nude mice, we injected MCF-7 cells carrying a YFP-tagged wild type ER( gene in one group, and a YFP-tagged K303R mutant ER( in the other. These mice were subjected to survival surgery when the primary tumor reached a size of ~600 mm3. The mice where then followed for signs of moribund behavior and sacrificed. The lungs, kidneys and lymph nodes where collected for analysis for the presence of metastatic lesions. Out of the 70 mice 34 % developed a primary tumor, and out of the mice that developed primary tumors 33 % of these developed metastatic lesions. When analyzed by student t test, the K303R mutation was not significant in the development of metastatic lesions when compared to the wild type receptor, but these studies need to be revalidated. nohistochemistry(IHC) studies are being conducted to verify these findings in both the primary tumor and metastatic site.

Contributors: Fernandez, Natalie Michelle; Corona, Arnoldo; Fuqua, Suzanne A.W.
Cytokinesis is the final step in cell division in which membrane furrows invaginate to partition the cell into two. Recently, membrane trafficking pathways have been recognized as essential for cytokinesis. Membrane addition occurs at the site of furrow invagination, but, more surprisingly, endocytosis has also been found in this region. The role of endocytosis, the coordination of endocytosis and exocytosis, how membrane trafficking controls furrow ingression dynamics, and the regulation of membrane trafficking during cytokinesis is still unclear. The central question I am interested in asking is how membranes move to drive furrow ingression during cytokinesis. I am using cellularization of the Drosophila melanogaster embryo as a model system to answer this question. Using imaging techniques including confocal time-lapse microscopy and electron microscopy, I am generating a quantitative description of membrane movement during furrow ingression in cellularization. This quantitative description is the basis for a mathematical model to test hypotheses for what drives furrow ingression in cellularization. Additionally, I am using the quantitative description as the basis for an RNAi screen to identify new membrane trafficking regulators of cellularization.

Contributors: Figard, Lauren; Sokac, Anna
SYNAPTIC PLASTICITY RESPONSES AT DIFFERENT CONVERGING PATHWAYS ON TO INDIVIDUAL NEURONS IN MOUSE VISUAL CORTEX

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We evaluated the induction of synaptic plasticity (long term potentiation or LTP and long term depression or LTD) in individual layer 2/3 pyramidal neurons in mouse primary visual cortex in response to simultaneous pairing of presynaptic activation of distinct sets of layer 4 afferents with direct postsynaptic activation. These experiments were carried out in order to understand whether the heterogeneous plasticity responses of individual layer 2/3 pyramidal neurons to an identical conditioning protocol are a property common to all synapses on a cell or are differentially expressed by distinct sets of synapses on the cell. We used acute brain slices of mouse primary visual cortex in conjunction with whole cell patch recording from visualized layer 2/3 pyramidal neurons, and stimulation of distinct sets of layer 4 afferents. The two stimulation sites were isolated by occlusion testing followed by alternative activation of each pathway to evoke a postsynaptic potential (PSP) every 10 seconds in an interleaved fashion. After a stable 10 minute baseline period, the activation of both pathways were simultaneously paired with direct postsynaptic activation that preceded the synaptic stimulation by 10 milliseconds resulting in 5-6 postsynaptic spikes at 0.1 Hz over a 10 min period followed by reversion to the interleaved stimulation protocol for an additional 20-30 minutes. The average peak amplitude of the evoked PSPs from activation of each convergent pathway compared between the pre-pairing control and post-pairing periods. Our preliminary results from 22 cells (with analysis of 44 pathways inputs – 2 onto each cell) validate in the mouse cortex our previous findings from other species demonstrating heterogeneous plasticity outcomes ranging from significant LTD to no change to significant LTP. Moreover, upon analysis of the plasticity outcomes of the separate pathways onto a common cell, the individual responses were not linked such that every set of plasticity outcomes were observed (shown is the percent change of the peak amplitude of the PSP 25 to 30 minutes after pairing from the 5 minute average of the PSP before pairing; statistical comparisons are made with a paired 2-tailed t-test)- both LTP (+40.1,+39.4; p<0.001,p<0.001); both no significant change(-1.4, +4.0; p=0.2, p=0.6); both LTD (-13.2, -47.1; p<0.001, p<0.001); LTP and LTD (+22.1, -26.9; p<0.001, p<0.001); LTP and no significant change (+24.8, -9.9; p<0.05, p=0.45); LTD and no significant change (-27.6, -22.4; p,0.001, p=0.115).

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O-GLCNAC REGULATES MITOSIS-SPECIFIC PHOSPHORYLATION ON HISTONE H3

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O-linked beta-N-acetylglucosamine, or O-GlcNAc, is a dynamic, monosaccharide glycan that cycles on and off serine and threonine residues of nucleocytoplasmic proteins. The processing enzymes responsible for the addition and removal of O-GlcNAc are O-GlcNAc transferase (OGT), and O-GlcNAcase (OGA), respectively. Many studies describe a reciprocal relationship between O-GlcNAc and phosphorylation on their target proteins, with both modifications capable of mutually inhibiting the other’s occupation on the same or nearby amino acid residue. There has also been an accumulation of evidence indicating the significant role of O-GlcNAc cycling in cell growth and cell cycle progression, although the precise mechanisms are still not well understood.

Here, we describe that histone H3, a chromatin-associated protein responsible for regulating DNA compaction and transcriptional activity, is modified with O-GlcNAc, and this modification regulates several mitosis-specific phosphorylations on H3. Several biochemical assays were used to show that H3 is modified with O-GlcNAc. In addition, mass spectrometry validated the occupancy of O-GlcNAc on H3, as well as identified threonine 32 as a novel O-GlcNAc site. O-GlcNAc also occupies H3 at higher levels during interphase than mitosis, inversely correlated with phosphorylation. Furthermore, O-GlcNAc was observed to inhibit mitosis-specific phosphorylations at serine 10, serine 28, and threonine 32 on histone H3. Lastly, inhibiting OGA activity prevents cells from properly transitioning between G2 exit to entering mitosis, showing a phenotype very similar to preventing mitosis-specific phosphorylation on H3.

Taken together, these data indicate that O-GlcNAc on H3 regulates mitosis-specific phosphorylations on H3, and this mechanistic switch is important for the G2-M transition of the cell cycle.

Contributors: Fong, Jerry; Nguyen, Brenda; Bridger, Robert; Medrano, Estela; Wells, Lance; Pan, Shujuan; Sifers, Richard.
DEFINING THE ROLES OF CELLULAR SENESCENCE IN OVARIAN TUMORIGENESIS

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Ovarian cancer is the most lethal gynecologic malignancy and the fifth most common cause of cancer death in women. While current therapies have improved, the five-year survival is still very poor with most women succumbing to death. Based on previous studies, defects in cellular senescence have been implicated as critical to understanding the pathogenesis of cancer. The ability of senescent cells to cease division is an important response to block the proliferation of cancer cells. Moreover, dysfunction in senescence pathways can lead to a decreased ability for cells to be removed and/or allow for aberrant proliferation. Our lab has been studying ovarian cancer in a mouse model lacking inhibin α (Inha). These Inha null mice exhibit dysregulation in important cell-cycle complexes and cellular senescence regulators, and develop fatal ovarian granulosa cell tumors. S-phase kinase protein 2 (SKP2), an E3-ubiquitin ligase, marks p27 and p21 for degradation. Degradation of these tumor suppressors allow for the up-regulation of CDK2 and the subsequent G1 to S-phase transition, generating proliferation and cell growth. Our microarray data showed that SKP2, N-MYC, L-MYC, CCNA2/E1/E2/CDK2, and CCND2/CDK4/6 mRNA is upregulated in Inha null granulosa cells prior to cancer formation. This information lends the hypothesis that cellular senescence pathways regulated specifically by SKP2, and proteins upstream (MYC family members) and downstream (cyclin/CDK complexes), control the development of granulosa cell cancers. The central hypotheses are that: 1) Cellular senescence in the ovary controls the balance of apoptosis, proliferation, and differentiation of ovarian cancer; and 2) Alterations in key components of senescence (i.e., SKP2, cyclinA2/E1/E2/CDK2) can influence ovarian tumorigenesis. To test these hypotheses, we plan to: (1) Elucidate the effects of cellular senescence on tumorigenesis and pathophysiology of gonadal tumors, and (2) Delineate the molecular mechanisms of cellular senescence in gonadal tumorigenesis. SKP2/Inha and CDK2/Inha double knockout (DKO) female mice will be created to study cellular senescence and cell-cycle regulation in granulosa cell proliferation and differentiation. Analysis of the SKP2/Inha and CDK2/Inha DKO female mice will allow us to observe the effects of modulating the senescence pathways associated with gonadal tumorigenesis.

By observing for known cell-cycle regulators and pathways involved in the senescence, we can gain a better understanding of the genetic dysregulation involved in ovarian carcinomas. Given the high mortality rate of patients, identification of the effects and roles of senescence and cell-cycle regulators on ovarian physiology and tumorigenesis is necessary.

Contributors: Fountain, Michael; Pangas, Stephanie; Nakayama, Kei-ichi; Kaldis, Philipp; Matzuk, Martin
Ronin is essential for embryonic stem cell self-renewal and acts independent of canonical pluripotency factors

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Embryonic stem (ES) cells have a remarkably fast cell cycle, which requires an increased biomass production. Our group has previously described a transcription factor, Ronin, which is expressed in embryonic stem cells and can promote embryonic stem cell self-renewal in an Oct4-independent manner. Moreover, Ronin was found to regulate different metabolic pathways, including protein biosynthesis and oxidative phosphorylation, differing from the canonical pluripotency factors, which tend to regulate development-related pathways. Here, we show that ablation of Ronin severely impairs ES cell self-renewal, while not affecting the expression of canonical pluripotency factors and germ layer marker genes.

To obtain Ronin ablation, we used an ERT2-Cre; RoninloxP/loxp mouse ES cell line, in which the Ronin gene is ablated upon treatment with tamoxifen for 4 days. After this treatment, cells were plated at a low-density and allowed to repopulate the cell culture to analyze the self-renewal capacity of Ronin KO cells. After 5 days, Ronin KO cell cultures contained around 25% of the cell number of control ES cell cultures. AP staining analysis revealed that Ronin KO cells form fewer colonies and tend to acquire a more differentiated morphology than control ES cells.

To assess Ronin effects on ES cell differentiation, we analyzed the expression of pluripotency factors Oct4 and Nanog, and germ layer marker Afp, Sox11, Nestin and Brachyury by RT-PCR, western blot and flow cytometry. Upon ablation of Ronin, the expression of pluripotency and germ layer marker genes did not significantly change, indicating that Ronin ablation does not cause differentiation. Moreover, Ronin KO cells were able to undergo retinoic acid-induced differentiation, although self-renewal was severely impaired. These results indicate that Ronin is essential for ES cell self-renewal and its ablation does not affect the expression of pluripotency factors and germ layer marker genes. These findings corroborate our previous studies that show an independent role for Ronin in the regulation of pluripotency.

Contributors: Freire, Pablo; Dejosez, Marion; Zwaka, Thomas
Similar to other sensory modalities, the visual system has evolved to process vital information in the organism’s natural environment, and thus it is believed to have adapted to its statistical properties. In contrast to primates, where we know a lot about the computational goals and important visual features for perception, including motion and faces, in mice we know little about which visual features different cortical areas represent.

The goal of this study is to identify the relevant features that the primary visual cortex of the mouse encodes. We have used miniature cameras mounted on the head of mice and recorded movies of their natural visual experiences. We have begun experiments where we are recording the activity of neurons from the primary visual cortex of both anesthetized and awake mice while they view these movies using two-photon imaging. Initially, we will identify the features that strongly modulate neuronal activity in their classical and extraclassical receptive fields. Manipulating the statistics of natural stimuli, and studying the effect on the activity of both single neurons and neural populations will allow us to build mechanistic models of how the mouse primary visual cortex processes information.
INVESTIGATION OF AMINO ACID VARIATION IN ACTIVITY AND EVOLUTION OF CTX-M FAMILY OF β-LACTAMASES.

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Antibiotics such as penicillins, and cephalosporins are the most commonly used anti-microbial agents in the world. They inhibit penicillin binding proteins (PBPs) which are necessary for microbial cell wall synthesis. The most common mechanism of antibiotic resistance to β-lactam antibiotics is mediated through production of beta lactamases. Beta lactamases are grouped into four classes A, B, C and D based on amino acid similarity. Class B are metallo-β-lactamases that are able to hydrolyze the beta lactam ring by using zinc coordinated water, and are structurally and mechanistically different than the other three classes. Class A, C and D beta lactamases utilize a catalytic serine as the nucleophile to break down beta lactams.

Newly emerging CTX-M enzymes represent a Extended Spectrum Beta Lactamases (ESBLs), which are a global threat to antibiotic therapy worldwide. CTX-M-type enzymes constitute a distinct lineage of molecular class A β-lactamases. They are easily transferred by plasmids, and have spread rapidly over the past decade. There are over 80 different types of CTX-M already characterized. They are divided into five distinct subfamilies based on primary amino acid sequence homology. Newly emerging CTX-M ESLBs are spreading rapidly among Enterobacteriaceae and other Gram-negative pathogens, and are capable of hydrolyzing broad-spectrum cephalosporins.

CTX-M enzymes are divided into five subfamilies based on primary sequence homology. Amino acid alignments were performed, and a microevolution is observed in each subfamily. Certain amino acid positions exhibit variation in several distinct subfamilies, and are hypothesized to be responsible for functional properties of the enzyme, such as stability/folding, and substrate specificity. In principle, this variation is a result of natural selection for altered properties of the enzyme. Substitutions at those amino acid positions will has been made in the model enzyme (CTX-M14). Following mutants will be then characterized on the basis of stability, substrate specificity, and their catalytic efficiency.

Contributors: Fryszczyn, Bartlomiej; Palzkill, Timothy
Autophagy is one of the two major catabolic pathways in a cell. Autophagy is characterized by the formation of a double membrane structure called autophagosome that sequesters intracellular cargo and delivers it to the lysosomes for proteolytic degradation. In times of cellular stress such as during nutrient deprivation, autophagy acts as a cell survival mechanism by recycling and re-allocating important amino acids. Autophagy is the only known biological process in which entire defective organelles within a cell can be degraded in addition to damaged proteins. Such a quality control mechanism has been proposed to be important in long-lived post-mitotic neurons and defects in autophagy have been implicated in several neurodegenerative diseases (Wong and Cuervo 2010).

We are interested in identifying novel genes involved in autophagy that affect neuronal function. We have generated a collection of X chromosome mutants looking for essential genes in Drosophila involved in neurodegeneration and synaptic transmission using electroretinogram as an initial read out. We re-screened this mutant collection for defects in starvation-induced autophagy and identified ten alleles of cacophony (cac) which encodes the pore-forming α1 subunit of voltage gated calcium channel (VGCC). The transmission electron microscopy (TEM) sections of mutant clones in the fly eyes of different alleles of cac and of its accessory subunit gene, straightjacket (stj) show age-dependent accumulation of autophagic vacuoles and abnormal looking mitochondria in the photoreceptor terminals, often observed in mutants of genes required in autophagy maturation (Rusten, Vaccari et al. 2007). We also saw an accumulation of poly-ubiquitinated proteins in laminae of cac flies, a phenomenon frequently observed in organisms defective in autophagy (Komatsu, Waguri et al. 2005; Juhasz, Erdi et al. 2007). Cac, its accessory subunits, the synaptic vesicle protein Cysteine String Protein (CSP) and exocytic SNARE complex are involved in neurotransmitter release through synaptic vesicle fusion by a calcium dependent mechanism and mutations in this highly conserved gene in mammals causes severe neurological diseases such as the polyQ disorder Spinocerebellar ataxia 6 (SCA6), episodic ataxia type 2 (EA2) and migraines (Imbrici, Jaffe et al. 2004; Rajakulendran, Schorge et al. 2010). Although calcium and certain SNAREs have been shown to be required for autophagy, a voltage-gated calcium channel has not previously been implicated in autophagy. It remains to be established whether Cac plays a role in autophagy by regulating the fusion of autophagosomes with lysosomes.
Single particle tomography (SPT) is an alternative to untitled single particle analysis (SPA), to produce 3D reconstructions of macromolecules. While traditional (SPA) can produce high-resolution maps from structurally homogeneous specimens, difficulties arise with structurally variable molecules. In some cases it is impossible for the computer to distinguish between changes in conformation and changes in orientation. The ambiguity is resolved by obtaining 3D information for each particle, allowing for the alignment of sub-volumes to produce structurally distinct low-resolution averages. This technique can also be used when the specimen is crowded, producing particle overlap in 2D projections, but where the particles can be separated in 3D. SPT thus offers the potential for ex-vivo reconstruction of molecules from cryoEM images of cells. We have implemented a graphical tool for 3D particle picking in EMAN2 and a full refinement pipeline for SPT, including sub-volume alignment, classification and averaging. Based on EMAN2’s modular approach, a variety of algorithms can be used at each stage of this process. CUDA acceleration is also implemented and can speedup alignments between 10 to 100x. Full distributed and threaded parallelism is also supported. Our method, which properly accounts for the effects of the missing wedge, was used for a 25Å resolution reconstruction of epsilon15 virus with icosahedral averaging. We also recently demonstrated a 26Å asymmetric average of TRiC. While the resolution provided by this technology is currently limited by the lack of robust CTF correction in cryoET, its unique capabilities make it a promising technique for continued development.

Contributors: Galaz, Jesus; Flanagan, John; Schmid, Michael; Frydman, Judith; Ludtke, Steven.
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G-protein coupled receptors (GPCRs) are encoded by more than 800 genes of the human genome. These receptors account for approximately 40% of current drug targets and are the most abundant family of transmembrane signaling proteins. GPCRs are involved in many cell signaling processes, among which is the regulation of intracellular calcium levels. Changes in the concentration and distribution of calcium ions play an important role in regulating all aspects of cell function.

Monitoring of intracellular calcium levels in real time using a fluorescent indicator dye revealed that an endogenous receptor in HEK-293 cells responds to the adrenergic agonist norepinephrine, by a delayed rise in intracellular [Ca2+]. Pharmacological analysis revealed that the response is blocked by ICI 118,551, a selective antagonist for beta-2 adrenergic receptor (β2-AR), and the relative potency of agonists, isoproterenol > epinephrine > norepinephrine, is consistent with β2-AR being the receptor responsible.

Treatment with thapsigargin, an inhibitor of the SERCA Ca2+ pump of the endoplasmic reticulum, or with extracellular EGTA, revealed that the source of the Ca2+ is release from intracellular stores, not entry from outside the cell. PKA involvement was assayed by subjecting cells to H-89 and KT5720 which are known PKA inhibitors. The Ca2+ signal was not significantly suppressed when adding either of these drugs, suggesting that PKA is not involved in this signaling mechanism.

These results reveal a novel pathway by which β2-AR, classically thought to signal primarily through cAMP production, can trigger release of Ca2+ from intracellular stores. Experiments in progress are directed at determining the molecular mechanisms by which this response occurs, and the biophysical properties of the underlying protein-protein, and small-molecule-protein interactions that mediate it.

Contributors: Galaz-Montoya, Monica; Rodriguez, Gustavo; Lichtarge, Olivier and Wensel, Theodore
Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a late-onset neurodegenerative disorder primarily affecting male premutation allele carriers of Fragile X Mental Retardation 1 (FMR1) gene. The current molecular model for the disease suggests the clinical features of FXTAS result from elevated expression of the expanded CGG repeat in the premutation FMR1 mRNA. Since there are rarely alterations in the level of the FMR1 gene product, FMRP, it is likely that the expanded CGG repeat in this RNA is toxic. Indeed, models in both fly and mouse have demonstrated that expanded CGG repeats in heterologous mRNAs are sufficient to confer degeneration of neurons with similar features. This observation has led to the hypothesis that aberrant interactions between the expanded CGG sequences and RNA-binding proteins may be responsible for the pathology in FXTAS, possibly manifested in changes in regulation of RNA splicing, transport or translation. We have previously described a Purkinje neuron specific transgenic mouse model of FXTAS that exhibits many human FXTAS clinical features including the formation of ubiquitin positive intranuclear neuronal inclusions and death of Purkinje neurons along with motor coordination and motor learning deficits. Our current efforts focus on understanding changes in translational profiles of diseased Purkinje neurons in our mouse model of FXTAS. Using the Purkinje neuron specific model, developed by the Heintz group at Rockefeller University, that expresses an EGFP-tagged ribosomal protein L10a, and the translating ribosome affinity purification (TRAP) methodology to affinity purify polysomal mRNAs, we have performed comparative analysis of gene translational profiles of various age groups of our FXTAS model using microarray technology. Our results suggest that translational profiles are altered as early as 4 weeks of age by the presence of expanded CGG repeat RNA. More than 400 mRNAs are significantly elevated or decreased in abundance on polyribosomes. The profiles reflect changes in genes involved in alternative splicing, mRNA transport, miRNA metabolism, translational regulation and programmed cell death. Preliminary data indicated that one candidate, Adcy7, is elevated nearly 16 fold over wildtype by four weeks. Work is in progress to validate additional genes from our microarray study of 8 and 12 week models. This study will identify many new candidate genes involved in the progression of FXTAS related pathology.
IDENTIFICATION OF GENES INVOLVED IN ANTI-INFLAMMATORY EFFECTS OF PROBIOTIC LACTOBACILLUS REUTERI IN VIVO

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Background: Probiotic Lactobacillus reuteri is a gram-positive bacterium that naturally inhabits the gut of mammals, birds and humans. Supplementation with L. reuteri strains has been found to be effective at ameliorating intestinal inflammation in patients with inflammatory bowel disease (IBD) and in rodent colitis models. Previous studies in our lab found that the low molecular weight (LMW) factors (<3kD fraction) secreted by L. reuteri ATCC PTA 6475 is able to suppress TNF production from activated monocytes. Meanwhile, thfs1 or LR0977 mutant strains of L. reuteri 6475 had diminished ability to inhibit TNF which could be partially recovered by complementation, indicating these genes may be involved in the anti-inflammatory effects of L. reuteri 6475.

Aims: To determine whether the thfs1 and LR0977 genes are responsible for the protective effects of L. reuteri 6475 in vivo.

Methods: Each 8-week old female BALB/c mouse was treated with two intraperitoneal (IP) injections of concentrated LMW factors from wild type L. reuteri 6475 or thfs1 or LR0977 mutants. Colitis was assessed two days after colorectal administration of TNBS (100 mg/kg body weight). Colonic inflammation and damage were graded according to the Wallace criteria. Serum amyloid A (SAA) protein quantities in plasma were measured by ELISA.

Results: TNBS-injected mice developed moderate colitis characterized by ulceration with inflammation at two or more sites with intestinal injuries extending greater than 1 cm. Macroscopic colitis scores after TNBS instillation were significantly reduced (P<0.001 vs media controls) in mice treated with LMW factors from wild type L. reuteri 6475 (0.5 vs 4.5 median score). This effect was diminished in mice treated with LMW factors from thfs1 (3.5 median score) or LR0977 (4.0 median score) mutants. Weight loss was significantly reduced in mice treated with LMW factors from wild type L. reuteri 6475 (7.8% vs 12.4%), but not in mice treated with LMW factors from thfs1 (13.9%) or LR0977 (10.6%) mutants. SAA proteins in serum were significantly reduced (P<0.001 vs media controls) in mice receiving LMW factors from wild type L. reuteri 6475 (2225 vs 6075 µg/ml serum), but not in mice receiving LMW factors from thfs1 or LR0977 mutant (7253, 6726 µg/ml serum, respectively). Also, as expected, the complemented L. reuteri strains of thfs1 or LR0977 mutants partially restored the anti-inflammatory activity of the wild type strain.

Conclusion: Using the TNBS induced mouse model of colitis, our study showed that secreted LMW factors from L. reuteri 6475 could ameliorate experimental colitis and the thfs1 and LR0977 genes contribute to the colonic anti-inflammatory properties of L. reuteri 6475 in vivo.

Contributors: Gao, Chunxu; Thomas, Carissa; Hemarajata, Peera; Spinler, Jennifer; Versalovic, James
A COMPARISON OF METHODS TO DETECT COMPLEX TRAIT RARE VARIANT ASSOCIATIONS IMPLEMENTING THE RAREPOWER TOOL

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There is currently great interest in detecting rare variant associations using next generation sequence data. A large number of association methods which aggregate variants across a region e.g. a gene have been developed specifically to analyze rare variant data. It is not clear which existing method is the most powerful and should be applied to test for associations using exome/genome sequence data. To compare rare variant association methods both realistic phenotype models and spectrum of variants across a region must be generated. The power was compared for 15 methods to detect associations for qualitative and quantitative traits. Power was evaluated for case-control, extreme quantitative trait sampling and population based study designs. For each method, power was determined for scenarios which included 1. analysis of a. only rare variants & b. rare (<1%) and low (1-5%) frequency variants; 2. detrimental and protective variants within a gene region; 3. misclassification a. exclusion of causal variants & b. inclusion of non-causal variants; 4. different underlying population demographic model for both Africans and Europeans and 5. gene size. It was observed that there is not a single method that is most powerful in all situations. The majority of rare variants methods had only small incremental difference in power. Rare variant association methods which were powerful in a variety of situations include the Variable Threshold method, Weighted Sum Statistic and Kernel Based Adaptive Cluster method. Those methods which were developed to detect associations when both protective and detrimental variants are within an associated region (e.g. C-alpha) are usually less powerful than more general rare variant association methods. The evaluated methods vary in their computation efficiency and ability to control for confounders. Additionally, we proposed optimizations on some of these methods, which proved to have boosted their performances. The RarePower tool with its user friendly graphical interface can be used to determine sample sizes which are necessary to detect rare variant associations under a large variety of complex trait and population demographic models.

Contributors: Wang, Gao; Leal, Suzanne M.
Proper brain function emerges from precise patterns of neuronal connectivity. Due to the complexity of nervous tissue, cracking the code of brain wiring has remained a significant challenge to both clinical and basic neuroscience. Understanding the fundamental mechanisms of synaptic wiring lies at the heart of elucidating this code. Recently, we have entered an experimental era that affords unprecedented opportunity to begin to dissect complex neuronal circuits with genetic, cellular, and molecular precision. With the expansion of molecular genetics, and its convergence with cell biology and electrophysiology, we are now poised to begin to uncover key mechanisms that govern brain wiring. Transsynaptic circuit tracing using genetically modified Rabies virus (RV) has recently emerged as a new technology for identifying patterns of synaptic connectivity. RV is a neurotropic virus that propagates via retrograde transsynaptic infection and can be engineered to express fluorescent markers. Complementing this technology, it has become routine to investigate the basic molecular and cellular properties of neuronal lineages derived from embryonic stem cell cultures in vitro. Here, we report the generation of a novel embryonic stem (ES) cell line that harbors the genetic elements to perform RV-mediated transsynaptic circuit tracing in mouse ES cell-derived neurons and their synaptic networks. To facilitate transsynaptic tracing, we have genetically targeted a tdTomato fluorescent reporter, the Rabies-G coat protein, and the avian TVA receptor into the ROSA26 locus. We demonstrate high-efficiency differentiation of our stem cells into functional neurons both in vitro and in vivo, show their capacity to ‘wire up’ with primary neuronal cultures as evidenced by immunohistochemistry and electrophysiological recordings, and show their ability to act as source cells for presynaptic tracing of neuronal networks using genetically modified RV. Together, our data highlight the potential for using genetically engineered stem cells to investigate fundamental aspects of brain wiring. Considering the widespread use of ES cells to generate mouse models of brain development and dysfunction, the broad array of available alleles expressed in the nervous system, and advancements in induced pluripotent stem (iPS) cell technology, in vitro experimentation that directly transfers from the culture dish to in vivo application is becoming feasible. Our main objective is to understand the basic molecular and cellular mechanisms that underlie synaptogenesis, circuit formation, and neuronal survival in the adult brain. Gaining further mechanistic knowledge in this area may provide new insight towards developing cell-based therapies to repair or replace damaged and diseased nervous tissue.

Contributors: Garcia, Isabella; Huang, Longwen; Ung, Kevin; Arenkiel, Benjamin
The initial vascular network of the mouse yolk sac, formed by embryonic day 8.0 (E8.0), is a primitive capillary plexus of vessels of uniform shape and size. At the initiation of blood flow (E8.5), this plexus undergoes vascular remodeling, in which endothelial cells establish a branched, hierarchical structure of large vessels and smaller capillary beds, evident by E9.5. Embryonic lethality due to failed remodeling is a common phenotype observed in over 80 single gene mutations, and may be due to either a primary defect in endothelial cell function or reductions in hemodynamic force. However, in a large number of yolk sac mutants, the direct causes of the observed vascular remodeling defects are still unknown.

FoxO1 germline null (−/−) mice have been reported to die by E11.0 due to abnormal vascular remodeling; however, the primary cause of the remodeling defect remains unclear. In this study, we conditionally delete FoxO1 in endothelial cells (ECs) and show that its function during embryonic development is critical for the formation of a remodeled yolk sac vasculature. Using live, rapid-time lapse imaging, we verify that the remodeling defects are not due to altered hemodynamic force. We observe that FoxO1 deletion in ECs in vivo leads to the downregulation of angiogenic genes, including connexins 37 and 40, which causes an increase in vascular permeability within the embryonic yolk sac. We observe a reduction in the density of ECs within the mouse yolk sac in both pre-remodeling and post-remodeling stages. Finally, combining time lapse confocal microscopy with embryo culture, we observe impaired vascular remodeling in FoxO1 conditional knockout mutants in vivo. These data suggest that FoxO1 expression in ECs is critical for the formation of a capillary plexus before the onset of hemodynamic force, resulting in impaired yolk sac remodeling and embryonic lethality.

Contributors: Sills, Tiffany M.; Udan, Ryan S.; Vadakkan, Tegy J.; DePinho, Ronald A.; Hirschi, Karen K.; Dickinson, Mary E.
PLATELET SUPERVILLIN INHIBITS THROMBUS FORMATION UNDER SHEAR STRESS IN HUMANS AND MICE

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High shear accentuates platelet adhesion to collagen during arterial thrombosis. We report here that genetic variants in SVIL, encoding nonmuscle supervillin (isoform 1) and muscle isoforms 2 and 3 (archvillin, SmAV), are associated with variations in platelet reactivity during shear-induced thrombus formation. Genome-wide genotyping and analyses of closure times in the Platelet Function Analyzer (PFA)-100, an assay that is both collagen- and shear-dependent, identified significant associations (p<5x10^-8) with 2 SNPs within the SVIL gene (chr 10p11.23) in healthy African-Americans. Expression analyses of platelet RNA and immunoblotting of platelet lysates confirmed the presence of SVIL isoform 1 and the absence of isoforms 2 and 3. SVIL mRNA levels in human platelets were inversely correlated with PFA-100 closure times and platelet volume. Mice lacking Svil isoform 1 were generated and back-crossed. Compared to controls, murine platelets lacking supervillin exhibited significantly larger volumes and enhanced platelet thrombus formation under high shear, but not low shear, conditions. We have shown for the first time that 1) platelets contain supervillin, 2) genetic variants in SVIL are associated with variant PFA-100 responses, and 3) murine supervillin regulates platelet thrombus formation at high shear. These data are consistent with a negative regulation by supervillin of integrin-based adhesions.

Contributors: Edelstein, Leonard C; Luna, Elizabeth J; Gibson, Ian B; Bray, Molly; Jin, Ying; Kondkar, Altaf; Nagalla, Srikanth; Hadjout-Rabi, Nacima; Smith, Tara C; Covarrubias, Daniel; Jones, Stephen N; Ahmad, Firdos; Kong, Xianguo; Fang, Zhiyou; Bergmeier, Wolfgang; Shaw, Chad; Leal, Suzanne M; Bray, Paul F.
The increase of antibiotic resistance has led to the search of new antimicrobial methods to treat bacterial infection. Here, we propose to engineer a bacterium with anti-microbial activity using deadly toxins delivered by conjugation. It has been suggested that one potential alternative is to use bacterial toxin-antitoxin (TA) systems. TA systems encode stable toxins and unstable anti-toxins, which inhibit their respective toxins. The toxin-antitoxin genes modules are always co-expressed to alleviate toxicity. The toxin is detrimental to the bacterium and will kill or inhibit cell growth when the antitoxin is depleted. To deliver the toxins into the pathogen, we will use conjugative plasmids allowing promiscuous and efficient genes transfer between different bacterial species. Our strategy consists of uncoupling the toxin/anti-toxin gene expression during conjugation by placing the toxin gene on the transfer plasmid and the anti-toxin gene on the bacterial host chromosome. Upon transfer of the plasmid, only the toxins will be expressed, killing the recipient bacterium. Previous research on TA systems has focused on the inhibitory effect of single toxins and the effect of targeting multiple cellular pathways with multiple toxins has not been investigated. Our work aims to investigate whether conjugative transfer of a plasmid encoding multiple toxins will result in the killing of recipient bacteria and how this toxic effect can be specific to pathogenic bacteria.
WHOLE-GENOME SEQUENCING TO IDENTIFY DISEASE CAUSING MUTATIONS IN HEREDITARY SENSORY AND AUTONOMIC NEUROPATHY

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Next-generation sequencing technologies and targeted capture have enabled high-throughput sequencing of exomes to discover pathogenic variants and identify new genes involved in mendelian diseases. However, exome sequencing is based in capture and enrichment of only the ~2% coding fraction of the genome to identify simple nucleotide variants (SNVs). Whole-genome sequencing allows the unbiased detection of all different types of variation in a given individual, from SNVs to copy-number variants (CNVs) that may be responsible or contribute to a disease phenotype.

Hereditary and sensory autonomic neuropathies (HSAN) affect primarily the peripheral nerves and are characterized by progressive loss of sensitivity in the extremities; the different subtypes vary in the degree of autonomic involvement. Several genes have been identified to cause the different subtypes of HSAN but the genetic cause of the disease in some individuals remains unknown.

We have used massively parallel sequencing by oligo ligation/detection technology, SOLiD, to perform whole-genome sequencing at ~30x average depth of coverage of a female patient affected by hereditary sensory and autonomic neuropathy (HSAN) who tested negative for mutations in known HSAN genes and other neuropathy associated genes. Comparison of the genomic sequence obtained to the human genome reference assembly identified 3.2 million single nucleotide polymorphisms (SNPs); 19,151 of these occur in coding regions of which 9,814 cause non-synonymous substitutions. Filtering of these variants for high quality calls and common variants present in dbSNP and observed by the 1000 Genomes Project, in order to look for novel variants, reduced the list of potential candidate variants to 232 nonsynonymous changes.

Considering that the patient is the daughter of a first cousin marriage, we undertook a recessive model of inheritance for her disease, focusing on homozygous variants, narrowing our list of candidate genes to nine.

Whole-genome sequencing can be applied to identify the genes and mutations responsible for mendelian phenotypes for which the molecular basis remains unknown, even if they are genetically heterogeneous. This technology will soon be performed routinely to achieve accurate genetic diagnosis and aid in clinical practice.

Contributors: Gonzaga-Jauregui, Claudia; Wiszniewski, Wojciech; Kurth, Ingo; Reid, Jeffrey; Gibbs, Richard A.; Lupski, James R.
CONJUGATIVE PROTEIN TRAR INDUCES STRESS RESPONSE PATHWAYS INDEPENDENTLY OF STRESSORS.

Elicia D Grace

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With the increased prevalence of antibiotic resistance in bacterial pathogens, a better understanding of how bacteria modulate horizontal DNA transfer under changing environments and what genetic factors are involved is needed. We have characterized the ability of TraR, present on both non-domesticated and lab-adapted conjugative plasmids, to upregulate general stress response pathways independently of stressors and provide pathways for these actions.

The non-domesticated pR1 and lab-adapted F plasmids were examined in Escherichia coli. Fluorescent microscopy traR::GFP fusions were used to determine natural expression patterns. Gene and protein expression of stress-related genes were examined utilizing beta-galactosidase assays and Western blots utilizing an inducible traR expression system. Cells containing F’ and F’(traR plasmids were examined for stress tolerance and biofilm formation in culture. Analysis of traR::GFP fluorescence demonstrated that traR is expressed heterogeneously in culture. In terms of the stress response, TraR is able to increase expression of (S via upregulation of the anti-anti-( factor, IraP, and the small non-protein-coding RNA, DsrA. Conversely, in the case of (E, TraR causes increased activity, but not increased expression of (E. Additionally, TraR increases biofilm production and fitness during temperature and ethanol stress. TraR’s non-uniform expression may be related to the conjugative efficiency of a population. This protein is able to strongly upregulate two major stress response pathways, independently of stressors, potentially increasing fitness of F’-containing cells. These activities are proposed to assist the F plasmid in responding to the stress resulting from formation and maintenance of the F pilus for bacterial mating. Increasing the survival during horizontal transfer not only may lead the spread of virulence factors and antibiotic resistance, but could play a key role in bacterial evolution.

Contributors: Grace, Elicia D.; Blankschien, Matthew D.; Herman, Christophe
Telomerase is an enzyme complex principally composed of a reverse transcriptase, TERT, and an RNA template, TERC. Telomerase maintains telomeric DNA that protects chromosome ends. Defects in telomerase impair DNA replication efficiency, leading to early cell senescence. Germline telomerase mutations characterize a spectrum of telomere biology disorders including pulmonary fibrosis, liver disease, aplastic anemia, myelodysplasia, and dyskeratosis congenita, a diagnosis that confers a 200-fold risk for AML and a 90% lifetime risk for bone marrow failure. These disorders have a variable and often subtle clinical phenotype, but pronounced telomere shortening is universal. Though germline telomerase variants occur in 2-3% of the general population, enrichment of specific variants occurs in adults with hematologic malignancies, including 6.5% of a large adult AML cohort, suggesting cancer susceptibility may be associated with variation in telomerase related genes. We are exploring the role of telomerase in AML development and treatment-related toxicities. We hypothesize that AML populations are enriched for telomere biology disorders, and exposure to chemotherapy or transplant, resulting in further telomere shortening, will accelerate disease phenotype manifestation.

We have performed an exploratory analysis to determine if (1) the frequency of telomerase variants observed in a pediatric AML population approaches the frequency reported in adults and (2) presence of these variants is associated with a telomere biology disorder phenotype. We are sequencing the exons and flanking intronic regions of TERT in a local pediatric AML cohort (n=100). Analysis of the first 57 subjects demonstrates germline heterozygous variants resulting in missense changes in 10.5%. Of the four missense changes found, 1 was novel and is being characterized by telomerase activity and processivity, and 3 are associated with reduced telomerase activity in vitro. None of these variants were found within 57 sex and race-matched controls. These cases were also reviewed for evidence characteristic of a telomere biology disorder. Amongst individuals with variants, additional diagnoses included bronchiolitis obliterans and myelodysplastic syndrome, and three died shortly after transplant. These findings suggest that, as in adults, presence of TERT hypomorphic variants may be a susceptibility locus in pediatric AML. AML falls within the spectrum of telomere biology disorders; however, children newly diagnosed with AML are not routinely screened for these disorders, despite the potential risk with these disorders for severe chemotherapy-related toxicities and morbidity with transplant. Our results suggest that further investigation of the disease phenotypes associated with these variants is warranted.
The Muscleblind-Like (MBNL) family of RNA binding proteins has three human paralogues (MBNL1, 2, 3), all of which have been shown to regulate alternative splicing. One well-characterized target is exon 11 of the Insulin Receptor (IR) gene which is activated by MBNL proteins.

To determine the mechanism by which MBNL family members activate splicing of IR exon 11, we first mapped the MBNL response element in the IR pre-mRNA. Using an in vitro gel shift assay, we showed that MBNL1 directly interacts with a 30 nt-long pre-RNA segment containing 3 MBNL binding motifs located 90-120 nt downstream of exon 11. Using transient co-expression of MBNL with IR minigenes, we showed that the binding sites serve as a response element since splicing activation was lost when the binding sequences were mutated. MBNL proteins contain 2 pairs of CCCH zinc-finger domains separated by approximately 100 residues. To identify the domains of MBNL1 required for splicing activation, we tested consecutive 40 amino acid deletion mutants from the N- or C-terminus and co-expressed the proteins with IR minigenes. A parallel analysis on deletion mutants was done on MBNL3. Both paralogues showed that the splicing activity domains are distinct from the RNA binding regions and that both MBNL1 and MBNL3 contain one activation domain between the two pairs of zinc-fingers and a second closer to the C-terminus.

The splicing activity domains may play a role in protein-protein interactions, so we are currently using assays to identify proteins that directly interact with MBNL1. Pulldowns with at least 4 different MBNL1 antibodies showed that MBNL1 does not form a stable complex with other proteins in HeLa whole cell extracts. We hypothesize that interactions between MBNL1 and proteins relevant to splicing regulation occur in the RNA context. An IR substrate is being used to isolate complexes associated with MBNL1 splicing activation. The substrate was shown to assemble spliceosomal complex A in vitro. Importantly, addition of recombinant MBNL1 enhances complex A assembly. Proteins will be identified by mass spectrometry and candidates will be tested using knockdown and overexpression studies in vivo.

Contributors: Grammatikakis, Ioannis; Goo, Young-Hwa; Echeverria, Gloria; Cooper, Thomas
Alzheimer’s Disease (AD) is the most common cause of dementia in the elderly. There are three pathological hallmarks of AD: 1) plaques formed by Aβ peptide deposition; 2) neurofibrillary tangles (NFT) caused by aggregation of hyperphosphorylated tau protein; 3) loss of neurons and synapses. AD patients not only suffer from impaired learning and memory ability, but also mood disorders, like anxiety and depression, and behavioral changes.

Aβ is one of the proteolytic cleavage products of Amyloid Precursor Protein (APP). PS1 and PS2 are components of γ-secretase which mediates APP cleavage and Aβ production. Genetic mutations in APP, PS1 or PS2 affect APP cleavage, leading to early onset of Familial Alzheimer’s Disease (FAD). While mouse tau does not develop NFT pathology, transgenic mice expressing genomic wild-type human tau (htau-tg) in the absence of mouse tau (mtau -/-) was shown to display AD-like tau pathology.

To better mimic human AD in mice and to avoid potential complications from transgene overexpression, we have generated three knock-in (KI) mouse models based on previously established KI alleles, including APPsl/Aβ/sl/Aβ single knock-in (SKI) which expresses the humanized Aβ sequence and carries the Swedish and London mutations, APPsl/Aβ/sl/Aβ; PS1M146V/M146V double knock-in (DKI), the latter contains the PS1 M146V FAD mutation, and APPsl/Aβ/sl/Aβ; PS1M146V/M146V; htau-tg; mtau -/- pseudo triple knock-in (pTKI). The SKI line serves as a pre-plaque model, DKI line serves as a plaque model, and pTKI line serves as a model to study both plaques and tangles. Behavioral tests revealed that all three lines exhibit increased anxiety levels at an early age (3–4 months) on elevated plus maze test. DKI and pTKI animals also showed increased freezing frequency in conditioned fear test. The Corticotropin Releasing Factor (CRF) levels and resting glucocorticoid levels in DKI animals were found to be higher than controls, suggesting that enhanced Hypothalamic-Pituitary-Adrenal (HPA) axis activity may contribute to the increased anxiety and freezing. We are currently investigating the possible changes in cognition, synaptic plasticity and evidence of neuronal and/or synaptic loss and correlate these changes as a function of age and neuropathology.
The p38 MAPK pathway is activated in response to stress and inflammatory stimuli and functions to modulate cytokine expression through post-transcriptional regulation. We have new evidence suggesting that this pathway also plays a role in aging in Drosophila melanogaster. We generated MAPK-Ak2, a viable deletion that removes Drosophila mitogen activated protein kinase-activated kinase 2 (MAPK-Ak2), a direct phosphorylation target of p38 MAPK. This deletion results in extended lifespan compared to wild type controls in two independent experiments. This phenotype was confirmed in two separate genetic backgrounds in both males and females. In order to test whether MAPK-Ak2 LOF is responsible for these phenotypes, we generated MAPK-Ak2 GR and MAPK-Ak2 KO transgenics. MAPK-Ak2; MAPK-Ak2 KO flies are long-lived compared to controls and their survival curve is nearly identical to MAPK-Ak2 flies in males and females in two independent backgrounds. These data suggest that MAPK-Ak2 LOF is the cause of the longevity phenotype. Longevity assays are in progress to test whether lifespan is returned to normal in MAPK-Ak2; MAPK-Ak2 GR flies. MAPK-Ak2 is homologous to mouse MK2. The MK2 /- mouse is susceptible to bacterial infection. Therefore, we are investigating whether MAPK-Ak2 mutants have immunity phenotypes. We have found that MAPK-Ak2 flies have increased expression of AMPs. We are currently testing whether mutants are also vulnerable to bacterial infection. Finally, we will conduct RNA-seq on MAPK-Ak2 mutants and controls to assay global changes in gene expression.
Mitochondria provide the majority of the energy needed for cellular functions. Therefore, mitochondrial diseases most severely affect tissues with high metabolic demand, such as muscles and neurons. Leigh Syndrome is one of the most devastating infantile mitochondrial diseases and is characterized by developmental delay, progressive neurodegeneration and muscle hypotonia. One of the genes mutated in Leigh Syndrome is Leucine-Rich Pentatricopeptide Repeat-Containing Protein (LRPPRC). LRPPRC encodes a mitochondrial protein that possesses approximately 20 RNA-binding PPR repeats and has been predicted to be involved in the posttranscriptional regulation of mitochondria-encoded RNA. Mutant human LRPPRC protein fails to be appropriately localized to the mitochondria, leading to altered RNA metabolism and mitochondrial function, exemplified by a deficiency of Complex IV of the mitochondrial respiratory chain. However, the underlying mechanism by which disruption of LRPPRC leads to mitochondrial dysfunction and disease is unknown. To elucidate the functions of PPR proteins in neuronal development, metabolism and maintenance, we are studying the Drosophila homologues of LRPPRC, namely CG14786 (dLRPPRC) and Bicoid Stability Factor (BSF). dLRPPRC has not been studied in Drosophila, while BSF is required for bicoid mRNA stability in the oocyte. We have identified multiple loss-of-function mutations in dLRPPRC from a forward genetic screen for X Chromosome genes that control neuronal maintenance and function. In parallel, we utilize a P-element insertion that disrupts the BSF open reading frame. Similar to LRPPRC, both fly homologues primarily localize to the mitochondria and disruption of either one results in prolonged larval development and pupal death. dLRPPRC mutants have aberrant mitochondrial morphology and decreased Complex IV enzymatic activity. Intriguingly, BSF is upregulated in dLRPPRC mutants, suggesting an interplay between the genes/proteins, perhaps to regulate RNA metabolism. In addition, the larval Neuromuscular Junctions (NMJ) in both BSF and dLRPPRC mutants are overgrown. We hypothesize that BSF and dLRPPRC coordinately regulate mitochondrial morphology and function that, in turn, fine-tune NMJ development and growth. Our results will help to understand the role of mitochondria in intercellular signaling during neuronal development and hopefully uncover the molecular and cellular aspects of Leigh Syndrome.
CHARACTERIZATION OF A NOVEL DUAL HISTONE MODIFICATION

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Eukaryotic chromatin is highly regulated by post-translational modifications of histone proteins. Histone modifications appear to form a decipherable code that is written by histone modifying enzymes and read by effector proteins. Translation of this code plays an essential role in multiple cellular processes including transcriptional regulation, gene silencing, DNA repair, and cell cycle progression. One such modification is histone H3 lysine 9 tri-methylation (H3K9me3), which plays a critical role in maintaining heterochromatin by creating a binding site for heterochromatin protein 1 (HP1). While binding of HP1 is essential for maintaining heterochromatin, removal of HP1 is required for the proper progression of mitosis. Ejection of HP1 is induced by phosphorylation of histone H3 serine 10 (H3S10p). Since H3K9me3 is maintained throughout the cell cycle, H3S10p functions as an on/off switch for HP1. We have identified another pair of modifications in human cells that potentially function in a similar manner given that they only occur in tandem at mitosis. Future experiments will be directed at determining the role of this pair of modifications in mitosis, and identifying the readers and writers of this dual modification.

Contributors: Hammond, Sharra L.; Martinez, Danielle; Medarano, Estela E.; Tyler, Jessica
Congenital genitourinary (GU) abnormalities such as hypospadias, cryptorchidism and ambiguous genitalia are among the most common human birth defects. They present difficult challenges for parents and physicians as care of the affected children is complicated by surgical, psychological, social and sexual concerns. Unfortunately, the etiology of these human reproductive disorders remains poorly understood. Submicroscopic chromosomal anomalies have been attributed as the molecular basis of genomic syndromes such as mental retardation, developmental delays and heart defects. We hypothesize that the inborn errors of urogenital tract development result from similar chromosomal aberrations that cannot be detected by a routine karyotype. Identification of such aberrations will help to define the molecular protagonists and regulatory pathways governing human GU development. A screen using clinically validated comparative genomic hybridization microarray identified significant copy number variants that evaded detection by high resolution karyotype in children born with GU abnormalities. Anomalies were scattered across the genome but notably clustered in gene-enriched subtelomeric loci. Confirmed de novo duplication and deletion events were significantly associated with GU defects (P=6.08x10^-12) when compared to 8951 non-GU patient controls. Among these variants, a gain on Xq28 was found in 2 unrelated patients and encompassed a single gene: vesicle-associated membrane protein 7 (VAMP7). Fluorescent in-situ hybridization confirmed the Xq28 gain and was ruled as a de novo event using DNA parental analysis. We found that VAMP7 is expressed in fetal and adult human and murine reproductive tissues. To unravel the precise function of VAMP7 in the development of the genital tract, VAMP7 knockdown was performed in NTERA-2 cells, which recapitulate the expression profile of endogenous markers of male sex determination and differentiation. Results indicate significant increase in the gene expression of HOXA13 and its downstream target FGF8 which are two key players in male external genitalia development. Gene expression microarray (Affymetrix Human U133 2.0 Plus) on VAMP7 knockdown NT2 cells further reveals important genes such as AMH and ARID5B that were significantly upregulated along with perturbations in estrogen signaling. Transgenic mice overexpressing VAMP7 variably displayed unilateral or bilateral cryptorchidism. In addition, large vacuoles, multinucleated cells and increased apoptosis were observed in the testis. Decrease in fertility was also evident in these transgenic mice. A VAMP7 knockout mouse was also generated to further assess the role of VAMP7 in GU defects. Taken together, the identification of clinically significant copy number variants will aid in elucidating the molecular mechanisms underlying the pathogenesis of human genital development and will define critical factors, such as VAMP7, in human male genitourinary development.
Dietary restriction (DR), a reduction in food intake without malnutrition, is a robust environmental intervention that increases lifespan. The fundamental mechanisms underlying this phenomenon, however, remain unknown. To uncover the potential role of innate immunity on the DR response in Drosophila melanogaster, we tested flies with mutations in different immune system components for the ability to respond to DR. We monitored the survival of mutant and control flies placed on food with varying nutritional content. Null mutants in the NF-κB-like transcription factor genes Relish and Dif do not respond to DR, indicating that Relish and Dif are necessary for the DR response. Surprisingly, null mutants in the gene coding for the canonical regulator of Relish, Ird5, have a functional DR response. This suggests that another protein(s) besides Ird5 is modulating Relish activity under different nutritional conditions. We are currently in the process of determining what other factor(s) may be interacting with Relish to affect the DR response.

Contributors: Hansen, Ingrid; Gendron, Christi; Pletcher, Scott D.
Background: While a first pregnancy before age 22 reduces life-time breast cancer risk, a first full-term pregnancy after age 35 doubles overall risk of breast cancer relative to age-matched nulliparous women. With the global increase in the age at which a woman chooses to have her first pregnancy, understanding the molecular mechanisms by which pregnancy stimulates breast cancer is critical for the development of effective strategies to lower breast cancer incidence. A pregnancy is associated with profound changes in the mammary gland, including reproductive hormone and growth factor levels, inflammatory cell infiltration, cellular hierarchy, and structural organization of the ductal tree. However, causal contributions of these factors to breast cancer risk have not been established. Moreover, though a pool of mutated cells accumulates as women age, it has not been shown that pregnancy can accelerate tumor initiation from these potential cancer precursor cells. If established, this mechanism could explain why pregnancy preferentially increases cancer risk in older women. We hypothesize that pregnancy stimulates tumorigenesis from mammary cells that have already acquired an oncogenic insult by modifying the response of the epithelial cells to oncogenic insult. Results: Pregnancy stimulates the progression of hyperplastic lesions to tumors in mouse mammary glands by promoting oncogenic cell survival via the PRLR-Jak2-STAT5 pathway. This pathway is both necessary and sufficient to modulate pregnancy’s stimulatory effect on breast tumorigenesis. Moreover, data from primary human samples confirm this finding. Conclusion: Based on our results, pregnancy at a later age leads to increased breast cancer risk by activating STAT5-mediated survival in oncogenic cells. Further, activated STAT5 might be a breast cancer risk factor in nulliparous women as well. Our study discovers invaluable targets for therapeutic advance and provides novel mechanistic insight into the juxtaposition of age, pregnancy, and breast cancer risk.

Contributors: Haricharan, Svasti; Dong, Jie; Reddy, Jay P; Du, Zhijun; Atkinson, Rachel; Hilsenbeck, Susan; Huang, Shixia; Woodward, Wendy; Li, Yi
Simian virus 40 (SV40) is a small DNA virus that disrupts critical cell cycle control pathways. The potent tumor virus has been shown to transform rodent and human cells in culture. SV40 induces tumors, including lymphomas, in the Syrian golden hamster model and was found in peripheral blood mononuclear cells (PBMCs) of immunocompromised monkeys. The virus was introduced into the human population as a contaminant of early Salk and Sabin poliovirus vaccines. SV40 has been detected in human PBMCs, in tonsils, and in non-Hodgkin lymphoma (NHL), suggesting that SV40 may be lymphotropic in humans. Infected lymphocytes could disseminate the virus throughout the body, leading to infections in various tissues and the development of occasional tumors. The research objective of this project is to evaluate the SV40 life cycle in human lymphoid cells. We hypothesize that SV40 establishes a persistent infection in B cells that is rarely productive.

Preliminary results using SV40-infected DG75 cells (human B cell line) suggest that viral genomes are maintained at low copy numbers and that intracellular T-antigen (T-ag) is expressed. In contrast, infection of CEM cells (human T cell line) results in the viral DNA being lost during cell passaging and no detectable T-ag expression. The goal is to determine the mechanism by which the SV40 genome is maintained in DG75 cells. It was previously reported that SV40 T-ag binds directly to the cellular protein TACC2, which is involved in microtubule stabilization during mitosis. T-ag binding leads to mitotic defects and chromosome missegregation. TACC2 strongly associates with the centrosomes during cell division and we speculate it could function in tethering T-ag-bound viral genomes to the chromosomes during cell division, allowing for proper segregation to each daughter cell. A tethering mechanism has been shown in papillomaviruses, where the viral protein E2 mediates tethering of the viral genome to mitotic chromosomes. Preliminary results indicate that the number of genome copies in SV40-infected DG75 cells decreases during siRNA knockdown of TACC2 compared to untreated virus-infected cells. Additional work will further characterize the effects of TACC2 knockdown in SV40-infected DG75 cells and its potential role in SV40 genome persistence.

Contributors: Harrigal, Lindsay J.; McNees, Adrienne L.; Butel, Janet S.
Background & Objective: Securin, also known as PTTG1 (pituitary tumor transforming gene 1), is an inhibitor of separase, a protease catalyzing the cleavage of sister-chromatid cohesions during mitosis. There is strong evidence for securin as a dynamic player involved in gene regulation, organ development and tumorigenesis. However, these earlier reports combined mutations and knockouts of other proteins such as p53, Retinoblastoma and p21 with securin. We want to understand how securin directly affects these processes. Our study focuses on the securin loss-of-function effects on mouse mammary gland development, gene regulation and tumorigenesis.

Methods & Results: Our lab generated a securin knockout (Sec-/-) mouse model that was normal with no decreased fecundity. Previous in vitro studies indicated that securin enhanced promoter activity of specific genes involved in proliferation (c-Myc, Cyclin D3, FGF-2), inhibition (p21) and extra cellular matrix modifiers (MMP2). Using quantitative real-time PCR (qRT-PCR) we analyzed Sec-/- mouse embryonic fibroblasts to reveal a significant reduction of the mRNA transcripts of matrix metalloproteinase 2 (Mmp2) and p21. Preliminary reports demonstrated that Mmp2 knockout mice had a disturbed mammary gland branch network, with increased lateral branching and decreased invasion of the developing ducts. We have completed developmental time points for 4, 7 and 13 wk old mice and our analysis shows that Sec-/- glands have super-numerated secondary and tertiary lateral branching as well as decreased invasion. Using BrdU incorporation to analyze proliferation, we have revealed that 7 and 13 wk old mice have increased proliferation in the branch network. To eliminate the systemic effect of the knockout, we performed mammary gland transplants and found similar results to the Sec-/-, indicating that the alteration of the gland is contained within the stem cell niche and not caused by a hormone-signaling imbalance due to the systemic knockout. Analysis of the glands by qRT-PCR reveals similarly low levels of mRNA transcripts in Mmp2 and p21. These Sec-/- mice also develop tumors exclusively in the breast (17%). Current studies are assessing the ability of MMP2-lentivirus to rescue the gland phenotype.

Conclusion: In gland development, securin is more than a regulator of mitosis; it controls the gene expression of both MMP2 and p21. The misregulation caused by the knockout increases proliferation and uncontrolled branching; a factor that is necessary for tumorigenesis. Therefore, securin is essential for gland architecture and tumor suppression.

Contributors: Dong, Jie; Li, Yi; Zhang, Pumin
Mitochondrial diseases affect thousands of children in America. They can be caused by primary deficiency of the electron transport chain (ETC) or by disturbances in the maintenance and/or replication of the mitochondrial DNA (mtDNA). Succinyl-CoA ligase, a component of the citric acid cycle, has recently been associated with mitochondrial disease, as deficiency of either SUCLA2, the ADP-forming beta subunit, and SUCLG1, the alpha subunit, have both been implicated in mitochondrial disease with mtDNA depletion (Rivera et. al, 2010; Carrozzo et. al. 2007; Ostergaard et. al. 2007). From a gene-trap screen in mouse embryonic stem (ES) cells previously performed in the lab, a gene trap allele in Sucla2 was isolated. Sucla2-deficient Mouse embryonic fibroblasts (MEFs) display mtDNA depletion. Current work in cell-based studies will characterize which domains of Sucla2 are necessary and sufficient for mtDNA maintenance using point and truncation mutants. Additionally, the means by which succinyl-CoA ligase interacts with other proteins the mtDNA nucleoid complex, a complex with mtDNA and interacting proteins, will be determined via Bimolecular Fluorescence Complementation (BiFC). Candidates will include proteins implicated in the mtDNA nucleoid complex such as mitochondrial transcription factor A (TFAM) and DNA polymerase subunit gamma (POLG, Bogenhagen et al., 2008), mtDNA depletion syndrome genes such as thymidine kinase 2 (TK2), deoxyguanosine kinase (DGUOK), and PEO1, which encodes the mtDNA helicase Twinkle. In Drosophila, a mutant of SCS(, the SUCLG1 ortholog, has been isolated through imprecise excision of a P-element inserted downstream. Preliminary analysis of these mutants reveal that they are homozygous viable but exhibit some neuromuscular dysfunction based on increased bang sensitivity. Full phenotypic characterization is in progress in compound heterozygotes to determine whether this mutant is a model for Suclg1 deficiency. The establishment and characterization of models of Succinyl-CoA ligase dysfunction promise to provide insights into mitochondrial diseases as well as into the biology of mtDNA maintenance.
GENETIC STUDIES TO GAIN INSIGHT INTO THE FUNCTION OF THE MECP2 DOMAINS IN VIVO

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Rett syndrome (RTT) is a debilitating neuropsychiatric disorder caused by mutations in the X-linked methyl CpG binding protein 2 (MECP2) gene, which encodes for a protein with the same name (MeCP2). Many of the RTT-causing mutations create a truncating null allele, suggesting that RTT is due to a loss of MeCP2 function. Interestingly, overexpression of MeCP2 due to a duplication spanning MECP2 also causes a progressive neurological syndrome that shares many features with RTT, including cognitive deficits, autistic features, motor abnormalities, seizures, and stereotyped behaviors. Studies in mice that either lack (RTT model) or express 2X MeCP2 (duplication model) reveal that the loss and gain of MeCP2 has opposing effects on excitatory synapses and gene expression, suggesting that the duplication causes the disorder by a “hyperfunction” mechanism. However, the challenge has been to understand exactly what function of MeCP2 is being exaggerated when it is overexpressed. Traditionally, MeCP2 was believed to be a transcriptional repressor, yet gene expression data from the animal models of RTT and duplication syndrome raise a question about this model in the nervous system, as loss of MeCP2 results in decreased expression of the majority of genes altered in the hypothalamus, whereas its gain leads to increased expression of the same genes. Newer hypotheses raised the possibility that MeCP2 normally dampens transcriptional noise such that in its absence, increased basal transcription throughout the genome might lead to decreased expression of neuronal genes. This model does not quite explain why doubling the protein will enhance expression of so many activity dependent neuronal genes. Another hypothesis proposes that overexpression of MeCP2 might titrate co-repressors, thus resulting in an increase of gene expression. I aim to test these hypotheses by generating and characterizing mice that overexpress MECP2 alleles that disrupt either of its two key functional domains. I propose that MeCP2 requires both its key domains to mediate the duplication phenotypes. To this end, I will study the in vivo consequences of three different RTT-causing mutations: T158M, which reduces methyl-CpG binding, R111G, which abolishes methyl-CpG binding, and R306C, which is in the transcriptional repression domain and whose precise effect on protein function is currently unknown. I have generated all the three mice and have begun characterization of these alleles in the context of both overexpression and in a MeCP2 null background to gain insight into the function of the two domains. I plan to investigate the molecular and biochemical consequences of these mutations by examining gene expression changes and the mechanisms leading to these changes, including testing specific histone marks and potential interactors.

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Latent Herpes Simplex Virus (HSV) infections are a special public health concern, as asymptomatic viral shedding is believed to be responsible for over 70% of primary infections, and has been shown to increase the rate of HIV transmission in patients with concurrent infections. The most common treatment for HSV includes the nucleoside analogue acyclovir. However, resistance to acyclovir is relatively common in immunocompromised individuals leading to the need for additional antivirals. Herpes Simplex Virus type-1 (HSV-1) is an enveloped mammalian virus approximately 2000 Å in diameter, consisting of a 1250 Å icosahedral capsid surrounded by tegument and envelope. In order for replication to occur, the HSV-1 capsid must interact with the host Nuclear Pore Complex (NPC); however, these interactions are poorly understood. In order to better understand these interactions, cryo-electron tomography (cryo-ET) will be used to demonstrate that the portal machinery vertex faces the NPC upon binding during viral genome release. We will utilize a two-pronged approach; first using single oocytes from Xenopus laevis ovaries injected with HSV-1 and isolated using an established protocol. Second, HSV-1 attachment to NPCs from purified rat liver nuclei will allow this process to be studied in a mammalian cell. HSV-1 capsids bound to NPCs will be visualized by cryo-ET and reconstructed to identify their respective orientations and reveal the nature of their interactions. Similar analyses will be carried out using mutant viruses, which have defects in DNA release in order to determine any structural differences that occur during the process of DNA release. Preliminary cryo-ET and confocal experiments of NPCs have been performed using Xenopus oocytes as a source of nuclear membranes. Further confocal experiments will be carried out in order to confirm binding of capsids purified from intact virions to the nuclear envelope of Xenopus oocytes. If our hypothesis is correct, we would expect the portal machinery to orient towards the NPCs and likely engage in specific interactions with it.

Contributors: Hecksel, Corey; Schmid, Mike; Rixon Frazer; Chiu, Wah
Environmental alterations can cause profound changes in organismal lifespan. One example is dietary restriction (DR), a reduction in caloric uptake without malnutrition, which can increase health and life span in different species across taxa, including worms, flies and rodents. Although a few studies have identified candidate genes required for DR-induced longevity in a variety of species, the molecular mechanisms remain largely elusive. In C. elegans, a widely used method of inducing DR involves the eat-2 mutant, which has reduced pharyngeal pumping and food intake. The C. elegans transcription factor, SKN-1/NF-E2, regulates DR-induced longevity from the pair of ASI neurons. This implies that downstream signals mediate the systemic physiological response to DR. Conceivably, this DR response could be communicated through hormonal signaling. If so, then specific hormones and their hormone receptors should be required for DR-induced longevity. To test this hypothesis, we are screening through candidate peptide hormones, G-protein coupled receptors, and nuclear receptors in an attempt to identify genes required for lifespan extension induced by DR. Currently, we have potentially identified a receptor necessary for DR-induced longevity and are testing for specificity using others forms of DR. Our studies will potentially unravel novel genes involved in a highly conserved biological process conferring increased lifespan.

Contributors: Heestand, Bree; Antebi, Adam
A HYPOTHETICAL PROTEIN IN LACTOBACILLUS REUTERI IS POTENTIALLY INVOLVED IN IMMUNOMODULATION

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Culture supernatant from human-derived probiotic L. reuteri ATCC PTA 6475 suppresses tumor necrosis factor (TNF) production by human myeloid (THP-1) cells. Transcriptomic data showed that a gene encoding a hypothetical protein, LR0977, was upregulated (>20-fold) in wild-type 6475 when TNF inhibition is most potent. LR0977 mutant lost the ability to suppress TNF and yielded diminished protective effect in a trinitrobenzene sulfonic acid (TNBS)-induced colitis mouse model compared to wild type. These findings suggested that LR0977 is involved in L. reuteri-mediated immunomodulation. In silico analysis of LR0977 sequence predicted that it might be involved in metabolism of cofactors or indole derivatives. The ability of the LR0977 mutant to suppress TNF production by THP-1 cells and the protective effect in TNBS-induced colitis model were partially restored by complementation with LR0977 gene. Transcriptomic profile of LR0977 mutant demonstrated downregulation of cellular pathways involved in vitamin B12 and folate biosynthesis. Reverse transcription quantitative PCR also showed significant downregulation of genes in the putative histidine decarboxylase (hdc) operon in LR0977 mutant compared to wild-type 6475. Histidine decarboxylase converts histidine to histamine, which has been identified as one of the immunomodulatory factors produced by wild-type 6475. These findings suggest that LR0977 may be involved in the regulation of histamine production and may play a role in immunomodulation. Ongoing studies will further characterize the structure and function of LR0977 using RNA sequencing, immunoblots and comparative proteomic analysis. This study will provide a better understanding of L. reuteri 6475-mediated immunomodulation, which may lead to novel therapeutics for inflammatory bowel disease (IBD).

Contributors: Hemarajata, Peera; Saulnier, Delphine; Zhang, Fan; Gao, Chunxu; Thomas, Carissa; Versalovic, James
Autism and autism spectrum disorder (ASD) are common neuropsychiatric conditions diagnosed by the presence of specific behavioral characteristics, including impairment in social interactions and communication, and stereotypic or inflexible behavior. Many mouse models for the disorder have been generated by knocking-out candidate genes, however, these genes are only causative for a very small portion of autism; in contrast, chromosomal abnormalities are thought to account for 10-20% of autism cases. In particular, microdeletion/microduplication syndromes and other copy number variations (CNVs) are associated with specific autistic-like, abnormal behavioral phenotypes. Potocki-Lupski syndrome (PTLS; MIM #610883) is caused by 3.7 Mb duplication of 17p11.2, and characterized by neurobehavioral and congenital abnormalities as well as developmental delay. Previous studies characterizing the cognitive and behavioral phenotypes of PTLS patients determined that ~70-90% of the subjects studied tested positive for autism or ASD. We previously generated a mouse model for PTLS (Dp(11)17/+ ) that harbors a duplication of a ~2 Mb region syntenic to the human PTLS region. The PTLS behavioral phenotypes were studied with a battery of experiments that have been established to evaluate core and associated autistic-like phenotypes, including tests for social abnormalities, ultrasonic vocalizations, perseverative and stereotypic behaviors, anxiety, learning & memory deficits, and motor defects. Alterations were identified in both core and associated ASD-like traits. We show that rearing mice in an enriched environment altered and rescued some of these neurobehavioral phenotypes, suggesting a role for gene-environment interaction in the determination of phenotypic severity.

Contributors: Melanie Heney Lacaria, Corinne Spencer, Wenli Gu, Richard Paylor, James R Lupski
Dictyostelium discoideum strains with different genotypes may coaggregate and develop into multicellular structures upon starvation. Only the spore cells in the chimeric fruiting body can propagate and the stalk cells die altruistically. Cheaters in D. discoideum are strains that make more spores than their fair shares when mixed with wild type cells. How the sociality of D. discoideum has evolved is intricate since cheaters are a constant threat to the maintenance of cooperation. One hypothesis of protecting cooperators from cheaters is through kin-recognition – the ability to recognize close genetic kin from non-kin. The lab has recently discovered a kin-recognition mechanism in D. discoideum, which is mediated by a pair of polymorphic adhesion proteins, TgrB1 and TgrC1. We hypothesize that kin-recognition could be a protective mechanism against cheaters in D. discoideum. My preliminary results support the hypothesis: I have found that cells with different tgrB-C alleles can escape the exploitation of chtA – one of the most potent cheaters known in Dictyostelium.
THE FORMATION OF THE AXON INITIAL SEGMENT IN DEVELOPING NEURONS

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Propagation of action potentials (APs) is essential for the function of the nervous system, and depends on axon-dendrite polarity and subcellular electrogenic compartments, including the axon initial segment (AIS) and nodes of Ranvier. The AIS is located near the cell body, and is enriched in voltage-gated sodium channels that initiate action potentials. Among the known proteins specifically enriched at the AIS, the scaffold protein ankyrinG (ankG) is the organizer for AIS assembly. After removal of ankG, action potential initiation and firing rates were impaired. Recent findings show another important role of the AIS: it is required for maintenance of neuronal polarity; however, whether the AIS is involved in the establishment of neuronal polarity remains unclear. Although the AIS is a critical structure in neurons, the timing of AIS formation during neural development has yet to be determined. Here we used in utero electroporation to study the temporal relationship between initiation of axon-dendrite polarity and the formation of the AIS, and showed ankG is not required to initiate neuronal polarity. To further study targeting of sodium channels to the AIS and nodes of Ranvier in vivo, we electroporated functional full-length sodium channels. We showed that a conserved glutamate residue within the AIS-targeting motif is essential for targeting of sodium channels to the axon initial segment and nodes of Ranvier, indicating that the AIS-targeting motif in sodium channels is also necessary for its localization to nodes of Ranvier.

Contributors: Ho, Szu-Yu; Gasser, Andreas; Dib-Hajj, Sulayman D.; Rasband, Matthew N.
**SERMS INHIBIT THE GROWTH OF BLADDER CANCER CELLS BY INDUCING APOPTOSIS VIA AN ER-DEPENDENT PATHWAY**

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**Introduction:** Bladder cancer is the fifth most prevalent type of cancer in the United States, with over 70,000 new cases anticipated for 2011. It is approximately three times more common in men than women, indicating a potential role for sex steroids and their receptors in the development and progression of this disease. Recent work by our group indicates the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene can moderately inhibit the growth of certain bladder cancer cell lines in vitro while showing marked tumor regression in a subcutaneous xenograft mouse model of bladder cancer. In this study, we examined the effects of tamoxifen, raloxifene and the pure antiestrogen ICI 182,780, as well as several estrogen receptor (ER) agonists on the growth of bladder cancer cell lines representing non-muscle invasive, invasive, and metastatic disease. We also explored whether SERM-induced growth effects were ER-dependent using the RT4 bladder cancer cell line.

**Methods:** Ligand-induced effects on proliferation were assessed in 8 different bladder cancer cell lines by MTS assay and Coulter counter. Effects were correlated to changes in cell cycle and apoptosis on select cell lines using flow cytometry and a cell death ELISA. Expression of cell cycle regulators as well as PARP and Caspase-3 cleavage were also evaluated. RT4 cells transiently expressing ER-targeted siRNA were used to determine ER-dependency of growth effects.

**Results:** None of the lines tested showed a significant response to ER agonist treatment, yet nearly all cell lines were growth inhibited by at least two antiestrogenic compounds. Long-term growth inhibition was accompanied by an increase in cell death and an accumulation of cells in the sub-G0 phase. PARP and Caspase-3 cleavage indicated an early apoptotic event in raloxifene-treated RT4 cells, an effect that was abrogated in ER knock-down conditions.

**Conclusions:** Antiestrogens inhibit the growth of bladder cancer cells through effects on apoptosis and cell cycle regulation, and may be effective treatments against bladder cancer.

**Contributors:** Hoffman, Kristi; Roethele, Joe; Foster, Estrella; Lerner, Seth; Smith, Carolyn L.
TAU LOSS REGULATES EXCITABILITY IN MOUSE AND DROSOPHILA GENETIC MODELS OF EPILEPSY.

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Advisor: Jeffrey Noebels, M.D./Ph.D.-Department of Neurology

For 25 years, Alzheimer’s disease (AD) has been recognized as a clinical risk factor for late onset seizures. AD and epilepsy share similar atrophic and metabolic changes in the human brain and most familial AD gene mutations lead to epilepsy. In addition, multiple AD mouse models overexpressing amyloid beta exhibit spontaneous seizures as well as remodeled circuitry similar to that seen in epilepsy. Aggregation of the microtubule binding protein tau is one hallmark of AD and recent work implicates a role for tau in the AD seizure phenotype. Deletion of the MAPT gene, which encodes tau, suppresses seizures and normalizes the synaptic excitation/inhibition imbalance in the J20 hAPP AD mouse model, suggesting that tau regulates hyperexcitability in AD mice. To better understand the relationship between tau and hyperexcitability, we are examining the role of tau in neuronal excitability in the non-AD brain using genetic knockout of tau in both mouse and Drosophila models of hyperexcitability.

Kcna1-/- mice have severe spontaneous seizures, early lethality, and megencephaly. We observe that Kcna1-/- mice with tau knockout have decreased seizures, increased survival, and decreased hippocampus volume. These results suggest that tau loss rescues seizures as well as the associated lifespan and megencephaly phenotypes in this seizure model. Bang sensitive Drosophila mutants are a second model of excitability, which have induced paralysis and seizures, as well as decreased neuronal seizure thresholds. We observe that tau reduction decreases bang sensitivity in multiple Drosophila bang sensitive mutants, which corroborates the results seen in the Kcna1-/- seizure mouse model.

Tau protein is known to affect many cellular processes ranging from microtubule transport to signal transduction. Our results provide evidence that tau plays a constitutive role in the regulation of neuronal excitability in the non-AD nervous system. Thus, the effects of tau loss on excitability extend beyond the reversal of amyloid beta-induced AD phenotypes to encompass a role in the regulation of epilepsy, where tau’s function can also be explored as a future therapeutic target.

Contributors: Holth, Jerrah; Reed, John Graham; Inoue, Taeko; Pautler, Robia; Botas, Juan; Noebels, Jeffrey
Osteogenesis Imperfecta (OI) is a spectrum of genetic disorders characterized by low bone mass and fragility. It is caused by dominant mutations affecting the synthesis and/or structure of type I procollagen or by recessively inherited mutations in genes responsible for the post-translational processing/trafficking of type I procollagen. In all but one class of OI, the histopathological and clinical features cannot predict the causative gene or functional defect among affected individuals. However, recessive OI type VI is characterized by a pathognomonic increase in the amount of unmineralized osteoid, thereby suggesting a distinct disease mechanism. OI type VI is of unknown etiology and identifying the disease gene will add to our understanding of matrix mineralization and identify potential therapies.

To identify the gene region, homozygosity mapping was carried out using the genomic DNA of 3 members of a consanguineous French Canadian family. A single region of homozygosity was shared by all three of these related OI VI patients defined by the markers rs8074026 and rs1362761. Next generation sequencing of one patient from this family identified homozygosity for a stop mutation in exon 4 (g.4130C>T, p.R99X) of serpin peptidase inhibitor, clade F, member 1(SERPINF1) which was confirmed by Sanger sequencing in all affected family members. The stop codon mutation was predicted to result in nonsense mediated decay (NMD) of the SERPINF1 transcript and realtime RT-PCR of patient fibroblasts confirmed that this is the case. Sanger sequencing the 8 exons of SERPINF1 in 10 unrelated patients identified additional mutations. Interestingly, the g.4130C>T mutation was identified in additional patients of French Canadian origin, suggesting the presence of a founder allele in the population. SERPINF1 encodes the 50kDa secreted protein, Pigment Epithelium-derived Factor (PEDF). Serum PEDF levels were undetectable in OI type VI patients in contrast to classical OI patients (caused by mutations affecting type I collagen) in whom PEDF serum levels were comparable to normal controls.

These data suggest that mutations in SERPINF1 resulting in loss of PEDF cause OI type VI. This provides an avenue for proper OI diagnosis of patients, whereas PEDF could be measured in serum for confirmation. In addition, PEDF is a secreted protein, suggesting that therapy with recombinant PEDF could be used as a potential therapy for OI type VI patients.
Nearly 100 years ago it was proposed that bacteriophage could be used as antibacterial agents to mitigate bacterial infections. Recently, interest in phage therapy has seen resurgence as an increasing number of pathogens are becoming multi-drug resistant. PRD1 phage, which is known to infect Salmonella typhimurium, is a good candidate for a possible antibacterial. PRD1 is a dsDNA bacteriophage with an inner membrane and belonging to the Tectiviridae family. As the virus structure has been previously solved by X-ray crystallography, it is known that the icosahedral capsid shell is composed of four structural proteins. However, the portal complex, which may consist of at least four proteins, plays an integral role in the packaging of the viral genome into the viral membrane vesicle through the icosahedral shell. In addition, five other proteins that are responsible for genome delivery during infection also remain elusive. The goal of this study is to reveal the structures of the genome transfer machinery of PRD1 and elucidate its working mechanism. We use single-particle cryo-electron microscopy (cryo-EM) to study both the mature and empty stages in solution state. We were able to reconstruct the icosahedral maps of the mature virion and the empty particle to ~6 Å resolution. For the empty particle, a tube tail structure, which protrudes at one of the five-fold vertices, can be visualized in the raw cryo-EM images. By symmetry-free reconstruction of the empty particle at ~20 Å resolution, we started to be able to resolve a tubular density at a 5-fold position and extra densities that are connected to the tube tail could also be seen. This might indicate the presence of the portal complex structure at the 5-fold position. This complex structure helps to elucidate the genome release mechanism of PRD1 and also provides guidance to reveal the packaging complex structure in the mature virion in the future.

Contributors: Hong, Chuan; Liu, Xiangan; Jakana, Joanita; Oksanen, Hanna; Bamford, Dennis; Chiu, Wah
It is proposed that Bacillus anthracis, the causative agent of anthrax disease, acquires heme-iron from host hemoglobin (Hb) during infection. This is accomplished by two secreted hemophores, IsdX1 and IsdX2, which harbor one or five NEAr-iron Transporter (NEAT) domains, respectively. NEAT domains are structurally conserved heme-binding modules found in several Gram-positive bacterial proteins. IsdX2, having five, non-identical NEAT domains, represented a novel, uncharacterized protein of which in silico analysis suggested possible heme-acquisition properties. Additionally, although heme scavenging from host Hb signifies the first step in bacterial-mediated heme acquisition, the specific mechanistic requirements needed for NEAT heme scavenging remained unidentified. We hypothesize that the NEAT domains of IsdX2 harbor distinct heme acquisition functions that can be utilized to determine the molecular mechanism of heme scavenging. We propose that the function of each IsdX2 NEAT domain is influenced by the amino acid composition of the heme-binding pocket. Guided by this principle, we defined the heme binding, heme extraction, and heme transfer function of each NEAT domain of IsdX2. Based on these findings, we propose a mechanistic model of heme-scavenging activity for bacterial NEAT proteins. The inhibition of heme-uptake systems represents a major entry point for the generation of new anti-bacterial drugs against several Gram-positive pathogens.
TARGETING THE TCF3 LOCUS WITH A REPORTER KNOCK-IN ALLELE

Jeffrey Matthew Howard  
Program in Translational Biology & Molecular Medicine/M.D.-Ph.D. Program  
Advisor: Hoang Nguyen, Ph.D.-Department of Molecular & Cellular Biology  
Mimi Leong, M.D.-Division of Plastic Surgery

The transcription factor Tcf3 is highly expressed in epidermal stem cells (SCs) and is critical to their function. Recent studies have also shown high levels of Tcf3 expression in the tumor-initiating cells (TICs) of cutaneous squamous cell carcinoma (SCCA), and evidence suggests that it may play an important role in SCCA pathogenesis. We hypothesize that Tcf3 expression is a defining feature of both normal epidermal stem cells and SCCA TICs. To test this hypothesis, we are constructing a knock-in reporter allele of Tcf3 containing eGFP and tamoxifen-inducible Cre recombinase (CreERT2) genes. The eGFP reporter will allow us to isolate live Tcf3-expressing cells from normal skin and SCCA by flow cytometry; we will then subject them to assays of stem cell function and tumorigenicity. The CreERT2 reporter will allow us to perform Cre/loxP-based lineage analysis by crossing knock-in mice with stop-floxed lacZ reporter mice (Rosa26R). This will allow us to observe the fate of Tcf3-expressing cells and their progeny in normal skin and tumors. Similarly, we will cross knock-in mice with mice carrying a stop-floxed diphtheria toxin gene, which will allow us to selectively kill Tcf3-expressing cells and observe the effect on epidermal homeostasis and tumor growth. Thus far, we have generated chimeric mice carrying the knock-in allele and are currently in the process of outcrossing them to obtain germline transmission.

Contributors: Howard, Jeffrey; Nguyen, Hoang
The ability to quickly establish microcirculation within a biomaterial scaffold which then anastomoses to the host circulation is critically important for engineering complex tissues. Pro-angiogenic growth factors, such as VEGF, FGF2, and PDGFBB, have been used to induce neo-angiogenesis. However, vessels induced by these growth factors alone are not stable and will regress rapidly. It has been demonstrated that the combination of PDGFBB and FGF2 has a synergistic effect on angiogenesis and could produce more stable vessel structures, even when PDGFBB and FGF2 were delivered transiently. Although the mechanism regulating vessel stability is still unclear, it has been shown that, depending on the tissue, macrophages can have either pro- or anti-angiogenic effects. The objective of this study is to understand the role of macrophages in vessel formation and stability during angiogenesis and develop angiogenic hydrogel scaffolds which can promote and sustain stable vasculatures for tissue engineering applications. A fluorescent reporter mouse model, which labels macrophages (c-fms-GFP) and endothelial cells (Flk1-myr::mCherry), was used to study the role of macrophages in vessel stability. Growth factors delivered by poly(ethylene glycol) diacrylate (PEGDA) hydrogels were used to evaluate the angiogenic potential and host responses in the cornea micropocket assay. Live, confocal microscopy was also performed to examine the dynamics of macrophages in response to different growth factors. Using PEGDA hydrogels, we have been able to confirm the synergistic angiogenic effect of PDGFBB and FGF2. Interestingly, our finding also indicates that the combination of PDGFBB and FGF2 not only induces more stable vessels, it also increases the density of macrophages before the induced vessels reach the hydrogel. This phenomenon suggests that instead of causing regression of VEGF-induced vessel, macrophages may contribute in stabilizing PDGFBB/FGF2-induced vessels. Vital imaging reveals that PDGFBB/FGF2 indeed results in the infiltration of circulating macrophages which migrate toward the implantation site. In summary, these data indicate that macrophages may play a role in stabilizing the neo-angiogenic response. Future studies, using vital imaging to study the macrophage dynamics in macrophage-deficient mice, will be used to further test whether macrophages are necessary and sufficient for the formation of stable vessels. We anticipate the second generation bio-scaffold design will incorporate activated macrophages or factors to directly activate these cells to optimize the angiogenesis response.

Contributors: Hsu, Chih-Wei; Poché, Ross; Saik, Jennifer; Ali, Saniya; Vadakkan, Tegy; West, Jennifer; Dickinson, Mary
As with many other viruses, the initial cell attachment of rotaviruses, major causative agents of infantile gastroenteritis, is mediated by interactions with specific cellular glycans. The distally located VP8* domain of the rotavirus spike protein VP4 mediates such interactions. The existing paradigm is that 'sialidase-sensitive' animal rotavirus strains bind to glycans with terminal sialic acid (Sia), whereas 'sialidase-insensitive' human rotavirus (HR) strains bind to glycans with internal Sia glycans such as GM1. Although the involvement of Sia in the animal strains is firmly supported by crystallographic studies, it is not yet known how VP8* of HRs interacts with Sia and whether their cell attachment necessarily involves sialoglycans. Using a glycan microarray screen comprised of 511 glycans, we found that VP8* of a HR strain specifically recognizes A-type histo-blood group antigen (HBGA) and that virus infectivity is abrogated by anti-A-type antibodies providing a novel paradigm for initial cell attachment of HR. These genetically determined glyco-conjugates present in mucosal secretions and on red blood and epithelial cells are recognized as susceptibility and cell attachment factors for gastric pathogens like H. pylori and noroviruses. Our crystallographic studies show that the A-type HBGA binds to the HR VP8* at the same location as the Sia in the VP8* of animal rotaviruses, and demonstrate how subtle changes within the same structural framework allow for such receptor switching. These results raise the possibility that host susceptibility to specific HR strains and pathogenesis are influenced by genetically controlled expression of different HBGAs among the world’s population.
MECP2 FUNCTION IS NECESSARY IN NEURAL CIRCUITS FOR CARDIORESPIRATORY REGULATION.

Teng-Wei Huang
Program in Developmental Biology
Advisor: Jeffrey Neul, M.D./Ph.D.-Department of Pediatrics

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder associated with loss of communication and purposeful hand skills as well as several autonomic deficits including abnormal temperature regulation, decreased heart rate variability, and respiratory deficits. These autonomic disorders may contribute to the sudden death observed in a fraction of people with RTT. Mutations in the gene encoding a transcriptional regulator, Methyl-CpG-binding protein 2 (MECP2), cause 95% of RTT cases, and mice lacking MeCP2 function exhibit the pathological features similar to RTT patients, including the autonomic deficits.

Our lab found that removing MeCP2 from caudal pons, medulla and spinal cord causes early death and autonomic phenotypes including decreased heart rate and abnormal respiratory response to hypoxia. In addition, re-expressing MeCP2 within this region is sufficient to rescue the lethality, heart rate, and respiratory response to hypoxia. This region contains various known components of the respiratory control circuitry including the nucleus of the solitary tract (NTS), the retrotrapezoid nucleus (RTN). To test the hypothesis that MeCP2 is necessary within spatially distinct components of the respiratory control circuitry for the respiratory response to hypoxia, we use transgenic mice with different Cre expression pattern to remove or re-express MeCP2 expression in different regions of respiratory control circuitry. In addition, stereotaxic viral delivery systems will be used to remove or re-expressing MeCP2 in specific neuron circuits that cannot be defined using Cre-based systems. Mice are analyzed by lethality, heart rate, and hypoxic response. From results, we found that remove MeCP2 function from the NTS and the caudal part of the medulla causes abnormal respiratory response, although the pre-Bötzinger complex, which is essential to the generation of respiratory rhythm in mammals, still have normal MeCP2 expression.

We also found that loss of MeCP2 in the region including the carotid bodies, the major organ that detects changes of the partial pressure of oxygen in the arterial blood, doesn’t cause increased hypoxic ventilatory response and early lethality. These results suggest that the abnormal breathing in MeCP2 deficient animals is resulted from the dysfunction of signaling processing in NTS, but not from the abnormal sensing or rhythm generating.

Contributors: Huang, Teng-Wei; Ward, Christopher; Neul, Jeffrey
THE NEW MTOR COMPLEX (MTORC2) IS SELECTIVELY REQUIRED FOR ACTINDYNA-MICS MEDIATED LONG-TERM SYNAPTIC PLASTICITY AND LONG-TERM MEMORY

Wei Huang
Department of Neuroscience
Advisor: Mauro Costa-Mattioli, Ph.D./M.S.-Department of Neuroscience

The reorganization of actin cytoskeleton is important for the late phase of long-term potentiation (LTP) and memory (LTM). However, the molecular mechanisms underlying actin dynamics-mediated changes in synaptic strength and memory storage remain poorly understood. The mammalian target of Rapamycin complex 2 (mTORC2), which contains mTOR and the key regulatory protein Rictor, was recently discovered. Thus, little is known about mTORC2’s function, up-stream regulation and downstream targets in the brain. Here we show that in slices from rictor forebrain KO mice (rictor fb-KO) - where rictor is conditionally deleted and mTORC2 activity is consequently abrogated - the late phase of LTP (L-LTP), but not the early phase of LTP (E-LTP), is impaired. Furthermore, hippocampus-dependent LTM is selectively blocked in rictor fb-KO mice, whereas short-term memory (STM) is spared. Finally we found i) the upstream synaptic events that activate mTORC2 and ii) that mTORC2 mediates these effects through regulation of Rac1-GTPase-mediated actin dynamics. In conclusion, our studies have identified a new regulatory complex (mTORC2) that accounts for the regulation of actin dynamics which controls the conversion from a short-term process (E-LTP and STM) into a long-term one (L-LTP and LTM).

Contributors: Huang, Wei; Zhu, Ping Jun; Zhou, Hongyi; Stoica Loredana; Costa-Mattioli, Mauro
Brainstem neurons control cardiorespiratory activity by generating synchronized cardiovascular and respiratory rhythm to match blood flow with the ventilatory movements. Lesions in the brainstem are responsible for life-threatening breathing conditions, including periodic apnea and central hypoventilation syndrome, and the sudden infant death syndrome (SIDS). To date the key brainstem nuclei responsible for rhythm generation are the Pre-Bötzinger Complex (preBötC), which is heavily innervated by rostral ventral respiratory group (rVRG) and parafacial respiratory group (pFRG) nuclei. Recently, our lab has identified the transcription factor Atonal homolog 1 (Atoh1) as a key developmental regulator for a multitude of brainstem nuclei, including the rVRG and pFRG. Moreover Atoh1 null mutants die at birth due to respiratory failure caused by brainstem nuclei. The overarching goal of this project is to identify the Atoh1-dependent neurons responsible for neonatal respiratory failure and lethality by conditionally removing Atoh1 from specific brainstem nuclei using the Cre-LoxP system.

Deletion of Atoh1 from the rVRG nucleus (using a HoxA4Cre transgenic line) resulted in only 15% of such perinatal lethality, suggesting the rVRG is a contributing but not the most essential component of respiratory-control neurons in the neonates. In contrast, when Atoh1 was deleted from the pFRG (using a Phox2bCreTg transgenic line) I observed 50% of neonatal lethality and ventral migration defects of the pFRG neurons, closely resembling the Atoh1 null phenotype. The 50% survivor mice present reduced CO2-evoked ventilatory response at three month of age, suggest the pFRG neurons are part of the central chemosensory circuitry. To understand the underlying transcriptional events, I identified the transcription factor Lhx2, as a downstream target of Atoh1 as well as a novel marker of pFRG neurons. Together these results provide the framework for understanding the physiological role of pFRG neurons, especially during neonatal breathing and the transcriptional regulation of pFRG development.

In future I plan to study the properties of pFRG neurons in Atoh1 conditional knockout mice using electrophysiological measurements and map the neuronal projections of the pFRG neurons to relate the observed phenotypes to the rhythm generation neuronal circuits. Moreover I characterize a conditional rescue allele of Atoh1, which I generated. This allele will allow me to restore Atoh1 expression in specific nuclei using the Cre-mediated recombination. This complementary approach will help to identify the mechanism by which Atoh1-dependent brainstem nuclei maintain neonatal breathing.

Contributors: Huang, Wei-Hsiang; Klisch, Tiemo; Zoghbi, Huda
Nitric oxide (NO) is a highly diffusive free radical produced in the nervous system in a calcium dependent manner in response to activity. NO has been shown to modulate the responses of neurons in various brain regions and facilitate signal detection by individual neurons in the visual cortex in vivo. The expression of nitric oxide synthase (NOS), the enzyme that produces NO, in both the pre- and postsynaptic compartments of both excitatory and inhibitory neurons and its ability to diffuse through cellular membranes have made it very difficult to determine the precise neuroanatomical compartments where NO is produced and/or acts. By studying synaptic function in a well-defined synaptic network within the visual cortex where NO is known to exert its effects, we are able to address these questions. We utilized whole-cell patch recording to study the effects of manipulating NO production by provision of the NOS substrate, L-arginine (L-Arg), on inhibitory postsynaptic currents (IPSCs) and excitatory postsynaptic currents (EPSCs) in layer 2/3 (L2/3) pyramidal neurons evoked by stimulation of layer 4 (L4) afferents in acute brain slices from guinea pigs. IPSCs were pharmacologically isolated with the AMPA glutamate receptor inhibitor, DNQX (20 μM), and the NMDA glutamate receptor inhibitor, APV (50 μM). EPSCs were pharmacologically isolated with the GABA receptor antagonist picrotoxin (15 μM). L-Arg increased the average peak amplitude of IPSCs (average percent change ± SEM = +32.6 ± 2.1%, p<0.001, n=10, paired t-test), but slightly reduced EPSCs (-13.0 ± 3.2%, p<0.01, n=10, paired t-test) suggesting that NO may modulate the balance between excitation and inhibition in visual cortex. The cell impermeable NO scavenger carboxy-PTIO (100 μM) was bath applied to determine if NO travels between cellular compartments to enhance inhibitory synaptic transmission. This extracellular, NO specific scavenger blocked the L-Arg mediated increase in IPSC peak amplitude (+1.3 ± 1.5%, p=0.3368, n=9, paired t-test) indicating that NO is acting as an intercellular messenger. To evaluate the site of the effects of NO on inhibition more directly, we tested the effects of L-Arg on unitary IPSCs (uIPSCs) recorded from L2/3 pyramidal cells in response to a single action potential evoked in individual identified L2/3 presynaptic inhibitory neurons with dual visualized whole cell patch recording. Our results suggest that uIPSCs evoked from a subset of inhibitory neurons are enhanced in the presence of L-Arg.

Supported by NIH grant R01-Ey12782 to MJF

Contributors: Huddleston, Allarhee; Friedlander, Michael
The neocortex is the most complex structure in the brain, consisting of billions of neurons with trillions of connections. In an effort to understand the circuitry of the neocortex and how it works neuroscientists have attempted to reduce it to smaller functional units. It has been proposed that ontogenetic microcolumn – vertical columns of pyramidal neurons derived from a single neural progenitor cell – are the elementary computational circuit modules of the neocortex. Indeed, it has been shown that neurons derived from the same progenitor are more likely to be synaptically connected to each other than to nearby, unrelated neurons. However, the functional significance of ontogenetic microcolumns remains unknown. Our goal is to study the function of microcolumns and elucidate the computations they perform.

The first step in this direction is to develop methods to label ontogenetic microcolumns in vivo. Here we describe a method using fluorescent markers that allow identification of clonally related neurons. A plasmid encoding Cre recombinase is delivered by in utero electroporation to the embryonic ventricular zone of floxed-STOP-EYFP mice during early neurogenesis. The mice are born naturally and survive to adulthood. The brains are fixed in 4% paraformaldehyde (PFA) and sectioned at 100 μm for confocal imaging. The distribution of labeled neurons is consistent with previous reports of microcolumns labeled by other methods. However, unlike other methods we are able to target specific regions of the ventricular zone by using electroporation. Using this labeling method enables us to study the functional and molecular composition of ontogenetic microcolumns in detail.

Contributors: Hughes, Cathryn; Tolias, Andreas
The Ku heterodimer, composed of Ku70 and Ku80, is a high affinity DNA end-binding (DEB) protein that is essential in humans for its role in telomere integrity. Ku is central in the protection of telomeres from homologous recombination (HR) yet its innate non-homologous end joining (NHEJ) function must be blocked to prevent deleterious chromosome end-to-end fusions. How any of this is achieved is unclear. Assumptions are that Ku functions directly by DEB, as in budding yeast, or by association with telomeric proteins. To target these properties, we mutated Ku. We found that a conditionally null Ku80flox/- cell line stably expressing a DEB defective Ku80 mutant (Ku80-DEB) was viable, signifying that Ku’s DEB ability is not vital for its telomeric role.

To examine Ku’s protein interactions, we mutated the (-helix 5 in Ku70 and Ku80, which is known to be required for Ku’s NHEJ and telomeric functions, respectively, in budding yeast. Co-immunoprecipitation (co-IP) assays showed the Ku70-(5 and Ku80-(5 mutants to be deficient in binding telomere specific factors TRF1 and TRF2, respectively. Ku80-DEB was proficient for binding TRF1 and TRF2 in a co-IP showing that Ku’s ability to bind these factors is separate of its ability to bind DNA. Using protein fragment complementation assays (PFCA) to assess Ku-TRF1 binding in vivo, we found that the interaction occurs primarily via Ku70 and not Ku80 and that the Ku70-TRF1 complex resulted in punctate foci. These foci co-localized with a telomeric marker, confirming this interaction occurred at telomeres. Further analysis of these mutants is planned.

In conclusion, Ku70-(5 is a conserved region crucial for NHEJ, which ironically telomeres are shielded from to avoid chromosome fusions. TRF2 is required to prevent telomere loss via HR, which also presumably occurs upon the loss of Ku. We propose that TRF1 binding to Ku70-(5 blocks cancer-inducing NHEJ at telomeres while TRF2 binding to Ku80-(5 prevents telomere loss via HR, thereby providing two mechanisms for how telomeres are protected from aberrant DNA repair.

Contributors: Indiviglio, Sandra; Bertuch, Alison
Multiple Sclerosis is a demyelinating disease of the Central Nervous System (CNS) with demyelination occurring in the brain, spinal cord and the optic nerve. It is slow progressing and ultimately results in debilitation of afflicted individuals. It is characterized by periods of relapse and remission of neurological symptoms that include decreased vision, muscle weakness and loss of coordination. The etiology of this disease is currently unknown, however, it understood that autoimmune mechanisms play a key role in the development of this disease. In order to study this debilitating disease, an animal model of MS has been developed and is called the experimental autoimmune encephalomyelitis (EAE) model. These animals exhibit MS-like symptoms by immunizing them against myelin. Using this model, elevations in reactive oxygen species (ROS) have been implicated in the development of lesions in this disease. Some of the major sources of ROS in MS are activated microglia and macrophages, as well as mitochondrial dysfunction. As a result, we hypothesized that lowering of ROS by overexpressing an endogenous antioxidant, superoxide dismutase 2 (SOD2) in a mouse model of EAE would result in decreased. After inducing EAE in SOD2 overexpressing and control mice, we found that SOD2 mice showed a trend towards decreased clinical symptoms and decreased immune infiltrates within the spinal cord indicating that lowering ROS levels may be neuroprotective in this mouse model of MS.

Contributors: Inoue, Taeko; Quick, Ann; Majid, Tabassum; Beeton, Christine, Ph.D.; Pautler, Robia G, Ph.D.
Cryo-electron tomography (cryo-ET) can reveal the low-resolution structure and location of nanomachines in a variety of cellular environments. This technique has been primarily used to characterize large structures, such as cells and organelles, and can also be used to average multiple copies of viruses or other large protein complexes. We recently showed, for the first time, that cryo-ET can be applied to study much smaller and heterogeneous specimens, such as nucleic acids. We successfully resolved the structure of a 132-nucleotide 42.8 kDa RNA from Moloney Murine Leukemia Virus (MoMuLV), which is to date the smallest specimen structurally characterized by cryo-ET. This RNA had been shown to be the smallest necessary and sufficient region that acts as a packaging signal.

We then applied this new method of examining nucleic acids to the putative functionally equivalent RNA from Human Immunodeficiency Virus (HIV-1) – a 158-nt monomeric fragment of the 5'-leader sequence. There is limited understanding of the mechanisms regulating the 5'-leader functions, mostly due to incomplete knowledge of the structure of the region.

Here we used cryo-ET and subvolume averaging techniques to study this small RNA, which according to secondary structure predictions is thought to contain four hairpins. The density map that we obtained via subvolume averaging has an overall size of approximately 150 Å in its longest dimension. The EM map showed a high density about halfway through, consistent with the anticipated location of a three-branched junction. A small density on one side of the junction suggested the location of one of the hairpins and the density on the other side splits, indicating the separation of two of the other hairpins. Our preliminary cryo-ET results also suggest that there is flexibility associated with the fourth hairpin. Our next step is to identify and resolve the structural variability associated with the inherent flexibility of that hairpin.

Contributors: Irobalieva, Rossi; Heng Xiao; Summers, Michael F.;
Attention-Deficit Hyperactive Disorder (ADHD) is the most commonly studied and diagnosed psychiatric disorder in children. Methylphenidate (MPH, Ritalin) is the most commonly prescribed treatment for ADHD, and it has been used for decades to treat ADHD, but little is known about its therapeutic mechanisms.

The hippocampus is a center for learning and memory, and synaptic plasticity is a measurable underlying mechanism for memory. This project examines the effects of MPH on synaptic plasticity in the hippocampus that correlates with and may contribute to learning and memory. We will measure synaptic plasticity in response to MPH in the perforant path to dentate gyrus pathway in the hippocampus by recording field potentials with permanently implanted electrodes in freely moving mice. These in vivo recordings offer a highly precise measurement of synaptic transmission, while preserving the overall circuitry and neurotransmitter systems that we ultimately wish to understand. The information gained in these studies will help us learn more about how MPH affects the hippocampus and provides its therapeutic actions.

Our hypothesis is that MPH will affect synaptic plasticity in the hippocampus that underlies learning, and the influence of MPH will arise from its ability to boost catecholamine signaling. The object of this proposal is to determine the effects of MPH on synaptic plasticity in the hippocampus of awake animals, and to more clearly elucidate its mechanism of action.

Contributors: Jenson, Daniel; Tang, Jianrong; Zhang, Lifen; Dani, John A.
THE ROLE OF THE ENDOCANNABINOID SYSTEM IN SYNAPTIC PLASTICITY AND CORTICAL MAP DEVELOPMENT

Christopher Paul Jew  
*Program in Developmental Biology  
Advisor: Hui-Chen Lu, Ph.D.-Department of Pediatrics*

Somatotopic cortical maps represent the topographical organization of sensory inputs within the central nervous system. Neurons of the ventral posterior complex of the thalamus, which encompasses a complete representation of the somatic sensory periphery, project their axons onto the primary somatosensory cortex in a highly preserved, stereotypic fashion. The barrel cortex comprises a subset of the rodent somatosensory cortex that represents the array of whiskers and sinus hairs found on the snout. During an organism’s critical period, the arrangements of the cortical maps are highly amenable to sensory input from the thalamocortical afferents (TCAs), thus delineating a specific time window to study cortical map plasticity.

The Endocannabinoid System (ECS) is classically referred to as the neuromodulatory system that mediates the psychoactive effects of cannabinoids, although the ECS is now known to have active roles in multiple neural developmental processes including axonal guidance, lineage specification, and neuronal differentiation. CB1R and GPR55, both G-protein coupled receptors, are the two main ECS receptors expressed throughout the CNS. Several lines of evidence generated from pilot studies conducted in our lab point to the presence of the ECS within the barrel system, and TCA fasciculation was altered in CB1R/null mutant mice.

Initial characterization of the CB receptors in the context of cortical map development will focus on studying thalamocortical brain slices that remain intact, living TC synapses. We have obtained CB1R KO mice as well as 2 strains of GPR55 mice that will be used to confirm the role of the ECS in TC synapses. Electrophysiological studies will focus on elucidating the functional properties of CB receptors using field potential recordings, whole-cell voltage clamp recordings, and multi-electrode array recordings. Manipulation of the ECS through pharmacological reagents will allow us to confirm and study CB1R and GPR55’s role in synaptic plasticity.

Contributors:
DIFFERENTIAL REQUIREMENT OF ZIC3 FUNCTION IN CARDIAC DEVELOPMENT AND X-LINKED HETEROXY

Zhengxin Jiang
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Heterotaxy (situs ambiguous), contributing to ~5% of congenital heart defects (CHD), arises from abnormal left-right patterning with abnormal symmetry or reversals of internal organs and cardiac chambers. Loss-of-function mutations of ZIC3 (Zinc finger protein of cerebellum 3), a member of the GLI transcription factor superfamily located on X chromosome, cause human X-linked heterotaxy (HTX1). A mouse model with targeted disruption of Zic3 exhibited ~50% prenatal lethality with additional ~30% die before weaning, and recapitulated the phenotype seen in human HTX1 patients. However, it is not known whether ZIC3 is required in a single developmental field or whether it has pleiotropic roles in multiple developmental processes, and the detailed mechanism remains elusive.

To dissect the differential function of ZIC3 in development, we generated a conditional allele of the murine Zic3 gene. Sox2-cre, Wnt1-cre and T-cre lines were utilized to delete Zic3 in epiblast, neural crest and mesoderm, respectively. Deletion of Zic3 in epiblast and mesoderm, but not in neural crest, led to ~50% para and postnatal lethality without apparent lethality before 15.5 dpc. Although at 9.5 dpc, ~50% of the Zic3 epiblast conditional embryos were significantly delayed with additional ~25% exhibited heart looping defects, similar to Zic3 null embryos; most of these delayed embryos were not lost during subsequent development in contrast with the delayed Zic3 null embryos. At later stages, ~10% of the delayed Zic3 epiblast conditional embryos developed multiple CNS defects similar to what were seen in ~10% of the surviving delayed Zic3 null embryos. But these CNS defects were not found in Zic3 mesoderm conditional embryos as they only exhibited heterotaxy associated visceral abnormalities including cardiac defects in ~25% of the mutants with no apparent developmental delay. Together these data suggest that Zic3 has pleiotropic roles in multiple developmental processes: 1) its function in extraembryonic tissues may be required for early embryogenesis before left-right patterning; 2) its function in epiblast, but not in mesoderm or neural crest derivatives, is required for early CNS development; 3) its deficiency in mesoderm, but not in cardiac neural crest, contributes exclusively to heterotaxy and its associated complex CHD; 4) its function in neural crest derivatives is not required for normal development and survival. In addition, whole heart gene expression microarray analysis of embryos at 15.5 dpc revealed a similar expression pattern between Zic3 null and epiblast conditional males, which was significantly different from control males. Perturbed expression of cardiac genes and direct targets of Zic3 suggested that BMP and TGF-β signaling might be affected in these mutants, and requires further investigation.

Contributors: Jiang, Zhengxin; Zhu, Lirong; Hu, Lingyun; Slesnick, Timothy; Pautler, Robia; Justice, Monica; Belmont, John
The rise of antibiotic resistance in bacteria and in human parasites is a major public health concern. One resistance mechanism that aids in antibiotic resistance is the bacterial SOS Response, a network of different pathways that help to repair damaged DNA. In some instances, the outcome of the SOS Response is the introduction of mutations into the bacterial genome by error-prone DNA replication. In order to combat this antibiotic resistance, new gene targets must be identified among the approximately one-third of all genes in the Escherichia coli that remain uncharacterized. The malaria parasite Plasmodium falciparum 3D7 causes the most deadly form of malaria in humans. Unlike in E. coli, a much greater number of genes in P. falciparum remain without annotation, and one important yet functionally unknown gene and antimalarial drug resistance marker is the exported protein 1 (exp-1). Exp-1 plays a potentially critical role in malaria pathogenesis and in human immune response. Here, we propose to analyze the E. coli SOS Response and P. falciparum exp-1 using in silico computational tools to identify novel SOS Response genes and to functionally annotate exp-1.

Contributors: Lisewski, Martin; Lichtarge, Olivier
CORRECTION OF PROCESSING OF MUTANT RNAS IN MYOTONIC DYSTROPHIES TYPE 1 AND TYPE 2 WITH RNA HELICASE

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Myotonic Dystrophies type 1 and type 2 (DM1 and DM2) are inherited, complex diseases that affect many organs, including skeletal and cardiac muscles, brain, eye and endocrine system. DM1 and DM2 are caused by non-coding expansions of CTG and CCTG repeats within the myotonic dystrophy protein kinase (DMPK) gene (DM1) and zinc finger factor 9 gene (DM2). Accumulation of DMPK and ZNF9 transcripts containing expanded CUG and CCUG repeats are toxic for cell function. The major goals of this project are to determine molecular mechanisms controlling processing and degradation of mutant CUG and CCUG-containing RNAs and develop approaches to neutralize toxicity associated with these RNAs. We have found that the mutant RNAs have increased stability. As the result, these RNAs do not properly degrade and accumulate in patients’ cells. We have focused on the identification of proteins that may accelerate processing and degradation of these mutant RNAs. To identify these proteins, we have applied several mammalian tet-regulated cell models expressing CUG and CCUG repeats. These cell models were used to examine the activities of CUG and CCUG RNA-binding proteins during accumulation and degradation of normal and mutant CUG and CCUG-containing transcripts. Several RNA-binding proteins with altered CUG and CCUG RNA-binding activities during degradation of mutant RNAs have been identified. One of these proteins, with a molecular weight of 75 kDa, was purified and sequenced. We determined that the 75 kDa protein is in fact p68 RNA helicase (DDX5), a member of the DEAD-box family of proteins. The levels of DDX5 are significantly reduced in muscle biopsies from patients with DM1 and DM2 and in the skeletal muscle of a DM1 mouse model (CTG transgenic mice). Experiments are currently under way to determine if the delivery of DDX5 into human muscle cells and into the muscle of CTG transgenic mice will accelerate processing and degradation of mutant CUG and CCUG repeats and correct DM1 and DM2 pathologies.

Contributors: Jones, Karlie; Jin, Bingwen; Iakova, Polina; Bugiardini, Enrico; Schneider-Gold, Christiane; Meola, Giovanni; Timchenko, Nickolai; Timchenko, Lubov
Noise is a major concern in circuits processing electrical signals, including neural circuits. There are many factors that influence how noise propagates through neural circuits, and there are few systems in which noise levels have been studied throughout a processing pathway. We recorded intracellularly from multiple stages of a sensory-motor pathway in the locust that detects approaching objects. We find that responses are more variable and that signal to noise ratios (SNRs) are lower further from the sensory periphery. SNRs remain low even when using stimuli for which the pathway is most selective and for which the neuron representing its final sensory level must integrate many synaptic inputs. Modeling of this neuron shows that variability in the strength of individual synaptic inputs within a large population has little effect on the variability of the spiking output. In contrast, jitter in the timing of individual inputs and spontaneous variability is important for shaping the responses to preferred stimuli. These results suggest that neural noise is inherent to the processing of visual stimuli signaling impending collision and contributes to shaping neural responses along this sensory-motor pathway.

Contributors: Jones, Peter; Gabbiani, Fabrizio
GENOME-WIDE ANALYSIS OF THE TARGETS OF THE SINE OCULIS TRANSCRIPTION FACTOR IN THE DROSOPHILA EYE

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Program in Developmental Biology
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The Drosophila eye provides a well-characterized system for studying the genetic basis of cell fate specification and differentiation in development. Drosophila eye formation depends on the highly conserved gene sine oculis (so), whose mammalian homologs play vital roles in eye and brain development. so encodes a DNA-binding homeodomain transcription factor, previously shown to be required for multiple processes in the developing eye, including cell survival and differentiation. Despite its importance in development, few direct targets of So are known. To gain a global view of the transcriptional targets of So, we have performed chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) to enrich for genome regions bound by So in eye discs, the larval precursors to the adult fly eye. We identified 7,566 regions (peaks) that are significantly enriched (p<1x10-5) for So binding, including regions in ten out of twelve previously identified So target genes. Gene Ontology (GO) analysis of So peak-containing genes shows enrichment for terms relevant to eye development, such as photoreceptor cell differentiation and neurogenesis. Many of the So-enriched regions map to or near genes that have not been previously studied in the Drosophila eye. We have obtained a collection of lethal mutations in a subset of such novel So-enriched genes, and are currently analyzing the effect of these mutations on eye development. We have identified several putative So target genes that are necessary for eye development, and cause a variety of defects – ranging from disorganized ommatidia to severely reduced eye size – when mutated. We plan to characterize a subset of these novel genes in detail and to test whether So regulates their expression in the developing Drosophila eye.

In parallel, we are performing high-throughput mRNA sequencing (RNA-seq) to compare gene expression levels between wild-type and so loss-of-function eye discs on a genome-wide scale. We predict that the overlap between So peak-containing genes and genes that are differentially expressed in response to so loss will allow us to identify novel direct targets of So. In summary, our experiments aim to expand our understanding of a transcriptional regulator that is vital to human as well as Drosophila development.

Contributors: Jusiak, Barbara; Karandikar, Umesh; Kwak, Su-Jin; Chen, Rui; Mardon, Graeme
Adoptive T-cell therapy has had considerable success in effectuating antitumor responses, however complete eradication of bulky disease is rarely observed. This limited efficacy is most likely due to the tumor stroma, which is not targeted by tumor-specific T cells. Cancer associated fibroblasts (CAFs), the central component of the tumor stroma, secrete inhibitory factors and nutrient depleting enzymes that are detrimental to effector T-cell function. In addition, CAFs promote angiogenesis and secrete extracellular matrix components, which act as a physical barrier. CAFs express fibroblast activation protein (FAP); a membrane bound serine protease, which is an attractive immunotherapeutic target. The aim of this project was to generate FAP-specific T cells and determine if targeting the tumor stroma with FAP-specific T cells has antitumor effects.

Methods: To generate FAP-specific T cells we took advantage of chimeric antigen receptors (CARs), which consists of antigen-specific single chain variable fragments (scFv) linked to T-cell receptor signaling domains. Using this approach we generated 2 CARs, which are specific for human FAP (hFAP) or human and murine FAP (mhFAP). T cells expressing hFAP-CARs or mhFAP-CARs were generated by retroviral transduction (hFAP- or mhFAP-T cells). Ex vivo, efficacy of hFAP- and mhFAP-T cells was determined by their ability to 1) secrete cytokines in coculture experiments with FAP-positive tumor or stroma cells, and 2) kill FAP-positive targets in cytotoxicity assays. To test in vivo, if selective targeting of FAP on tumor stroma prevents the development of human tumors in a xenograft model, we took advantage of lymphoblastoid cell lines (LCL), which are FAP-negative.

Results: hFAP-CARs or mhFAP-CARs were successfully expressed on T cells as judged by FACS analysis. hFAP- T cells recognized FAP-positive human tumor and stroma cells as judged by cytokine production and cytotoxicity assays. In addition to recognizing human FAP positive targets, the mhFAP-T cells also recognized and killed murine FAP targets. To evaluate if targeting the tumor stroma, prevents the development of tumors, FAP-negative, luciferase-expressing LCLs, were mixed with hFAP, mhFAP-, or nontransduced T cells prior to the s.c. injection into flanks of SCID mice. While LCLs tumor readily established in mice injected with LCL/hFAP-T cells or LCL/NT-T cells; LCL tumor growths was 10 to 100 fold slower in mice injected with LCL/mhFAP-T cells as judged by serial bioluminescence imaging.

Conclusions: Our results indicate that targeting FAP-positive stroma with T cells delays the development of tumors in human xenograft models. Thus, targeting the tumor stroma in addition to cancer cells with FAP-specific T cells has the potential to improve current immunotherapy approaches for cancer.
Metabotropic glutamate receptors (mGluRs) are important for modulating signaling by glutamate, the main excitatory neurotransmitter in the central nervous system. The mGluRs have been implicated in protection from neuronal excitotoxicity and in learning and memory. These receptors have multiple functional sites: the “venus flytrap” ligand binding module of the N-terminal domain, the transmembrane domain, and the dimerization interfaces. There have been few studies of which residues may be important for ligand specificity differences between Group1 and Group2 (mGluR1, 2, 3, 5) and Group3 (mGluR4, 6, 7, 8). Specially, the endogenous ligand, L-serine-O-phosphate shows much higher binding affinity toward Group 3 mGluR over Group1 and Group2. To determine important residues for L-SOP binding, the Lichtarge group and ours have used the Evolutionary Trace to analyze a number of sequences from the class C GPCR family, based on the known crystal structure of the amino terminal domain of mGluR. The results have identified G319, Q170, L342, N415, S344 and S189 that may be functional residues that uniquely characterize that ligand binding. As the first step to test these residues, I have prepared expression plasmids for c-myc-tagged mGluR1 and mGluR4. As the expression system, I used HEK-293 cells transiently coexpressing Gα15 protein that yield Ca2+-mobilization detectable by a high-throughput fluorescence assay, in response to activation of many GPCR (G-protein-coupled receptors). I tested for mGluR expression by assaying for Ca2+ mobilization in response to glutamate, and by immunoblotting. I also successfully detected cell surface expression of mGluR1 and mGluR4 by immunocytochemistry. Then I generated reciprocal mutants for mGluR1 and mGluR4 respectively. mGluR4 mutants, S319G, P342L, D415N and R344S showed decreased response for L-SOP, a ligand of mGluR4. Two mutants, A189S and R344S showed decreased response to another ligand, L-Glutamate. For mGluR1 mutants, L342P and S344R showed increased response to L-Glutamate but G319S, S189A and Q170M didn’t show response for L-SOP indicating these residues may be not enough to generate new function, gaining affinity for L-SOP. Currently, the surface expression level detection assay is in progress to validate the surface expression of mutants is similar to their wild-type’s expression level.
THERMODYNAMICS OF OLIGOGLYCINE IN WATER

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Intrinsically disordered proteins (IDPs) are proteins that do not fold into a stable, three-dimensional, native structure, except possibly when bound to a ligand, partner protein, etc. Evidence suggests that IDPs function by varying the thermodynamics of recognition and binding by proteins, and thus affect the affinity and specificity with which the proteins bind to their partners. For example, the ultrabithorax (Ubx), a member of the Hox family of proteins, binds to DNA via a homeodomain motif, but the remainder of the protein comprises largely of disordered regions. Removal of the disordered regions decrease the affinity with which Ubx binds to DNA. Sequentially IDPs tend to be rich in glycines, since glycine has the most degree of freedom allowing the protein the largest degree of flexibility. For example, in Ubx, glycine has a 17% occurrence. The protein also has a sequence of 13 consecutive glycines in the disordered, functionally relevant region. Thus oligoglycine is an ideal model to study the thermodynamics of the folding/unfolding transitions in an IDP.

The solubility of oligoglycine in water decreases as its length increases until, when the peptide contains 5-6 glycines, it aggregates and falls out of solution. However, previous work in our lab shows that the solvation free energy of oligoglycine increases as its length increases from 2 to 5 residues. We hypothesise that in longer sequences, the intermolecular interactions between oligoglycines are favoured over interactions between oligoglycine and water, leading to their aggregation. In a similar manner very long sequences of oligoglycine, such as those observed in Ubx, fold and form intramolecular interactions with itself that are favoured over interactions between oligoglycine and water. We use an oligoglycine containing a sequence of 25 consecutive glycines as a model to analyse the thermodynamic and structural changes that occur when the polypeptide in placed in a box of water.

We have performed the simulation of a long oligoglycine of 25 (Gly25) residues in a box of water for 100ns. The oligoglycine explores a wide range of conformations during the simulation. We have analysed the thermodynamic and structural changes that occur across the various conformations that Gly25 assumes in the course of the simulation.

Contributors: Karandur, Deepti; Pettitt, B M
Background and Objectives: Dendritic Cell (DC) vaccines have been developed to exploit the cells’ awesome potential for anti-tumor immunity. Our lab has focused on augmenting DC vaccines using an inducible MyD88/CD40 signaling switch (iMC) capable of stimulating DC activation. In ex vivo pre-established tumor studies, DCs pulsed with tumor-specific antigen (TSA) and manipulated to express the iMC significantly decreased tumor growth in mice relative to DCs pulsed with TSA alone.

Ex vivo modification and re-delivery of DCs is highly specialized and costly, limiting its utility as a primary cancer-fighting agent. Therefore, we have developed a strategy capable of TSA and iMC delivery to in vivo DCs using adenovectors. Previous data showed that treatment of tumor-bearing mice with adenovectors expressing only TSA was effective in significantly decreasing tumor growth. Co-injection with iMC in a separate vector yielded no improvement in anti-tumor efficacy. We hypothesized that this failure was due to the strategy’s reliance on simultaneous transgene expression by two separate vectors in the same cell. We observed that such double expression occurs in only about 30-40% of relevant cells. Therefore, we developed a bicistronic adenovector (BAdv) encoding both TSA and the iMC.

Methods: Peripheral blood, tumor resident lymphocytes, and splenocytes of BAdv treated mice were assessed for antigen specificity and function using MHCI-restricted tetramer analysis and IFN-γ ELISpots, respectively. Additionally anti-tumor responses were tested using mice bearing pre-established B16-LacZ tumors treated with footpad injection of the BAdv.

Results: Peripheral blood tetramer analysis showed the development of significant antigen specific immune responses. Mice treated with either Ad-LacZ or BAdv yielded similar levels of tetramer positive CD8+ T-cells. However, when these cells were assayed for functionality using an ELISpot assay, a significantly higher amount of CD8+ T-cells from BAdv versus Ad-LacZ treated mice secreted IFN-γ. Additionally, when tumors of mice injected with Ad-LacZ or BAdv were analyzed, a significantly higher amount of antigen specific CD8+ T-cells were found in the tumors of BAdv mice. Finally, treatment of B16-LacZ tumor-bearing mice with BAdv resulted in significantly enhanced tumor reduction versus Ad-LacZ alone.

Conclusions: Dual delivery of iMC with TSA in a single adenoviral vector enhances anti-tumor immune responses compared to treatment with Ad-TSA alone. Mechanistically the iMC is able to enhance the functionality of antigen specific CD8+ T-cells induced by antigen production presumably via localized IL-12 secretion and enhanced relevant DC activation.

Contributors: Kemnade, Jan Ole; Seethammagari, Mamatha; Narayanan, Priya; Levitt, Jon; Spencer, David
DEPLETION OF α/β T CELLS ATTENUATES OBESITY-ASSOCIATED INFLAMMATION AND METABOLIC ABNORMALITIES

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Vijay Nambi, M.D.-Department of Medicine

Recent investigations have linked obesity with low-grade chronic inflammation in adipose tissue (AT), which causes AT dysfunction, thus contributing to obesity associated metabolic abnormalities. Diet-induced obesity is correlated with accumulation of “classically activated” (M1) macrophages in AT, which secret proinflammatory cytokines and impair insulin signaling and lipid storage in adipocytes. In addition, obesity alters T cell numbers in AT. However, the role of T cells in metabolic complications of obesity is less defined. Using mouse model of diet-induced obesity, we found that both αβ and γδ T cells reside in perigonadal AT of lean mice. However, only αβ T cells were significantly increased in AT of obese mice compared with lean controls. Therefore, we studied the role of αβ T cells in AT inflammation and metabolic dysfunctions using TCRβ-null mice, deficient in α/β T cells, with C57BL/6J as WT controls. Obesity was induced by high fat diet (HFD; 21% fat) feeding with mice on normal diet (ND; 5% fat) as lean controls. TCRβ-null mice on HFD gained similar amount of body weight, but had significantly heavier perigonadal fat pads with enlarged adipocytes. Nevertheless, we observed lower fasting glucose levels and improved glucose and insulin tolerance in obese TCRβ-null mice compared to obese WT. Compared to obese WT, obese TCRβ-null mice had reduced levels of IFN-γ, a Th1 cytokine, and decreased M1 macrophage contents with concomitant decreases in levels of cytokines (TNF-α) and chemokines (MCP-1, RANTES) in AT, indicating that HFD-induced AT inflammation was blunted in the absence of α/β T cells. Obese TCRβ-null mice also had significant reduction in triglyceride content and inflammation in skeletal muscle. In conclusion, αβ T cells, which are increased in AT with obesity, play an essential role in obesity-associated AT inflammation and metabolic complications.

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CONSEQUENCES OF VITAMIN D RECEPTOR ACTION IN TMPRSS2:ERG OVEREXPRESSING PROSTATE CANCER CELLS

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Treatment with the active metabolite of vitamin D (1,25D), or the less calcemic analog of 1,25D (EB1089) decreases growth of many prostate cancer (PCa) cell lines both in vitro and in vivo. The actions of 1,25D are mediated by vitamin D receptor which is a hormone activated transcription factor. Recently, it was discovered that many and perhaps even the majority of PCa contain some form of TMPRSS2:ETS factor fusion gene (T/E) where the promoter region of the androgen regulated transmembrane protease serine 2 (TMPRSS2) is fused with the coding regions of E26 transformation specific (ETS) transcription factors. The most commonly occurring T/E in human PCa is TMPRSS2:ERG. This fusion promotes growth, invasiveness and motility of PCa cells. Surprisingly, in TMPRSS2:ERG containing VCaP PCa cells, both 1,25D and EB1089 induced the TMPRSS2:ERG expression. Despite this induction, EB1089 still inhibited growth of VCaP cells in vitro. EB1089 reduced the activity of an ERG responsive luciferase reporter in vitro, which suggests that even though EB1089 induces expression of the growth stimulating TMPRSS2:ERG fusion gene, it may inhibit cell growth by inhibiting selected ERG activities. Next, an in vivo study was performed to confirm the in vitro findings. VCaP PCa cells stably expressing luciferase were injected into SCID mouse prostate, and mice were treated with vehicle (sesame oil) or EB1089 for five weeks via oral gavage. The tumor growth was monitored by measuring the intensity of luciferase activities. EB1089 induced TMPRSS2:ERG expression in the tumors as seen in vitro, but it also induced the TMPRSS2:ERG target gene expression which suggested that EB1089 failed to inhibit the TMPRSS2:ERG activity in vivo. Furthermore, EB1089 did not inhibit the tumor growth in vivo suggesting the possible harmful effects of vitamin D signaling in vivo. Despite the importance of studying prostate cancer in a TMPRSS2:ERG containing background, the lack of model systems limits the research. To overcome this problem, two different inducible stable cell lines, type III + 72 ERG (3+72 ERG) and type VI + 72 ERG (6+72 ERG), the most common fusions, in LNCaP PCa cells were generated. The induction of 3+72 ERG or 6+72 ERG in a dose dependent manor upon treatment with doxycycline was successful. The functional activity of these 3+72 ERG and 6+72 ERG was validated using an ERG responsive luciferase reporter. Despite the overexpression of 3+72 ERG or 6+72 ERG, 1,25D still inhibited the cell growth. To our surprise, overexpression of 3+72 ERG or 6+72 ERG also reduced cell growth. Furthermore, increasing expression of 3+72 ERG or 6+72 ERG caused super induction of the vitamin D receptor target gene, CYP24, upon 1,25D treatment indicating the possible cooperation of ERG with vitamin D receptor at the transcriptional level. Our studies suggest that vitamin D receptor action in TMPRSS2:ERG containing PCa may not be beneficial.

Contributors: Kim, Jung-Sun; Weigel, Nancy
REACTIVE STROMA STEM/PROGENITOR CELLS IN PROSTATE CANCER: MECHANISMS OF TGF-(1 PROMOTED MYOFIBROBLAST DIFFERENTIATION

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Reactive stroma in prostate cancer is typified by the co-evolution of myofibroblasts and carcinoma-associated fibroblasts (CAFs), yet the origin and biology of myofibroblast stem/progenitor cells are not known. TGF-(1 is overexpressed in prostate cancer cells and affects myofibroblast differentiation, a key step in formation of a tumor-promoting reactive stroma. However, specific mechanisms of how TGF-(1 regulates recruitment, activation and differentiation of reactive stroma stem/progenitor cells are poorly understood. Reactive stroma stem/progenitor cell lines were initiated from organ cultures of normal human prostate gland and characterized by a novel 3D co-culture model. Differentially engineered LNCaP cells to overexpress active TGF-(1 were cultured as organoids on CM-inserts and progenitor cells were co-cultured with engineered LNCaP cells either in direct contact or on laminin-coated coverslips in the bottom chamber. 3D organoids grown in serum-free media for 72 hours were analyzed by IHC, ELISA and qRT-PCR. In addition, shRNA gene knockdown experiments were performed in reactive stroma stem/progenitor cells to identify key transcription factors involved in myofibroblast differentiation. Reactive stroma stem/progenitor cells were CD44+/CD90+ and showed characteristics and gene expression similar to human mesenchymal stem cells. In the 3D model, LNCaP cells and reactive stroma stem/progenitor cells self-organized into a free-floating organoid. The organoids exhibited a periphery of LNCaP cells and a core of reactive stroma-appearing cells. Significantly, LNCaP cells induced myofibroblast differentiation of reactive stroma stem/progenitor cells and this was promoted by LNCaP expression of active TGF-(1. Gene expression analysis identified the RUNX1 and ID1 transcription factors as upregulated in reactive stroma stem/progenitor cells by TGF-(1. Knockdown of RUNX1 and ID1 are being used to assess the role and mechanisms of how these transcription factors regulate myofibroblast differentiation. Together, these data suggest that the genesis and co-evolution of reactive stroma in prostate cancer results from activation and differentiation of CD44+/CD90+ endogenous reactive stroma stem/progenitor cells via upregulation of specific transcription factors by TGF-(1. Defining key mechanisms that recruit, activate and differentiate the origin of myofibroblasts/CAFs could provide clues to develop new strategies to target the microenvironment niche.

Contributors: Kim, Woosook; Barron, David; Rowley, David
CHARACTERIZATION OF CLAUDIN-LOW SUBTYPE OF BREAST CANCER

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Breast cancer is a highly heterogeneous disease, with multiple subtypes identified. In collaboration with the Medina laboratory we developed a transplantable p53-null GEM model with a signature that corresponds to the rare claudin-low subset of breast tumors. This subset is enriched in Tumor-Initiating-Cells (TICs), which are thought to be responsible for drug resistance and recurrence. One of the pathways that contribute to drug resistance in claudin-low tumors is PI3K/Akt pathway. This is an important signaling pathway in TICs, and is now a major target for new breast cancer treatments. Other pathways that regulate the progression of claudin-low tumors still remain to be elucidated. TICs also exhibit decreased expression of certain microRNAs, shown to be critical for Epithelial-Mesenchymal Transition (EMT). Members of the miR200 family are down-regulated in TICs, and represent a part of the feedback loop with inducers of EMT, such as Zeb1 and Snail. The goal of this project is to characterize the signal transduction pathways of claudin-low tumors, specifically the role of miR-200 family members and how they affect tumor progression and therapeutic resistance.

Contributors: Knezevic, Jana; Herschkowitz, Jason; Rosen, Jeffrey
We demonstrated previously that leukocyte function associated antigen 1 (LFA-1), comprised of the integrins CD11a and CD18, is required for recruitment of T helper type 2 (TH2) cells and induction of immunopathologic disease states attributable to accelerated TH2 responses. Two experimental examples of such TH2-dependent immune dysfunction include uncontrolled infection with the cutaneous protozoan parasite Leishmania major and asthma-like allergic lung disease. We identified polymorphisms in the metal ion binding domain of CD11a between Balb/c and C57BL/6 mice and hypothesized that such polymorphisms mediate functionally significant changes in CD11a mediated adhesion in TH2 cells. Congenic Balb/c-background mice expressing the C57BL/6 CD11a allele (BalbC57) that were infected with L. major showed significantly reduced progression of the cutaneous infection when compared to wild-type Balb/c mice, as defined by footpad swelling and parasite burdens. Dissemination of parasites to the spleen was also significantly reduced, indicating that C57BL/6 CD11a allele confers enhanced disease control. Similarly, in the allergic lung disease model, BalbC57 mice showed attenuated allergic disease, whereas congenic C57BL/6 mice with the Balb/c CD11a allele (C57Balb) showed enhanced allergic disease when compared to genotype-matched wild type animals. Corresponding directly with these in vivo observations, the Balb/c CD11a allele conferred relatively greater T cell adhesion to ICAM-1 under dynamic flow conditions in vitro relative to the C57BL/6 allele. Moreover, adoptive in vivo T cell transfer experiments confirmed the greater potential of T cells possessing the Balb/c CD11a allele to home to lungs during induction of allergic lung disease. Finally, analysis of human CD11a polymorphisms revealed a single nucleotide polymorphism that significantly correlated with clinically relevant markers of allergic and atopic diseases. These results demonstrate that polymorphisms in CD11a critically modulate TH2 cell homing and expression of diverse TH2-dependent immunopathologic states and suggest that similarly important polymorphisms exist in humans.

Contributors: Corry, David; Lee, Seung-Hyo
Polarity is a property of all cells and is required for proper functioning and development of organisms. While the loss of apical-basal cell polarity is associated with carcinogenesis, a similar possible cancer link for loss of anterior-posterior (AP) cell polarity has not been examined. Tumor viruses have long been used as tools to reveal molecular mechanisms for human cancers. The primary oncogenic determinant of human adenovirus type 9 is the 125-residue E4-ORF1 protein, which targets several different cell polarity proteins, including Dlg1, PATJ, and ZO-2. Dlg1 and PATJ are chief regulators of AP cell polarization, which is necessary for cell functions such as directed migration, T-cell activation, and stem cell renewal. Unpublished data from our lab suggests that E4-ORF1 inhibits AP polarization and directed migration of rodent fibroblasts. Thus, I hypothesize that the tumorigenic potential of Ad9 E4-ORF1 stems in part from its ability to block AP cell polarization by interfering with the polarity functions of Dlg1, PATJ, and possibly ZO-2. To test this hypothesis, I have chosen to use human MCF10A breast epithelial cells in scratch wound assays (Aim 1) to determine whether E4-ORF1 blocks AP polarity by binding to Dlg1, PATJ, and ZO-2; (Aim 2) to identify domains and activities of Dlg1, PATJ, and ZO-2 required for anterior-posterior polarity establishment; and (Aim 3) to link the tumorigenic activities of E4-ORF1 to its effects on these polarity proteins. Immunofluorescence studies showed that whereas Dlg1 localization at the leading edge membrane is similar between E4-ORF1-expressing and control non-expressing cells, PATJ localization at the leading edge cannot be seen in E4-ORF1 cells, hinting that the interaction between E4-ORF1 and PATJ may decrease AP cell polarization. Preliminary data comparing E4-ORF1-expressing and control non-expressing human MCF10A cells at the wound edge also suggests that E4-ORF1 may reduce AP cell polarity establishment (62% of control cells versus 40% of E4-ORF1 cells). The latter data was obtained by manual scoring of AP polarity. I am in the process of employing an automated, high-throughput system to score repositioning of the microtubule organizing center (MTOC), a measure of AP polarity establishment. The power of this system is underscored by its rapid ability to calculate the angle between the MTOC and wound edge for thousands of cells. Future work will focus on using this automated system to quantify the effect of E4-ORF1 on AP cell polarization and demonstrate statistical significance. I will also test whether knockdown of Dlg1, PATJ, or ZO-2 expression likewise inhibits AP cell polarization. These studies may provide new insights into the molecular mechanisms by which defects in cell polarity establishment provoke neoplasia in people.

Contributors: Kong, Kathleen; Newberg, Justin; Taruishi, Midori; Hilsenbeck, Susan; Javier, Ronald T.
BAG6 is a member of the BAG family proteins that plays an important role as a co-chaperone. Recently, it was shown that BAG6 forms a stable complex with UBL4A and GET4 and functions in membrane protein targeting and degradation of mislocalized and misfolded proteins through the ERAD pathway. However, the BAG6 sequence contains a canonical nuclear localization signal and localizes predominantly in the nucleus. The function of BAG6 complex in the nucleus is not known. We have identified BAG6 as a potential substrate for checkpoint kinase ATM/ATR in a proteomics screen, suggesting that BAG6 complex may participate in DNA damage response. In this work, we provide evidence that BAG6 is phosphorylated in an ATM-dependent manner at serine-212 residue within an ATM/ATR phosphorylation consensus (S/TQ) motif. Depletion of UBL4A by siRNA results in a delay of BAG6 phosphorylation upon IR treatment. Moreover, BAG6 appears to be the central component of the complex, as depletion of BAG6 leads to the loss of the other two components of the BAG6 complex. UBL4A and GET4, which mainly localize in the cytoplasm, translocate to the nucleus upon exposure to DNA damaging reagents. Importantly, depletion of BAG6 causes disruption of DNA damage-induced BRCA1 foci and increased sensitivity to DNA damage. Taken together, our results show that BAG6 complex is a new component of the ATM-mediated DNA damage response pathway.

Contributors: Liu, Shangfeng; Shi, Yi; Yucer, Nur; Kim, Beom-Jun; Odejimi, Ore Abiola; Qin, Jun; Wang, Yi.
(P)PPGPP REGULATES GTP LEVELS TO ALLOW FOR SURVIVAL OF B. SUBTILIS

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The unusual nucleotide (p)ppGpp is known to affect bacterial physiology in numerous ways and is known to be essential for growth during nutrient limitation, however, exactly how (p)ppGpp allows for survival during nutrient stress remains unknown. To determine the critically important physiological effect of (p)ppGpp, we conducted an unbiased suppressor screen for mutants of a strain that is unable to produce (p)ppGpp, (p)ppGpp0, which cannot grow without amino acid supplementation. We identified loss-of-function mutations in the genes codY, guaB, guaA and gmk, which enable growth of the (p)ppGpp0 strain during amino acid limitation. These suppressors rescue the (p)ppGpp0 strain by decreasing GTP levels, indicating that reduction of GTP levels by (p)ppGpp is the key physiological event that allows for cell survival during nutrient limitation in B. subtilis. We also reveal a surprising requirement for (p)ppGpp during growth in environments where external purine sources are unbalanced. Low levels of (p)ppGpp are sufficient to control GTP homeostasis by inhibiting the salvage pathway of GTP biosynthesis, thus protecting cells from high levels of external guanosine. We conclude that (p)ppGpp is an obligatory link in the control of intracellular GTP levels in response to external fluctuations in nutrient availability and we demonstrate the importance of GTP homeostasis for cell survival.

Contributors: Kriel, Allison; Liu, Kuanqing; Tehranchi, Ashley; Zou, Winnie; Rendon, Samantha; Chen, Rui; Wang, Jue
Membrane-bound compartments or organelles exist in well-defined sizes and copy numbers in a given cell type. Transitions in organellar size and number are apparently determined by the fundamental processes of membrane fission and fusion. However the control mechanisms governing fusion of vesicles and its coordination with the antagonistic fission activity are largely unexplored. It is worthwhile to investigate this tight regulation since a deviation from fusion-fission equilibrium may lead to accumulation of structural and functional defects in organelles and ultimately impaired organelle homeostasis. While intracellular membrane fusion is largely mediated by SNARE proteins and tethering complexes, fission needs the presence of dynamin or dynamin-related proteins. Mutations in dynamins are known to be associated with a wide range of neuromuscular disorders including Charcot-Marie-Tooth disease. Studying membrane dynamics in native yeast vacuoles, we found that the yeast dynamin homolog Vps1, a multidomain large GTPase, is not only an essential part of the fission machinery but also controls membrane fusion. It does so by generating an active Qa SNARE pool that is capable of undergoing trans-SNARE formation. In the absence of Vps1, the Qa SNARE Vam3 does not associate with the tethering complex HOPS leading to fusion-incompetent organelles. Moreover, fusion of vacuoles is inhibited in vivo and in vitro by the dynamin inhibitor Dynasore that prevents the release of Vps1 from the vacuolar compartment and impedes trans-SNARE formation. We propose that dynamins, besides their well-established function in membrane fission, also control membrane fusion by directly acting on SNARE proteins.
Sexually attractive characteristics are often thought to reflect an individual’s condition or reproductive potential, but the underlying molecular mechanisms through which they do so are generally unknown. Insulin signaling (IIS) is known to modulate aging, reproduction, and stress resistance in several species and to contribute to variability of these traits in natural populations. Here we show that insulin/insulin-like signaling (IIS) determines sexual attractiveness in Drosophila through transcriptional regulation of genes involved in the production of cuticular hydrocarbons (CHC), many of which function as pheromones. Using traditional gas chromatography/mass spectrometry (GC/MS) together with newly introduced laser desorption/ionization orthogonal time-of-flight mass spectrometry (LDI-MS) we establish that CHC profiles are significantly affected by genetic manipulations that target insulin signaling. Manipulations that reduce IIS also reduce attractiveness, while flies with increased IIS are significantly more attractive than wild-type animals. Insulin signaling influences CHC through pathways that are likely independent of dFOXO and that may involve the nutrient-sensing Target of Rapamycin (TOR) pathway. These results suggest that the activity of conserved molecular regulators of longevity and reproductive output may manifest in different species as external characteristics that are perceived as honest indicators of fitness potential.
THE IMPACT OF TUMOR-ASSOCIATED FIBROBLASTS ON THE FUNCTIONAL STATUS OF TUMOR INITIATING CELLS IN BLADDER CANCER

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Bladder cancer is the second most common urological malignancy in the US with 69,250 new cases estimated for 2011. The incidence of bladder cancer has increased by 1.6 times in the past three decades, while the cure for muscle invasive bladder cancer remains limited. A potential reason for ultimate progression is likely due to the properties of bladder cancer tumor-initiating cells (TICs) and their active interactions with tumor microenvironment. Tumor associated fibroblasts (TAFs) are considered to be a predominant component of tumor microenvironment in epithelial cancers and implicated to many aspect of tumorigenesis. TAFs can affect survival, differentiation, proliferation and migration of tumor cells by direct contact-induced mechanisms and paracrine effect of various secreted molecules, such as cytokines and components of extracellular matrix.

Our preliminary experiments confirmed the presence of activated fibroblasts (co-expressing vimentin, alpha-smooth actin and tenascin C) in close proximity to CD44+CD49f+ TICs in muscle-invasive bladder cancer. Using primary explant model we isolated several fibroblast lines co-expressing vimentin and alpha-smooth actin directly from patient-derived tumor samples. Interestingly, xenografted tumors formed from co-injection of TICs with ex-vivo expanded TAFs showed high collagen I deposition and change in growth characteristics. We confirmed by RT-PCR that TAFs are the major source of collagen I. We hypothesize that TAFs, characterized by co-expression of fibroblast activation markers can affect the biological properties of neighboring TICs via secreting and modulating extracellular matrix components, such as collagen I. We are currently testing whether collagen I secreted by TAFs can affect TICs expansion, proliferation status and metastatic potential. We anticipate that detailed understanding of TICs-TAFs interactions mediated by collagen I will help to rationalize the choice of potential drug targets. Additionally, we predict that possible prognostic significance of activated TAFs can be used to improve the risk stratification strategy in bladder cancer.

Contributors: Kurtova, Antonina; Chan Keith
NEW TARGET FOR THE OPTICAL IMAGING OF LYMPHANGIOGENESIS

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Advisor: Eva Sevick-Muraca, Department of Radiology

Lymphangiogenesis, the process of forming new lymphatic vessels, is thought to play a crucial role in an array of diseases including cancer metastasis and chronic inflammation.

Despite the importance of lymphangiogenesis, there are no validated markers for the process of lymphangiogenesis as there are for angiogenesis. In our project, we seek to validate the first markers for lymphangiogenesis and to develop an imaging agent to visualize lymphangiogenesis in vivo. We hypothesize that ITGA9, an integrin which has been linked to lymphangiogenesis in mouse models and in vitro experiments, is a potential marker for lymphangiogenesis. In the work presented herein, we establish that ITGA9 expression is changed during inflammation-induced lymphangiogenesis to promote cell migration and proliferation and we are developing an optical agent targeting ITGA9 able to identify tissue regions in which lymphangiogenesis is underway in vivo.

Using a model of oxazolone-induced acute inflammation we show use non-invasive, near-infrared fluorescence imaging with a non-specific lymphatic contrast agent (indocyanine green) to observe dramatic changes in lymphatic architecture with inflammation. The lymphatic vessels associated with inflammation are leaky, enlarged, and do not conduct the dye possibly indicative of lymphatic remodeling was occurring. To further characterize the molecular mechanism of lymphangiogenesis, we have developed a flow cytometry based assay to assess the number of proliferating lymphatic endothelial cells (LECs) in vivo, to identify markers on the cells surface, and to isolate LECs from mouse tissue. This novel method will be used to characterize LECs in mouse models of lymphangiogenesis. Using these validated models of lymphangiogenesis, we seek to image the process of lymphangiogenesis with molecularly specific agent that targets ITGA9.

A peptide motif, EIDGIEL, specifically recognize the ligand binding site of ITGA9 was synthesized, labeled with a near-infrared fluorescent dye, IRDye800, and shown to possess biological activity through binding to SW-480 cells overexpressing human ITGA9 in vitro and a Kd of 10µM. Labeled peptides displayed binding inhibition in the presence of Tenascin C and VCAM-1, two ligand of ITGA9. To further characterize the imaging agents we are currently assessing testing the peptides in vivo in the inflammation model described above. While preliminary in vivo data suggests specific binding, further characterization is underway. Supported in parts by R01 HL092923, R01 CA128919, and the Canadian Institute of Health Research Pre-doctoral Fellowship.

Contributors: Hazen, Amy; Sevick, Eva.
Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for many patients with hematological malignancies. However patients are immunocompromised to maximize chances of successful transplantation. This condition leaves them dangerously susceptible to common virus infections such as cytomegalovirus (CMV). Anti-viral drugs are either ineffective or have adverse side effects. Thus, alternative methods of protecting patients against these infections would greatly improve HSCT.

Adoptive T cell therapy can be a promising option. Our group has already developed modified T cells against EBV, Ad, and CMV-pp65 from CMV sero-positive (SP) and CMV sero-negative (SN) donors as well as cord blood (CB). These T cells had effective anti-viral activity regardless of their source. Interestingly, T cells derived from naïve sources (cord blood and SN) recognized different epitopes of CMV antigen pp65 than those recognized by T cells derived from SP. Whether this is characteristic of all naïve T cells or restricted to the CMV pp65 antigen is unknown.

We hypothesize that T cells specific for other antigens generated from naïve T cells will also recognize different epitopes of latent viruses from those recognized by seropositive donor derived T cells. We will test this hypothesis with the following aims: Aim 1 Determine CMV-IE1 epitope usage of naïve T cells IE-1 is another CMV antigen and importantly, it is the first protein to be expressed upon CMV reactivation. We have successfully generated T cells specific for pp65 and IE-1 from SP donors. We are in the process of generating IE-1 specific T cells from naïve sources, SP and CB. Aim 2 Evaluate the typical and atypical epitope usage of CB, SN, and SP T cells for antigens from other latent viruses Atypical epitope usage of naïve T cells may be a phenomenon exclusive to the herpesvirus family or is it an aspect of the T cell response to viral infection? We will study epitope usage of another virus family, human immunodeficiency virus-1 (HIV) by generating HIV-gag specific T cells from SP (currently on standard anti-retroviral therapy), SN, and CB donors. Currently we have generated HIV-gag specific T cells from 1 out of 3 SN donors as shown by ELIspot. Our goal is to successfully generate virus-specific T cells with a dual purpose (1) so that we can study atypical epitope usage of naïve T cells beyond CMV-pp65 and (2) our virus specific T cells can have therapeutic potential in the future.
THE REGULATION OF UTERINE RECEPTIVITY BY EGFR

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The establishment of the appropriate embryonic-uterine interactions necessary for successful implantation and pregnancy requires the coordinate actions of growth factors and their receptors. The spatiotemporal expression of Erbb ligands and receptors in the periimplantation uterus and embryo suggests that they play a role in reproduction. Numerous Erbb ligands have overlapping expression patterns in the uterus during pregnancy, and gene ablations of individual Erbb ligands do not show a major uterine phenotype due to compensation by other family members. We have identified EGFR (ERBB1) as being induced during the implantation period and hypothesize that EGFR signaling regulates the ability of the endometrium to support embryo implantation. This hypothesis is being tested in vivo using mouse models and in vitro using primary human endometrial stromal (HES) cell culture and a novel high-throughput microscopy assay.

In order to circumvent the embryonic lethality of Egfr-/- mice, conditional ablation of Egfr was achieved by crossing an Egfr floxed (Egfrf/f) mouse with a progesterone receptor cre (PRCre) mouse, resulting in Egfr deletion in all PR positive cells (PRCre/+Egfrf/f; Egfrd/d). Egfrd/d mice are severely subfertile and exhibit reduced implantation and abnormal embryos. Mutant mice demonstrate an ablation of artificial decidualization including defects in morphology, proliferation, apoptosis, differentiation and gene expression. The use of microarray analysis has identified approximately 4,500 uterine genes to be misregulated in the absence of Egfr (+/- 1.5, p<0.01). This analysis has identified genes that are well known to play a critical role in uterine differentiation to be downstream of Egfr including Bmp2 and Wnt4.

EGFR is also induced during in vitro HES cell decidualization and the use of EGFR knockdown impedes decidualization. Furthermore, these results have been confirmed using a novel, automated image-based microscopy assay. These results strongly indicate that Erbb signaling plays a critical role in female reproduction. Continuation of ongoing studies determining the mechanism of EGFR action and the role of Erbb2 will provide valuable insight and allow further elucidation of complex pathways and the orchestrated molecular interactions vital to reproductive health. This research was supported by NIH Grant RO1CA077530 (to J.P.L.), by NIH R01 HD057873 (to JW. J), and by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement U54 HD07495 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

Contributors: Large, Michael J.; Hartig, Sean M.; Creighton, Chad; Kovanci, Ertug; Mancini, Michael A.; Threadgill, David W.; Lydon, John P.; Jeong, Jae-Wook; DeMayo, Francesco J.
Myotonic dystrophy type 1 (DM1) is an RNA-dominant disease caused by abnormal transcripts containing expanded CUG repeats. The CUG transcripts aggregate in the nucleus to form RNA foci and lead to nuclear depletion of Muscleblind-like 1 (MBNL1) and stabilized expression of CUGBP Elav like family 1 (CELF1), both of which are splicing regulatory proteins. The imbalance of these proteins results in misregulation of alternative splicing and neuromuscular abnormalities. Here, we report the use of antisense oligonucleotides (ASOs) as a therapeutic approach to target the pathogenic RNA in DM1. We designed chimeric ASOs, termed gapmers, containing modified nucleic acid residues to induce RNase H-mediated degradation of CUG-repeat transcripts. The gapmers selectively silence expanded CUG transcripts and are sufficient to disrupt RNA foci both in cell culture and mouse models for DM1. Furthermore, combination of gapmers with other ASOs that help release binding of MBNL1 to the toxic RNA can potentially enhance the silencing effect. Our study provides an alternative strategy for the use of ASOs in DM1 therapy.

Contributors: Lee, Johanna; Bennet, Frank; Cooper, Thomas
Upon activation, CD4+ T cells can differentiate into T helper (Th) cells, which play a central role in orchestrating the immune system by coordinating the function of both innate and adaptive immune cells. Different types of immune responses elicit different cytokine milieus, giving rise to particular types of Th cells with specific function and tissue distribution. Until recently, Th17 cells have been identified as potent inducers of tissue inflammation capable of eradicating pathogen infections as well as mediating autoimmune diseases. Therefore, the proinflammatory properties of Th17 cells have made them a potential therapeutic target to develop novel strategies to either fight infectious diseases or to ameliorate autoimmune disorders. Th17 differentiation is a tightly regulated process mediated by a network of cytokines and transcription factors but the exact mechanisms are still far from being completely understood.

ELF4 (E74-like factor 4) is a transcription factor known to modulate proliferation and differentiation of many cell types. ELF4 induces cell cycle arrest in CD8+ T cells during homeostasis and restrains memory formation upon immunization. A robust homeostatic expansion of not only CD8+ but CD4+ T cells in the spleens of aged Elf4-null mice suggested a potential role of ELF4 in CD4+ T cells. Our preliminary data showed that, in contrast to its role in CD8+ T cells, ELF4 is dispensable for the proliferation of CD4+ T cells both in vitro and in vivo. However, Elf4-null CD4+ T cells generated more IL-17+ cells and secreted more IL-17 under Th17 polarizing conditions while showing no apparent defect in differentiating into Th1, Th2, or Treg cells. Therefore, we hypothesize that ELF4 negatively regulates Th17 cell differentiation in vivo. As expected, more Th17 cells were found in lamina propria of the small intestine of naïve Elf4-null mice and in draining lymph nodes of immunized Elf4-null mice. By using EAE (experimental autoimmune encephalomyelitis), a mouse model of Th17-mediated pathogenesis, we found that Elf4/- mice showed an earlier onset and moderately more severe disease, suggesting that ELF4 also inhibits Th17 differentiation in vivo. To understand the underlying mechanism, we examined whether ELF4 affects responsiveness to Th17-polarizing cytokines IL-6 and TGF. We found that Elf4-null CD4+ T cells showed a lower requirement of these cytokines and elevated phosphorylation of the transcription factor STAT3 induced by IL-6. Further studies are required to link STAT3 hyperphosphorylation with increased Th17 differentiation in Elf4-null CD4+ T cells both in vitro and in vivo. Collectively, our study reveals a novel regulator of Th17-mediated immunity, which may be targeted pharmaceutically to treat autoimmune inflammatory disorders such as multiple sclerosis.

Contributors: Lee, Ping-Hsien; Lacorazza, Daniel
Mutations in Drosophila rumi result in a temperature-sensitive loss of Notch signaling. Rumi is a protein O-glucosyltransferase that adds glucose to EGF repeats with a C-X-S-X-P-C consensus sequence. Eighteen of the 36 EGF repeats in the Drosophila Notch receptor contain the consensus O-glucosylation motif. However, the contribution of individual O-glucose residues on Notch to the regulation of Notch signaling is not known. To address this issue, we carried out a mutational analysis of these glucosylation sites and determined their effects on Notch activity in vivo. Our results indicate that even though no single O-glucose mutation causes a significant decrease in Notch activity, all of the glucose residues on Notch contribute in additive and/or redundant fashions to maintain robust signaling, especially at higher temperatures. O-glucose motifs in and around the ligand-binding EGF repeats play a more important role than O-glucose motifs in other EGF repeats of Notch. However, a single O-glucose mutation in EGF12 can be compensated by other O-glucose residues in neighboring EGF repeats. Moreover, time-course cell aggregation experiments using a rumi-null cell line indicate that complete lack of Rumi does not affect Notch-Delta binding at high temperature. In addition, rumi fully suppresses the gain-of-function phenotype of a ligand-independent mutant form of Notch. Altogether, our data suggest that at physiological levels of Notch, the combined effects of multiple O-glucose residues on this receptor allow productive S2 cleavage at high temperatures and thereby serve as a buffer against temperature-dependent loss of Notch signaling.

Contributors: Leonardi, Jessica; Fernandez-Valdivia, Rodrigo; Li, Yi-Dong; Simcox, Amanda; Jafar-Nejad, Hamed
To identify novel genes that affect hearing, we screened a collection of mutations in essential genes on the X chromosome for morphological defects in the fly auditory organ, the Johnston’s Organ (JO). We used the FLP/FRT system to create mosaic animals to bypass lethality and isolated mutations in a gene that cause extra neurons and scolopale cells in the JO. This gene encodes a Drosophila homolog of the mammalian UBR3 proteins, which are E3 ubiquitin ligases. Previous studies have shown that these ligases target ubiquitination of aminoterminal amino acids of specific proteins and control their degradation.

To better understand the function of this UBR3, we explored the phenotype in different organs. Interestingly, we observe apoptosis in the eye and wing imaginal discs in mutant cells. This apoptosis can be suppressed when we overexpress p35, an inhibitor of DrICE/Dcp1. However overexpression of Diap1, a more upstream inhibitor of apoptosis, cannot rescue the apoptotic defects. This indicates that UBR3 may regulate apoptosis of proteins that function between Diap1 and DrICE/Dcp1. Apoptosis in the eye disc seems to induce proliferation in adjacent wild type cells, possibly to compensate for the loss of the dying cells.

We also observe a decrease in Ci155 in the morphogenetic furrow of the eye disc. Ci is the key downstream transcription factor of the Hh pathway. It has an active form, Ci155, and a truncated repressive form, Ci75. Consistent with a loss of Ci155 in the morphogenetic furrow, there is a delay in photoreceptor differentiation, a phenotype typically associated with the loss of Hh. Overexpression of Ci155 cannot rescue this delayed differentiation, suggesting that the loss of Ci155 is not causative. We are currently testing whether the loss of UBR3 affects the ability of the cells in the morphogenetic furrow to interpret Hh signaling or affect Ci processing leading to increased levels of the Ci75 form. We are also trying to identify the targets of UBR3.

Contributors: Li, Tongchao
C. ELEGANS NECROTIC CELLS ACTIVELY ATTRACT PHAGOCYTES THROUGH A
PS-EXPOSURE MECHANISM MEDIATED BY THE ABC TRANSPORTER CED-7

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Cells injured pathologically undergo necrosis, a type of cell death distinct from apoptosis in both morphology and mechanism. Like apoptotic cells, necrotic cells must be swiftly removed from animal bodies for prevention of harmful inflammatory and autoimmune responses. In the nematode Caenorhabditis elegans, gain-of-function mutations in certain ion channel subunits result in necrotic-like cell death of six touch neurons. Necrotic touch neurons are engulfed and degraded inside engulfing cells. It is unclear how necrotic cells are recognized by engulfing cells. Phosphatidylserine (PS) is an important apoptotic cell surface signal that attracts engulfing cells. Using ectopically expressed MFG-E8, a high-affinity PS-binding protein, we observed that PS was present on the surface of necrotic touch neurons. In addition, phagocytic receptor CED-1, whose function is needed for the efficient clearance of necrotic cells, clusters around necrotic cells. The extracellular domain of CED-1 specifically associates with PS but not other membrane phospholipids in vitro. We further found that a necrotic cell-specific function of CED-7, the worm homolog of mouse ABC1 transporter, was necessary for PS-exposure on necrotic cell surfaces. In engulfing cells, CED-7 contributes another distinct activity to facilitate engulfment. We demonstrate that necrotic cells, like apoptotic cells, rely on cell surface PS as an “eat me” signal to attract CED-1. Furthermore, unlike previously thought, necrotic touch neurons actively present PS through a CED-7-mediated mechanism instead of passively leaking out PS.

Contributors: Venegas, Victor; Li, Zao; Raghavan, Prashant; Zhou, Zheng
Nuclear factor kappa B (NFkB) is a family of dimeric transcription factors consisting of subunits with a highly conserved Rel homology domain and a DNA binding protein that recognizes the consensus κB site. In the resting state, NFkB is bound by its inhibitor IkB protein in the cytoplasm and is transcriptionally inactive. Activation of NFkB involves phosphorylation-induced degradation of IkB, which releases NFkB and enables it to enter the nucleus and regulate gene expression. NFkB signaling is involved in both physiological and pathological conditions in the central nervous system (CNS) such as learning and memory and neuroinflammation. However, its exact function and the molecular mechanisms remain largely unknown. This project aims to uncover the role of NFkB in the CNS by using mice with conditional inactivation of IkBα. IkBα is the main inhibitor of NFkB and also a target gene of activated NFkB. The autoregulatory loop between IkBα and NFkB is essential for the regulation of the NFkB signaling pathway. Germline knockout and conditional knockout of IkBα in myeloid lineage and B cells all lead to constitutive NFkB activation. Thus the IkBα conditional knockout mice serve as good models for studying the consequences of NFkB activation in CNS. We have generated mice that have IkBα knocked out in brain cell populations (IkBα cKO: IkBofl/-;Nestin-Cre). In contrast to the germline deletion, mice with inactivation of IkBα in the brain are viable and apparently healthy. Their brain morphology is overtly normal. However, microarray analysis of the mutant mice revealed a list of genes that shown altered expression when compared with the littermate controls. The expression change of two candidate genes has also been validated by real-time PCR. In addition, aged knockout mice show enhanced astrogliosis and microgliosis. Further analysis of the phenotypes at molecular, cellular and functional levels will help us to better understand the mechanism by which NFkB regulates the CNS function.

Contributors: Lian, Hong; Shim, David; Zheng, Hui
THE ROLE OF FKBP10 IN RECESSIVE OSTEOGENESIS IMPERFECTA

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Osteogenesis Imperfecta (OI) is the most commonly inherited form of brittle bone disease and displays a spectrum of severity from mild phenotypes to severe early lethality. Key clinical features of OI are bone fragility and low bone mass, whereas patients may also display blue sclera, dentinogenesis imperfecta, joint and skin laxity, hearing impairment and/or wormian bones on the skull. Specifically, this project focuses on a gene in which mutations result in recessive OI as well as Bruck Syndrome termed FK506 Binding Protein 10 (FKBP10) that encodes the FKBP65 protein. The FKBP65 protein complexes with Heat Shock Protein 47 (HSP47) and together they are thought to be involved in chaperoning of collagen in the endoplasmic reticulum. Currently, we do not understand the consequences of FKBP10 loss and its role in collagen and ECM formation. Therefore our goal is to elucidate the role of FKBP10 in the skeleton and how null mutations lead to progressively deforming OI. In other types of recessive OI, the biochemical modifications of collagen are altered, in particular by the complex of proteins consisting of Prolyl-3-Hydroxylase-1 (P3H1), Cartilage Associated Protein (CRTAP) and prolyl cis-trans isomerase cyclophilin-B (PPIB). In addition, in vitro studies of human cells with FKPB10 loss show ER stress, fragmented Golgi, procollagen aggregates, and increased numbers of autophagosomes. This suggests that there may be strong cellular phenotypes and the unfolded protein response pathway may be affected. We hypothesize that due to changes in intracellular conditions owing to Fkbp10 loss, disruptions in cellular and possibly biochemical phenotypes lead to common mechanisms that may exist between different causes of OI due to the similarity in phenotypes. We aim to test this hypothesis by determining the in vivo phenotypes of FKBP10 loss on skeletal and connective tissues, assessing the biochemical and cellular phenotypes of Fkbp10 null cells, and evaluating cell signaling defects in the absence of Fkbp10. This research is important for establishing common mechanisms of disease which may provide potential therapeutic targets for patients with OI and Bruck syndrome, especially through addressing altered cell signaling and altered collagen biochemistry which may affect it.

Contributors: Lietman, Caressa; Homan, Erica; Grafe, Ingo; Baldridge, Dustin; Kelley, Brian; Bertin, Terry; Jiang, Ming Ming; Chen, Yuquing; Yeng, Tao; Lee, Brendan
GENETIC INTERACTION BETWEEN CDC14B AND CDH1 IN DNA DAMAGE REPAIR AND IN TUMORIGENESIS

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Cdc14 is a dual specificity phosphatase, whose essential function for mitotic exit was first described in budding yeast. There are two mammalian homologs, Cdc14A and Cdc14B. Cdc14B was implicated in G1 phase control and G2/M DNA damage checkpoint, while it was also suggested dispensable for cell cycle progression. Upon these somewhat controversial findings, transgenic mice are necessary to elucidate Cdc14B’s physiological function. Our lab generated Cdc14B knockout mice, which developed early onset aging phenotypes, i.e. cataract and kyphosis. MEF (mouse embryonic fibroblast) cultures suggested an essential role of Cdc14B in DNA damage repair. As deficient DNA damage repair confers high risk of tumorigenesis, we will investigate potential role of Cdc14B in tumorigenesis. Statistical analysis of tumor incidence, either spontaneous or induced by $\gamma$-irradiation, in Cdc14B -/- and +/+ mice will be carried out to determine if Cdc14B is a tumor suppressor.

To fully understand the mechanism underlying Cdc14B’s function in DNA damage repair and tumorigenesis, it is important to figure out potential targets of this phosphatase. Our preliminary study with Cdc14B/-/Cdhl+/- mice showed a higher incidence of cataract, indicating Cdhl may be involved in Cdc14B’s function, especially with the notion that Cdc14 could dephosphorylate Cdhl. To test if Cdhl mediates Cdc14B’s function in DNA damage repair and tumorigenesis, Cdc14B -/- and +/- MEFs will be analyzed for DNA damage repair under the conditions of Cdhl overexpression or knocking down, while Cdc14B/-/Cdhl+/- and Cdc14B/-/Cdhlfl/fl/ER-Cre mice will be generated to analyze aging and tumor phenotypes.

Contributors: Lin, Han; Wei, Zhubo; Zhang, Pumin.
(P)PPGPP-REGULATED GTP LEVELS IN BACILLUS SUBTILIS

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GTP is an important molecule for all living organisms, as it serves as a building block of DNA and RNA, provides energy for many cellular processes, and functions as a signaling molecule. Deregulated GTP biosynthesis has been implicated in several severe human diseases, including cancer and human retinitis pigmentosa. However, the mechanisms by which GTP levels are regulated, and the importance of regulation of GTP levels remains under-explored. GTP is synthesized via the de novo and salvage pathways. The de novo pathway synthesizes GTP from purine precursors, whereas the salvage pathway does so by recycling intra- or extracellular purine nucleobases, such as guanosine. GTP biosynthesis is regulated by negative feedback inhibition and by transcriptional regulation of purine-biosynthesis genes. We recently found that the unusual nucleotides, guanosine tetra- and penta-phosphate, collectively named (p)ppGpp, is also critical for regulation of GTP biosynthesis under different nutrient conditions. (p)ppGpp exists ubiquitously in bacteria and plants, and possibly even in higher organisms. We found that GTP levels elevate both under amino acid starvation and upon addition of external guanosine in the absence of (p)ppGpp. To elucidate how (p)ppGpp regulates GTP pools, we performed in vitro enzymatic assays and found that (p)ppGpp inhibits IMPDH and HprT, two key enzymes of the de novo and salvage pathways, respectively. Furthermore, metabolic profiling experiments suggested that conversion of GMP to GDP is also inhibited by (p)ppGpp. We purified the enzyme that catalyzes this step, GMK, and found that it is potently inhibited by (p)ppGpp. Our study therefore reveals a new mechanism by which (p)ppGpp regulates GTP levels.

Contributors: Kriel, Allison; Bittner, Alycia; Jue, Wang
Complex genomic rearrangements (CGR) consisting of two or more breakpoint junctions have been observed in genomic disorders. Recently, a chromosome catastrophe phenomenon termed chromothripsis, in which numerous genomic rearrangements are apparently acquired in one single catastrophic event, was described in multiple cancers. Here we show that constitutionally acquired CGRs share similarities with cancer chromothripsis. In the 17 CGR cases investigated we observed localization and multiple copy number changes including deletions, duplications and/or triplications, as well as extensive translocations and inversions. Genomic rearrangements involved varied in size and complexities; in one case, array comparative genomic hybridization revealed 18 copy number changes. Breakpoint sequencing identified characteristic features, including small templated insertions at breakpoints and microhomology at breakpoint junctions, which have been attributed to replicative processes. The resemblance between CGR and chromothripsis suggests similar mechanistic underpinnings. Such chromosome catastrophic events appear to reflect basic DNA metabolism operative throughout an organism’s life cycle.

Contributors: Liu, Pengfei*; Erez, Ayelet*; Nagamani, Sandesh C. Sreenath; Dhar, Shweta U.; Kołodziejska, Katarzyna E.; Dharmadhikari, Avinash V.; Cooper, M. Lance; Wiszniewska, Joanna; Zhang, Feng; Withers, Marjorie A.; Bacino, Carlos A.; Campos-Acevedo, Luis Daniel; Delgado, Mauricio R.; Freedenberg, Debra; Garnica, Adolfo; Grebe, Theresa A.; Hernández-Almaguer, Dolores; Immken, LaDonna; Lalani, Seema R.; McLean, Scott D.; Northrup, Hope; Scaglia, Fernando; Strathern, Lane; Trapane, Pamela; Kang, Sung-Hae L.; Patel, Ankita; Cheung, Sau Wai; Hastings, P. J.; Stankiewicz, Pawel; Lupski, James R.; Bi, Weimin.
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The Role of DIDO1 in Stem Cell Self-renewal

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Embryonic stem (ES) cells have the unique properties of self-renewal and pluripotency, which make them promising resource for regenerative medicine. However, the mechanism underlying self-renewal and pluripotency remains elusive. To investigate the mechanism, we carried out bimolecular fluorescence complementation screen to identify interacting proteins for important ES cell factors NANOG, OCT4 and SOX2. We further did a functional screen for the interacting proteins and found DIDO1, when overexpressed, can inhibit differentiation under condition normally don’t promote self-renewal. We then withdrew LIF from DIDO1 overexpressing ES cells for 5 days and injected them into the nude mice, ES cells overexpressing DIDO1 differentiated into different germ layers in vivo. DIDO1 is expressed in ES cells. We are currently investigating the role of DIDO1 in the self-renewal and differentiation.

Contributors: Liu, Yinyin; Kim, Hyeung; Liang, Jiancong; Songyang, Zhou
INVESTIGATE THE PATHWAYS FOR RESPONDING TO GRAM-NEGATIVE BACTERIA IN DICTYOSTELIUM DISCOIDEUM

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Background: In higher organisms, innate immunity serves the first line of defense against microbial pathogens. Interestingly, genetic and transcriptional evidence suggests that Gram-positive and Gram-negative bacteria can elicit differential innate immune-like response in social amoeba D. discoideum. In contrast to its normal growth on Gram-positive bacteria, the growth of tirA- mutant is impaired on Gram-negative bacteria. On the other hand, cells grown on Gram-positive or Gram-negative bacteria show distinct transcriptional profiles. We hypothesize that the signaling pathway for responding to Gram-negative bacteria is mediated by TirA, a protein containing TIR (Toll/IL-1 Receptor) domain, whose function in innate immunity is shared by both mammals and plants. The goal of this research is to investigate the tirA pathway by isolating and characterizing the TirA-interacting proteins.

Methods: We identified TirA-interacting proteins by immunoprecipitation followed by mass spectrometry (IP/MS). Protein IP was conducted in D. discoideum cells expressing various fusion proteins (GFP-TirA full length, TirA1-983-GFP and TirA176-1273-GFP). LC/LC-ESI-LTQ was run to identify the IP proteins. Common candidates emerging from independent IP/MS experiments with different GFP-TirA fusions were knocked out in D. discoideum. The growth phenotype of knockout mutants on Gram-negative bacteria was characterized.

Results: Overexpression of GFP-TirA rescued the growth defect of tirA- on Gram-negative bacteria. IP/MS yielded 75 proteins associating with GFP-TirA full length, 38 proteins with TirA1-983-GFP and 36 proteins with TirA176-1273-GFP. Among these, two genes were repeatedly isolated from cells expressing GFP-TirA full length and TirA1-983-GFP. Knocking out another candidate, DDB_G0271970, which encodes an extracellular polysaccharide (EPS) depolymerase, caused delayed growth on Gram-negative bacteria.

Conclusions: (1) We have isolated candidate genes that may signal through TirA for responding to Gram-negative bacteria in D. discoideum. (2) The GFP-TirA fusion is functional in vivo. (3) EPS may be the bacterial component for D. discoideum cells to recognize the Gram-negative bacteria.

Contributors: Liu, Zhiyi; Nasser, Waleed; Chen, Guokai; and Kuspa, Adam
Interleukin 13 receptor alpha1 (IL-13Rα1) binds to IL-13 and is required for IL-13 signaling. Although clearly expressed on non-immune cells, the presence and function of IL-13Rα1 on T cells is still controversial. Our laboratory generated IL-13Rα1-/- mice and studied the role of IL-13Rα1 in T cell function and allergic lung disease. As expected from prior studies, compared with identically treated wild type control mice, IL-13Rα1-/- mice challenged with the spores of Aspergillus niger failed to acquire an asthma-like phenotype, most likely due to the lack of IL-13 signaling in the lung. Surprisingly, we further discovered significantly fewer T helper type 2 (Th2) cells in the lung in IL-13Rα1-/- mice after spore challenge, suggesting that IL-13Rα1 may have a direct role in regulating Th2 cell function. To understand this phenomenon further, we used qRT-PCR to define the expression of IL-13Rα1 on distinct T helper effector subsets, finding IL-13Rα1 mRNA only expressed in differentiated Th2 cells. Further studies revealed that Th2 cell-intrinsic IL-13Rα1 is dispensable for Th2 cell lineage commitment, but is required for Th2 cell homing to the lung using an adoptive T cell transfer system. Thus, although further studies are required, our findings suggest that IL-13Rα1 is selectively upregulated in Th2 cells and mediates Th2 cell homing to lung in allergic lung disease. These findings potentially reveal a novel mechanism by which IL-13 regulates allergic responses.

Contributors: Lu, Wen; Yang, Tianshu; Liu, Xiaoling; Kheradmand, Farrah; Corry, David
ROLE OF FRAGILE X RELATED PROTEINS IN MAMMALIAN CIRCADIAN BEHAVIORS AND GLUCOSE HOMEOSTASIS

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Fragile X syndrome, the most common form of inherited developmental disability, results from the absence of the fragile X mental retardation 1 (FMR1) gene product FMRP. FMR1 has two paralogs in vertebrates: fragile X related gene 1 and 2 (FXR1 and FXR2). One of the behavioral symptoms observed in fragile X patients is the increased occurrence of sleep disorders. Fmr1 or Fxr2 knockout (KO) mice display a shorter free-running period of locomotor activity in total darkness (DD) compared to wild-type mice, while mice lacking both Fmr1 and Fxr2 (double knockout) exhibit complete loss of rhythmic activity in both the light:dark cycle (LD) and DD. DKO mice also display significant alterations in the cyclical patterns of abundance of core clock component messenger RNAs in the liver, but not in the suprachiasmatic nucleus. These findings suggest that Fmrp and Fxr2p are acting downstream of the central clock to control rhythm in mice, but it is unclear how the FXRs affect the peripheral outputs, such as in the liver.

Locomotor assays with restricted feeding demonstrate that the Fmr1/Fxr2 DKO mice were able to adjust their rhythm to food availability. Remarkably, these mice also had a significantly higher mortality rate (~60%) in the first three days of restricted feeding, which suggests a difference in their physiological response to food restriction. Circadian cycling in the liver is known to contribute to glucose homeostasis, and we are testing for the specific involvement of FXRs in this system. In mice of similar body weight, body fat is significantly lower in the Fmr1/Fxr2 DKO mice compared to WT. They had consistently low levels of glucose, cholesterol, and leptin over a 24hr cycle, while the cycling of the other metabolic markers in the blood- adiponectin, glycerol, free fatty acids, and triglycerides, but not insulin- is out of phase. The DKO mice also exhibited exaggerated clearance of a bolus of glucose, and hypersensitivity to insulin, even as both insulin production and gluconeogenesis appear normal.

The close association of food input to the circadian system and the timing of sleep and wakefulness, together with the typical disturbances of circadian behavior and sleep in Fragile X syndrome, open up a new perspective for the investigation and treatment of patients suffering from this disorder.

Contributors: Lumaban, Jeannette G.; Nelson, David L.
MiR-155 is quickly becoming one of the most studied microRNAs in dendritic cells (DCs). However, studies have shown that the star-form partner, miR-155*, is the dominant form of the microRNA immediately after activation of DCs. The two microRNAs, 155 and 155*, are both derived from the same pri-miRNA and their production is regulated by the RNA associated protein KHSRP, which acts as a toggle. Approximately four hours after DC activation, induction of KHSRP leads to a sharp decrease in miR-155* while miR-155 levels increase. During the brief window of miR-155* dominance after activation, miR-155* directly targets and inhibits IRAKM production, a process which our lab has shown may increase longevity and mobility of dendritic cell vaccines in a previous report. However, permanent suppression of IRAKM in DCs facilitates the over-production of type I interferons and interferes with later stages of DC activation, such as IL-12 secretion, which may hinder an effective T cell response. Therefore our lab aims to modify DCs in a non-permanent manner in order to extend the window of miR-155* influence while leaving later stages of DC activation intact. So far, knockdown experiments with silencing RNAs against KHSRP have been promising, but transient transfection methods can be toxic or pre-maturely activate the dendritic cells. Through collaborations at Rice University, we are able to manufacture gold nanoparticles with short RNAs conjugated to their surface. Similar particles have been shown to effectively deliver silencing RNAs, resulting in knockdown of proteins of interest. In our own studies, we have observed the nanoparticles to be an extremely gentle method of delivery to DCs, as gold is an inert metal and does not activate the DCs nor is it toxic. In time, we hope to optimize gold nanoparticle delivery for silencing of KHSRP as a method of increasing the efficacy of dendritic cell vaccines against cancer.
All proliferating cells face the task of coordinating DNA replication with cell division to preserve genomic stability. Consequently, DNA replication factors are among the most highly conserved proteins across all domains of life. Control of replication initiation is fundamentally similar in all organisms: Cell cycle-regulated DNA binding proteins catalyze open complex formation at replication origins, allowing for replisome assembly and chromosomal duplication. Our lab uses E. coli as a genetically tractable model organism to study DNA replication in order to better understand this fundamental and highly conserved regulation.

Recent findings indicate that localization of oriC within the cell may direct initiation of DNA replication. Immediately prior to initiation, fluorescently labeled replication proteins form a focus at midcell, while oriC resides at one of the cell quarter positions. Concomitant with initiation of DNA replication, oriC moves to midcell. Replisome components form foci normally in anucleate cells, suggesting that replisome localization precedes oriC localization or any direct signal from the DNA. Additional redundant levels of control may help explain some experimental observations, such as the observation that cells overexpressing wild type DnaA maintain a 1:1 ratio of initiation to cell division.

In order to test if oriC position directs initiation, I have developed a method to disrupt normal oriC positioning in vivo, using fluorescent transcription factor fusion proteins. This model system results in a growth defect and cell filamentation, however my newest results indicate that this phenotype is the result of defects in replication elongation. Modifications of the model system or alternative model systems are being considered, as well as future studies using the current system as a model to study replication roadblocks. I am also studying the effect of temperature sensitive mutants arrested at different stages in initiation on oriC localization, to determine at what step oriC movement fits into the already described initiation process. Future studies will include a more in depth study of the events leading up to replication initiation, including more accurate correlations between oriC movement and replication.

Contributors: Magnan, David; Bates, David
Naïve CD8 T cells are quiescent and remain locked in G0 stage of the cell cycle until they encounter a cognate antigen. Stimulation through the T cell receptor leads to proliferation and differentiation to short-lived effector and long-lived memory cell subsets. Memory population comprises two distinct but interconvertible subsets – central memory and effector memory – that can persist for the entire lifespan of the host. Understanding the mechanisms governing differentiation of a single naïve CD8 T cell to the two memory subpopulations is pivotal for designing better vaccines and treating immune disorders.

The ETS family transcription factor ELF4 is crucial for maintaining quiescent state of mouse naïve CD8 T cells. Upon TCR stimulation ELF4 is rapidly downregulated thus allowing CD8 T cells to enter cell cycle and start their differentiation program. ELF4-null CD8 T cells show augmented homeostatic proliferation as well as enhanced magnitude of primary expansion and memory production in response to stimulation with peptide-pulsed dendritic cells (DC).

To examine the role of ELF4 in mounting CD8 T cell response to bacterial infection in mice we adoptively transferred ELF4-/- CD8 T cells together with wild type competitors to C57BL/6 hosts and challenged recipients with a sublethal dose of intracellular pathogen Listeria monocytogenes. In contrary to the DC vaccine model we found that ELF4 is required for proper production of effector CD8 T cells and subsequent development of effector-memory population during anti-bacterial response. ELF4-null CD8 T cells show reduced differentiation to CD62Llow effector cells and less effector memory than wild-type competitors. To gain insights on ELF4 function we have purified CD8 T cells throughout the immune response and analyzed the expression pattern of ELF4. ELF4 protein is greatly upregulated in effector cells and slowly declines during contraction. Loss of ELF4 results in lower proliferation upon priming and more intense apoptosis without altering expression of cytokines and cytotoxic molecules. These data suggest that ELF4 supports expansion and survival of effector CD8 T cells during inflammatory response to intracellular pathogens.

Contributors: Yamada, Takeshi; Park, Chun-Shik; Lacorazza, Daniel
A CONSERVED ROLE OF NUCLEAR RECEPTOR LRH-1/NR5A2 IN ENDOPLASMIC RETICULUM STRESS RESOLUTION

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Endoplasmic reticulum (ER) stress is a common event in the life of a cell, occurring in response to a wide range of triggers including nutrient deprivation, increased protein expression, viral infection, and exposure to endogenous or exogenous toxins. A highly conserved set of signaling pathways termed the unfolded protein response (UPR) exists to protect the cell against ER stress. Genetic studies have determined that even partial loss of UPR functioning results in cell damage or death and may initiate development of multiple human diseases, including neurodegenerative diseases, diabetes, and familial metabolic disorders. We have discovered a novel role in ER stress resolution for the orphan nuclear receptor liver receptor homolog-1 (LRH-1/NR5A2), previously studied for its roles in bile acid homeostasis, cholesterol transport, and development. Initial experiments demonstrated an inability of mice with liver-specific loss of Lrh-1 to resolve ER stress induced by the chemical agent tunicamycin, much as was seen in previous studies challenging mice deficient in core components of the UPR with tunicamycin. Additionally, control mice were able to resolve chemically-induced ER stress much more rapidly upon treatment with the LRH-1 agonist DLPC. Parallel experiments performed in C. elegans also suggest that the Lrh-1 ortholog nhr-25 is required for resolution of ER stress as assessed by survival following treatment with tunicamycin; strikingly, nhr-25 is also responsive to the Lrh-1 agonist DLPC, with agonism significantly decreasing developmental defects caused by ER stress. Microarray studies indicate that induction of target genes of the PERK/ATF4 branch of the UPR following tunicamycin treatment is lost upon Lrh-1 ablation; congruously, LRH-1 agonism specifically increases expression of the same class of target genes under ER stress conditions. Since the final output of the PERK/ATF4 branch of the UPR is induction of target genes by the ATF family of transcription factors, yet these transcription factors are present but fail to induce target gene expression upon loss of Lrh-1, we hypothesize that LRH-1 physically interacts with ATF family members upon ER stress. Future studies will determine full extent of LRH-1 protein-protein interactions and transcriptional activity in ER stress conditions. Conservation of the role of Lrh-1/nhr-25 in UPR protein interactions and transcriptional induction will also be studied extensively using C. elegans. Benefits of LRH-1 agonism will be determined in mouse models of chronic ER stress, including models of alpha1-antitrypsin deficiency, diabetes, and Wolcott-Rallison syndrome. In conclusion, we report an unexpected but conserved role of Lrh-1 in ER stress resolution and are optimistic that LRH-1 agonism may soon be applied to specifically treat human disorders resulting from chronic ER stress.

Contributors: Mamrosh, Jennifer; Lee, Jae Man; Wagner, Martin; Cooney, Austin; Sifers, Rick; Wang, Meng; Moore, David
During definitive hematopoiesis, a subset of the vascular endothelium is thought to give rise to the first multi-lineage hematopoietic stem and progenitor cells. In the murine yolk sac (YS), this hemogenic subpopulation of the endothelium is characterized by Hoechst dye-efflux capacity (side population or SP cell phenotype), expression of the transmembrane receptors Flk-1 and c-Kit, and lack of CD45 expression (Flk1+c-Kit+CD45- SP cells) (Goldie et al., 2008). Raldh2-/- mice lack the enzyme that converts dietary retinol into retinoic acid (RA), resulting in decreased expression of key hematoendothelial genes, including Runx-1, c-Myb, and c-Kit, loss of vascular remodeling, and impaired development of yolk sac hemogenic endothelium, prior to the onset of definitive hematopoiesis. Restoration of RA signaling in RA-deficient mutants rescues the hemogenic endothelial cell defect, revealing a critical role for RA signaling in the specialization of yolk sac hemogenic endothelium from primitive vascular endothelium (Goldie et al., 2008).

We have localized cells responsive to active RA signaling to the vascular endothelium of the murine yolk sac during the developmental stages that encompass definitive hematopoiesis in this tissue. Subsequent analysis of gene expression levels via qPCR indicates that early hematoendothelial genes are upregulated in RA-responsive endothelium relative to the non-RA-responsive fraction. We therefore hypothesize that three candidate genes, Runx-1, c-Myb, and c-Kit, act downstream of retinoic acid signaling to induce hematopoietic potential within a subset of primitive vascular endothelium. To test this hypothesis, we have generated lentiviral expression vectors designed to restore candidate gene expression in Raldh2-/- embryos cultured ex vivo. We are currently assessing the ability of each candidate gene in rescuing hemogenic endothelium formation and function in developing mouse yolk sac via hematopoietic colony formation assays and gene expression analyses.
Mesothelin (MSLN) is a cell surface glycoprotein overexpressed in several cancer types, including pancreatic cancer, lung cancer, mesothelioma, and ovarian cancer. Our laboratory has previously reported that MSLN overexpression results in increased proliferation and migration in pancreatic cancer cells. We have also found that MSLN overexpression is accompanied by a down-regulation of several cellular microRNAs. In this study, we found that miR-198 is a key regulator involved in a reciprocal regulatory loop with mesothelin (MSLN), several homeobox domain transcription factors (TFs), and multiple tumorigenic factors in a functional network.

We found that overexpression of MSLN correlates negatively with miR-198, which is downregulated in a majority of PC cell lines and patient tumors. MSLN overexpression leads to NF-κB-mediated induction of repressive TFs including the octamer protein OCT-2 (POU2F2) and zinc finger E-box binding homeobox 1 (ZEB1), which suppress the miR-198 promoter. We confirmed that miR-198 targets several tumorigenic factors: MSLN itself, which is uniquely targeted by miR-198 at multiple sites within its coding region; Pre-B cell leukemia TF 1 (PBX-1); and Valosin-containing protein (VCP). The dysregulation of these factors contributes to the pathogenesis of MSLN overexpression. Reconstitution of miR-198 reduces the tumorigenic properties and pro-survival effects conferred by MSLN, PBX-1, and VCP, including proliferation, migration, and invasion in vitro and reduced tumor growth and metastasis in vivo. Our study reveals a novel role for miR-198 in PC and indicates therapeutic potential for other cancers where MSLN, OCT-2, ZEB1, PBX-1 and VCP play important roles.

Contributors: Bharadwaj, Uddalak; Li, Min; Chen, Changyi; Yao, Qizhi.
The voltage-dependent anion channel (VDAC or porin) is an integral membrane protein present in the mitochondrial outer membrane (MOM). VDACs are the predominant determinant of MOM permeability but also integrate mitochondrial function and other cellular pathways by interacting with various mitochondrial and cytoplasmic proteins; however, many details of these functions as well as the interacting pathways remain poorly understood. VDACs have also been implicated to play a pathogenic role in several human diseases including cancer, diabetes, Alzheimer’s, and cardiac ischemia-reperfusion injury. Flies mutant for porin (the predominant VDAC in Drosophila) demonstrate energy metabolism defects, neurologic dysfunction with abnormal mitochondrial distribution in motor neurons and male infertility. The ultimate goal of this project is to exploit the genetic versatility of Drosophila to dissect and identify VDAC functions and interacting pathways by identifying modifiers of porin mutant phenotypes using both candidate genes and unbiased genetic screens.

Aconitase activity analysis from isolated mutant mitochondria suggests that porin deficiency results in increased mitochondrial reactive oxygen species (ROS). A microarray analysis of gene expression has identified significant altered expression of a subset of cytochrome P450 genes in porin mutants. Given that cytochrome P450 monoxygenases can produce ROS through NADPH dependent oxidation reactions and that porin mutants have increased ROS, these P450 genes are potential candidate modifiers of porin mutant phenotypes. To evaluate their potential as genetic modifiers of porin, cytochrome P450 genes with confirmed altered expression in porin mutants are being screened through systematic overexpression and knockdown in the porin mutant background to observe potential modifying effects on porin mutant phenotypes. Additionally, the relationship of oxidative stress and changes in cytochrome P450 genes is being explored both chemically using various antioxidants as well as genetically using a GAL4-inducible transgene of the mitochondrial isoform of Superoxide Dismutase (SOD). In addition, a pilot deficiency screen has identified multiple deletions that suppress male infertility in porin mutants, including one deletion that also suppresses neuronal dysfunction in porin mutants. Using deletion mapping, the suppressor region has been narrowed down to a 25 kb region and individual genes in this region are now being tested. Identification of suppressors of porin mutant phenotypes will provide insight into VDAC functions as well as identify potential novel therapeutic targets and strategies for multiple human diseases.
DNA methylation is an important epigenetic modification in vertebrate genomes. DNA methylation is catalyzed by a family of DNA methyltransferase enzymes, Dnmt1, Dnmt3a, and Dnmt3b, and occurs at the cytosine in CpG dinucleotides. Cytosine methylation is generally associated with gene repression. The Dnmt3s are de novo methyltransferases that are essential to normal embryonic development. When both Dnmt3a and Dnmt3b are knocked out in mouse ES cells, the cells are able to replicate but lose differentiation capacity as they are passaged. DNMT3A is mutated in ~20% of human acute myeloid leukemia (AML) and DNMT3A mutations have been identified in ~8% of myelodysplastic syndrome (MDS) patients. Little is known about the normal targets of DNA methyltransferases, and it is thought that methyltransferase targets in somatic cells may be distinct from their targets in ES cells. Both Dnmt3a and Dnmt3b are expressed in HSCs, and neither Dnmt3 is expressed appreciably in progenitors or differentiated cells. Our lab has used a conditional knockout of Dnmt3a to study its effects on the balance between self-renewal and differentiation in hematopoietic cells. When purified HSCs were transplanted into lethally irradiated recipients, there was no difference in peripheral blood contribution of the knockout cells compared to controls. However, since there is no known demethylase to remove the previously established methylation marks, cell division may be required to passively remove the methylation, so serial transplantation was used to force the cells to divide. Through serial transplantation, absence of Dnmt3a leads to an increase in self-renewal at the expense of differentiation in HSCs. Dnmt3a-KO HSCs have both hypo- and hypermethylated sites after transplantation. Conditional Dnmt3ab-dKO cells have not been studied as extensively, but have a similar phenotype to the single knockout cells. I have transplanted Dnmt3ab-dKO cells and am performing a time course analysis to characterize their function and methylation status. We hypothesize that the dKO cells are undergoing apoptosis at the start of differentiation, and that hypomethylation will be observed.
Background: Hepatocellular carcinoma (HCC) is the 3rd lethal malignancy worldwide. In-depth analysis of key molecular mechanisms of HCC development is necessary to develop effective new therapies. Our lab has previously shown that extracellular ATP-mediated activation of P2 purinergic receptors was sufficient to induce cell cycle progression in primary hepatocytes in vitro. Furthermore, hepatocyte proliferation in response to 70% partial hepatectomy is impaired in P2 receptor knockout (P2Y2-/-) mice, suggesting a role for purinergic signaling in hepatocyte proliferation in regenerating livers. Our preliminary studies reveal increased P2Y2 receptor protein expression in human HCC lesions as compared to uninvolved areas of the liver. However, the functional significance of purinergic signaling in the pathogenesis of HCC remains unknown. Therefore, we tested the hypothesis that dysregulation of purinergic signaling facilitates aberrant cell proliferation and migration underlying hepatocellular carcinogenesis.

Methods: Huh7 hepatoma cells, maintained in serum-free conditions, were treated with nucleotides (ATPγS, ATP or UTP; 100uM) for 24hrs. Effects on cell proliferation (BrdU, Cyclin D1) and induction of key mediators of cell migration (uPA, PAI-1, MMP-9) were evaluated by Western blotting of total cell lysates. P2 purinergic receptor expression (mRNA, protein) was evaluated in livers from an established mouse model of HCC (mst1/2-/- vs WT) and HCC patients (tumors vs uninvolved areas).

Results: Nucleotide treatment alone was sufficient to induce Huh7 cell proliferation, as determined by increased BrdU incorporation (1.7-fold) and Cyclin D1 (3-fold) protein expression. Similarly, nucleotide treatment induced uPA (2.6-fold), PAI-1 (2.0-fold) and MMP-9 (2.3-fold) protein expression. Implicating P2 purinergic signaling in the induction of HCC, P2Y2, Y6, Y14, X2, X5, X6 and X7 mRNA as well as P2Y1, Y2, X6 and X7 protein expression were significantly elevated in mst1/2-/-, as compared to WT livers. The temporal profile of P2 receptor expression suggests that absence of mst1/2-/- alone was sufficient to induce P2X expression, detectable in both mst1/2-/- with no tumor burden and with heavy tumor burden, whereas P2Y upregulation may be secondary to increased tumor burden. Interestingly, over 40% of liver tumors from HCC patients displayed at least 2-fold increase in P2Y1, Y2, Y4, X6 and X7 mRNA expression. Conclusion: Our in vitro data highlight the role of P2 receptors as key players in transformed hepatocyte cell cycle progression, with potential roles in ECM remodeling. Our in vivo findings both in mice and humans have uncovered the translational relevance of these receptors, as potential biomarkers and novel therapeutic targets in the management of HCC.

Contributors: Maynard, Janielle P.; Thevananther, Sundararajah
Rett syndrome (RTT) is a postnatal neurological disorder caused by mutations in MECP2, encoding the epigenetic regulator methyl-CpG-binding protein 2 (MeCP2). The onset of RTT symptoms during early life together with findings suggesting neurodevelopmental abnormalities in RTT and mouse models of RTT raised the question of whether maintaining MeCP2 function exclusively during early life might protect against disease. We show by using an inducible model of RTT that deletion of MeCP2 in adult mice recapitulates the germline knock-out phenotype, underscoring the ongoing role of MeCP2 in adult neurological function. Moreover, unlike the effects of other epigenetic instructions programmed during early life, the effects of early MeCP2 function are lost soon after its deletion. These findings suggest that therapies for RTT must be maintained throughout life.

Contributors: McGraw, Christopher M.; Samaco, Rodney C.; Zoghbi, Huda Y.
Francisella tularensis (Ft) is a ubiquitous gram-negative bacterium that is considered one of the most infectious bacteria ever characterized. This, combined with previous efforts to develop it into a biological weapon, has resulted in its classification as a Category A Select Agent. Current diagnostic methods include both PCR- and ELISA-based techniques, but these are slow and inefficient. This work proposes a new diagnostic assay that incorporates DNA-protein chimeras for the detection of very low numbers of Ft molecules in complex biological samples, such as blood, thus giving clinicians the ability to diagnose an infection earlier in its course with greater sensitivity.

The DNA-protein chimeras, known simply as “tadpoles,” consist of a short DNA oligonucleotide (the tail) conjugated to a protein (the head) engineered to tightly bind a desired target. In one approach, we have created a tadpole using a chimeric protein designed to bind mammalian antibodies, which were used to detect the presence of antibodies generated in response to 12 specific Ft antigens. These data also indicated that these tadpoles have the ability to detect targets at attomolar (10^-18) concentrations - approximately one million times more sensitive than traditional ELISA.

We are also engineering “head” ligand proteins using a human antibody single chain variable region (scFv) phage display library, which allows us to select, isolate, and purify human antibody fragments that specifically bind to any target. We are using surface or secreted Ft proteins as targets for these phage display experiments. Multiple Francisella protein targets have been used for these experiments, but one of these, FTT0715, has the most promise moving forward. This protein (ChiA) is a chitinase, is known to be involved in virulence, is secreted actively by Francisella, and is immunogenic. Most importantly, it is very unique to the Francisella species. We have selected for and isolated an antibody specific to this protein, and using multiple techniques, we showed its KD to be ~100 nM. In an effort to improve this binding, alanine scanning and random mutagenesis has been done, and binding affinity has improved to ~50 nM. We are now in the process of creating tadpoles for testing in clinically applicable settings, as well as using our knowledge to platform this diagnostic tool to other pathogens.

Contributors: McWilliams, Brian; Burbulis, Ian; Weinstock, George; Resnekov, Orna; Petrosino, Joseph
TARGETING THE REST NETWORK IN TRIPLE NEGATIVE BREAST CANCER

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Program in Cell & Molecular Biology

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While targeted therapies have improved survival for ER+ and HER2+ breast cancer patients, targeted treatments for patients with aggressive ER-, PR- and HER2-breast cancer (triple negative breast cancer, TNBC) have not been developed in part due to a poor understanding of the signaling pathways controlling TNBC. We hypothesize that the REST tumor suppressor pathway is a negative regulator of TNBC. We seek to identify the signaling networks that regulate REST function and exploit these pathways to inhibit TNBC proliferation.

REST (RE-1-Silencing Transcription Factor) was originally discovered as a master repressor of neuronal gene expression. Recently, we identified REST as a tumor suppressor in multiple epithelial tissues. The REST locus is frequently inactivated by genetic or epigenetic mechanisms in several epithelial malignancies. However, these alterations do not occur in human breast cancers. Interestingly, we have found that REST protein expression is undetectable in 42% of TNBCs which suggests that REST is post-transcriptionally lost in these cancers. The pathways that regulate REST protein levels, however, have not been elucidated.

Recently, we have shown that REST protein is highly regulated by the ubiquitin ligase SCFbetaTRCP and proteasomal degradation. REST must be phosphorylated prior to degradation, suggesting that REST stability is regulated by a network of kinases and phosphatases. To identify this signaling network, we have conducted an RNAi-based screen for genes that govern REST degradation. We have identified a signaling network that regulates REST protein abundance, including PLK1. By utilizing RNAi and pharmacological inhibition, we have shown that PLK1 loss of function leads to an increase in relative REST protein abundance. PLK1 physically interacts with REST indicating that it may be a direct REST kinase. Additionally, using our newly developed lentiviral expression system, pINDUCER, we have demonstrated that ectopic PLK1 expression results in a significant decrease in REST protein levels. Our future work will further characterize the PLK1-REST interaction in order to determine whether targeting the REST regulatory network is antiproliferative in TNBC.

Contributors: Meerbrey, Kristen; Kessler, Jessica; Migliaccio, Ilenia; Hu, Guang; Fang, Justin; Schmitt, Earlene; Huang, Jian; Schiff, Rachael; Osborne, C. Kent; Hilsenbeck, Susan; Shaw, Chad; Elledge, Stephen; Westbrook, Thomas.
ROLE OF γδ T CELLS IN MURINE DIETARY OBESITY

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Obesity often results in a state of chronic low-grade systemic inflammation. Studies have shown that there is an infiltration of lymphocytes and macrophages, and an increase in pro-inflammatory cytokines in the adipose tissue in diet-induced obesity in mice. T cell numbers in the epididymal fat pad of C57BL/6 mice, fed a diet high in saturated fatty acids ("Western" diet, 41% Kcal milk fat) for five to ten weeks, have been found to be greater than that in mice on regular chow (6.5% Kcal fat). γδ T cells make up about one-third of this T cell population in the epididymal adipose tissue in the normal as well as obese state, and apparently play an important role in inflammatory response in adipose tissue. We have shown that TCRδ/-/- mice lacking γδ T cells failed to recruit macrophages into adipose tissues or increase expression of cytokines such as MCP-1, IL-6, IFN-γ, IL-10 even though they ate similar amounts of food and showed similar weight gain pattern as wild-type mice. In additional studies, γδ T cell receptor was blocked in wildtype mice by i.p. administering an anti-TCRδ antibody. A decrease in macrophage marker F4/80 and IL-10 was observed in these mice on the high fat diet (HFD) as compared to saline-treated control mice on HFD. We hypothesize that γδ T cells contribute to inflammation in adipose tissue by producing inflammatory cytokines that could recruit and activate other immune cells. γδ T cells were shown to produce IFN-γ in obese mice by flow cytometry where a fraction of γδ T cells present in the stromal vascular fraction obtained by collagenase digestion of the fat pad showed intracellular IFN-γ staining and were CD27+, a marker for IFN-γ-producing γδ T cells in the periphery. A number of subsets of γδ T cells exist but little is known about which ones play a role in regulating inflammation in adipose tissue. The γδ T cell subsets observed in epididymal adipose tissue of WT mice were found to be Vγ2,4,6 and Vδ3,4 which were also present in mice on HFD for 5 weeks. In contrast to the reduced inflammatory response, adiposity of TCRδ/-/- mice on HFD was equal to that of wild type mice. Analysis of adipose tissue, though, revealed smaller adipocytes in the TCRδ/-/- with a greater number/unit area and also lower total body fat mass by quantitative magnetic resonance studies. Recent studies have shown that obesity also results in inflammation in the skeletal muscle and liver, which along with adipose tissue inflammation could play a role in leading to insulin resistance. We showed that obese TCRδ/-/- mice have reduced expression of markers of inflammation in the soleus muscle and liver as compared to wild type mice on HFD. Thus, it appears that the γδ T cell knockout mice in an obese state have markedly reduced inflammation compared to obese wild type mice.

Contributors: Mehta, Pooja
Angelman syndrome is a neurodevelopmental disorder mostly attributed to deficiency of UBE3A at 15q11.2. Unlike ordinary autosomal genes, UBE3A is subject to genomic imprinting with mono-allelic expression only from the maternal chromosome in neurons. It is unknown how the imprinting status of UBE3A is established, since no differential DNA methylation has found to be associated with its promoter. However, an antisense ncRNA named UBE3A-ATS overlapping UBE3A locus has been identified and hypothesized to suppress UBE3A in cis. By studying a mouse model carrying a 0.9kb genomic deletion of the paternal Snrpn major promoter, we found reduction of Ube3a-ATS associated with partial activation of paternal Ube3a. Such activation is not a result of imprinting defects because DNA methylation and expression of upstream imprinted genes remain normal, suggesting a direct repressive role of Ube3a-ATS in mediating paternal Ube3a silencing. To further validate the result, a polyA cassette was inserted downstream of Ube3a to terminate the transcription of Ube3a-ATS. In this mouse model, no other transcripts, but only the part of Ube3a-ATS overlapping Ube3a locus is removed. On the Angelman syndrome background (maternal Ube3aKO), paternal Ube3a is shown to be completely activated. Behavior tests will be further performed to assess the recovery of Angelman behavior defects by the STOP allele. Overall, our studies show ncRNA Ube3a-ATS functions to suppress paternal Ube3a expression. Targeting this antisense RNA to activate the silenced paternal Ube3a may provide novel insights into therapeutic development for Angelman syndrome.
RNA SECONDARY STRUCTURE PREDICTION INCLUDING K-NONCROSSING PSEUDOKNOTS

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Pseudoknots are cross-serial interactions between different sections of a RNA that are functionally important in many classes of noncoding RNA. Current methods of predicting RNA secondary structure fall short in at least one of the following areas:

Unable able to predict pseudoknot motifs, which are expected to play a role in structural stability for larger RNA structures.

A small, obscure output class. For example, pknotsRG (Reeder, Steffen et al. 2007) was only able to produce, due to algorithmic constraints, a little over half of the pseudoknot structures in Pseudobase (van Batenburg, Gultyaev et al. 2000).

Extremely computationally expensive such that their use on ncRNA longer than 50 bases rendered them infeasible (Huang, Peng et al. 2009).

Overly simplistic calculation of pseudoknot thermodynamic contributions (Andronescu, Pop et al. 2010), typically only accurately representing H-type (the most simple) pseudoknots.

Our method, built to overcome these deficiencies, offers a discernible and large output class, multiple (suboptimal predictions), guaranteed optimal solutions given a set of thermodynamic parameters (not heuristic), and computational efficiency via parallelization using the OpenCL framework. This new, robust, cross-platform method will provide researchers investigating RNA with a powerful tool to aid in challenges such as building 3D structure models, predicting functional switchable elements, locating conserved structural motifs that are undetectable when looking at the primary or secondary structure alone, and predicting the effects of mutation.

Contributors: Menlove, Kit; Ma, Jianpeng
Transgenic animal models of Alzheimer’s disease have revealed significant deficits in the proliferation and survival of adult-born neurons in the hippocampal dentate gyrus. Mounting evidence supports a role for these neurons in episodic memory formation and their loss in cognitive deficits. Yet, the experimental systems currently available prevent studying their functions without inevitably destroying them in the process. The Jankowsky laboratory is in the final stages of developing a novel transgenic mouse, which will allow selective silencing of any genetically defined neural circuit in a spatially and temporally restricted manner. Neuronal silencing in this mouse is based on the cre-induced expression of a ligand-gated chloride channel (GluCl) that is activated by the commonly used anti-parasitic drug ivermectin. By crossing the floxed-stop GluCl mice to a line expressing creER only in neuronal precursors (nestin-creERT2), we will be able to reversibly hyperpolarize (and therefore acutely inactivate) adult-born neurons using the GluCl-specific agonist ivermectin. By preventing these cells from participating in the hippocampal circuit, we will be able to directly test the role that adult hippocampal neurogenesis plays in learning and memory and that its loss may contribute to cognitive decline in Alzheimer’s disease.
ROTAVIRUS INDUCES RAPID POLYCLONAL ACTIVATION AND ANTIBODY PRODUCTION

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The immune system responds to many pathogens by nonspecific proliferation and differentiation of B and T lymphocytes (polyclonal response), which helps maintain immunologic memory. The mechanisms of induction and the roles of polyclonal responses in pathogen immunity are not well defined. However, polyclonal responses might increase antibody production to pathogens through non-canonical pathways by enabling rapid B cell activation and antibody production in a T cell independent fashion. Rotavirus, the leading cause of acute gastroenteritis in young children worldwide, induces early antibody production in a T cell independent manner. The murine model of rotavirus infection induces rapid and massive B cell activation and increased numbers of IgA+ cells in the local lymphoid tissues, but the majority of these B cells are not rotavirus specific. These data suggest that rotavirus induces a polyclonal immune response but the molecular pathways and implications of this response in rotavirus immunity are not known. Since early B cell activation by rotavirus is T cell independent, I hypothesize that dendritic cells modulate a rapid polyclonal B cell response to rotavirus. The large increase in surface IgA expression, suggests that rotavirus induces hypergammaglobulinemia (Hlg). To determine induction of Hlg, mice were mock or rotavirus inoculated and serum and stool samples assessed for concentrations of total immunoglobulin (Ig) by ELISA. At 21 days post inoculation total Ig concentrations in sera of rotavirus inoculated mice were approximately two fold greater than the mock infected mice. Total IgA antibody concentrations in stool were also elevated at early time points post infection. To assess whether rotavirus induces production of non-specific antibodies, stool were also assessed for antibody to an unrelated antigen, E. coli lipopolysaccharide (LPS) by ELISA. Compared to mock inoculated mice, stool from rotavirus inoculated mice had elevated levels of antibodies to LPS. Thus, rotavirus inoculation induces Hlg and production of non specific antibodies implying the induction of polyclonal responses. Further experiments will determine the (1) kinetics of the sera and stool Hlg and LPS antibody responses as well as autoimmune antibodies, (2) relationship between the polyclonal and antigen specific B cell response to rotavirus, (3) need for MyD88, TGF-β and BAFF signaling in the polyclonal response. Understanding whether an acute viral infection induces polyclonal antibody responses and Hlg prior to a specific antibody response and whether this response is critical for the induction of the pathogen specific antibody response will provide important insights into the mechanisms of immune protection that could be utilized during vaccine development as well as inducing and maintaining immunologic memory.

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ATP-binding cassette (ABC) transporters can translocate a broad spectrum of molecules across the cell membrane such as lipids, peptides, amino acids, ions, and xenobiotics. ABC genes are known for the role they play in resistance towards anticancer agents in chemotherapy of cancer patients. The soil amoeba Dictyostelium discoideum possesses 68 members of the ATP-binding cassette (ABC) super family of genes. The function of most of these transporters is not known in Dictyostelium or in other higher organisms. We hypothesize that the ABC transporters have functional similarities in correlation with the structural similarities they show. The functional similarity is reflected in their effect on survival and cell fate determination during the development of Dictyostelium discoideum. We propose to test these hypotheses through a systematic study of mutations in these transporters.

We are currently categorizing ABC transporters based on the transcriptional profile of the respective mutants. Transcriptional profiling using second generation sequencing yields very high resolution phenotypes for mutant analysis. Transcriptional profiling has been performed for 48 ABC genes to functionally classify them and to obtain a genetic network. Morphological characterizations of ABC mutant strains support transcriptional phenotyping classification. We see evidences supporting our hypothesis that the functional similarities obtained for a cluster of ABC transporters through transcriptional phenotyping, will be manifested through their cellular and molecular phenotypes. Through these analyses we hope to characterize the physiological functions of these evolutionarily conserved ABC genes.

We are also currently studying the specific role of ABC transporters in cAMP signaling. Dictyostelium employs cAMP as a chemoattractant and as a central extracellular signaling molecule during development. The mechanism of secretion of cAMP in Dictyostelium is unknown. We are testing the role of specific ABC transporters in cAMP export through genetic and biochemical approaches. We have knocked out our candidate genes and are testing the ability of these mutants to secrete cAMP. Generation of multiple gene knockouts within the same strain and expression of candidate genes in other systems to test their ability to export cAMP is underway to identify cAMP exporters.

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MUTATION OF SF_SWAP RESULTS IN MICE WITH VESTIBULAR AND COCHLEAR DEFECTS

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Sfswap is a splicing factor believed to be important for exon skipping. Random insertion of a tyrosinase minigene in the 4th intron of Sfswap results in mice with vestibular and cochlear defects. Analysis of mutant Sfswap cDNA shows that many aberrant splice forms of Sfswap are produced, the most common of which includes 111 bases of vector inserted into the RNA. This insertion disrupts an essential RNA binding domain, likely leading to a mouse hypomorphic for Sfswap. Behavioral assays indicate that homozygous mutants are hyperactive and have dramatic circling behavior as well as other abnormal behaviors associated with vestibular defects. Deficits in Auditory Brainstem Response (ABR) and Distortion Product Otoacoustic Emissions (DPOAE) assays indicate the mutants also have a significant and moderate degree of hearing loss at the level of the cochlea. Histological analysis reveals P0 mutant mice have a reduction in outer hair cells and support cells as well as supernumerary inner hair cells. In addition, mutants have shorter cochleas and smaller neural regions of the vestibular system. This data is consistent with the degree of hearing loss observed with in ABR and DPOAE. Future studies will focus on identification of genes downstream of Sfswap that are important for proper development of the cochlea and vestibular apparatus.

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Hedgehog signaling is important in organogenesis and stem cell function, and misregulation contributes to various cancers, including breast cancer. Breast cancer patients with ductal carcinoma in situ and invasive breast cancer show decreased expression of Patched 1 (Ptch1) (a negative regulator of signaling) and increased expression of Smoothened (Smo) (the main activator of signaling) [1]. A transgenic model with an activated Smo allele in the mammary epithelium showed hyperbranched and hyperproliferative mammary glands but decreased stem cell function. Previous studies of Ptch1 in the mouse mammary gland using hypomorphic or null mutant alleles demonstrated that epithelial, stromal, and systemic Ptch1 is required for normal development [2, 3] with morphology and proliferation profoundly altered in these mutants. However, the specific roles of Ptch1 and Smo in these different compartments with respect to normal development and stem cell function have not been dissected fully.

We hypothesize that inhibition of hedgehog signaling in both epithelium and stroma is required for normal mammary stem cell function and gland development. To address this hypothesis, I will examine the epithelial and stromal roles of Ptch1 in regulating stem cell function using a conditional knockout mouse model, and address whether phenotypes observed are due to canonical (via Smo) or non-canonical mechanisms.

We have found that in vivo ablation of Ptch1 in fibroblasts results in an adult virgin gland with filled, stunted ducts and reduced proliferation. Studies are in progress to understand whether phenotypes are due to local or systemic effects, and determine whether stem cell function is compromised. Preliminary data from adenovirus-Cre treated wild-type and Ptch1fl/fl mammary epithelial cells transplanted into a wild type fat pad suggest a requirement for epithelial Ptch1 for normal duct formation. Finally, conditional loss of Smo in the mammary epithelium has not produced a ductal phenotype, suggesting that epithelial Smo is dispensable for normal development of the adult virgin gland. Studies are in progress to investigate phenotypes in lactation and involution, and evaluate effects on stem cell function.
ENGINEERING A SELECTION FOR STRESS-INDUCED GROSS CHROMOSOMAL REARRANGEMENTS IN ESCHERICHIA COLI

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Gross chromosomal rearrangements (GCRs) including copy-number variations underlie most variation between human genomes, promote cancer and evolution, and are induced by stress in E. coli. When E. coli cells are placed under stress such as starvation, their mutation rate increases transiently until a mutation occurs that allows the cell to escape the stress. In a well characterized assay, E. coli carrying a leaky lac frameshift allele are starved on lactose-only medium. After several days, some cells in a subpopulation acquire compensating mutations that restore the ability to utilize lactose, either compensatory frameshift (“point”) mutations, or amplifications of the leaky allele to 20-50 copies, which confers sufficient enzyme activity for growth. These are distinguished by plating on rich medium with X-gal dye, on which point mutants give solid blue colonies and amplified clones form sectored colonies. The amplifications form by micro-homologous recombination and provide a model system for study of the mechanisms of GCR. We would like to understand the molecular mechanism of formation of these GCRs. Amplification is difficult to study because even after seven days starvation, most of the colonies are point mutants, and the sectored colony assay is laborious. Therefore, I designed an assay that selects amplification directly during starvation stress. I am constructing E. coli cells with an argA gene deletion and the lac frameshift allele flanked by a 5’- and a 3’-truncated argA gene, with varying degrees of homology (3 bp to 300 bp) between them, in which micro-homologous recombination could occur to restore argA+. We will starve the cells on lactose without arginine. Only through amplification of the leaky lac, including micro-homologous recombination between the truncated argA copies, can these cells escape the starvation stress and form colonies. We will use this new assay to measure rates of homologous and nonhomologous (micro-homologous) recombination as the cells transition from growth to starvation, to select amplifications, and further elucidate the requirements of stress-induced amplification.

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Background: We have previously shown that overexpression of metastasis associated protein 2 (MTA2) can render estrogen receptor (ER)-positive breast cancer cells hormone-independent through posttranscriptional effects on ER acetylation and phosphorylation. Recently we discovered that MTA2 overexpression in ER-negative MDA-MB-231 breast cancer cells enhances invasion and metastasis in athymic nude mice, which involves activation of the Rho pathway. It is also known that the androgen receptor (AR) is frequently expressed in breast tumors, however its role in proliferation, invasion, or breast cancer metastasis is poorly understood.

Materials and Methods: MTA2 was stably overexpressed in MDA-MB-231 and its levels validated using Western blot analysis. To identify downstream effects due to MTA2 overexpression, we employed reverse phase protein arrays, and Affymetrix expression arrays. Significant changes in gene expression were identified using dCHIP software, and student t tests. Invasion was examined using modified Boyden chamber growth assays, proliferation was assayed using soft agar growth and MTT assays, and metastasis was measured as tumor growth in athymic nude mice.

Results: MTA2 overexpression in ER-negative cells enhanced distant and skin metastases in athymic nude mice. In an attempt to identify effectors of MTA2-mediated metastasis, we used RNA microarray and protein array screens, and found that AR RNA and protein were significantly increased. As a control, we employed a rescue of the highly metastatic MTA2-MDA-MB-231 cell phenotype via stable transfection and add-back of Rho GDI(, a gene that was concomittantly down-regulated with MTA2 overexpression. AR levels were significantly reduced with Rho GDI( add-back. To determine if AR was functional in these cells, we used ARE-luciferase reporter assays and discovered that both basal, and agonist (R1881)-induced transcriptional activity were significantly enhanced. The AR antagonist, bicalutimide, was effective at significantly decreasing the invasive potential of MTA2-overexpressing. In vivo experiments in nude mice with bicalutimide treatment are underway.

Discussion: AR was significantly overexpressed with the aggressive phenotype conferred via MTA2 overexpression. This suggests that blockade of AR action might provide a new therapeutic target to inhibit metastasis of breast cancer cells.

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Expanded triplet repeats have been identified as the basis for a number of neurological and skeletal disorders. Zinc-finger nucleases use a tandem array of zinc fingers fused to the Fok1 endonuclease to direct double-strand breaks in a site-specific manner. We developed zinc-finger nucleases to recognize and cleave CAG repeat sequences and examined the contribution of double-strand break repair to CAG•CTG repeat instability in mammalian systems. Using our previously described tissue culture assay for identifying modifiers of CAG repeat instability, we found that treatment with the nucleases induced up to a 15-fold increase in changes of the CAG repeat. Analysis of the individual colonies arising after treatment revealed a spectrum of events dominated by repeat contractions. Additionally, we found expression of a dominant form of RAD51, in combination with the zinc finger nuclease, reduced the effect of the nuclease. This finding suggests double-strand break-induced repeat instability is mediated, in part, through homology directed repair. These studies identify the zinc finger nuclease as a useful reagent for characterizing the effects of double-strand breaks on CAG repeats in cells. In addition, that the induction of a double-strand break could be a novel therapeutic approach for treating triplet repeat disorders by contracting a long, disease causing repeat to a shorter, nonpathogenic length.

Contributors: MITTELMAN, DAVID; MORTON, JASON; SYKOUDIS, KRISTEN; LIN, YUNFU; TRAUTMAN, JONATHAN; CARROL, DANA; WILSON, JOHN
Norwalk virus (NV), the prototype human Calicivirus, is the leading cause of acute gastroenteritis worldwide. The NV protease cleaves the polyprotein, which is encoded by ORF1, at five sites (with only three types of sites: Q/G, E/G, and E/A) to release six non-structural proteins essential for replication. The requirement of protease for successful viral replication makes it a prime target for antiviral drug design. The goal is to delineate the structural determinants that govern the multi-substrate recognition and specificity in the NV protease to facilitate rational design of small molecule protease inhibitors. The X-ray structure of an NV protease construct with an active site mutation was determined to 2.2 Å resolution. In the structure the C-terminal tail of one of the protease molecules, representing a native substrate P1-P5, is inserted into the active site cleft of the neighboring symmetry related protease molecule providing visualization of substrate recognition by the protease in unprecedented detail. This structure provides new insights for the design of substrate-based inhibitors with unsaturated carbonyl groups. Peptide-based inhibitors with sequences mimicking substrate and cleavage sites in the NV protease were synthesized and screened for their inhibition potency. Crystallographic structures of the NV protease in complex with each of the three most potent inhibitors, including one structure at ~1.6 Å resolution, have been determined. These structures exhibit conformational changes surrounding the S1 pocket of the protease to accommodate variations in the P2 site of the substrate/inhibitor, which could be a mechanism for how the NoV protease recognizes multiple sites in the NV polyprotein with differential affinities during virus replication. These structures further indicate that the mechanism of inhibition by these inhibitors involves covalent bond formation with the side-chain of the conserved cysteine in the active site by nucleophilic addition and such substrate-based aldehydes could be effective protease inhibitors. We acknowledge support from NIH (PO1 AI057788 and P30DK5638), and the Robert Welch Foundation (Q1279).
BACKGROUND Cytomegalovirus (CMV) is a leading cause of congenital disability, including sensorineural hearing loss, developmental delay, and mental retardation. Thus, CMV is a leading vaccine priority.

OBJECTIVES To determine the seroprevalence and associated risks of CMV infection in adolescent females.

METHODS Females (12-17 years) were recruited from Cincinnati, Galveston, Houston, and Nashville from 6/2006-7/2010. Participants (N=1585) responded to questions regarding potential exposures using a computer assisted self interview. For those with young children in the home (N= 858), additional questions were asked about feeding and changing diapers, and for those > 14 years of age (N= 1162), questions regarding sexual activity were asked. Serum was evaluated for CMV Ab using a commercial IgG assay.

RESULTS The prevalence of CMV was 49% in 12 year old participants and ranged from 46-51% through age 17. In the univariate analyses, the CMV seroprevalence was significantly higher among African Americans (OR 2.06, p<0.001), those with children < 3 years of age in the home (OR 1.88, p<0.001), and those with a history of oral, anal, or vaginal intercourse (OR 1.31, p=0.02). Among those with young children in the home, the odds ratio further increased in females who fed them (OR 2.06, p=0.005) or changed diapers (OR 1.55, p=0.001). Kissing and only having vaginal intercourse was not associated with CMV.

CONCLUSIONS
By age 12, CMV infection was common. Seroprevalence was related to African-American race and household contact with young children. The role of sexual exposure remains unclear in this population.

Contributors: Stadler, Laura; Bernstein, David; Callahan, Steve; Turley, Christine; Munoz, Flor; Ferreira, Jennifer; Simone, Gina; Patel, Shital; Edwards, Kathy; Rosenthal, Susan.
ACTIVATION OF WNT SIGNALING ARRESTS EFFECTOR DIFFERENTIATION IN HUMAN PERIPHERAL AND CORD BLOOD-DERIVED T LYMPHOCYTES

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The canonical Wnt-β catenin signaling pathway plays an important role in thymocyte development and T cell migration but little is known about its role in naïve-to-effector differentiation in human peripheral T cells. We show that activation of Wnt-β catenin signaling arrests human peripheral blood and cord blood T lymphocytes in the naïve stage and blocks their transition into functional T effector cells. Wnt signaling was induced in polyclonally activated human T cells by treatment either with graded doses of synthetic GSK3β inhibitor TWS119 or the physiological Wnt agonist Wnt-3a and this preserved a naïve phenotype (CD45RO– CD45RA+ CD62L+) in both CD4+ and CD8+ peripheral T cells. These Wnt-induced phenotypically naïve cells also showed reduced effector T cell function measured by degranulation and IFN-γ production in response to polyclonal T cell activation and measured by tumor cell lysis in antigen-specific redirected T cells. In addition, Wnt signaling impaired T cell activation by inhibition of proximal TCR signaling and significantly blocked T cell division. The block in T cell division may be attributed to the reduced IL-2Rα expression in TWS119-treated T cells that lowers their capacity to utilize autocrine IL-2 for expansion. Similar effects were observed in T cells derived from cord blood, which can be considered a better source of immature T cells compared to those circulating in peripheral blood. However, Wnt-induced T cells could be rescued from arrest in proliferation by exogenous γ chain cytokines allowing them to become effector T cells. We also found that Wnt signaling induced by TWS119 does not imprint on the T cell phenotype and functions as these cells acquire proliferative and effector function when restimulated in the absence of TWS119. Altogether, our data suggest that canonical Wnt signaling is a negative regulator of naïve-to-effector T cell differentiation in human T lymphocytes. In future experiments, the mechanism by which Wnt-β catenin signaling interacts with the TCR signaling pathway and specifically inhibits T cell activation and differentiation will be further investigated. The arrest in T cell differentiation induced by Wnt signaling might have relevant clinical applications such as to preserve the naïve T cell compartment in antigen-specific T cells generated ex vivo for adoptive T cell immunotherapy.

Contributors: Muralidharan, Sujatha; Hanley, Patrick; Liu, Enli; Chakraborty, Rikhia; Bollard, Catherine; Shpall, Elizabeth; Rooney, Cliona; Savoldo, Barbara; Rodgers, John; Dotti, Gianpietro
EMAN2 AND FREALIGN: COMPARISON OF CRYO-ELECTRON MICROSCOPY RECONSTRUCTION SOFTWARE

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Cryo-electron Microscopy (Cryo-EM) has proven to be a successful way to resolve biological structures to high resolution. As imaging technology improves, it is important to ensure that the software used to analyze and solve these high-resolution structures is held to a high standard. To that end, we compare EMAN2 and FreAlign, two software suites for high-resolution refinement of 3D reconstructions from Cryo-EM images.

We examine both reconstruction and refinement of particle parameters, and compare the final 3D structures generated by these two programs. Furthermore, we examine the methodology used by the two programs to refine and reconstruct the single particle images. We use both asymmetrical samples and specimen showing symmetry for testing and upon examination, we see comparable performance from EMAN2 and FreAlign with some notable differences.

Contributors: Murray, Stephen C.; Ludtke, Steven J.;
Growth differentiation factor 3 (GDF3) is a member of the TGF-β family expressed in adipose tissue and its expression is up-regulated under high fat diet (HFD) conditions. GDF3 knock out mice are protected against HFD-induced obesity compared to wildtype mice. In contrast, adenoviral transfer of GDF3 in mice results in enhanced sensitivity to HFD including increased adipocyte size and adipose tissue mass relative to wildtype mice under the same diet conditions. These results suggest a possible role of GDF3 in regulation of differentiation and/or function of adipocytes. We hypothesize that under HFD conditions, loss of GDF3 function results in increased lipolysis via effects on (-adrenergic receptor signaling or other mechanisms, or defects in lipogenesis resulting in protection from obesity. We differentiated 3T3-L1 cells into mature adipocytes and treated the cells with GDF3 recombinant protein. We used quantitative RT-PCR to assess the expression of mature adipocyte genes and glycerol and non-esterified fatty acid assays to measure lipolytic activity. There is a time and concentration-dependent increase in free glycerol in the media of differentiated cells treated with GDF3 while there is no change in undifferentiated cells. Expression of lipogenic genes, fatty acid synthase, diacylglycerol acyl transferase and stearoyl coenzyme A desaturase increased from 4 hrs to 24hrs. However, after 24 hours, the same genes showed a decrease in expression with a corresponding increase in GDF3 concentration. Together, these results suggest that GDF3 has lipolytic effects in mature adipocytes in vitro. We are currently examining the cell autonomous effects of GDF3 on differentiated mouse embryonic fibroblasts and preadipocytes from wildtype and Gdf3/- mice. Finally we are examining the effects of lipolysis via (-adrenergic receptor signaling.

Contributors: Namwanje, Maria; Bournat, Juan; Huan, Lihua; Brown, Chester
The Aurora family of serine/threonine kinases is required for regulating mitosis and is frequently overexpressed in human tumors. They are maximally activated in the G2/M phase of the cell cycle and regulate multiple events during M-phase. Most vertebrate organisms possess two such mitotic regulators, Aurora A and B. Aurora A regulates centrosome maturation and mitotic spindle bipolarity, while Aurora B regulates chromosome congression, the spindle assembly checkpoint, central spindle structure, and cytokinesis. In this report, we identify DHX9 and Ku70, two components of a putative heterochromatin formation complex, as in vitro interacting partners for Aurora B kinase. Of which DHX9 is phosphorylated on the arginine/glycine rich C terminal domain by Aurora B kinase in vitro. Additionally, we show that Aurora B kinase localizes to sites of DNA replication in interphase nuclei. Furthermore, inhibition of Aurora B kinase activity with ZM447439 during early to mid S phase delayed mitotic progression due to a failure in chromosome congression to the metaphase plate. This investigation provides a novel and surprising function for Aurora B in the S/G2 phase, preceding its well-established activity in M-phase, which are both essential for chromosome congression.

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BACTERIAL DISCRIMINATION IN THE SOCIAL AMOEBA DICTYOSTELIUM DISCOIDEUM

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The social amoeba Dictyostelium discoideum inhabits the forest soil and feeds on bacteria. We hypothesize that Dictyostelium amoebae possess mechanisms that enable them to discriminate among different bacteria, allowing the amoebae to respond to changes in the soil microbiota, achieve optimal feeding, and avoid exploitation by pathogens. To identify candidate genes required for handling different bacterial species, we performed a forward genetic screen where we selected for mutants that can grow on one bacterial species but not on a different bacterial species. From this initial screen, we identified genes that are required for Dictyostelium growth on Gram-positive bacteria, but not Gram-negative bacteria, as well as other genes with inverse phenotype. Using the Illumina Solexa Sequencing technology, we also identified genes that are differentially expressed when wild-type amoebae are grown on different bacteria. Our results indicate that Dictyostelium amoebae have distinct physiological responses to different types of bacteria. We derived a transcriptional profile of wild-type amoebae growing on Gram-positive and Gram-negative bacteria. We applied this transcriptional profile to examine changes in the transcriptional landscape in mutants with a severe growth phenotype on Gram-positive or Gram-negative bacteria. We identified at least two distinct pathways in amoebae for handling Gram-positive bacteria. The first pathway seems to involve a metabolite of glucose that acts as an internal signal to sense Gram-positive bacteria. This signal controls the induction of lysozymes and hydrolases that degrade peptidoglycan. The other pathway seems to involve a receptor glycoprotein. On the other hand, the growth of amoebae on Gram-negative bacteria seems to involve a more complex pattern, with the strongest response reserved to Enterobacteriaceae. We expect that this work will contribute to an understanding of the innate immune responses in other organisms, and help illuminate the natural ecology of soil microorganisms, some of which are important human pathogens.

Contributors: Nasser, Waleed; Parikh, Anup; Santhanam, Balaji; Miranda, Roshan; Dinh, Chris; Juneja, Kavina; Chen, Rui; Shaulsky, Gad; Kuspa, Adam.
Dyskeratosis congenita (DC) is a bone marrow failure and cancer predisposition syndrome. Underlying DC is a defect in the maintenance of telomeres, the essential nucleoprotein structures located at the ends of linear chromosomes. Reflecting this, six of the seven genes mutated in DC encode components or factors required for the assembly or activity of telomerase, the enzyme that replenishes terminal telomeric repeats following DNA replication. Fifteen percent of patients with DC have mutations in the seventh gene, TINF2, which encodes TIN2, a central member of the telomeric binding complex shelterin. The vast majority of TINF2 mutations are de novo, and patients with these mutations have shorter telomere lengths and earlier age of disease onset in comparison to other DC patients, underscoring the severe disruption in telomere function conferred by these mutations. Both a short (TIN2S) and a long (TIN2L) isoform of TIN2 are expressed in human cells. TIN2L associates with the telomeres and the nuclear matrix, indicating that it is unlikely to have identical function to TIN2S. All DC-associated TINF2 mutations are heterozygous and map to a region of unclear significance (the DC-cluster), located between its known binding regions and a C-terminal extension unique to TIN2L. The goal of this project is to determine how DC-associated TINF2 mutations result in such severe telomere shortening. We hypothesize that DC-associated TINF2 mutations result in such severe telomere shortening because they specifically impact on a unique function of TIN2L that is absent from the short isoform. This hypothesis is supported by co-immunoprecipitation studies that showed TIN2L interacted more robustly with TRF2, a member of the shelterin complex, than TIN2S and that this increased interaction required the DC-cluster region and a conserved region of TIN2L. We will use a combination of TINF2 mutant DC patient derived lymphoblastic cell lines, protein mass spectrometry, and peptide array to further elucidate the unique functions of TIN2L that are absent from TIN2S and the role of these functions at the telomere and in the devastating telomeric disease dyskeratosis congenita.

Contributors: Nelson, Nya D; Bertuch, Alison A.
IDENTIFICATION OF RHEUMATOID ARTHRITIS PATIENTS USING AN ADMINISTRATIVE DATABASE: A VETERANS AFFAIRS STUDY

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Introduction: Computerized administrative databases are increasingly being used to identify patients for research studies. Data pertaining to database utility for rheumatoid arthritis (RA) is sparse and variable. We assessed the utility of a Veteran Health Administration (VHA) database to identify patients with rheumatoid arthritis.

Methods: Using the ICD code for RA, 544 patients were randomly identified and stratified based on disease modifying anti-rheumatic drugs (DMARD), rheumatologist visit and rheumatology visit diagnosis status. RA was deemed to be present when 4 or more of American College of Rheumatology (ACR) diagnostic criteria for RA are met, when there is a positive anti-cyclic citrullinated peptide (CCP) positivity or when DMARD are prescribed for RA by outside rheumatologists. All charts were reviewed for the clinicians’ diagnoses and clinical criteria documentation and the correlation with the ICD coding.

Results: The positive predictive value for RA with two ICD 9 codes of 714 made six months apart is 32.6%. On the whole, having DMARD increased the PPV to 64.1%. The highest PPV of 91.4% was when patients were on DMARD, seen by Veteran Affairs Medical Centre (VAMC) rheumatology and a diagnosis of RA made at the last VAMC rheumatology visit. This number dropped to 75% when RA was diagnosed at any VAMC rheumatology visit. For the group not on DMARD, PPV ranged from 0.0 to 15.0%. Almost half of the patients diagnosed with RA by primary care physicians (PCPs) have no ACR RA criteria documentation. Approximately one-third of the patients with RA per PCP diagnosis were not being treated with DMARD.

Conclusions: While it is clear that using single ICD 9 codes in database studies to identify RA subjects is not sufficient, we have found that having two ICD 9 codes six months apart was also not acceptable. Adding DMARD as an inclusion criterion increased the accuracy of the RA diagnosis. Charts have to be reviewed to confirm the diagnosis. Under-documentation remains a formidable problem in electronic databases. Greater need of PCP education about RA is needed to improve documentation of RA criteria and RA knowledge. Coding training and better practices are essential to improve accuracy of database based studies.

Contributors: Ng, Bernard; Aslam, Fawad; Petersen, Nancy; Hong-Jen, Yu; Suarez-Almazor, Maria
TOWARDS PHASE 2/3 TRIALS FOR EPSTEIN - BARR VIRUS (EBV)-ASSOCIATED MALIGNANCIES

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Advisor: Cliona Rooney, Ph.D.-Department of Pediatrics
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About 30% of lymphomas arising in immunocompetent individuals as well as >90% of undifferentiated nasopharyngeal carcinomas (NPC) carry the EBV genome. We have targeted two of the expressed EBV latent antigens (LMP1 & 2) in clinical trials for the treatment of refractory lymphoma and NPC. LMP-specific T cells had minimal toxicities and produced tumor responses in over 60% of lymphoma patients. In patients with NPC 15/34 (45%) of infused patients had tumor responses including 5 CRs, which were predominantly observed in patients with locoregional disease (4/5).

Our current method of generating LMP-specific T cells presents several critical barriers to its transition to pivotal phase 2 and 3 clinical trials, including the use of live virus (EBV) and viral vectors (adenovirus), difficulty in generating autologous antigen presenting cells, and competition between our antigens of interests and more dominant EBV antigens.

To overcome these problems, we have developed a new approach to manufacture type 2 latency antigen-specific T cells by replacing adenovirus and EBV with overlapping peptide libraries (20 amino acids overlapping by 15) spanning the entire protein sequences of LMP1, LMP2 and EBNA1, as well as a novel system of antigen presenting cells by combining autologous activated T cells (T-APCs) that express HLA class I and class II antigens together with an HLA-negative cell line (K562) expressing costimulatory molecules to prevent anergy induction.

Results: In both healthy donors and NPC patients, this combination of antigen and antigen-presenting cells produced significantly better antigen-specific T cell frequencies and expansion without reducing the T cell repertoire, which remains broad with subsequent stimulations. Competition between peptides within the libraries ensures that the avidity of the responding T cells remains high. By eliminating the requirement for EBV-LCLs we have reduced the minimum generation time from 10 weeks to ~30 days. There were no detectable differences in the phenotype or functions of these T cells, but the specificity was more focused on relevant antigens. In patients with relapsed NPC the frequency of LMP/EBNA1-specific T cells was increased in the presence of blocking antibodies to PD-L1. This phenomenon was not found in healthy donors, suggesting that type 2 latency antigen-specific T cells might be anergic in NPC patients.

Contributors: Ngo, Minhtran Charlotte; Ando, Jun; Ennamuri, Sravya; Helen Heslop, Carl June, Ann Leen, Stephen Gottschalk and Cliona Rooney
Steroid Receptor Coactivator-3 (SRC-3/AIB1) is a potent transcriptional coregulator for nuclear receptors and other transcription factors and is often amplified or overexpressed in tumors. The growth promoting effects of SRC-3 involve its ability to integrate extracellular signals into discrete patterns of gene expression. SRC-3 is regulated by various post-translational modifications, including multiple phosphorylations that define a combinatorial code that tailors SRC-3 activity in response to specific signaling stimuli. HER-2 is a member of the ErbB receptor tyrosine kinase family of growth factor receptors and is often associated with cancer. Clinical studies show that breast cancer patients with tumors expressing high levels of both HER-2 and SRC-3 have reduced disease-free survival, and SRC-3 phosphorylation is influenced by HER-2. The current study seeks to define the effect of HER-2 signaling events on SRC-3 activity in breast cancer cells. HER-2 knock down using siRNA affects multiple kinase pathways, as evidenced by decreased AKT and c-Raf phosphorylation. HER-2 knockdown causes the isoelectric point of SRC-3 to shift, suggesting that post-translational modification of SRC-3 is actively altered by HER-2 signaling. Reduction of SRC-3 phosphorylation in HER-2-depleted cells also was evident using a phospho-specific antibody to SRC-3. Expression microarray analysis revealed that many genes are altered by HER-2 knockdown. Parsing the microarray with SRC-3 ChIP-seq data identified genes that are coordinately regulated by a HER-2/SRC-3 signaling axis. Collectively, this work describes transcriptomic and cistromic mechanisms for oncogenic cooperation between HER-2 and SRC-3 and identifies key genes that contribute to proliferation of breast cancer cells.

Contributors: Smith, Carolyn L.
Gastrointestinal (GI) infections are the second leading cause of death worldwide, with almost 80 million cases of food-borne illness occurring each year in the US alone. Enterohemorrhagic Escherichia coli (EHEC) and other pathogenic E. coli strains, such as the strain that caused the recent outbreak in Germany, continue to cause severe outbreaks with devastating social and economic outcomes. During infection, enteric pathogens are exposed to large quantities of bile, which is secreted into the GI tract to aid in digestion. Direct catabolites of heme are a major component of bile pigments. This includes bilirubin which is added to bile before secretion into the GI tract. Analysis of the structure of bilirubin suggests it may have antioxidant properties due to a highly conjugated system of electron acceptors, and some preliminary work from others suggests these properties may have biological relevance.

We propose that bilirubin modulates the host-pathogen interaction within the mammalian GI tract. Specifically, we hypothesize that bilirubin serves as an antioxidant to protect pathogenic E. coli from environmental and host induced reactive oxygen species (ROS), thereby allowing bacterial colonization and pathogenesis.

Here, we report that bilirubin, but not its close analog biliverdin (which lacks radical absorbing ability) or bilirubin-glucuronate (a soluble form of bilirubin), protect EHEC against superoxide in culture. Further, EHEC efficiently transported both bilirubin and biliverdin, but not bilirubin-glucuronate, into the cell. This suggests a mechanistic model by which bilirubin localization to the bacterial membrane and neutralizes ROS through its inherent radical absorbing properties. Finally, mice treated with bilirubin showed higher levels of EHEC colonization than untreated animals.

Taken together, these data support the hypothesis that bacterial pathogens utilize heme catabolites for protection from ROS. Future studies will determine if this effect is a mechanism used by enteric bacteria to negate ROS generated by the host immune system.
Introduction: Intrauterine growth restriction (IUGR) has been observed to be associated with the risk of dysregulated lipid metabolism, glucose homeostasis, insulin resistance, and atherosclerotic disease later in life. These effects can be observed across generations and are thought to occur in association with modified epigenetic regulatory mechanisms. Animal models of IUGR from our laboratory and others reveal that these epigenetic changes include modified CpG dinucleotide-specific DNA methylation patterns as well as histone acetylation in the IUGR animal, such as with reprogramming of Igf1. Moreover, we have previously demonstrated that diet supplementation of essential nutrients (ENS) can prevent adult metabolic disorders in a transgenerational uteroplacental insufficiency rat model of IUGR. However, it is not clear to what extent associated epigenetic changes in Igf1 are inherited across generations and whether these changes can be prevented by dietary interventions. We hypothesized that ENS supplementation would prevent transgenerational effects of adult metabolic disease in rats resulting from maternal IUGR in association with epigenetic dysregulation of Igf1.

Methods: Sprague-Dawley P1 dams underwent bilateral uterine artery ligation (n 8) or sham surgery (n 8) on e19, and resultant F1 litters yielded IUGR (n 64) or control lineages (n 64), respectively. On d21, weaned F1 were allocated to ENS (Teklad8640+folic acid, choline, B12, betaine, L-methionine, L-arginine, zinc) or control diet (Teklad8640). F1 pairs were mated by d80, and resultant F2 (n 512) were weaned to their parental diet. We interrogated the differential epigenetic regulation of Igf1 in response to dietary intervention through examination of the DNA methylation status of the second promoter (P2) of the Igf1 gene in fetal liver tissue utilizing bisulfite modification and sequencing. To understand functional dysregulation, we employed Igf1 mRNA quantitative PCR.

Results: Significant differences in mean birthweight were observed among F2 offspring of IUGR lineages (maternal 6.1g, paternal 6.5g, both 5.6g) when compared with shams (8.0g), regardless of maternal diet (6.0g vs 6.1g). However, by d160 these progeny of IUGR animals on control diet exceeded sham body weights (496g vs 411g) while ENS fed had not (340g vs 390g). Accompanying these phenotypic variations, IUGR lineages demonstrated reduced DNA site-specific methylation of the Igf1 promoter 2 in male F2 offspring at day 21 of life relative to sham-operated control animals (p<.04). In contrast, dietary supplementation (ENS) was associated with a significant increase in methylation of the Igf1 promoter 2 in females at day 21 (p<.02). We also found significant changes in the mRNA expression of Igf1 in d21 F2 offspring (Igf1 mRNA in ENS fed sham males 20-40% of control, and in ENS fed sham and IUGR females 30-65% of control, p<.03).

Conclusions: We conclude that prevention of the adverse transgenerational effects of maternal IUGR on metabolism by giving a methyl donor-rich diet occurs (in part) in association with maintaining DNA methylation of Igf1.

Contributors: O’Neil, Derek; Suter, Melissa; Dasso, Joe; Hu, Min; Shope, Cynthia; Showalter, Lori; Lane, Robert; Aagaard-Tillery, Kjersti
Sarcoplasmic Reticulum Mitochondria Associated Membranes (SR-MaMs) are sites of physical attachment between the Sarcoplasmic Reticulum (SR) and mitochondria. These sites are important for Ca2+, reactive oxygen species (ROS), and lipid signaling between the two organelles and are believed to play a role in both cell homeostasis as well as apoptosis. While it has been hypothesized that disruption of these structures is involved in both skeletal muscle dysfunction and heart failure, the exact makeup of these structural tethers, including the identity of the primary Ca2+ release channel, remains incomplete. By modifying a protocol to isolate Endoplasmic Reticulum-MaMs from mouse livers for use in skeletal and cardiac muscle, we have purified SR-MAMs and have demonstrated that they contain calreticulin, calnexin, sigma-1 receptors and a number of other proteins. We also find both ryanodine receptors and IP3 receptors suggesting that we are probably isolating a mixture of SR-MaMs and ER-MAMS with different Ca2+ channels.

Contributors: Lee, Chang Seok; Joshi, Aditya; Hamilton, Susan
Both progesterone (P4) and prolactin (PRL) are required for proliferation of the mammary epithelium and for alveologenesis. Classical endocrine ablation and gene knock out studies show that neither hormone alone is sufficient. Gene microarray experiments with mammary glands of acute P4 treatment of ovariectomized mice, and PRLR knockout (KO) mice, have identified a common set of proliferation gene targets regulated by P4 and PRL. Whether these genes are simply redundant targets or whether regulation requires cooperative interactions between P4 and PRL signaling is not known. To study the mechanisms of hormonal regulation of these genes we have developed 3D culture systems of primary mouse mammary epithelial cells (MECs) embedded in Matrigel that form polarized acini composed of luminal epithelial cells and myoepithelial cells and maintain expression of endogenous PR. Similar to the mammary gland in vivo, PR expression is heterogeneous in luminal epithelial cells and is expressed in non-proliferating cells as determined by Ki67 serial section staining. PR is also functional, mediating a proliferative response to P4, and P4 induction of several of the common in vivo identified target genes of P4 and PRL including Wnt4, RANKL, and amphiregulin. Since RANKL is an important paracrine regulator of P4 proliferation in the mammary gland in vivo, we have focused on this gene to analyze potential cooperation between P4 and PRL signaling. P4 and PRL alone each induced RANKL expression and the two hormones together gave an additive induction under certain conditions. To define whether RANKL is a direct target of PR or Stat5 (downstream target of PRL signaling) transient co-transfection experiments in mouse NMuMG cells were done with PR, PRLR, Stat5 and six different RANKL enhancer regions as reporter genes linked to luciferase. Three enhancer regions were activated by R5020 or PRL. By ChIP assay in a mouse mammary cell line, HC11, and primary MECs, progesterone-dependent binding of PR and Stat5 to these same RANKL enhancers was detected, suggesting PR and Stat5 are required together for progesterone mediated DNA binding. Experiments are ongoing to define the actions of both hormones together, determine the interplay between PR and Stat5 at the RANKL enhancer and to scale up the 3D MEC system to perform ChIP-Seq analysis to define the genomic PR and Stat5 binding sites on the endogenous RANKL gene in response to single and multiple hormones.

Contributors: Obr, Alison E.; Grimm, Sandra L.; Lydon, John P.; Bishop, Kathleen A.; Pike, J. Wesley; Edwards, Dean P.
17-β-ESTRADIOL DECREASES INTERICTAL SPIKE FREQUENCY AND SEIZURES IN A GENETIC MOUSE MODEL OF X-LINKED INFANTILE SPASMS

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X-linked Infantile Spasms Syndrome (ISSX) is a childhood epilepsy syndrome characterized by seizures and motor spasms in the first year of life often associated with mental retardation and autistic features. Currently there is no treatment for either the seizures or developmental deficits. The most common cause of ISSX is a triplet repeat expansion in Aristaless-related homeobox gene (Arx), a transcription factor with complex roles in neurodevelopment. Mutations in Arx have also been linked to dystonia, autism, and lissencephaly. A mouse model, engineered with the same mutation (“Arx(GCG)10+7”), recapitulates many hallmarks of the human syndrome, including epilepsy, cognitive deficits, and interneuronopathy. Arx(GCG)10+7 mice have reduced numbers of Arx+ and calbindin+ GABAergic interneurons in neocortex, dentate gyrus and striatum; NPY+ interneurons in striatum; and ChAT+ interneurons in striatum. Since estrogen receptor activation can influence interneuron development and have antiepileptic effects, I am examining the hypothesis that 17-β-estradiol, the most potent endogenous estrogen receptor agonist, can alter the epilepsy and behavioral deficits in Arx(GCG)10+7 mouse by possibly modulating interneuron development. The data suggest that the anti-epileptic effect of 17-β-estradiol in the Arx(GCG)10+7 mouse is age dependent. Male mutants that received daily 17-β-estradiol injections during the perinatal period (from P3 through P10) had significantly fewer interictal spikes and no seizures during the recorded period, whereas mutants that received 17-β-estradiol from P33-40 did not show a significant reduction in either interictal spike rate or in seizure occurrences compared to vehicle-injected mutants. In addition, the number of striatal ChAT+ cholinergic interneurons seems to be restored in the estradiol-treated mutants in comparison to the vehicle-treated group. These results suggest that estradiol suppresses cortical hyperexcitability in the Arx(GCG)10+7 mouse by modulating perinatal neurodevelopment. Moreover, this effect might be associated a restoration of interneuron populations that are reduced in the Arx mutant brain.

Contributors: Olivetti, Pedro R.; Noebels, Jeffrey L.
TLR4 IS REQUIRED FOR PROTEINASE-MEDIATED ALLERGIC LUNG DISEASE.

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Background: Asthma is a lung inflammatory disorder afflicting ≈300 million people worldwide, characterized by the presence of T-helper type 2 (Th2) cells in the lung that produce the signature cytokines interleukin 4 (IL-4), IL-13, and IL-5. Recently, our lab has shown that active fungal proteinases are requisite for robust Th2 cell generation and allergic airway inflammation that protects against fungal invasion during airway infection. Macrophages (MΦs), the most numerous cells in the naïve lung, interact with inhaled pathogens and control airway infections via germline-encoded receptors such as toll like receptor (TLRs) family, suggesting that macrophage TLRs participate in host defense against inhaled fungi.

Hypothesis: We tested the hypothesis that fungal proteinases activate one or more TLR in MΦs, resulting in allergic lung disease and highly effective fungal killing through MΦs.

Methods: Wildtype C57BL6 mice and mice deficient in specific TLRs (TLR4) and molecules required for most TLR signaling pathways (MyD88–/– and TRIF–/– mice) were intranasally infected with viable conidia of Aspergillus niger. Airway resistance in response to increasing doses of acetylcholine, total lung IL-4 producing cells, and airway eosinophilia were assessed to characterize the asthma phenotype. Bone marrow cells were also harvested from TLR4–/– and wildtype mice to assess for differentiation of MΦs by determining relative RNA expression of canonical markers. These macrophages were then cultured with the viable conidia of A. niger to determine their effect on fungal growth.

Results: TLR4–/– mice failed to develop asthma-like disease while MyD88–/– and TRIF–/– mice had normal or exaggerated responses. Fungal proteinase induced differentiation of naive MΦs into highly differentiated cells that profoundly inhibited fungal growth in vitro in a TLR4 dependent manner.

Conclusions: These studies demonstrate that proteinase-dependent induction of the murine asthma phenotype and the MΦ fungistatic response are coordinated through TLR4 signaling involving a novel signaling mechanism. Because TLR4 ligands such as endotoxin are known to suppress Th2 cells and allergic responses in part by promoting Th1 responses, our findings imply the existence in mammals of alternate TLR4 ligands analogous to the protein Spaetzle, which activates the insect TLR4 homologue Toll to induce host defense. Our findings therefore suggest a “Biased Agonism” model of TLR4 signaling in which distinct ligands may activate alternative signaling pathways that give rise to entirely different immune responses. Confirmation of our findings will clarify our understanding of fundamental immunoregulatory pathways and suggest novel means to diagnose and treat asthma.

Contributors: Ongeri,V.; Byrd, T.; Abramson, S.L.; Corry, D.B.
Neuroendocrine-type ATP-sensitive K+ (KATP) channels (SUR1/Kir6.2) play an integral role in glucose homeostasis. KATP channels are metabolic sensors that couple the ATP/ADP ratio to membrane excitability (Vm). Modulation of Vm regulates Ca2+ influx through voltage-gated Ca2+-channels and thus exocytosis of pancreatic hormones and neuropeptides. Mutations in both SUR1 and KIR6.2 can increase channel openings to cause neonatal diabetes (ND) by reducing, i.e., hyperpolarizing, Vm. Nucleotides bind to, and inhibit, openings of Kir6.2, while binding and presumably hydrolysis of ATP on SUR1 antagonizes this action. The SUR1Q1178R ND mutation produces hyperactive channels by “over-stimulating” Kir6.2 and thus hyperpolarizing β-cells to reduce insulin release. Interestingly, this form of ND responds to sulfonylureas like glibenclamide (GBC) used to treat type 2 diabetes, albeit higher doses are required. SUR1 is an ABC (ATP Binding Cassette) protein with three transmembrane domains (TMDs) and two cytosolic nucleotide-binding-domains (NBDs 1 & 2). Binding of 2 ATPs induces formation of an NBD1/2 dimer and reorientation of the TMDs. ATP binding at NBD1 is Mg-independent, while NBD2 binds and presumably hydrolyses MgATP. The MgADP bound, post-hydrolytic SUR1 intermediate is argued to stimulate openings of ATP-inhibited Kir6.2. GBC inhibits channel openings by stabilizing a pre-hydrolytic conformation of SUR1. I used [3H]-GBC binding as a sensitive probe of SUR1 conformational states. In the absence of ATP, wildtype SUR1 binds GBC with nM affinity. MgATP, but not ATP4-, has a negative allostERIC action, which correlates with NBD dimerization and TMD reorientation that reduces the affinity of SUR1 for GBC. Q1178R, and several neighboring ND mutations, increase the apparent affinity for MgATP. Displacement (IC50) of [3H]-GBC from SUR1Q1178R was left shifted ~260-fold by MgATP and ~7-fold by MgADP, versus WT. Comparison of the allostereISM between nucleotide and GBC binding for WT vs SUR1Q1178R showed a reduction in Kd, 98±66 vs 19±6 nM, respectively, for non-hydrolyzable MgADP-BeFx. The increase in affinity for nucleotide was confirmed by photolabeling with 8-N3-[α32P]-ADP. Surprisingly, ATP4- also will displace GBC from SUR1Q1178R with an IC50 ~2.9 mM, while showing no significant effect on WT SUR1 at >100 mM. The estimated Kd’s for ATP4- binding to SUR1Q1178R are 10 (NBD1) and 600 (NBD2) μM. Q1178R is in TMD2, removed from either NBD. The results imply changes in the TMDs can affect NBD dimerization to reduce affinity for GBC, consistent with the need for higher patient dosage, and to increase the fraction of SUR1 that stimulates openings of Kir6.2 to hyperpolarize Vm and thus reduce insulin secretion.

Contributors: Ortiz, David; Voyvodic, Peter; Quast, Ulrich; Bryan, Joseph
Stress granules (SG) are cytoplasmic aggregates of RNA and protein that form during the stress response. They are sites of mRNA storage and sorting, and are thought to sequester stalled translation complexes so that stress response proteins can be preferentially translated during stress. SG formation also inhibits apoptosis, allowing the cell time to overcome various insults. More specifically, recent research has shown that stress granule modulation is a recurrent theme in viral infection. Poliovirus (PV) has been shown to induce, and subsequently disassemble, SGs. Although it is unknown how PV induces SG formation, disassembly of SGs is caused by the cleavage of RasGAP SH3 domain-binding protein (G3BP) by the viral proteinase 3C (3Cpro). Inhibition of SG formation is also important for complete replication of PV. Coxsackievirus B3 (CVB3), a human pathogen and close relative of PV, has also been reported to cleave Ras GTPase-activating protein (RasGAP), an inhibitor of Ras, which is not seen during PV infection. We propose to determine the mechanism and function of SG modulation by CVB3, as well as to understand the role of RasGAP cleavage in CVB3 infection.

Contributors: Ottarson, David; Lloyd, Richard
Background: Biofilm infections are frequently caused by S. epidermidis, are resistant to antimicrobial agents and adversely affect patient outcomes. Farnesol, the Candida quorum sensing molecule, has antibacterial effects.

Hypothesis: Farnesol will inhibit S. epidermidis biofilms, in vitro and in vivo.

Methods: We evaluated ED50, ED75 and ED90 (drug concentrations causing 50%, 75% and 90% inhibition respectively) of farnesol and evaluated synergy with nafcillin and vancomycin. Farnesol’s effects on morphology of S. epidermidis biofilms were analyzed using confocal microscopy and real-time changes using a bioluminescent strain of S. epidermidis, Xen 43. In mice, effects of farnesol treatment on subcutaneous catheter biofilms, cultures of blood, kidney, catheter and peri-catheter tissues and bioluminescence in strain Xen 43 were evaluated.

Results: Farnesol inhibited biofilms (ED50 ranged from 0.625 to 2.5 mM) and was synergistic with nafcillin and vancomycin at most combination ratios. Farnesol significantly decreased biovolume, substratum coverage and mean thickness of S. epidermidis biofilms. In mice, farnesol significantly decreased viable colony counts of S. epidermidis from blood, kidney, catheter and peri-catheter tissues and decreased Xen 43 bioluminescence.

Conclusions: We confirmed the anti-biofilm effects of farnesol both in vitro and in vivo, in a bioluminescent strain and its synergy with antibiotics. Farnesol has great potential for the treatment of clinical S. epidermidis biofilm infections.

Contributors: Pammi, Mohan, Liang, Rong, Hicks, John, Barrish, Jim, Versalovic, James
BAYLOR COLLEGE OF MEDICINE

B CELL EXPRESSION OF HMGB1 MODULATES ANTIBODY PRODUCTION AND NEUROINFLAMMATION

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Department of Pathology & Immunology

Advisor: Shuhua Han, M.D.-Department of Pathology & Immunology

John Rodgers, Ph.D.-Department of Pathology & Immunology

High Mobility Group Box 1 (HMGB1) is a ubiquitously expressed protein that has been linked to central nervous system inflammation. HMGB1 expression is elevated in the serum and tissues of patients with multiple sclerosis (MS). HMGB1 functions as a danger signal and imparts proinflammatory activity. The role of HMGB1 in lymphocyte activation during MS is incompletely understood. To study the role of HMGB1 in immune responses and understand the mechanisms by which HMGB1 mediates neuroinflammation we have generated a transgenic mouse on the C57BL/6 background to conditionally over-express HMGB1 in CD21+ cells with an IRES-eGFP reporter. CD21 is expressed predominately by mature B cells. Transgenic mice show GFP expression by B cells in lymphoid tissue and normal B cell development. To investigate the role of HMGB1 in neuroinflammation, experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis, was induced by immunization with myelin oligodendrocyte glycoprotein (MOG). Clinical scores and incidence were determined. MOG specific antibodies and proliferative responses were analyzed. Cytokine secretion after MOG re-stimulation was tested. Transgenic mice exhibit increased clinical scores during the induction and recovery phases of disease. IgM and IgG antibody titers were similar between littermate controls and transgenic mice at the peak of disease and IgG antibodies were reduced at the end of disease. Transgenic mice show significantly reduced proliferation after in-vitro MOG re-stimulation compared to controls. IFNγ and IL-17 cytokine production have been shown to attribute to EAE pathogenesis. Increased severity of disease by transgenic mice is associated with increased IFNγ and IL-17 cytokine production at basal and low levels of MOG stimulation. These data show that HMGB1 regulates EAE disease recovery. HMGB1 appears to be involved in regulating inflammation in EAE by influencing maintenance of antigen specific antibody responses, antigen specific proliferation and proinflammatory cytokine production. This study uses a novel mouse model and reveals new insights into the inflammatory activity of the danger signal HMGB1 during neuroinflammation.

Contributors: Parillon, Xyanthine; Guo, Lingie, Han, Shuhua
Human brain consists of ~10 billion neurons and there are ~60 trillion synapses. However, how these billion neurons, which receive trillion inputs, are structured and how they shape our perception and memory is still unclear. Neuroscientists have approached this question by identifying neuronal classes and studying their anatomical input connectivity, their physiological properties and functional properties. Here I intend to approach a smaller question: How does one class of neurons integrates its numerous dendritic inputs to generate an overall response in vivo? We will focus on studying pyramidal neurons in layer 2/3 of area V1, which provide the main relay input from V1 to higher visual cortex. Specifically, we are interested to study mouse V1 L2/3 neuronal receptive field properties before and after selective dendritic ablation. To address the question, we have developed a selective ablation approach during which we can selectively prune the dendrites of layer 2/3 neurons using a chameleon Ultra-II laser. This will result in the loss of the synaptic input from the targeted (ablated) dendrites. We will monitor the functional properties of the ablated neurons using i) in vivo two photon guided patch clamping, and ii) calcium indicator dyes.

Contributors: Park, Jiyoung; Meyer, Jochen Fritz Henry; Smirnakis, Stelios Manolis
CHARACTERIZING AND PREDICTING DEEP VENOUS THROMBOSIS IN CHILDREN WITH STAPHYLOCOCCUS AUREUS OSTEOMYELITIS

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Clinical Scientist Training Program  
Advisor: Sheldon Kaplan, M.D.-Department of Pediatrics

Children with Staphylococcus aureus osteomyelitis (vs. other bacterial types) are at increased risk for the developing deep venous thrombosis (DVT). Currently, when children with suspected osteomyelitis are evaluated, they are not routinely screened for DVT. Most DVTs are found incidentally when imaging is performed for diagnosis of osteomyelitis. Symptoms of DVTs such as pain and swelling mimic those seen in children with osteomyelitis and DVT may not be suspected immediately. Increased mortality, morbidity, and utilization of healthcare resources occur in children with Staphylococcus aureus osteomyelitis who develop DVT. In 2007, Hollmig et al, reported the duration of hospitalization (30.6 days for children with DVT vs. 9.5 days for children without DVT), admission to the intensive care unit (60% of children with DVT and osteomyelitis were admitted to an intensive care unit vs. 3% in children with osteomyelitis and without DVT) and mortality was much higher in children with Staphylococcus aureus osteomyelitis who develop DVT than in those without DVT.

There are very few studies characterizing the epidemiology, pathogenesis and treatment or prophylaxis with anticoagulation for these children. Completing the proposed research may lead to changes in clinical practice and potentially decrease morbidity and mortality from DVT associated with Staphylococcus aureus osteomyelitis. The aims of the proposed prospective research would be to:

1. Determine the incidence of deep venous thrombosis (DVT) in children with osteomyelitis at presentation to the hospital.

2. Determine if a biomarker panel (CRP, d-dimer, VWF antigen, TAT complexes and Factor 8 levels) at time of presentation predicts presence of DVT or risk of developing DVT while hospitalized.

3. Determine if anticoagulation within 48 hours of presentation decreases length of hospitalization, need for intensive care utilization, duration of antibiotic therapy, and time to resolution of DVT and Staph aureus infection in patients anticoagulated for DVT compared to those not anticoagulated within 48 hours of admission

Contributors:
Patel KN
ERK AND mTOR PATHWAY ACTIVATION AND INTERACTION IN CORTICAL DYSPLASIA AND EPILEPSY

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Program in Developmental Biology
Advisor: Hugo Bellen, Ph.D./M.B.A.-Department of Molecular & Human Genetics

RATIONALE: Cortical dysplasia (CD) is commonly associated with drug resistant epilepsy and microarray studies of cytomegalic neurons found in CD have shown activation of mammalian target of rapamycin (mTOR) pathway. The cause of mTOR pathway hyperactivity is not known and one explanation for hyperactivation of mTOR pathway is phosphorylation of upstream regulator, TSC2 at the S664 site by extracellular regulated kinase (ERK). Though, the interaction between ERK and mTOR is well characterized in cancer biology, it has not yet been evaluated in neurons. In the studies presented we sought to characterize ERK and mTOR pathway activation and interaction in epilepsy.

METHODS: Human brain tissue sections obtained after resection from epilepsy surgery were stained with antibodies against the phosphorylated ERK (pERK), TSC2 (ERK phosphorylation site S664; pTSC2), eIF4E (ERK phosphorylation site S209; peIF4E) and S6 (phosphorylation site S235/236; pS6). Staining data was correlated with the neuropathological and clinical history. Furthermore, we evaluated mTOR pathway activation subsequent to ERK phosphorylation in HEK293 cells and mice cortical neurons along with its effect on mice cortical neuronal morphology by transfecting them with constitutively active MEK1 (caMEK1) copGFP. Activation of ERK and mTOR signaling in these cells was determined using immunofluorescence (IF) and western blotting (WB) against abovementioned phosphoantibodies. Dendritic morphological analysis of transfected mouse cortical neurons also was performed using Neurolucida software.

RESULTS: IHC analysis of brain sections obtained from individuals undergoing epilepsy surgery showed aberrant pERK, pTSC2, peIF4E and pS6 labeling of cytomegalic neurons. IF and WB showed that activation of ERK correlates with increased levels pTSC2 and pS6 in HEK293 cells (WB: p<0.001, n=5) and mouse cortical neurons. Pilot experiment of dendritic morphological analyses of cultured neurons with high levels of pERK showed a decrease in the number of dendrites (p<0.001, n=5) and dendritic branching (p<0.001, n=5) compared to controls expressing copGFP. This effect was reversed by 200nM rapamycin treatment of cultures for 4 days.

CONCLUSIONS: Our findings suggest that ERK and mTOR pathways are aberrantly activated in cortical dysplasia. Additionally, there is ERK and mTOR pathway cross-talk at the level of TSC2 in neurons under physiologic conditions and in epilepsy. Preliminary studies suggest interplay between these two pathways in the CNS and may provide support for targeting both pathways in epilepsy.

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DEFINING LIGAND BIOPOTENCY AND RECEPTOR SPECIFICITY OF GDF9 AND BMP15

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The transforming growth factor β (TGFβ) superfamily is the largest family of secreted proteins in mammals, and members of TGFβ family are essentially involved in every developmental and physiologic process. Because the number of TGF-β ligands greatly exceeds the number of type I and type II receptors, there must exist ligand structure features to confer specificity for receptor interactions. Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are key oocyte-secreted members of the TGFβ superfamily in the regulation of female fertility in mammals, and their signaling pathways are conserved from mouse (m) to human (h). The focus of our ongoing research is to define ligand biopotency and receptor specificity of GDF9 and BMP15 in both mouse and human. Using genetic engineering, we produced recombinant mouse and human GDF9 and BMP15 proteins, and discovered that only mGDF9 and hBMP15 have the biopotency to stimulate cumulus cell gene expression in an in vitro granulosa cell (GC) assay. We also demonstrated that an arginine in the pre-helix loop of mGDF9 is responsible for the majority of the high ligand biopotency in the GC assay. However, switching the same residue or even the whole pre-helix loop between mBMP15 and hBMP15 does not change their bioactivity. It indicates that BMP15 has other mechanism/s to define its biopotency, which remain independent from the pre-helix loop. Our future directions are to determine the mechanism governing ligand biopotency of BMP15 by protein structure prediction and test receptor specificity of mutant ligands in different TGFβ family receptor null cells. Since GDF9 and BMP15, play critical roles in oocyte developmental competence and fetal survival in mammals, characterization of the bioactivity of recombinant GDF9 and BMP15 have important translational implication for improving in vitro maturation (IVM) in assisted reproductive technology (ART) clinics.

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Alcohol is one of the most prevalent addictive substances in the world. Cessation from alcohol and nicotine produce a large number of overlapping withdrawal signs and symptoms. For example, increases in anxiety a commonly reported withdrawal symptom for both alcohol and nicotine, and has been shown to play an important role in relapse for both drugs. Evidence suggests that alcohol interacts directly with the nicotinic cholinergic system. Gene target studies have recently suggested a strong correlation between the $\alpha_5$, $\alpha_3$, $\beta_4$ gene cluster and both alcohol and nicotine dependence. Since our lab has shown that the $\alpha_5$ and the $\beta_4$ nAChR subunits play an important role in nicotine withdrawal, we want to test the hypothesis that these receptor subunits are also involved in alcohol withdrawal. Both these subunit have overlapping expression in the medial habenula (MHb) and the interpeduncular nucleus (IPN), which our lab has previously shown to play a role in nicotine withdrawal. We hypothesize that the MHb/IPN axis plays a role regulating withdrawal induced by behaviors.

Wild type, $\alpha_5$ null and $\beta_4$ null mice received a daily alcohol injection over the course of 9 days. These mice were then tested in the open field arena and the marble burying test to investigate the effects of the $\alpha_5$ and $\beta_4$ null mutation on ethanol withdrawal induced anxiety-like and compulsive-like behavior. In addition, we examined somatic signs of withdrawal under spontaneous conditions. To examine the role of the MHb/IPN axis wild type mice received a microinjection of the non-selective nicotinic receptor antagonist, mecamylamine, into the MHb or IPN 4 hrs after the last ethanol injection. Somatic signs of withdrawal were then measured for 20 min. Unlike their wild type littermates, $\alpha_5$ null and $\beta_4$ null mice do not exhibit increases in anxiety-like behavior in the open field arena or increases in compulsive-like behavior in the marble burying test during ethanol withdrawal. In addition, increases in somatic signs of withdrawal are not observed in $\alpha_5$ null or the $\beta_4$ null mice. Mecamylamine microinjections into the MHb or IPN are able to precipitate increases in somatic signs of withdrawal only in alcohol treated mice.

Overall our data suggests that $\alpha_5$- and $\beta_4$-containing nAChR play a role in the manifestation of affective and somatic signs during ethanol withdrawal. Our data also highlight the role of the MHb/IPN as an important circuit for alcohol withdrawal. Overall these results highlight possible drug targets for alcohol cessation therapies.
Alzheimer’s disease (AD), the most common form of dementia, is an incurable and terminal progressive neurodegenerative disease. The two predominant pathophysiological hallmarks of AD include amyloid plaques and neurofibrillary tangles. However, detecting either of these hallmarks in vivo has proven difficult. Amyloid accumulation before plaque formation is also believed to be a causative factor in AD. Therefore, imaging techniques that can detect amyloid accumulation prior to plaque formation could be greatly beneficial for diagnosis and monitoring of AD. Magnetization Transfer Contrast (MTC) is a Magnetic Resonance Imaging (MRI) technique to specifically detect changes in macromolecule concentration. In this work, we show that MTC MRI is sensitive to large changes in amyloid concentration as seen in an AD mouse model: the Tg2576 mouse model. The Tg2576 mouse overexpresses a mutated form of amyloid precursor protein with a familial AD mutation and exhibits accumulation of amyloid as early as 4 months and eventual plaque formation as early as 10 months of age. This mouse model does not present other hallmarks of AD like neurofibrillary tangles and neurodegeneration. Importantly, our work shows that changes in MTC can be seen as early as 6 months of age well before plaque formation and even before learning and memory deficits. These results suggest that MTC MRI, a technique already used clinically, could potentially serve as an early diagnostic test for AD.
Telomeres are DNA-protein complexes at the end of eukaryotic chromosomes that protect chromosome termini. Maintenance of Telomeric Capping 5 (Mtc5) was identified as having a potential role in telomere maintenance in S. cerevisiae because a synthetically sick phenotype was observed in a strain bearing mutations in mtc5Δ and cdc13-1, a temperature sensitive allele of the telomere end protection factor, Cdc13. Further, a high-throughput study identified Mtc5 as interacting with Ku, a protein that is required for telomere structure and function. Recently, Mtc5 was identified as a component of the SEA complex, a vacuolar complex thought to be involved in intracellular trafficking. Vacuolar proteins have been implicated in telomere length regulation and epistatically linked to the Ku and telomerase pathways, though the nature of these interactions is unknown. Thus, we hypothesized that Mtc5 was a vacuolar protein that impacted telomere length via Ku. Tetrads from mtc5Δ/YKU80Δ diploids showed two populations of viable mtc5Δ haploid mutants. One population had wild type colonies and telomere length, whereas the other had small colonies and slightly short telomeres. Telomeres in mtc5Δ kus80Δ double mutants had a stable but variable telomere length defect, thus placing Mtc5 and Yku in different pathways for telomere length maintenance. Random spore analysis (RSA) revealed decreased viability of mtc5Δ haploids derived from mtc5Δ/MTC5 diploids. Notably, when RSA was performed with mtc5Δ/MTC5 kus80Δ/YKU80 and mtc5Δ/MTC5 tlc1Δ/TLC1 diploids the viability of mtc5Δ kus80Δ and mtc5Δ tlc1Δ double mutants was significantly greater than mtc5Δ single mutants. Further, tetrads from the aforementioned diploids showed rescue of the small colony phenotype seen in mtc5Δ mutants from mtc5Δ/MTC5 diploids. This rescue of colony size was not seen when mtc5Δ was combined with a mutation in lig4Δ, a member of the non-homologous end-joining pathway, suggesting the rescue is specific to Mtc5’s relationship with telomeric proteins. Successive culturing revealed mtc5Δ tlc1Δ mutants were not able to form survivors as readily as tlc1Δ mutants. Since survival in the absence of telomerase can be achieved by telomere recombination via break induced replication (BIR), we examined the impact of an mtc5Δ mutation in a strain that assays BIR and saw that mtc5Δ mutants were significantly less viable than wild type. Further, there was a shift in the types of repair products formed. Interestingly, introducing a yku70Δ mutation rescues the mtc5Δ’s inviability. In contrast to previous reports, Yku80 and Mtc5 did not interact in co-immunoprecipitation assays. Further studies are underway to elucidate the mechanisms underlying the telomeric phenotypes and genetic relationships seen between Mtc5 and Ku and telomerase.
In eukaryotes transcription occurs on a chromatin template and altering chromatin architecture is used to modulate expression levels. Historically it was thought that gene expression depended on recruiting RNA Polymerase (RNAP). However, recent global analyses of RNAP binding profiles and RNA sequencing studies demonstrate that the transition from initiation to elongation phase of transcription is critically important. The mechanism by which histone modifications impact the exit of RNAP from the post initiation pause into active elongation remains largely unknown.

We use Drosophila dosage compensation (DC), which is mediated by the Male Specific Lethal (MSL) complex as a model system to interrogate the modulation of transcriptional output via large scale chromatin remodeling. A speculative model in the field posits that DC is mediated by modulating transcription elongation. We carried out two genetic screens that used an easily scored visual read-out of the dosage compensation complex to find components that link these two processes. We identified novel mutations in msl1 and mle, encoding subunits of the MSL complex, illustrating that the screen is effective in isolating relevant factors. We also isolated mutations in Spt5, a pause-release factor that regulates the proximal pausing of RNAP, as a haploinsufficient, male-specific suppressor of eye pigmentation pattern. We hypothesized that MSL complex interacts with Spt5 during active transcription of X-linked genes in male flies and that this interaction is crucial for effective dosage compensation. In support of this idea, we found that Spt5 interacts with the MSL complex physically and genetically. Both MSL complex and Spt5 are conserved in humans. We have mapped direct physical interaction between the most conserved portions of MSL1 and Spt5 and expect that this interaction is likely to be conserved in other organisms as well. We also observed that Spt5 and MSL complex colocalize on many sites on the X-chromosome. The identification of Spt5 is the first molecular link known so far between elongation machinery and the MSL complex and substantiates the idea that dosage compensation in flies is achieved by modulating elongation.
Purpose: To engineer a knock-in mouse model that can be used to monitor the effects of treatments on destabilization and mislocalization caused by a common disease-causing mutation in the human rhodopsin gene, P23H. The goal was to introduce a gene encoding a protein expressed at low levels, in order to avoid rapid retinal degeneration, and with a tag attached that makes it readily visible and easy to distinguish from wild type rhodopsin.

Methods: Using gene targeting in mouse ES cells, we replaced one copy of the endogenous mouse rhodopsin gene with a mutant human rhodopsin gene that encodes P23H-rhodopsin fused to eGFP at its C-terminus. P23H-rhodopsin is a common cause of autosomal dominant retinitis pigmentosa (ADRP). The gene includes a LoxP site in the sequence corresponding to the 5' -untranslated region, which greatly reduces translation efficiency. We characterized the resulting heterozygous and homozygous P23H-hRho-GFP mouse lines for mRNA and protein expression, P23H-rhodopsin localization in rod cells, effects on visual function, and retinal degeneration with age.

Results: Expression studies show that P23H-hRho-GFP mice transcribe the knock-in gene at about the same level as the endogenous mouse allele, but they express much less mutant protein, as expected. Because of the reduced expression of mutant protein, the retinas of heterozygous P23H-hRho-GFP mice are morphologically and functionally very similar to those of wild type mice, and they display little cell death over time. Despite this similarity, P23H-hRho-GFP mislocalizes to the inner segment, nuclear and synaptic regions of rod photoreceptors, consistent with aberrant trafficking as a fundamental property of the mutant rhodopsin, rather than a consequence of a dysfunctional cell.

Conclusions: The P23H-hRho-GFP knock-in mouse is a sensitive model to evaluate the consequences of the P23H mutation without the confounding effects of degeneration. The P23H-hRho-GFP mice provide a valuable tool to determine the efficacy of potential therapies for ADRP that influence protein levels or affect trafficking of the mutant protein, such as gene knock-out or knock-out, chemical chaperones, proteases or protease inhibitors, among others.

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Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of adult T cell leukemia (ATL). The HTLV-1 oncoprotein, Tax-1, can induce transformation both in cell culture and in animal models. In contrast, HTLV-2, which is 70% (nucleotide level) identical to HTLV-1, has not been associated with any human cancer. A new retrovirus, HTLV-3, which is 61.4% (nucleotide level) identical to HTLV-1, was recently identified in African primate hunters. The prevalence of HTLV-3 is currently unknown, but independent identification of four isolates suggests that it may be widespread. The Tax-3 protein encoded by the HTLV-3 2026ND isolate is 75.4% (nucleotide level) identical to Tax-1 and contains sequences that are conserved with several functional domains of Tax-1. Of interest, Tax-3, but not Tax-2 contains a sequence similar to a C-terminal sequence of Tax-1 that has been shown to function as a PDZ binding motif (PBM). PBMs have been shown to be critical for the transforming potential of other viral oncoproteins. Therefore, we hypothesized that, similar to Tax-1, the Tax-3 protein will possess oncogenic properties mediated by its PBM. The ability of Tax-1 to interact with cellular proteins and modulate their function is thought to play an important role in its transforming potential. Specifically, the PBM of Tax-1 is known to interact with and modulate the function of hDLG, a PDZ-containing cellular protein. Interestingly, Tax-3 expressing cells exhibited an altered subcellular distribution of hDLG, similar to what has been reported for Tax-1, suggesting that Tax-3 may also interact with hDLG. We have found that Tax-3 is diffusely localized throughout the nucleus and cytoplasm. We have also determined that Tax-3 can activate CREB, NF- B, and SRF-dependent promoters. To further assess the transcriptional potential of Tax-3, we are using high throughput qPCR to analyze the ability of Tax-3 to regulate a subset of cellular genes known to be differentially regulated in Tax-1 expressing cells. Since HTLV-3 appears to be emerging as a new human retrovirus, it will be critical to determine the potential of this virus to cause disease in humans.

Contributors: Pryor, Kendle; Marriott, Susan
Voltage dependant anion channels (VDACs) are pore-forming proteins present across the mitochondrial outer membrane (MOM) that form complexes with other proteins and serve as the main pathway for metabolite transport across the MOM. We are interested in investigating the role of VDAC isoform VDAC2 in apoptosis, since we had previously shown that VDAC2 directly interacts with the pro-apoptotic BAK protein in MOM. VDAC2-/- mice exhibit embryonic lethality and a heart-specific conditional knock-out develops a dilated cardiomyopathy. We have also observed that BAK accumulates in the endoplasmic reticulum (ER) and that there is a blunted response to ER stress in VDAC2-/- cells.

We performed a microarray expression analysis between WT and VDAC2-/- MEFs and, amongst all genes that showed significant variation, we have observed a consistent alteration in the transcript levels of many key players in the ER-stress response pathway. Follow-up studies with quantitative-PCR and Western blotting have confirmed this pattern in several of the genes identified using microarray analysis. The up-regulation of P21 and other P53-regulated transcripts suggests a specific, coordinated impact on the transcription of certain genes in the VDAC2-/- cells, although the mechanism has yet to be defined. Further analysis of Bak-/- and VDAC2/Bak-/- MEFs suggests a Bak-dependant dysregulation of p53 function. Moreover, a double knockout of BAK and VDAC2 appears to rescue the aforementioned lethal phenotype. With the above evidence, we hypothesize that absence of VDAC2 leads to a blunted ER-stress response due to a variety of factors, including the accumulation of BAK in an inactive confirmation in the ER, the down-regulation of ER-stress related genes and, possibly, their upstream effectors.
NR4AS REGULATE LINEAGE BIAS POTENTIAL OF DISTINCT HEMATOPOIETIC STEM CELL SUBTYPES TO PREVENT AML DEVELOPMENT

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Advisor: Orla Conneely, Ph.D./M.S.-Department of Molecular & Cellular Biology

The NR4A subfamily of orphan nuclear receptors (NR4A1, NR4A2, and NR4A3) function as nuclear transcription factors that transduce diverse extracellular and stress signals into altered gene transcription to coordinate apoptosis, proliferation, cell cycle arrest and DNA repair. We previously discovered that two of these receptors, NR4A1 and NR4A3, are potent tumor suppressors of acute myeloid leukemia (AML); they are silenced in human AML and abrogation of both genes in mice results in extremely rapid early postnatal development of AML.

To address the cellular and molecular mechanisms by which NR4As regulate myeloid homeostasis we have generated tamoxifen regulated conditional knockout mice to interrogate the preleukemic effects of NR4A1/3 deletion in hematopoietic cells. We find that acute deletion of NR4As leads to an abrupt lineage priming crisis within hematopoietic stem cells (HSCs). These preleukemic HSCs exhibit abnormal frequencies of lineage biased subpopulations reflected in a selective decrease in lymphoid-biased HSCs (Ly-HSCs) and relative overproduction of myeloid-biased HSCs (My-HSCs). My-HSC lineage skewing, coupled with excessive cycling of NR4A1/3 deleted HSCs, results in overproduction of myeloid progenitor progeny ultimately leading to emergence of leukemia initiating cells (LICs) and AML transformation. Furthermore, retroviral rescue of NR4A1/3 expression after AML development is sufficient to drive transient translineage reprogramming of LICs but inhibits their long-term engraftment and ability to transplant AML disease. Our data indicate that NR4As are intrinsic transcriptional regulators of HSC homeostasis that are essential for coordination of lineage development priming of HSCs.

Contributors: Boudreaux, Seth; Zhang, Shuo; Conneely, Orla
ROLE OF SERUM OPACITY FACTOR IN REVERSE CHOLESTEROL TRANSPORT

Puanani Rebeiro  
Program in Cardiovascular Sciences  
Advisor: Henry Pownall, Ph.D./M.S.-Department of Medicine

Cardiovascular disease (CVD) is the number one cause of death in the United States. Certain types of CVD, which are concentrated in developed countries, include atherosclerosis, the formation of hardened plaques comprising fat and cholesterol in the vascular wall. As part of this process, modified low density lipoproteins-cholesterol accumulates in the arterial wall. In contrast, high density lipoproteins (HDL) perform the opposite function, a process known as reverse cholesterol transport. Circulating HDL takes up excess cholesterol from the vessel walls for transport to the liver for recycling and degradation. Individuals with CVD typically display low concentrations of HDL and thus have less efficient reverse cholesterol transport.

Serum opacity factor (SOF) is a virulence factor from S. pyogenes and opacifies mammalian plasma by its action on HDL. HDL comprises specialized proteins, apolipoproteins, which solubilize lipids including free cholesterol, cholesteryl esters, triglycerides, and phospholipids. There are three products formed from the SOF opacification reaction with HDL: CERM, neoHDL and Lipid Free ApoA-1. These products are hypothesized to enhance reverse cholesterol transport. One of our goals is to better understand the mechanism of opacification while exploring the therapeutic potential of the reaction of SOF with HDL. Our efforts will concentrate on the functional potential of neoHDL both in vivo and in vitro.

Contributors:
DATA-MINING EXPERIMENTAL PIPELINES WITH EMEN2

Ian Rees
Program in Structural and Computational Biology an Molecular Biophysics
Advisor: Steven Ludtke, Ph.D.-Department of Biochemistry & Molecular Biology

Cryo-electron microscopy is an emerging technique for determining the 3D structure of biological macromolecular assemblies in near-native states at sub-nanometer resolution without the need for crystals. A typical cryo-EM study requires a number of steps: purification, freezing, imaging, image processing, reconstruction, and modeling. Data sets can be very large and interconnected, well-documented parameters are critical for reproducibility of the sample and micrographs, and achieving high resolution requires understanding the numerous experimental factors that can influence the quality of the image data.

To address these needs, we have developed EMEN2, a “Web 2.0” object-oriented scientific database and electronic notebook. In contrast to a relational database, EMEN2 uses a flexible schema based on plain text descriptions of experimental protocols, allowing the investigator to quickly add new or modified methods. Additionally, protocols, parameters, and records can be arbitrarily connected together for rich queries, including built-in statistics and visualization. EMEN2 can also import data from other packages, and we are using this capability to improve the structural resolution of the skeletal muscle Ca2+ channel RyR1 by tracking individual particles across the entire reconstruction pipeline. While originally developed to serve the needs of the cryo-EM community, we believe EMEN2’s architecture provides an excellent foundation for many other scientific endeavors.

Support for EMEN2 development was provided by P41RR02250 and Ian Rees was supported by NIH training grant 5T15LM007093 through the Gulf Coast Consortia.

Contributors: Langley, Edward; Chiu, Wah; Ludtke, Steven
RATIONALLY DESIGNING PEPTIDES TO DISRUPT PROTEIN-PROTEIN INTERACTIONS

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Across the landscape of drugs used in modern medicine, there is a noticeable dearth of compounds targeting protein-protein interactions (PPI), despite their relevance to many diseases. Drug development has historically been based on serendipitous discoveries and the imitation of nature. Protein-protein interactions tend to be too large and complex to approach this way.

One promising approach to expanding the realm of PPI-targeting drugs is the use of specially designed peptide mimetics. Theoretically, any given PPI interface comprises two sets of amino acid functional groups (one for each interacting protein) specifically arranged in space and time. A molecule that mimics the arrangement of groups on one partner should thus compete for binding to the other. This type of inhibition has been successfully demonstrated in several systems.

To design this type of inhibitor, two factors must be considered: selection of a “scaffold” to present the mimetic residues, and selection of those residues themselves. Many diverse solutions have been demonstrated for the former, the most common being short \( \alpha \)-helical peptides; identification of the key residues, however, has proven difficult. We wish to develop a platform to expedite this process.

We can use information about the evolutionary importance of PPI residues, gained by correlating sequence variations with evolutionary divergence, to select amino acids to mimic. We have previously used this evolutionary trace (ET) information, along with an algorithm that optimizes \( \alpha \)-helical scaffolds, to design inhibitors of a G protein-couple receptor kinase (GRK) and of germ cell nuclear factor (GCNF). Building on these results, my goal is to further develop our tools into a streamlined platform enabling rapid design of peptide inhibitors for any given protein-protein interaction.

As my first step, I am using the homodimerization of the E. coli protein orotidine-5’-monophosphate decarboxylase (ODCase) as a test system for refining and expanding our methods. ODCase is well studied, easy to work with, and its catalytic activity is directly dependent on proper dimerization. I have designed peptides mimicking evolutionarily important residues in an \( \alpha \)-helix in the dimer interface. While holding those positions constant, I varied the parameters used to optimize the scaffold – hydropathy and helicity. We hypothesize that the ideal inhibitor will be hydrophilic and have high helicity. Testing this will allow us to improve our design methods, eventually making them generalizable to a broad array of PPI targets.

Contributors: Regenbogen, Sam; Katsonis, Panagiotis; Marciano, David; Wilkins, Angela; Morgan, Daniel; Palzkill, Timothy; Lichtarge, Olivier
ROLE OF RYR2 PHOSPHORYLATION BY CAMKII DURING HEART FAILURE PROGRESSION

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Advisor: Xander Wehrens, M.D./Ph.D. - Department of Molecular Physiology & Biophysics

Rationale: Increased activity of calcium (Ca2+)/calmodulin-dependent protein kinase II (CaMKII) is thought to promote heart failure progression. However, it remains unclear whether increasing diastolic sarcoplasmic reticulum (SR) Ca2+ leak and the resulting cardiac failure depend on CaMKII phosphorylation of the ryanodine receptor (RyR2).

Objective: We tested the hypothesis that specific inhibition of CaMKII phosphorylation of RyR2 prevents diastolic Ca2+ leak and improves cardiac contractility in mice with experimental heart failure.

Methods and Results: Knock-in mice in which CaMKII phosphorylation site serine 2814 (S2814) was inactivated by mutation S2814A exhibited improved contractility and reduced cardiac dimensions following transverse aortic constriction compared to wildtype (WT) littermates. Moreover, S2814A mice were protected from developing signs of heart failure such as pulmonary congestion and increased levels of atrial natriuretic factor (ANF). Cardiomyocytes from S2814A mice exhibited significantly lower SR Ca2+ leak and improved SR Ca2+ loading compared to WT mice. Interestingly, these protective effects on cardiac contractility were not observed in S2814A mice following experimental myocardial infarction. Similarly, in human patients with non-ischemic heart failure, we observed increases only at the CaMKII phosphorylation site on RyR2.

Conclusions: Our results suggest that increased CaMKII phosphorylation of RyR2 plays a critical role in the development of pathological SR Ca2+ leak and heart failure progression in a mouse model of pressure overload but not following myocardial infarction. Therefore, the upregulation of CaMKII expression and activity in mice and in humans with heart failure, implicates it as a possible treatment option in the future of molecular medicine.

Contributors: Jonathan L. Respress, Ralph J. van Oort, Na Li, Angela C. de Almeida, Darlene G. Skapura, Xander H.T. Wehrens
TO DETERMINE WHETHER GERMLINE EPIMUTATIONS PLAY A ROLE IN THE OBSERVED LOSS OF MLH1 AND MSH2 EXPRESSION IN NON-OBSTRACTED AZOOSPERMIC MEN.

Alex David Ridgeway  
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Advisor: Dolores Lamb, Ph.D.-Department of Urology

The ability to produce progeny is a required trait for the survival of all mammalian species and as such infertility is considered by nature to be a genetically lethal state. The advent of assisted reproductive techniques, specifically intracytoplasmic sperm injections (ICSI), has aided in achieving pregnancies in couples with severe male factor infertility. Currently, ICSI births account for a significant number of children worldwide. There are concerns, however, that the use of ICSI for men with non-obstructed azoospermia (NOA) may possess implications for the offspring ranging from birth defects to inherited systemic defects. Preliminary studies within our lab have suggested that an intrinsic defect in DNA replication and repair via abnormal or absent expression of hMLH1 and hMSH2 may be an underlying cause of NOA in some men. Therefore, the elucidation of the molecular basis of mismatch repair deficiencies in NOA men may better help assess the risk involved in ICSI and its consequences for their offspring.

I propose to investigate the possible role of germline epigenetic alterations in NOA men with MLH1 and MSH2 deficiencies. Alterations in a genes epigenetic code (histone modifications, CpG island methylation and alterations in the ability of nuclear proteins to bind chromatin) can affect its expression. These alterations can result in changes in gene expression, independent of gene mutations, and may provide a novel mechanism in copying a disease phenotype when coupled with ICSI. For example, hypermethylation of the MLH1 promoter has already been shown to downregulate MLH1’s expression in Hereditary Non-Polyposis Colon Cancer. Therefore, I will determine whether loss of MLH1 gene expression in NOA men correlates with DNA methylation of the MLH1 promoter. If methylation of the MLH1 promoter is present in both the somatic and testis tissue of NOA men with absent MLH1 expression this may indicate a germline event that can be transmitted to potential offspring.

Contributors:
Prostate cancer (PCa) is a leading cause of cancer deaths in American men. Evidence suggests that the majority of prostate cancers contain a chromosomal rearrangement that links the 5' untranslated region of the androgen regulated TMPRSS2 gene to the coding region of ETS family of transcription factors such as ERG or ETV1. Androgen response elements in the promoter of TMPRSS2 allow for the overexpression of ETS family members in PCa. The most common fusion found in PCa is the TMPRSS2:ERG fusion resulting in increased growth and invasiveness. Data from our lab and others have shown that the biologically active metabolite of vitamin D, 1α25-dihydroxyvitamin D3 (1,25D), decreases proliferation and invasiveness in PCa cell lines in vitro as well as tumor growth in vivo. In these cell lines, which do not express TMPRSS2:ERG, 1,25D treatment results in the downregulation of c-Myc, although the mechanism for downregulation is unknown. Our laboratory has found that 1,25D induces TMPRSS2 in multiple cell lines and TMPRSS2:ERG in VCaP cells. Because the TMPRSS2:ERG fusion is associated with increased growth and especially, invasiveness, 1,25D action may differ in cells with this re-arrangement. The PCa cell line, VCaP, contains the TMPRSS2:ERG fusion and our lab has shown that despite inducing the expression of ERG, treatment with 1,25D results in reduction of cell growth. Furthermore, we have shown that 1,25D reduces c-Myc, a key ERG target, regulating growth. This bipotential effect can be harmful in cases where the TMPRSS2:ERG fusion is present, or compensatory considering the reduction in cell growth regardless of ERG induction. Invasion assays are currently being used to assess whether induction of TMPRSS2:ERG by 1,25D increases invasiveness of VCaP cells or whether there are favorable actions of 1,25D that prevent an increase or even reduce invasiveness. This method is also being used for our LNCaP cell line modified to overexpress ERG in response to doxycycline. In these cells, early data suggests that induction of ERG expression leads to increased invasion and treatment with 1,25D reduces invasion comparable to control cells. Recently, sarcosine was identified as a potential biomarker for prostate cancer progression and as a contributor to invasiveness of PCa cells. LNCaP cells are more invasive and contain high levels of sarcosine compared to benign cells. Since treatment of LNCaP cells with 1,25D reduces invasiveness, we are interested in testing whether sarcosine treatment blocks 1,25D mediated reduction in invasiveness or whether 1,25D inhibits invasiveness independent of sarcosine action.

Contributors: Kim, Jung-Sun; Weigel, Nancy
The herpes family of viruses is the cause of some of the oldest documented human infections, with descriptions of the infections dating back more than 5000 years. Herpes Simplex Virus type I (HSV-1), is an enveloped dsDNA, approximately 200nm in diameter, comprised of glycoproteins, tegument and a dsDNA filled capsid. While high-resolution structural studies of large spherical viruses have become a routine procedure using single-particle cryo-electron microscopy (cryo-EM), one limitation of this technology is studying the small structural components of viruses that are not icosahedrally organized. As such, many of these protein complexes remain unsolved for very large viruses like HSV-1.

Using a state-of-the-art Zernike Phase Contrast electron cryo-microscope, we have for the first time solved the structure of the native HSV-1 B-capsid at 24Å, without the assumption of icosahedral symmetry. This instrument is one of a handful of electron cryo-microscopes in the world equipped with a thin carbon film phase plate and is capable of phase-shifting electrons scattered by the sample. As a result of this phase shift, the individual images show a dramatic improvement in the overall information content of the collected data. Images of the HSV-1 B-capsid collected on this microscope were reconstructed without the restraint of icosahedral symmetry, revealing the genome packaging apparatus (the portal protein) situated beneath one of the capsid’s 12 pentameric vertices. This study unambiguously reveals the location and stoichiometry of the portal complex, thereby resolving the current uncertainty over its true location and nature. As HSV-1 is by far the most prevalent form of the herpes family of viruses in humans, with global estimates for latent infection in adults reaching nearly 90%, a detailed understanding of the proteins involved in dsDNA loading, at sub-nanometer resolutions, may identify potential targets for future therapies directed against HSV-1 infection.

Contributors: Ryan Rochat, Xiangan Liu, Kazu Murata, Nagayama Kuniaki, Frazer Rixon, Wah Chiu
Osteosarcoma (OS) is a potentially fatal bone malignancy principally affecting adolescents and young adults and overall survival rates are still significantly below most other pediatric malignancies. Therefore, it is critical to increase our understanding of the molecular pathogenesis of this disease in order to determine novel targets for therapeutic intervention. Among genes found aberrantly expressed in OS is the transcription factor Runx2, a vital gene regulator in bone. A recent report showed that Runx2 was the most significantly gained gene in copy number, resulting in a 9-fold over-expression in human OS tumors. Furthermore, upregulation of Runx2 has been associated with an increase in metastatic potential.

We have hypothesized that Runx2 has a pronounced role in osteosarcoma development and progression. To study the role of Runx2, we investigated whether ablating the expression of Runx2 affected the phenotype of osteosarcoma cells and tumors. We have generated OS cell lines with stable knockdown of Runx2 and appropriate controls. We noticed that ablation of Runx2 expression results in a significant decrease in OS cell and tumor growth rate in vitro and in vivo, respectively. Microarray analysis suggests that knockdown of Runx2 results in decreased expression of genes that positively regulate the cell cycle and cell growth. Future experiments are planned to investigate the role of Runx2 in regulating the expression of genes that control OS cell growth.

We have also shown that Runx2 transcriptional activity is increased in OS, and gene expression analysis from primary osteosarcomas reveals upregulation of multiple known Runx2 target genes. We also hypothesize that Runx2 co-factors play a significant role in activation of Runx2 target genes, and aid in disease progression. Therefore, in conjunction with the BCM Proteomics Core, we have set forth to study Runx2 interacting proteins in primary and metastatic osteosarcoma cells. Due to their over-representation in the dataset, we decided to validate and investigate the interaction between Runx2 and 14-3-3 proteins, which are critical regulators of numerous cellular functions. Future experiments will focus on investigating whether 14-3-3 proteins are altering the activity of Runx2 in OS.

Contributors: Kirsten Johnson, Jason Yustein MD,Ph.D, Lawrence Donehower Ph.D
Cystic Fibrosis (CF) is the most common autosomal recessive disease affecting the Caucasian population and is associated with a high mortality rate. There is no cure for CF which is caused by mutations in the cystic fibrosis conductance regulator (CFTR) gene. Adenovirus serotype (Ad5) vectors have been used extensively for CF gene therapy but without success because of inefficient transduction of the airway epithelium due to the absence of the virus receptor, CAR, on the apical cell surface. It has been reported that Ad5F35, a chimeric Ad5 vector containing the Ad serotype 35 fiber, can mediate a 10-fold improvement in transduction of primary human airway epithelia via the apical cell surface in vitro compared to Ad5 presumably due to the apical location of its CD46 receptor. Additionally, a variant, Ad5F35++, bearing two mutations (Asp207Gly, Thr245Ala) in the Ad35 fiber has been shown to increase the vector’s affinity for CD46 60-fold. Thus, we have produced two helper-dependent adenoviral vectors, HDAd5F35LacZ and HDAd5F35++LacZ, and tested them for apical transduction in the NuLi-1 (normal human bronchial epithelia) cell culture system. Contrary to the published report, our results suggest that their apical transduction efficiencies were comparable to HDAd5. Intrapulmonary aerosolization of HDAd5F35, HDAd5F35++, or HDAd5 into nonhuman primates resulted in only modest transduction of the respiratory airway epithelium. Surprisingly, extensive transduction of type II alveolar epithelial cells was observed for HDAd5F35 and HDAd5F35++, but not HDAd5. Thus, while these vectors may not be ideal for CF gene therapy, they may be very useful for gene therapies where the target cells are alveolar type II cells, such as surfactant deficiencies.

Contributors: Rosewell, Amanda; Hiatt, Peter; McConnell, Ruth; Dang, Dianne; Vetrini, Francesco; Grove, Nathan; Palmer, Donna; Finegold, Milton; Beaudet, Arthur; Ng, Philip
Huge populations of viruses are present in the human gut and at other body sites. We are seeking to establish a method of identifying and evaluating these populations in order to study the role these viruses (the human “virome”) might play in health and disease. The identification of new viruses (those phylogenetically distant from currently described viruses) has been, until recently, quite difficult. Viral identification has been dominated by the use of cell culture and, more recently, by molecular characterization. However, many (if not most) viruses cannot be grown in culture, and molecular methods are usually very specific, making the identification of novel viruses difficult.

We have elected to use high-throughput sequencing (Illumina) to evaluate the viral populations in stool samples collected as part of the Human Microbiome Project. This method is sensitive and does not rely on culture. Our first dataset contains 8 stool samples collected from individuals at 3 time-points each. With these samples we hope to begin to answer some basic questions about the human gut virome: What viruses are typically present? Do these change over time? Are there viruses that are always present at a particular site? Are there viruses that many/most people have in common? How does phage present at a site correlate to the bacterial populations at that site? We believe these studies are necessary to establish groundwork on which to evaluate the role many of these viruses likely play in human health and disease.

Contributors: Holder, Mike; Qin, Xiang; Petrosino, Joseph
Osteoarthritis (OA) is a common clinical condition characterized by degeneration of articular cartilage. It is a major cause of disability that affects more than half of the population over the age of 55 years, resulting in an annual cost of over 100 billion dollars in the United States. Most genetic and therapeutic studies in OA to date have been hampered by the lack of robust OA models in mice as well as paucity of candidate genes for potential therapy. The Proteoglycan 4 (PRG4) loss of function mutation has been reported to cause early onset OA in Camptodactyly-Arthropathy-Coxa Vara-Pericarditis Syndrome. In addition, PRG4 knockout mouse develops early OA. Therefore, we hypothesized that PRG4 over-expression in cartilage would be protective in OA. To test our hypothesis, we generated a transgenic mouse over-expressing PRG4 in articular cartilage. To recapitulate the predominant form of human OA, we developed a microsurgical procedure that specifically transects the cruciate ligaments in the murine hind limbs with minimal damage to the surrounding tissues. We also developed a novel approach that combines phase contrast optics and high-resolution micro computed tomography and can now comprehensively quantify cartilage volume and surface area in the joints. We used these techniques to evaluate the development of OA in the PRG4 transgenic mice after transection of the cruciate ligaments. Finally, to evaluate the potential therapeutic effects of PRG4, we injected helper-dependent adenoviral vectors expressing PRG4 (HDAd-PRG4) into the articular capsules of wild type mice after transection of cruciate ligaments. Our results showed that by either transgenic technology or vector delivery, mice with PRG4 overexpression were protected against the development of OA. Thus, we have established a novel mouse model for OA and developed new imaging techniques that allow systematic, quantitative evaluations of OA. Moreover, we identified a novel target for chondroprotection that could potentially lead to a new treatment modality for OA.
Healthy uterine function requires the precise action of the ovarian steroid progesterone (P4) acting through its receptor, the progesterone receptor (PR). Microarray analysis has shown that Gata2 is regulated by P4 through its receptor in the mouse uterus. Gata2 belongs to a zinc finger containing family of transcription factors. Gata2 is well known for its role in hematopoiesis, while also important in regulating the development of a variety of tissues. Mice that are null for Gata2 are embryonic lethal. We have examined the expression of Gata2 and PR in the pre-implantation uterus by immunofluorescence and expression peaks in the luminal epithelium on day 2.5 of pregnancy, mirroring that of the PR. Due to the temporal and spatial expression of Gata2, we hypothesize that Gata2 is a critical mediator of P4 action in the regulation of endometrial function.

Conditional ablation of Gata2 in the uterus was achieved by crossing a progesterone receptor driven cre (PRcre) mouse with one that contained a floxed Gata2 (Gata2f/f) gene. This generated a mouse with conditional ablation of Gata2 in the uterus (Gata2d/d). Gata2d/d mice are infertile, with defects in implantation and decidualization. Microarray analysis demonstrated that the Gata2d/d mice have a defect in the induction of 91% of PR target genes in response to an acute P4 stimulus. The expression of the PR itself is reduced indicating a reciprocal regulation between itself and Gata2, which is corroborated through in vitro transient transcription assays and through direct binding of Gata2 to the PR promoter via in vivo ChIP analysis.

We have performed ChIP-seq on whole uterus for the PR and Gata2. A database containing our high-throughput sequencing with that of our microarray data has given us a new tool which can combine high-throughput arrays into a dataset that can reveal real biological relevance. This tool allows us to elucidate not only which genes are regulated in the uterus under hormone control, but will identify those that correspond to actual transcriptional regulation through DNA binding in vivo. The power of this database will be its ability to be updated with future datasets giving greater understanding of the role of steroid hormones and their targets in uterine biology.

Contributors: Lanz, Rainer B, Franco, Heather L, Jeong, Jae-wook, Lydon, John P, DeMayo, Francesco J
The inner ear develops from the otic placode, an ectodermal thickening that is induced by FGF signaling and gives rise to the cochlea and vestibular system of the inner ear. Development of the inner ear begins at the end of gastrulation, when the otic placode is induced from the pre-placodal region, an area of ectoderm between the neural plate and early epidermis which gives rise to the cranial sensory placodes. We have previously shown that only ectoderm in the pre-placodal region (PPR) can be induced to become otic placode in response to FGF signals. However, what makes PPR cells uniquely competent to respond to FGF is unknown.

The PPR is marked by expression of several transcription factors, such Eya2, Six1, and Foxi3. In contrast to other PPR genes, we have recently shown that Foxi3 is necessary for otic development, as Foxi3 null mice completely lack inner ears. These mice also have severe craniofacial defects. Initial observations show that the otic placode is not induced in Foxi3 nulls, as indicated by a lack of Pax2 expression, the earliest ear marker in mice. We hypothesize that Foxi3 may be a competence factor required for otic placode induction.

We are assessing the effect of loss of Foxi3 in both mouse and chick embryos. We are currently studying the expression of PPR and early ear markers in Foxi3 mutant mice to determine when ear development fails. This may be at the level of Foxi3 competence, PPR defects, or at another point during the molecular sequence of placode induction. In addition to the mouse experiments, we are using Foxi3 knockdown in early chick embryos to further elucidate Foxi3’s role in conferring FGF competence in otic induction in vivo and in vitro.

Contributors: Mayle, Ryan; Ohyama, Takahiro; Edlund, Renee; Groves, Andrew
CLINICAL IMPLICATIONS OF HEMOPTYSIS IN PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION

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Clinical Scientist Training Program  
Advisor: Mark Entman, M.D.-Department of Medicine

Background

Pulmonary arterial hypertension (PAH) is a disabling disease that may lead to hemoptysis. Commonly used strategy to control hemoptysis include bronchial artery embolization (BAE). However, the effect of BAE on hospital length of stay, and morbidity and mortality related to BAE are not well defined.

Methods

An analysis was conducted at The Methodist Hospital of the outcome of BAE and conservative management in PAH patients presenting with hemoptysis. Medical records from 145 admissions in 116 PH patients were reviewed. Twenty-one PAH patients presenting with hemoptysis for 37 admissions were included in this analysis. Fourteen patients were in the BAE group and seven in the conservative treatment group (37 admissions). Demographic, hemodynamic, procedural, laboratory and clinic data was reviewed.

Results

There were no significant differences in baseline demographic, hemodynamic, and laboratory values between the two groups except for age (p=0.03) and blood urea nitrogen (p=0.01). BAE successfully terminated hemoptysis in 96% (29 of 30) of the cases. There was recurrent hemoptysis in 43% of patients treated with BAE. Patients treated with BAE had non-statistically significant shorter length of stay (4.0 days ± 4.0 vs. 13.7 days ± 22.5; p= 0.26). One-year mortality was 21% (3 of 14) in the BAE group and 57% (4 of 7) in the conservatively treated group (p= 0.16).

Conclusions

BAE was successful in rapidly terminating hemoptysis with no additional morbidity or mortality when compared to supportive care. There was a trend favoring BAE in reducing hospital length of stay and lowering mortality.

Contributors: Frost, Adaani
EVALUATING THE SAFETY OF PIGGYBAC MEDIATED GENE TRANSFER IN HUMAN CELLS

SUNANDAN SAHA

Program in Translational Biology & Molecular Medicine
Advisor: Matthew Wilson, M.D./Ph.D.-Department of Medicine
Cliona Rooney, Ph.D.-Department of Pediatrics

PiggyBac has been successfully used for modification of primary human cells and cell lines with transgene(s) of interest and holds promise as an effective non-viral gene delivery method. Currently, limited information exists about the safety of the piggyBac system for the modification and generation of clinical grade human cells. In this study we began to evaluate the safety of piggyBac for modification of human cells. PiggyBac works through a “cut and paste” mechanism thereby delivering a transgene of interest flanked by inverted repeat sequences into the genome. We found that piggyBac leads to stable transgene integration and transposase expression is undetectable after 7 days in a mixed population of human cells. Although there are no sequences in the human genome with complete similarity to the 17bp terminal repeat sequence (TRS) of the piggyBac transposon inverted repeats (IR), there are sequences with 16, 15 or 14 bp similarities all of which end in the tetranucleotide TTAA required for transposition. The potential of these sequences mimicking the TRS was assessed by replacing the TRS of the 5’Inverted repeat with these genomic sequences and looking at transposition efficiency using a colony count assay. None of the 14 tested sequences were able to effectively act as a terminal repeat sequence. In addition, these sequences also failed to excise from transposons in presence of the transposase, as measured by an excision assay. To assess the safety of delivering the transposase and transposon from a single vector, we isolated clones derived from transfections using the transposase and the transposon cassettes in cis (on the same plasmid vector) and found that all clones had residual transposase expression. In contrast, stable clones generated with transposase delivered in trans from a separate non-integrating plasmid showed no residual transposase expression. The transposon in absence of any promoter can drive residual reporter gene activity which may be attributed to the 5’IR, but the level of reporter gene activity driven by a strong promoter like the CAGS promoter is not significantly altered by the transposon. Additional studies are ongoing to further evaluate the potential genotoxicity of using piggyBac in human cell. Thus, piggyBac appears to be a promising and potentially safe non-viral gene delivery system for therapeutic genetic modification of human cells.

Contributors: Saha, Sunandan; Kaja Aparna; Rooney, Cliona M.; Wilson, Matthew H.
The underlying genetic basis of many childhood cancers remains largely unknown, which places a significant focus on the discovery and understanding of cancer susceptibility genes. Further knowledge of these genes can also lead to the elucidation of the mechanisms behind sporadic cancers. Additionally, the information gathered will assist clinicians and researchers in determining the best methods for prevention and treatment of patients and their at-risk family members. Our research is focused on the identification of novel high risk childhood cancer susceptibility genes through next generation sequencing methods and functional assays.

Found within small cohorts of childhood cancer families, one kindred contained four individuals diagnosed during childhood with lymphocytic leukemia or lymphoma that presented with an autosomal dominant pattern of inheritance. Whole exome sequencing was performed on the constitutional DNA of three of the patients diagnosed with childhood cancer and one non-transmitting parent as an internal control. This analysis resulted in over 1000 unique single nucleotide variant (SNV) sites shared among the three affected family members. Of these SNVs, 95% were also found in dbSNP and excluded. The list of potential disease causing SNVs was further narrowed down by eliminating the SNVs also found in the non-transmitting parent and through systematic bioinformatics analysis. This resulted in a short list of seven missense SNVs predicted to be functionally important. Within this list is the missense mutation L254P of Human Cytosolic 5’ Nucleotidase (NT5C1A). NT5C1A has a role in nucleoside metabolism as this protein catalyzes the production of adenosine through the dephosphorylation of AMP. Adenosine metabolism is essential for lymphocyte viability, making NT5C1A an attractive leukemia susceptibility candidate to pursue further. Previous studies have shown overexpression of wild type NT5C1A in HEK293 cells improves cell survival after treatment with purine and pyrimidine analogs also used in leukemia treatment. These cytostatic or cytotoxic analogs work through inhibition of replication or the activation of apoptosis. Our lab is investigating the functional impact of the L254P mutation through the creation of multiple HEK293 cell lines that stably overexpress either wild type or mutant NT5C1A protein. These cells are being tested for quantitative changes in NT5C1A activity as measured by changes in cytotoxicity to the nucleoside analogs, Cladribine and Gemcitabine. We hypothesize the L254P protein will be deficient in mediating resistance to these analogs and decrease the IC50 of cells expressing mutant versus wild type protein. Our results will provide valuable insight into the function of the mutant NT5C1A found in this familial leukemia family. This single nucleotide variant may alter sensitivity of leukemia cells to nucleoside analogs currently in use for treatment of ALL and lymphoma. Similar functional assays will be performed to further interrogate other rare mutations identified through whole exome sequence analysis of childhood cancer families.

Contributors: Saliba, Jason; Trevino, Lisa R; Meng, Qingchang; Zabriskie, Ryan; Hicks, Stephanie; Kimmel, Marek; Chang, Kyle; Cheung, Hannah; Muzny, Donna M; Reid, Jeffrey G; Wheeler, David A; Gibbs, Richard A; Plon, Sharon E
We have been developing and studying knock-in mouse models for testing gene-based therapies to treat retinal degenerative diseases. Mice were engineered to have one copy of the mouse rhodopsin gene substituted by either a wild type or mutant form of the human rhodopsin gene encoding an EGFP fusion at the C-terminus. Appearance of EGFP fluorescence is an easy way to detect the expression and localization of rhodopsin, even at the single cell level, providing us with an exquisitely sensitive assay to detect repair events.

Our mouse model has a nonsense mutation at position Q344, which causes an autosomal dominant form of the blinding disease retinitis pigmentosa in humans. As determined by nuclear staining, the homozygotes of these mice degenerate at a fast rate and lose all the photoreceptors by six months. In contrast, heterozygotes degenerate very slowly, only losing 20-30% of the photoreceptor nuclei by 11 months. Examination of retinas by fluorescence microscopy has revealed the presence of an average of approximately one rod per retina (out of ~ 6 million) that has undergone spontaneous mutation correcting the premature stop codon and giving rise to bright EGFP fluorescence. These mice are an excellent system for testing treatments to stimulate gene repair frequency above this surprisingly high background of spontaneous mutations.

Zinc finger nucleases (ZFNs) are artificial proteins engineered to bind and cause a double strand break at a specific DNA sequence. ZFNs have been designed that successfully target the genome at a location close to our Q344ter mutation site in mammalian cells cultures. Adeno-associated virus vectors (AAV) have been used to deliver these ZFNs to photoreceptor cells in our knock-in mice. Although no homologous recombination events have been detected yet we have indication that our ZFN can be indeed used to cause DBS at a specific gene in vivo in neurons. Further work will focus on optimizing conditions to promote gene repair events.

Contributors: Sandoval, Ivette M.; Price, Brandee; Gross, Alecia K.; William Hauswirth; Porteus, Matthew; Wilson, John; Wensel, Theodore G.
A GFP-BASED ASSAY FOR CAG REPEAT INSTABILITY

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Myotonic dystrophy, Huntington disease, and several spinocerebellar ataxias are members of a group of disorders that correlate with CAG trinucleotide repeats (TNRs) that increase in length (expand) in specific genes. Despite their long term study and their severe effects on patients, these diseases still lack effective treatment strategies. Our lab is trying to define the mechanisms that underlie CAG repeat instability, with the ultimate therapeutic goal of devising ways to prevent expansion or promote contraction.

In order to efficiently search for modifiers of instability, we have generated a human cell assay carrying an unstable CAG87 repeat within a GFP reporter. A large contraction within the CAG tract will yield a functional GFP transcript, giving rise to a green cell detectable by flow cytometry. Through cell sorting experiments we have shown that the intensity of green fluorescence is dependent on the size of the repeat tract. This allows us to detect a range of contraction sizes and therefore instability events. Furthermore, the ability to sort and subsequently isolate cells enables us to rapidly characterize the entire spectrum of length changes at the TNR locus, including expansions and contractions. Finally the frequency of events is high enough that the system should be amenable to large-scale screens.

This system will give us the ability to uncover the major genetic and environmental effectors of TNR instability using small interfering RNAs or drug treatments.

Contributors: Santillán, Beatriz A.; Wilson, John H.
AUXILIARY TRANSCRIPTION FACTOR DKS A IS REQUIRED FOR HIGH FIDELITY TRANSCRIPTION

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Advisor: Christophe Herman, Ph.D.-Department of Molecular & Human Genetics

Differences between the information encoded in the DNA and the corresponding transcripts have been reported in many biological systems. Errors during RNA synthesis are common and we are only starting to realize their consequences. At the rate of 10-5 per nucleotide errors in transcription occur about 10,000 times more frequently than errors in DNA. Unfaithful transcription results in transcript heterogeneity, which may destabilize genetic networks and lead to stochastic differentiation, affecting entire lineages rather than single cells. We have previously shown that errors of transcription of the lac repressor gene can switch the lac phenotype of Escherichia coli. The altered state is then epigenetically maintained by the intrinsic positive feedback of the lac operon. Transcription fidelity is ensured by RNA polymerase (RNAP) and auxiliary transcription factors GreA and GreB. The Gre factors interact directly with RNAP without binding DNA and their elongated coiled-coil domains protrude into the secondary channel of RNAP and reach the active site where they modulate transcription. The absence of GreA and GreB decreases transcript fidelity in the cell, which is reflected in a dramatic increase in phenotypic switching as compared to WT. DksA shares structural similarity with the Gre factors and is involved in transcription initiation and elongation, yet its role in transcription fidelity has not been reported to date. Here we demonstrate that DksA is, similarly to GreA and GreB, a bona fide transcription fidelity factor. In the absence of DksA we observe elevated leakiness of a polar lacZ allele, which indicates a drop in fidelity of transcription. The reduced RNA fidelity results in a significant increase in the lac phenotype switching. These results clearly suggest that DksA is critical for maintaining epigenetic stability by ensuring high transcript fidelity.

DksA requires the alarmone ppGpp to be capable of RNAP binding; yet a series of super DksA mutants were shown to be able to overcome this requirement. We genetically dissected the connection between RNAP, DksA and ppGpp and isolated RNAP mutants that either do not bind DksA or bind it regardless of ppGpp. Our results enhance our understanding of the complicated relationship of RNAP, DksA and the alarmone ppGpp, and identification of ppGpp-independent RNAP mutant provides grounds for further analysis of this connection.

Contributors: Satory, Dominik; Gordon, Alasdair; Halliday, Jennifer; Herman, Christophe
Elucidating signaling events between the diverse cell types that comprise tumors and their microenvironment is a major challenge in understanding and treating cancer. Drosophila melanogaster has emerged as an important tool for modeling tumors and genetically dissecting the circuits they rely upon. Cells mutant for the tumor-suppressor gene scribble are neoplastic but are eliminated by cell competition unless they acquire additional mutations that confer increased fitness or prevent their removal. Cell competition is a process by which cells that are viable in homotypic situations die when juxtaposed to cells of a different genotype and the mechanisms underlying this event are beginning to emerge. scribble cells in that evade competition in imaginal discs undergo excess proliferation fueled by activity of the growth-promoting transcriptional co-activator Yorkie. Activity of the JNK and Hippo pathways in and around scribble cells is known to be important in determining whether these tumorigenic cells are eliminated or survive. The proliferation-regulating JAK/STAT pathway has been shown to be important in mediating the hyperproliferation and metastasis that results from cells losing scribble and gaining an active form of Ras. However, whether JAK/STAT signaling plays a role in mediating the survival of scribble cells facing cell competition remains unknown. We show that JAK/STAT signaling is crucial for normal cells to out-compete tumorigenic neighbors but is dispensable for the overgrowth of scribble cells protected from competition. These data provide an important step forward in understanding the complex network of signals operating in and around tumorigenic cells and identify JAK/STAT activity as a critical determinant of whether or not a tissue will be successful in protecting itself from inappropriate growth or will be overtaken by abnormal cells.

Contributors: Schroeder, Molly C.; Chen, Chiao-Lin; and Halder, Georg.
The human brain is estimated to contain over 100 trillion synapses, which constitute the major mode of communication between neurons. Glutamatergic synapses, the major class of excitatory synapses in the brain, are primarily housed in small protrusions called dendritic spines. Upon repetitive stimulation of these synapses, synaptic currents become larger and spines undergo cytoskeletal and morphological alterations. These modifications are believed to form the cellular basis of learning and memory, and will henceforth be referred to as long-term potentiation (LTP). While the final outcome of LTP is synaptic strengthening, a mechanistic account of how this rapid strengthening initially occurs (LTP induction) is absent. Spine actin polymerization, spine enlargement, and elevation of synaptic AMPAR content are tightly correlated in time after LTP stimulation. We hypothesize that these spine alterations have a causal relationship to synaptic strengthening, and thus the Rho GTPases (i.e. Rac, Rho, Cdc42) that control these spine alterations are a necessary signaling component of LTP. We propose to: 1) identify the exchange factors that activate Rho GTPases and induce these cytoskeletal and morphological alterations 2) determine the necessity of these alterations in LTP induction.
Embryonic stem cells (ES cells) provide a potentially powerful tool for regenerative medicine due to two unique properties. Self-renewal allows them to divide continuously in culture without differentiating, while pluripotency enables them to differentiate into all three germ layers. Additionally, ES cells are very well protected from spontaneous mutations and maintain robust DNA repair capacity, despite their prolific nature. Although robust DNA repair capacity may be integral to the low mutation rate of ES cells, the mechanisms underlying transcriptional control of key DNA repair pathways in ES cells remain elusive. A better understanding of this control may give insight into how these cells protect their genome. Recently, we have identified a transcription factor named Ronin that is essential to ES cell self-renewal by transcriptionally regulating its targets via recruitment of chromatin modifying enzymes. Here we show Ronin controls a transcriptional program specific to ES cell DNA repair. Mapping promoter binding sites for Ronin in ES cells (using ChIP-Seq technology) indicate it to bind genes involved in multiple DNA repair pathways including nucleotide excision repair, post-replication repair, and homologous recombination. Conditional loss of Ronin via tamoxifen-inducible Cre recombinase results in a 6-fold increase in sensitivity to UV-C irradiation and γ-irradiation, decreased repair kinetics of DNA strand breaks, and a 1.5 fold increase in sister chromatid exchange frequency. In contrast, constitutive overexpression of Ronin results in enhanced DNA repair and enhanced survival after DNA damage. As our data suggest Ronin to regulate transcription of its bound DNA repair genes, we propose this regulation to be one component of the mechanism underlying the low mutation rate in ES cells. Ultimately, our studies may give insight into the spontaneous mutation rate of adult stem cell populations, the mutation rate within the developing embryo, and allow us to propagate ES cells under more stable conditions.
The primary goal of my thesis is to decipher the role of Notch signaling in adult neural stem cell proliferation. The unique capacity of adult hippocampus to generate new neurons throughout life is of great scientific and medical interest, as adult neurogenesis has been linked to learning and memory and a variety of neuropsychiatric disorders, including depression, epilepsy, and schizophrenia. We have barely begun to untangle the molecular and cellular mechanisms that encompass adult neurogenesis. Those studies mostly rely on morphology and confocal stereology to quantify specific group of cells under different circumstances, allowing measurements of the response of the neurogenic cascade to a particular stimulus. Several transgenic mouse models are used, such as Sox2-GFP, Nestin-GFP, and GFAP-GFP. These mouse models allow labeling of the specific groups of cells within the neurogenic niche. Unfortunately, none of them have been able to label specific progenitor cell subtypes such as the Quiescent Neural Progenitors (QNP) which represent the primary cells that give rise to newly born population of cells in the adult hippocampus. Upon extensive expression database search (Allen Brain Atlas, GENSAT, Eurexpress), we have found multiple candidate genes specifically expressed in QNP-like cells. One of these candidates is Lunatic Fringe, which encodes for a glycosyltransferase and modifies Notch signaling. Here, we present our data on characterization of the Lfng-eGFP transgenic mouse and demonstrate that this gene is indeed specifically expressed in QNP's in the adult dentate gyrus. Thus, Lfng-eGFP is a novel reporter mouse model which can be used for studying early stages of neurogenic cascade and specific properties of QNP fate and function in the adult hippocampus.
Neurodegenerative diseases are characterized by an age-dependent, progressive disruption in neuronal structures and/or loss of neuronal function, which ultimately results in death of neuronal cells. Hence, identification of genes that function in neuronal maintenance will enable us to better understand the pathogenesis of neurodegenerative disorders. We performed a large scale, unbiased, forward genetic screen on the X- chromosome of Drosophila to identify lethal mutations that result in age-dependent degeneration of the structure and function of photoreceptor neurons. Here, we describe mutations in flapwing (PP1\(\beta\)9C), the catalytic subunit of non-muscle myosin phosphatase PP1. Flapwing plays a novel role in the maintenance of photoreceptor neurons and its disruption leads to neurodegeneration which we document with electrophysiology and transmission electron microscopy (TEM). flw mutant photoreceptors display reduced ability to sense light upon aging, according to electroretinogram recordings. In addition, TEM analysis shows that in flw retinas the glial layer surrounding the photoreceptors is thicker and contains an increased number of vesicular structures in comparison to wild type retinas. Moreover, photoreceptor synaptic terminals at the lamina are abnormally enlarged in young flw mutants, with respect to wild type terminals. Importantly, both retina and lamina show signs of age-dependent degeneration, as evident by the altered structure of the glia capitate projections, which is a sign of loss of synaptic activity, and by the ultimate loss of photoreceptors. Flapwing is an integral component of the mechanical force –generating cellular machinery that regulates intracellular trafficking. Thus, perturbed intracellular trafficking affects neuronal viability in flapwing mutants. We are currently trying to elucidate the pathways that are affected.
Promyelocytic leukemia protein (PML) is an interferon-inducible protein essential for the formation of nuclear organelles known as PML nuclear bodies (PML NBs), which are targeted for disruption by many viruses, suggesting its role in cellular antiviral responses. Murine gammaherpesvirus 68 (MHV68) is a rewarding model system for investigating the consequences of the PML-NB disruption by herpesviruses in vivo because it infects laboratory mice and shares genomic homology to human gammaherpesviruses. Our previous studies demonstrated that the MHV68 tegument protein ORF75c, which is conserved in all gammaherpesviruses, induced the degradation of PML protein by a proteasome-dependent mechanism, resulting in more robust viral replication in cell culture. However, the precise mechanism by which ORF75c mediates PML degradation and the consequences of PML reduction in the animal host during infection remain unclear. To address these issues, we characterized the functions of ORF75c in vitro and in vivo. We found that ORF75c interacted with PML transiently but sufficiently to increase poly-ubiquitination of PML in cell culture, even though this was not dependent on two known PML stability regulators, casein kinase II and HPV E6-associated protein (E6AP). Highly purified ORF75c increased the formation of ubiquitin chains in vitro and demonstrated an auto-ubiquitination activity with no requirement for E1 and E2 enzymes. These findings suggest that ORF75c is an E3 ubiquitin ligase that can directly modify PML for degradation. Results in animals show that mice infected intranasally with MHV68 encoding an ORF75c protein unable to degrade PML had a delay in development of splenomegaly and possibly delayed viral clearance from the lungs, suggesting that PML might also have a role in modulating host adaptive immune responses. A better understanding of ORF75c from this study will provide new insights into gammaherpesvirus pathogenesis and the role of PML in modulating herpesvirus infection.
EFFECTS OF GHRELIN RECEPTOR ABLATION ON COCHLEAR AND HEARING FUNCTION IN LEPTIN DEFICIENT MICE

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Hearing loss is the most common sensory impairment, affecting nearly 10% of the adult population and 50% of those over age 75. Presbycusis is the most common cause of hearing loss, involves age-related degeneration of inner ear function, and results in a sensorineural hearing loss beginning at the high frequencies and progressing to the middle and low frequencies. Several medical conditions associated with advancing age are implicated in presbycusis, including cardiovascular disease, hyperlipidemia, diabetes mellitus and immune function impairment. However, the relationships of these conditions with hearing loss are not well characterized or understood. Mouse models of deficiencies in the appetite-stimulating hormone ghrelin, its receptor (the ghrelin receptor, GHS-R), and the appetite-suppressing hormone leptin have the potential to address some of the issues of presbycusis because these mouse models have alterations in feeding behavior, glucose homeostasis, lipid metabolism, and induction of thymopoiesis. Indeed, ablation of the ghrelin receptor in leptin deficient mice improves insulin sensitivity, adiposity and serum lipid profiles. These findings led us to hypothesize that ablation of GHS-R in leptin deficient mice will improve auditory function, reduce cochlear cholesterol levels and minimize age-related degeneration of the cochlea. We are currently performing experiments to assess these outcomes with age (young and old), as well as in the context of long-term feeding studies to alter nutritional lipid intake. Collectively, these studies will provide valuable information regarding how common alterations in the metabolic milieu with age affect cochlear and hearing function and, thus, may influence the medical management of these conditions to preserve audition.

Contributors: Sun, Yuxiang; Pereira, Fred A.
Androgen ablation therapy is the most common treatment for advanced prostate cancer (PCa). However, a majority of patients will eventually develop castration-resistant prostate cancer (CRPC) for which there is no effective treatment. CRPC is androgen independent but androgen receptor (AR) dependent. AR is a nuclear receptor whose transcriptional activity is regulated by hormones binding to the ligand-binding domain (LBD) in the C-terminus. This hormone binding induces a conformational change in the receptor allowing for dissociation of cytoplasmic chaperones, dimerization of receptors, and interaction with transcriptional cofactors. Recently, the identification of multiple alternatively spliced AR isoforms in CRPC has gained interest. A common characteristic of these isoforms is the truncation of the regulatory C-terminal region. Previously, it has been shown that experimentally induced deletions of the LBD yield constitutively active receptors. Interestingly, the AR-V7 splice variant, which has been found in tissue samples of CRPC patients and androgen-independent PCa cell lines, lacks the LBD and is constitutively active.

My project is focused on understanding the differences between wild-type AR and alternatively spliced AR isoforms (AR-V7 and AR-NTD) in PCa. This will involve investigating the alterations in gene regulation and cell biology that occur when PCa cells begin expressing splice variants. I have determined optimal doxycycline levels needed to express AR-NTD comparable to the full-length AR in the LNCaP-NTD stable cell line. At optimal levels of induction, the variants stimulate expression of AR target genes and increase cell growth; while at high levels of variant expression, the induction of target genes is blocked. AR has a shorter half-life than NTD and V7 in the presence and absence of hormone. In the future, RNA-Seq and ChIP-Seq will allow for a global view into the role of splice variants in gene regulation. The expression of constitutively active variants indicates that new methods to inhibit AR activity that do not rely on inactivating the hormone binding domain are needed. For example, AR antagonist, MDV3100, decreases AR activity but does not affect variant activity. Full length AR requires a series of chaperone proteins for activity. I have begun to test the requirement of hsp chaperones on AR variants’ expression and function. I have found that hsp90 inhibitors (Geldanamycin and STA9090) decrease full-length AR expression and do not affect expression of variants in LNCaP and HeLa cells. The hsp90 inhibitors strongly inhibit full-length AR activity and have a modest effect on variant activity in HeLa and LNCaP cells. Preliminary studies suggest that Nocodazole, a microtubule-targeting drug, decreases AR and variant expression. In the future, the role of AR splice variants in tumorigenesis will be examined using in vivo experiments. These studies will elucidate the role of splice variants in PCa and potentially yield new therapeutic approaches to treat CRPC.

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‘CONTROL AND CONTAIN’ MECHANISM FOR BLOCKING PROTEIN AGGREGATION IN HUNTINGTON’S DISEASE

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Mutant Huntingtin (mHtt) fibrillogenesis and aggregation has been strongly implicated in Huntington’s disease. The eukaryotic chaperonin TRiC is composed of two heteromeric 8-subunit rings, and traditionally assists in protein folding via encapsulating the target protein. Biochemical and cell biological studies have shown that mHtt aggregation can be blocked by the chaperonin TRiC [1].

To assess the structural basis of how TRiC inhibits Htt aggregation, we performed in vitro aggregation assays and cryo-electron tomography (cryoET) in the presence or absence of TRiC. The specimens were preserved by “rapid-freezing” in liquid ethane, without chemical fixation or staining, at time-points 0.75h, 2h, 4h and 14h post-initiation of aggregation. Tilt series of the frozen-hydrated specimens were collected in a cryo-electron microscope and computer processed to generate 3D tomograms.

We found that mHtt fibrils form increasingly thick fiber bundles over time, and that interaction with TRiC delays this process. We observe free-standing TRiC and TRiC bound to tips of the mHtt fibrils in our tomograms. To investigate structural details, we further processed the sub-tomogram volumes containing TRiC attached to the end of a mHtt fibril, and a separate group of sub-tomogram volumes containing free-standing TRiC.

Our results thereby suggest that cryoET and sub-tomogram data processing can be used as a direct structural assay for the effect of TRiC-based therapeutics in sequestering precursor forms of mHtt aggregates and/or preventing growth of mutant Htt fibrils.

*Both authors contributed equally to this work.

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Contributors: Shahmoradian, Sarah1,2*; Galaz, Jesús2*; Cong, Yao2; Chen, Bryan3; Schmid, Michael2; Frydman, Judith3; Ludtke, Steven2; Chiu, Wah1,2
Our long-term goal is to understand how apoptotic cells, which are generated in large numbers during development and in adulthood, are removed using the nematode C. elegans as a model organism. Previously, our lab found a novel role of clathrin, an endocytic structural protein, in promoting the engulfment of apoptotic cells in C. elegans. We also identified a critical link between clathrin and actin polymerization, the driving force of pseudopod extension. Recently, I have mapped and cloned a C. elegans gene epn-1, whose mutations cause inefficient engulfment of apoptotic cells. epn-1 encodes a homolog of mammalian epsin, an adaptor that links clathrin with certain transmembrane receptors and induces receptor-mediated endocytosis. We propose that EPN-1 associates with the phagocytic receptor CED-1, which specifically recognizes apoptotic cells, and recruits a clathrin scaffold underneath the phagocytic cup for the actin cytoskeleton to assemble upon. This research project aims to test our hypothesis. My three specific aims are: (1) To establish EPN-1’s role in the engulfment of apoptotic cells. (2) To examine whether EPN-1 recruits clathrin to phagocytic cups in response to the CED-1 activation. (3) To determine whether EPN-1 and clathrin act to promote actin reorganization around apoptotic cells. Our study, which will elucidate how apoptotic cells are efficiently engulfed and degraded, is highly related to cancer research, since it is critical to understand whether and how the apoptotic tumor cells generated during chemo- and radiation-therapies are efficiently removed. Inefficient removal may induce inflammatory and autoimmune reactions. Our study will provide novel mechanistic insight into how the actin cytoskeleton is reorganized underneath plasma membrane to promote the removal of apoptotic cells.

Contributors: Shen, Qian; He, Bin; Zhou, Zheng
FOXM1 AND CDC5L ARE TRANSCRIPTION FACTORS CRITICAL FOR THE GROWTH OF BASAL-LIKE BREAST CANCER

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Transcriptional profiling of human breast tumors results in distinct subtypes - including luminal, HER2, and basal-like, which have differing prognoses and treatments. Patients suffering from luminal or HER2 tumors have greatly benefited from therapies which target estrogen receptor alpha (ER) or HER2, respectively. Basal tumors typically lack these drivers of growth and, therefore, do not respond to targeted therapies. To identify potential therapeutic targets for basal breast cancer it is important to identify the key drivers of their growth. I hypothesize that specific transcription factors (TFs) which regulate basal breast cancer can be identified by analyzing the TF binding motifs of genes which are highly expressed in basal as compared to other breast tumor types, and that siRNA knockdown of the specific TFs will identify TFs that can be utilized for targeted therapeutics. To identify these TFs, I have used transcriptional profiling of tumor mRNA, which identifies large sets of genes which are differentially regulated between the basal tumors and other types of breast cancer. By comparing TF binding motifs found within the promoters of genes which are associated with the basal subtype I have identified TF binding motifs which are enriched among basal genes. Among the TFs associated with these motifs, several are also highly expressed in basal tumors when compared to luminal breast tumors. Using siRNA against these differentially expressed TFs, I have identified two TFs, FOXM1 and CDC5L, which are critical for growth of basal breast cancer cell lines. FOXM1 and CDC5L are critical regulators of basal breast cancer growth and potential candidates for targeted treatment of this aggressive disease.

Contributors: Shepherd, Jonathan; Mazumdar, Abhijit; Tsimelzon, Anna; Hilsenbeck, Susan; Brown, Powel.
Cohesion between sister chromatids is essential for faithful chromosome segregation and accurate DNA double strand break repair. This requires a four-subunit cohesin complex (Smc1, Smc3, Scc1/Mcd1 and Scc3) that forms a ring-like structure to entrap sister chromatids. How sister chromatid cohesion is established remains a long-standing question. Previous studies have shown that this requires Eco1, a replication fork associated lysine acetyl-transferase, which acetylates the Smc3 subunit of cohesin at two conserved residues (K112-K113 in yeast and K105-K106 in human cells). Both Eco1 and acetylation of Smc3 are essential for cohesion and cell survival in wild-type yeast. However, both become non-essential when the anti-establishment factor Wpl1 is absent. In this study, we showed that Smc3 acetylation by Eco1 is required for physical binding between cohesin rings and such an interaction is required for cohesion. This interaction is also strengthened either in the presence of an eco1 ts suppressor mutant of Scc1 or in the absence of Wpl1. We also found that deacetylation of Smc3 is required for binding of cohesin ring onto chromatin. These results led us to propose a two-step model for the establishment of sister chromatid cohesion in budding yeast: 1) a deacetylated singular cohesin ring is loaded onto a sister DNA molecule or chromatid and 2) acetylation of Smc3 by Eco1 promotes physical binding between a pair of cohesin rings each entrapping a sister DNA molecule to allow establishment of cohesion.
PINC INTERACTS WITH A PRC2 COMPLEX THAT INCLUDES RBAP46 AND REGULATES MAMMARY EPITHELIAL CELL DIFFERENTIATION

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Pregnancy-Induced NonCoding RNA (PINC) and retinoblastoma associated protein 46 (RbAp46) were identified as genes potentially involved in the protective effect of an early pregnancy against tumorigenesis, and are expressed in parity-induced mammary epithelial cells (PI-MECs) that remain in the regressed lobules following involution. These cells are thought to function as alveolar progenitor cells that rapidly differentiate into milk-producing cells in subsequent pregnancies, but it is unknown whether PINC and RbAp46 are involved in maintaining PI-MECs in the involuted gland. Here, we show that in the mouse mammary gland, mPINC expression increases throughout pregnancy and then sharply declines in early lactation. In HC11 cells, mPINC expression is significantly decreased in response to hormone-induced differentiation, while overexpression of mPINC1.0 and mPINC1.6 blocks differentiation-induced expression of β-casein. Finally, using RNA immunoprecipitation (RIP), we find that both mPINC1.0 and mPINC1.6 interact with RbAp46, as well as other members of the polycomb repressive complex-2 (PRC2), in HC11 cells. Taken together, our data suggest that mPINC inhibits terminal differentiation of alveolar cells and mPINC downregulation is necessary during lactation to allow alveolar cells to undergo secretory activation. Additionally, a PRC2 complex that includes mPINC and RbAp46 may provide the epigenetic modifications that maintain a partially differentiated population of MECs committed to the alveolar fate in the involuted gland.

Contributors: Shore, Amy; Kabotyanski, Elena; Roarty, Kevin; Rosen, Jeffrey
Background: Previous work in our lab showed that introduction of the activated ErbB2 oncogene to the adult mammary gland (MG) resulted in early lesions exhibiting elevated apoptosis compared to fully developed tumors, suggesting that apoptosis represents an anti-proliferative barrier that is overcome during tumor formation. One potential mediator of anti-proliferative barriers (including apoptosis and senescence) in the adult mammary epithelium is the ARF (alternative reading frame) tumor suppressor, which has been shown to respond to oncogene-induced replicative stress by signaling to p53, a key regulator of anti-proliferative processes. It is unclear if and how ARF promotes apoptosis/senescence following aberrant oncogene activation within adult mammary epithelium in vivo.

Experimental design and methods: To determine whether ARF is necessary for the apoptotic/senescence response following oncogene-activation in the adult mammary gland, we utilized 12-14 week old mice that were null, heterozygous, or wild-type for ARF. Nipple injection of the ErbB2 oncogene resulted in early lesions that were examined for oncogene-induced apoptosis by TUNEL assay, as well as for oncogene-induced senescence using the senescence-associated-β-galactosidase assay. Expression of p16 and p21 were also examined in these lesions using immunohistochemistry.

Results: Compared to early lesions from ARF-WT MGs, early lesions from ARF-null MGs did not exhibit a significant difference in levels of apoptosis, but did exhibit significantly decreased senescence and p16 expression. Levels of p21 appeared low across all groups.

Conclusion: ARF appears to be necessary at least for the senescence response after oncogene activation within the adult mammary epithelium in vivo.

Contributors: Sinha, Vidya; Lee, Jae Ho; Li, Yi.
SAFE AND EFFECTIVE TREATMENT OF VITAMIN D DEFICIENCY IN PEDIATRIC DIALYSIS PATIENTS

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Background/Purpose: We and others have shown that vitamin D deficiency (vit D def) is highly prevalent in Pediatric (ped) dialysis pts. However, no treatment (tmt) guidelines exist for ped dialysis pts. Purpose of this study was to establish a safe and effective tmt regimen for vit D def in ped dialysis pts and assess effects of tmt on biochemical mineral parameters.

Methods: 10 month prospective study of 58 ped pts (median age 15y; range 1-24y; 28 Hispanic, 16 Black, 13 White, 1 Asian; 31 HD/25 PD /2 home HD). Vit D def was diagnosed by serum 25-hydroxy vitamin (25vitD) <30 ng/dL. KDOQI guidelines for tmt of vit D def in adults with chronic kidney disease (CKD) were followed: if serum 25vitD level <15 ng/mL, oral ergocalciferol 50,000 units weekly x 4 followed by monthly doses x 6 or 4000 units daily x 12 weeks; if 25vitD level 16-29, 50,000 units monthly x 6 or 2000 units daily for 12 weeks. 28/31 HD pts were treated in-center with either weekly or monthly ergocalciferol. Serum 1,25-dihydroxy vit D (1,25vitD) was assessed in 55 pts pre- and end tmt.

Results: 54/58 pts (93%) had vit D def with 13/58 (23%) severe (serum 25vitD <7 ng/mL). 54/58 pts were taking IV or oral calcitriol; 4/58 received IV paracalcitol. Ergocalciferol tmt improved mean serum 25vitD from 15.5 to 30.1 ng/mL (p=0.0001, Table). Tmt response did not differ by modality or pt ethnicity. Serum Ca, P, PTH & 1,25vitD were not different at end of tmt compared with pre-tmt.

Conclusions: 1) Vit D def can be treated safely and effectively with oral ergocalciferol in ped dialysis pts as young as 1 y old using adult CKD KDOQI guidelines. 2) If pt adherence is a concern, in-center tmt with weekly or monthly doses is safe and not associated with hypercalcemia. 3) Serum PTH was not different at end ergocalciferol tmt, so oral ergocalciferol improves vit D def in ped dialysis pts, but does not by itself correct high serum PTH.

Contributors: Srivaths, Poyyapakkam; Brewer, Eileen
Members of the Steroid Receptor Coactivator (SRC) family are important and promiscuous mediators of nuclear receptor and transcription factor activity. Highlighting their importance, dysfunction of SRCs has been observed in several pathologies including cancer, inflammation, heart disease, and metabolic disorders. Particularly, loss of SRC-2 is well known to perturb liver metabolic function through aberrant regulation of gluconeogenesis and bile acid export. Recently, we also found that loss of SRC-2 significantly disrupts the central circadian clock causing abnormal diurnal locomotor activity and a possible insensitivity to exogenous light entraining cues. The circadian clock is essential for maintaining energy synchronization in many metabolic tissues, and circadian clock disruption leads to inapt energy regulation similar to that found in Metabolic Syndrome. Loss of critical circadian genes including the transcription factor Bmal1 is detrimental not only to the central clock but also metabolic synchrony. To better understand the overarching effects of SRC-2 in energy homeostasis, we thus investigated the molecular impact of SRC-2 ablation on the circadian clock. SRC-2 ablated primary hepatocyte cultures show a significant impairment in the cell autonomous clock with loss of rhythmicity of three prominent circadian transcription factors: Bmal1, Npas2, and Clock. With the known diurnal regulation of metabolic genes, we looked further into the possible connection between SRC-2 and Bmal1. We have shown that SRC-2 coactivates RORα on the Bmal1 promoter, binds to the Bmal1 promoter, and coactivates the Bmal1:Clock heterodimer on certain clock-controlled genes. With the increasing prevalence of Metabolic Syndrome, it is imperative that we understand the molecular mechanisms linking both circadian rhythm and metabolism. As a well-established energy coregulator, SRC-2 may serve as a primary regulator of the circadian clock synchronizing the central clock and metabolism. Therefore, I hypothesize that SRC-2 is a critical coactivator in the coupling of circadian rhythm with energy homeostasis.
Alzheimer’s disease (AD) is the leading cause of dementia and the 6th leading cause of death in the United States. Methods of diagnosis and treatment of AD are popular of research, but are challenging to move to clinic because of the complications in monitoring their efficacy. The identification and characterization of a biomarker for early AD progression is an important step towards better treatment and diagnosis. Nicotinic acetylcholine receptors have been linked to learning and memory and their concentration can be used as a biomarker for AD.

The α7 nicotinic acetylcholine receptor (nAChR) has been shown to change in concentration in AD in both mouse models and in human samples. The precise correlation between AD and the α7 nAChR has yet to be determined, but studies have demonstrated its ability to be used as an AD biomarker. I am developing a method to measure α7 nAChR concentration in vivo in the brain of an AD mouse model using magnetic resonance imaging (MRI). My hypothesis is that α7 nAChR changes are indicative of AD onset and progression, and that measuring these levels will enable the analysis of AD treatment efficacy. The initial goal of this study is to characterize the change of α7 nAChR levels in vivo in a mouse model of AD with a targeted contrast agent.

To make an α7 nAChR targeted contrast agent I have attached a chelate to α-bungarotoxin, a 74 amino acid peptide with an ~10nmol affinity for the α7 nAChR, that binds gadolinium III which provides contrast in the MRI. We have demonstrated specific binding of this contrast agent to membranes containing the muscle-type nicotinic receptor. We have also demonstrated that this agent has similar contrast to that of the FDA approved Gd3+-DTPA agent Magnevist. Future work will be done with α7 nAChR expressing cells before this agent is moved into mice.

With this agent and those that I develop after, I plan to determine in vivo α7 nAChR variation in AD and identify its role in early AD onset. I also plan to determine its ability to measure the efficacy of AD treatment and diagnosis methods.

Contributors: Stinnett Gary R., Pedersen Steen E. Pautler Robia G.
We are defined as individuals by our capacity to learn, integrate data from new experiences, and make stored representations of that information as memories. Two general types of memory storage mechanisms have been described: short-term memory (STM), which lasts minutes, and long-term memory (LTM) lasting days, weeks, or even a lifetime. This distinction in behavior is reflected in specific forms of synaptic plasticity, as well as specific molecular requirements. At the cellular level the most studied form of synaptic plasticity is long-term potentiation (LTP), which refer to long-lasting increases in synaptic strength. Like memory, LTP occurs in two temporally distinct phases: an early (E-LTP) and a late phase (L-LTP). It is well established that both LTM and L-LTP require de novo protein synthesis. The mammalian Target of Rapamycin (mTOR) complex 1 (mTORC1) integrates information from various synaptic inputs and its best-characterized function is to promote translation rates. However, mTORC1 direct role in L-LTP and LTM is unclear. Given that mTOR knock out mice die in utero, and the brain-specific deletion of mTOR up-stream targets results in death within the first postnatal weeks, the study of mTORC1 in the adult brain has been limited. To overcome this problem, we decided to use a "pharmacogenetic" approach that relies on the synergistic action of a drug (rapamycin) and a genetic manipulation (mTOR heterozygotes, mTOR+/-) on the same signaling pathway/target to specifically and temporally inhibit mTORC1 activity in the hippocampus and then study long-lasting changes in synaptic strength, learning and memory. Several advantages are inherent to this methodology: a) it allows the study of mTORC1 function in the adult, b) it “directly” targets the mTORC1 complex because rapamycin forms a complex with the immunophilin FKBP12, which directly binds to and inhibits mTORC1 but not mTORC2 and c) because a low (normally sub-threshold) concentration of rapamycin is used, it is less likely to have off-target effects. Although L-LTP and LTM are normal in mTOR+/- mice, we found that application of a low concentration of rapamycin – one that is subthreshold for WT mice – prevented L-LTP and LTM only in mTOR+/- mice. Furthermore, we demonstrate that mTORC1-mediated translational control is required for memory reconsolidation. These data indicate that mTORC1 positively regulates long term memory storage.

Contributors: Loredana Stoica, Ping Jun Zhu, Wei Huang, HongYi Zhou, Sara Kozma, Mauro Costa-Mattioli.
Information processing in the brain takes time due to the architecture of the brain. An important question that has interested scientists and philosophers for decades is how neural delays affect sensory perception. Does the brain compensate for delays in sensory signals to perceive the world in real time? If it does, what are the neural mechanisms? This question has been addressed in an experimental paradigm called flash-lag illusion, where a briefly flashed stimulus appears to spatially lag behind a predictably moving stimulus at the instant when both are in physical alignment. Multiple hypotheses have been put forward to explain this illusion. Here, we consider two of the hypotheses – differential latency and motion bias - for experimental test. Differential latency hypothesis posits that the neural signals from moving objects reach ‘perceptual endpoint’ earlier than those of flashed objects. In contrast, the motion bias model predicts that latencies of neural signals of moving and flashed objects are the same and that the illusion arises because position judgments of moving stimuli are biased by motion signals that stream in during the ~80 msec period following the judgment trigger. To test the predictions of these theories systematically, we are collecting neural data from the first cortical level on the visual information processing hierarchy, the visual area V1, in macaque monkeys. In a two alternative forced choice paradigm, we are training monkeys to report the spatial alignment of a moving bar relative to a flashed bar. Behavioral results from two monkeys indicate that similar to humans, monkeys perceive robust flash-lag illusion of comparable magnitude. In the near future, neural data from single and multi-units will be collected from area V1 using chronic multi-tetrode arrays in awake monkeys. Since statistical regularity is a key parameter that is manipulated in our experiment, the results will give important clues for understanding how statistical regularities are utilized by the brain to make effective perceptual judgments.

Contributors: Subramaniyan, Manivannan; Ecker, Alexander; Berens, Philipp; Tolias, Andreas
A major obstacle in the genetic therapy of inherited metabolic disease includes host immune responses to the therapeutic protein. This is best exemplified by inhibitor formation in the protein therapy for hemophilia A. An approach to overcoming this is the stimulation of immunological tolerance to the therapeutic protein. Tolerogenic dendritic cells (DCtols) have been reported to induce tolerance and cytokines such as IL-10 and TGF-β1 are known to induce a tolerogenic response. To model protein therapy, we used ovalbumin (OVA) as antigen in BALB/c and their transgenic derivative, DO11.1 mice. In this study we show that adoptive transfer of dendritic cells (DCs) treated with a combination of IL-10 and TGF-β can suppress antibody response in mice. Adoptive transfer of cytokine-conditioned DCs in pre-immunized mice results in reduction of antibody response in the mice. Furthermore, the effect is antigen-specific, as the recipient mice were able to mount a potent antibody response to the control antigen. Analysis of the contribution of IL-10 and TGF-β to the DCtol phenotype shows that IL-10 treatment of DCs is sufficient in inducing antigen specific tolerance. This report demonstrates that autologous cell therapy for antigen-targeted immune suppression may be developed to facilitate long-term therapy.
The dysfunction of p27 has been implicated in many types of human cancers, mainly showing as degradation and mislocalization of p27 protein, which is highly regulated by its phosphorylation state. Although several kinases of p27 have been found, no phosphatase has been reported yet. In our preliminary study using genomic phosphatase screening, we found that PPM1G, a PP2C family phosphatase, could reduce phospho-Thr198 of p27 protein in HEK293T cells. In further studies, we found PPM1G could co-precipitate with p27 in HEK293T cells and they could also interact with each other in the in-vitro pull-down assay. Moreover, PPM1G could directly dephosphorylate p27 at Thr198 in vitro. This indicated PPM1G is a potential phosphatase of p27 protein.

Phospho-Thr198 has been shown to associate with both the stability and localization of p27, which has been modulated in several types of cancer cells. Our further studies will focus on how PPM1G functions in normal and cancer cells through dephosphorylation of p27 at Thr198 and we expected to define its role in tumor development.

Contributors: Sun, Chuang; Wrighton, Katharine; Lin, Xia and Feng, Xin-hua*
Membrane proteins play crucial roles in various physiological processes and are also associated with many kinds of human diseases. Membrane proteins are main targets of currently approved drugs, however, there are technical difficulties for determining high-resolution protein structures. As an alternative approach, computational methods have been developed to model membrane protein structures for better understanding their functions.

FGFR (fibroblast growth factor receptor) belongs to the RTK (receptor tyrosine kinases) family, which represents a large class of transmembrane receptor. Many missense mutations in FGFRs can disrupt the dynamic equilibrium by stabilizing receptors dimerization and activate downstream signaling cascades constitutively in a ligand-independent manner. One of these mutations is FGFR3 A391E in the transmembrane domain (TMD) has been identified to link to Crouzon syndrome and bladder cancer.

Polar residues are frequently over-represented in many diseases-associated mutants of transmembrane interfaces and many of them are proposed to play an important role for stabilizing helical dimerization. Therefore, the main purpose for this project is to develop a new generation of drugs that can block or attenuate FGFR activity with high specificity and affinity. The RosettaMembrane program has been implemented to model the unknown FGFR3 A391E mutant TMD structure and design de novo new membrane soluble peptide inhibitors. The significance of our study is that successfully designing an inhibitor against the A391E mutant will set the stage for designing inhibitors against a large diversity of dysfunctional receptors, which exhibit hydrophobic to polar mutations in the TMD regions.

To test the first generation of inhibitors, we used ToxRed assay, which was developed to study transmembrane helices dimerization in the inner membrane of E.coli. One interesting design, SW64, has a strong inhibitory effect on FGFR3 A391E and it also achieves a high specificity. In order to improve this design, combinatorial library generated by random mutagenesis will be used to discover novel inhibitors with high specificities and affinities by natural selection. Complementary fragments of murine dihydrofolate reductase (mDHFR) to study protein-protein interactions will be used for this high throughput selection. Besides, biophysical methods such as FRET will be performed in order to quantitatively determine the free energy of SW64 for the TMD dimerization with mutant FGFR3 in lipid vesicle.
Identifying a New Tumor Suppressor Network in Human Breast Cancer

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Advisor: Thomas Westbrook, Ph.D.-Department of Biochemistry & Molecular Biology

Breast cancer is a collection of diseases with distinct clinical behaviors and underlying genetic causes. Triple-negative breast cancer (TNBC) is a common subtype of breast cancer that confers a particularly poor prognosis and is refractory to current targeted therapies. Unfortunately, the molecular determinants driving this aggressive malignancy are poorly understood. Using an unbiased genetic screen, we have identified a novel tumor suppressor network that governs proliferation and transformation of TNBCs in vitro and in vivo. We define PTPN12 as a core component in this network and a commonly inactivated tumor suppressor in TNBC. PTPN12 is a potent suppressor of human mammary epithelial cell proliferation and transformation. PTPN12 function is frequently compromised in human TNBCs by inactivating mutations, deletion, or loss of protein expression. Mechanistically, PTPN12 is a tyrosine phosphatase that suppresses cellular transformation by interacting with and inhibiting several oncogenic receptor tyrosine kinases including HER2, EGFR, and PDGFR. Notably, the tumorigenic and metastatic potential of PTPN12-deficient TNBCs is severely impaired by restoring PTPN12 function or by inhibiting kinase targets of PTPN12, suggesting that TNBCs are dependent on the proto-oncogenic tyrosine kinases constrained by PTPN12. Collectively, these data identify PTPN12 as a commonly inactivated tumor suppressor and provide a rationale for combinatorially targeting tyrosine kinases in TNBC and other cancers based on their profiles of tyrosine phosphatase activity.

Contributors: Sun, Tingting; Meerbrey, Kristen; Kessler, Jessica; Zhao, Chunshui; Migliaccio, Ilenia; Nguyen, Don; Pavlova, Natalya; Botero, Maria; Huang, Jian; Bernardi, Ronald; Schmitt, Earlene; Creighton, Chad; Shaw, Chad; Gibbs, Richard; Wheeler, David; Osborne, Kent; Schiff, Rachel; Elledge, Stephen; Westbrook, Thomas
The D2 dopamine receptors (D2Rs) and 5-HT2A serotonin receptors (5-HT2ARs) both belong to the class A subfamily of G protein-coupled receptors. Both receptors are expressed in the central nervous system and serve as potential targets for antipsychotic drugs. Although their binding sites are predicted to be structurally similar, they are able to discriminate between the neurotransmitters dopamine and serotonin and mediate distinct physiological processes. Previous study using Difference Evolutionary Trace (difference-ET) identified two sets of residues with high evolutionary importance that differ between D2R and HT2AR. By ET-residue swapping, it was found that replacing the ET-residues at the ligand-binding pocket in D2R with the corresponding ET-residues from HT2AR led to higher serotonin affinity and lower dopamine affinity. The other set of ET-residue swaps, which are located outside the binding pocket exhibited a significant enhancement of serotonin-stimulated $G_\alpha16$ protein activation or reduced dopamine responsiveness. Currently, we are working on the effects of combined ET-residue swaps and on the effects of residue swaps on downstream signaling bias. The combined mutations chosen are predicted to be covariant during evolution by ET analysis. We are able to determine the level of $G_\alpha_i$ activation induced by agonist-stimulated D2Rs by membrane potential assays in which activated $G_\alpha_i$ would lead to the opening of the TRPC4β channel and cation influx across plasma membranes in HEK cells. To gain insight into the link between ligand binding and downstream response, dose response curves coupled with controls will also be generated to determine the limiting step occurring between the agonist-binding to D2R and the production of the response in each downstream signaling pathway.

Contributors: Sung, Yun-Min; Rodriguez, Gustavo J.; Wilkins, Angela Dawn; Lichtarge, Olivier; Wensel, Theodore G.
The Steroid Receptor Coactivator (SRC) family selectively controls the transactivational potency of nuclear receptors, including the estrogen receptor (ER) and progesterone receptor (PR). Each SRC member exerts diverse regulatory effects, from controlling mammary morphogenesis to regulating metabolic homeostasis. Importantly, deregulation of SRC family members is recognized as a causal factor in the etiopathogenesis of many mammalian target tissues. In the case of the endometrium, clinical studies reveal that SRC-2 and SRC-3 are significantly upregulated in endometrial hyperplastic tissue and cancers, suggesting a causal link between deregulation of these coregulators and the emergence of these pathologies. Moreover, SRC-2 and SRC-3 levels have also been found to be upregulated in the endometrium of women with polycystic ovarian syndrome (PCOS) which is one of the most common endocrine disorders in women that is characterized by an increased risk for endometrial cancer.

Therefore, I hypothesize that increases in SRC-2 and/or SRC-3 levels accelerate estrogen receptor dependent and independent endometrial proliferation, which leads to the development of endometrial hyperplasia and cancer.

The following specific aims will test this hypothesis: (1) To define the aberrant endometrial cellular responses that arise from deregulated expression of SRC-2 and/or SRC-3. Using a lentiviral approach, SRC-2 and/or SRC-3 expression levels will be increased in primary or immortalized human endometrial epithelial cells in a three dimensional culture system; (2) To determine that unscheduled overexpression of SRC-2 and/or SRC-3 in the human endometrium is causal for severe estradiol-induced endometrial hyperplasia and cancer. An innovative human xenograft mouse model in combination with lentiviral approaches will be used to conditionally overexpress SRC-2 and/or SRC-3 in human endometrial cells transplanted beneath the renal capsule of immunocompromised mice in the absence or presence of hormone; and (3) Establish that targeted upregulation of SRC-2 and/or SRC-3 in ER/PR positive cells of the murine endometrium perturbs normal proliferative responses to estradiol exposure. An inventive cre/loxP engineering strategy will induce SRC-2 and/or SRC-3 expression in ER/PR positive cells in the endometrium.

Results from these specific aims will provide much needed cellular and molecular insight regarding the implicated individual and combined roles of SRC-2 and SRC-3 in both hormone-dependent and hormone-independent endometrial hyperplasia and cancer progression.
Background: Diet-induced obesity has become an epidemic in Western societies. Obesity induces a program of systemic inflammation, and cytokines released from adipose tissue play a major role in the pathogenesis of a spectrum of disorders associated with obesity. Extracellular nucleotides via activation of P2 purinergic receptors have the potential to influence acute and chronic inflammatory responses, but their effects on low-grade, chronic inflammation orchestrated in metabolic tissues in response to excess nutrients and energy, central to obesity is not well-understood. Therefore, the purpose of this study was to test the hypothesis that P2 purinergic receptors play a critical role in the induction of diet-induced obesity.

Methods: Wild-type (WT) male, P2Y2, and P2X7 knockout (KO) mice (5 weeks) were fed a high fat/high sucrose diet (42% calories from fat/42% calories from sucrose; HF), or a standard chow (SC). At the end of the feeding period (10 weeks), tissues were harvested and serum was collected. Percentage of fat and lean mass was assessed by Dual-emission X-ray Absorptiometry (DEXA). Total RNA was isolated from epididymal fat pads and analyzed for inflammatory cytokine (TNFα, IL-6, IL-1β) expression by qRT-PCR. Serum cytokine and adipokine concentrations were analyzed by Multiplex assay.

Results: WT mice on HF diet for 8 weeks had increased fat mass (2 - 2.5 fold; p<0.05), as compared to mice on SC. Fat mass gain in response to HF diet was significantly attenuated in P2Y2 KO and P2X7 KO mice (0.3, 0.4-fold respectively; p<0.05), as compared to WT (1.0). Correspondingly, HF diet-induced increases in organ weights (liver and adipose tissue weight/body weight ratio) were attenuated in KO mice. Lean mass gain was comparable between HF and SC diet in the WT. However, P2Y2 KO and P2X7 KO mice on HF diet had increased lean mass (1.1 fold; p<0.05), as compared to WT. WT mice on HF diet had elevated TNFα mRNA expression (3.5 - 4-fold; p<0.05) in adipose tissue (epididymal fat), whereas, P2Y2 KO and P2X7 KO mice exhibited an attenuated increase (0.6, 0.4-fold respectively; p<0.05), as compared to WT. Serum levels of leptin were increased (29-fold; p<0.05) in WT mice on HF as compared to SC, but P2Y2 KO and P2X7 KO mice on HF diet showed significant attenuation in the induction of leptin (0.5, 0.6-fold respectively; p<0.05), as compared to WT (1.0).

Conclusions: High fat diet-induced changes in body composition and inflammatory response were attenuated in the P2Y2 KO and P2X7 KO mice, suggesting a potential role for P2 purinergic receptors in metabolic inflammation—a low grade, chronic inflammation in response to excess nutrients and energy. These findings highlight a hitherto unrecognized role of extracellular nucleotides and their cognate P2 purinergic receptors in the pathogenesis of diet-induced obesity.

Contributors: Tackett, Bryan; Mani, Arunmani; Thevananther, Sundararajah
Background: The ryanodine receptor type 2 (RyR2) is the principal mediator of sarcoplasmic reticulum calcium release in cardiomyocytes and plays a key role in excitation-contraction coupling. RyR2 activity is modulated by Ca2+/calmodulin-dependent protein kinase type 2 (CaMKII) dependent phosphorylation of serine 2814. Abnormal regulation of RyR2 by CaMKII has been associated with intracellular calcium leak and contractile dysfunction in heart failure. Although previous studies have demonstrated that CaMKII associates with RyR2, the precise reciprocal binding domains remain unknown. Moreover, CaMKII activation state capable of interacting with RyR2 is not clear.

Methods: We were able to recapitulate the binding of RyR2 and CaMKII both in vitro and in vivo using lysates from HEK293 cells co-transfected with CaMKII and RyR2 expression vectors and from mouse hearts. To identify the precise CaMKII binding site on RyR2, we engineered eight RyR2 truncations fused to an N-terminal FLAG tag. These truncations have been expressed in HEK293 cells and subjected to binding assays with full-length CaMKII. To assess the activation state at which CaMKII is capable of associating to RyR2, we generated four CaMKII-HA fusion mutants: a catalytic inactive, an autophosphorylation deficient, a constitutively active autophosphorylation and a calmodulin-binding disabled mutant.

Results and conclusions: Binding assays suggest that CaMKII interacts with RyR2 N-terminus and that CaMKII constitutively active autophosphorylation mutant associates two folds more to RyR2 than wild type CaMKII. We anticipate that our study will elucidate the requirements for CaMKII phosphorylation of and interaction with RyR2, which could ultimately advance the development of more specific and directed therapies for cardiac diseases.

Contributors: Terrón-Díaz, María E.; Wang, Guoliang; Dixit, Sayali; van Oort, Ralph J.; Xander H.T. Wehrens
AMONG nonhuman primates commonly used for AIDS research, pig-tailed macaques (PTMs) are uniquely susceptible to HIV-1 infection, although the infection does not persist and is rapidly cleared. This unique susceptibility of PTMs is due to the absence of the restriction factor TRIM5α, and because novel TRIM5 isoforms expressed by PTMs do not inhibit HIV-1 infection. Since other restriction factors in PTMs, such as APOBEC3 (A3) family proteins, can be inactivated by the SIV encoded viral infectivity factor protein (Vif), we hypothesized that an HIV-1 derivative (Pt-HIV-1) encoding the vif gene from PTM-adapted SIVmne would replicate in PTMs. Interestingly, Pt-HIV-1 replicated to high-levels in PTM peripheral blood mononuclear cells in vitro. In juvenile PTMs, Pt-HIV-1 persistently replicated for nearly 2 years, producing low but measurable plasma viral loads and persistent proviral DNA in PBMCs. It also elicited strong antibody responses. However, there was no decline in CD4+ T-cells or evidence of disease. Surprisingly, Pt-HIV-1 was rapidly controlled in newborn PTMs. These results suggest that substitution of SIV vif enables persistent replication of HIV-1 in pigtailed macaques.

We have identified three notable differences between Pt-HIV-1 and pathogenic SIVmne. First, SIV Vif does not associate with Pt-HIV-1 viral particles. Second, Pt-HIV-1 does not degrade either Pt A3G or A3F or prevent their inclusion in virions to the extent of SIVmne. Thus, while SIV Vif expression from Pt-HIV-1 is adequate for replication in PTM CD4+ T-cells in vitro, improvements in its expression may be required for robust replication in the host. Third, our results indicate that unlike pathogenic SIVmne, Pt-HIV-1 may be unable to escape interferon alpha induced restriction in pig-tail CD4+ T-cells.

We also identified a major problem of substitution of HIV-1 Vif with SIV Vif gene. Our results show that overlapping open reading frame for Vpx protein in the SIV Vif gene interferes with the expression of HIV-1 Vpr protein. We introduced mutations in the SIV vif gene at the SIV vpx start codon as well as two ATG codons upstream of the Vpr codon to make Pt-HIV-1 to express Vpr protein (Vpr+ Pt-HIV-1). We observed that Vpr+ Pt-HIV-1 viruses replicate with improved kinetics in human macrophages and PTM CD4+ T-cells than Vpr- Pt-HIV-1 viruses. Interestingly, we observed that Vpr- Pt-HIV-1 viruses show higher G to A mutations during infection of PTM CD4+ T-cells compared to Vpr+ Pt-HIV-1 viruses, demonstrating the positive influence of Vpr expression on Pt-HIV-1 replication. The data suggest that improved expression of Vpr may enhance Pt-HIV-1 replication in the PTM host.

Overall, our results support the hypothesis that overcoming the effects of innate restriction factors is critical for cross-species transmission of HIV-1.

Contributors: Polacino, Patricia; Ruan, Hongmei; Yu-Kimata, Monica; Siwak, Edward; Anderson, David; Wang, Weiming; Arora, Reetakshi; Wen, Michael; Zhou, Paul; Hu, Shiu-Lok
LEPTIN SIGNALING ABNORMALITIES IN MOUSE MODELS OF RETT SYNDROME

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Department of Molecular & Human Genetics  
Advisor: Paolo Moretti, M.D.-Department of Neurology

Rett Syndrome (RTT) is a neurodevelopmental disorder. The majority of RTT cases are caused by mutations in Methyl CpG Binding Protein 2 (Mecp2). Human patients with Mecp2 dysfunction have growth and weight abnormalities, as well as various manifestations of autonomic dysfunction. Our lab has preliminary evidence suggesting that mice with a mutation in Mecp2 (Mecp2KO) also have phenotypes suggestive of autonomic dysfunction including abnormalities in weight, body length, and body temperature. One of the brain regions critical for autonomic regulation is the hypothalamus, and a key regulator of hypothalamic function is leptin. Leptin is a pleiotropic circulating hormone primarily produced in adipocytes that modulates neuronal function in adults, and is important for development of hypothalamic neurons in the mouse. Plasma leptin levels are significantly increased in human RTT patients, and preliminary data in our lab shows that plasma leptin levels are also significantly increased in Mecp2KO mice at three weeks of age. Weight studies in these mice also suggest complex abnormalities in leptin signaling, as Mecp2KO initially gain weight at a slower rate than wildtype mice but at later ages become heavier than controls. This suggests early leptin signaling abnormalities followed by acquisition of leptin resistance. Furthermore, we have observed molecular abnormalities in the dynamic response of leptin signaling in the hypothalamus in the absence of abnormalities of the static components of the pathway. Using a variety of molecular and genetic techniques, we are working to understand the nature of the leptin signaling abnormalities we have observed in Mecp2 mutant mice, and to understand the downstream functional consequences of alterations in this pathway.

Contributors: Thomas, Amanda; Moretti, Paolo
Synesthesia is a perceptual phenomenon in which normal sensory stimulation triggers an anomalous sensory experience. One common form of synesthesia is characterized by an automatic perception of color in response to members of over-learned sequences such as letters, numbers, weekdays, or months. We call this form colored sequence synesthesia (CSS). To elucidate the neural activity underlying CSS, we used neuroimaging to localize grapheme-sensitive brain regions in 20 synesthetes and 20 controls. A contrast of graphemes with scrambled graphemes revealed regions in right and left temporal lobes that are similar in the synesthetic and control groups, with the difference that synesthetic grapheme activity bleeds into the right fusiform gyrus, overlapping the traditional color region, V4. In the second step of the neuroimaging, participants listened to a dynamic presentation of graphemes in the form of audio clips from children’s television. By performing functional connectivity analyses with several seed regions, we found that the grapheme regions in synesthetes were most negatively correlated with color regions in the fusiform gyrus, unmasking a novel relationship between the grapheme and color regions in synesthetes. We are currently using dynamic causal modeling to probe the directional relationship between these seed regions, while using graph theory to examine whole-brain networks that are involved in processing the audio clips. Finally, we are searching for the genetic basis of CSS. We present data from our ongoing family linkage analysis (Tomson et al. 2011), which implicates a region on chromosome 16 containing over 100 genes expressed in the brain. We are in the process of sequencing all family members for a subset of these genes. In summary, we are combining neuroimaging studies and genetic linkage analyses to build a richer picture of the neural basis of synesthesia, an understanding which will serve as a guide to the normal and abnormal operations of neural cross-talk.

Contributors: Bray, Molly; Gibbs, Richard A; Leal, Suzanne; Eagleman, David M;
Excitatory Synapse Development Requires Bidirectional Regulation of Rac GTPase Signaling

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Most excitatory synapses in the brain are located on small, actin-rich protrusions called dendritic spines. Spines are dynamic structures that undergo actin-dependent remodeling important for synapse maturation and plasticity. Conversely, aberrant spine morphogenesis is associated with numerous neurodevelopmental and psychiatric disorders, suggesting that proper spine development is critical for normal brain function. The Rho GTPase Rac1 plays a central role in directing the formation and growth of spines by modulating actin cytoskeletal dynamics. To function properly, Rac1 requires precise spatio-temporal regulation, however, the mechanism that dynamically regulates Rac1 signaling at synapses remains poorly understood. Previously, we identified the Rac-GEF (guanine nucleotide exchange factor) Tiam1 as a critical mediator of EphB- and NMDA receptor-dependent spine development. Here, we report that the Rac-GAP (GTPase-activating protein) Bcr interacts and colocalizes with Tiam1 at synapses and blocks Tiam1-induced Rac1 signaling. The complex between Tiam1 and Bcr may serves as an "on-off switch" that dynamically regulates Rac1 signaling at synapses. To investigate this possibility, we examined Bcr's role in hippocampal neurons and found that both in vitro and in vivo, Bcr and the highly homologous protein Abr restrict spine and synapse formation and growth. The exuberant spine phenotype caused by disruption of Bcr/Abr function was rescued by inhibiting Tiam1, suggesting that Bcr and Tiam1 do act together as a binary switch to regulate Rac-dependent spine development. Interestingly, EphB receptor tyrosine kinases appear to regulate this switch by binding to and phosphorylating Tiam1 and Bcr and transiently disrupting their interaction in neurons. Furthermore, like Tiam1, Bcr appears to be required for proper EphB-mediated spine development, since Bcr/Abr loss converts ephrinB-induced spine formation and growth into spine retraction due to excessive Rac-dependent EphB receptor internalization. Bcr and Abr may therefore normally serve to restrict active Rac levels to within an optimal range that promotes appropriate excitatory synapse formation and growth while preventing inappropriate EphB internalization and spine retraction. Taken together, these results suggest that Bcr and Tiam1 cooperate to tightly regulate Rac1 activity in spines, which is essential for proper excitatory synapse development.

Contributors: Um, Kyongmi; Niu, Sanyong; Liu, Feng; Tolias, Kimberly
While epigenetic and immunotherapeutic approaches to cancer have been promising, responses to each tend to be transient at best. Several epigenetic drugs not only have direct anti-tumor effects but also reverse multiple tumor mediated immune evasion mechanisms such as inhibition of the antigen-presentation machinery, inhibition of effector T cells and induction of T regulatory cells. Therefore in the presence of these epigenetic drugs, tumor-specific effector T cells should have enhanced anti-tumor activity. However, the same drugs also directly inhibit effector T cell functions and so the potential immune benefit is lost. The ultimate goal of my research will be to realize the immunological benefits of epigenetic modification with histone deacetylase inhibitors (HDAC-Is) that upregulate tumor antigen presentation on HLA class I and class II molecules, using tumor-specific T cells.

Histone deacetylase inhibitors (HDAC-Is) have produced both direct anti-tumor effects for example, by downmodulating anti-apoptotic molecules as well as potentially proimmunogenic effects in the tumor environment: HDAC-Is increase tumor antigen (TA) presentation by inducing autophagy and upregulating MHC molecules and costimulatory molecules and can repress inhibitory cell types and activate local professional antigen-presenting cells (APCs). Unfortunately, most HDAC-Is also inhibit effector T cells so their pro-immune potential is largely wasted. We will therefore discover if TA-specific T cells can be combined with HDAC-Is to enhance anti-tumor activity.

Aim 1: To determine how various classes of HDAC-Is modify the tumor microenvironment.

Aim 2: To determine how various classes HDAC-Is alter TAA CTL effector functions.

Aim 3: To determine if combining HDAC-Is and immunotherapy adjunctively or in sequence can produce synergistic anti-tumor effects.

Contributors: Um, Peter; Rooney, Cliona
NOTCH AND TGFβ ENFORCE A RECIPROCAL POSITIVE REGULATORY LOOP TO SUPPRESS PROSTATE BASAL STEM/PROGENITOR ACTIVITIES

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The Notch pathway is an evolutionarily conserved signaling cascade that is required for the appropriate development of multicellular species. Previous studies have shown that Notch-1 expressing cells are critical to prostate development and that loss of Notch-1 at the embryonic stage results in a lack of terminal differentiation. However, it remains unclear whether active Notch signaling is required to maintain the status of the cellular lineages that comprise the prostate. To test this question we utilized a mouse model in which expression of RBP-J is disrupted in the adult prostate, leading to inactivation of Notch signaling. RBP-J floxed (RBP-Jfl/fl) mice were mated with Probasin Cre (PbCre/+) mice resulting in specific deletion of RBP-J in basal and luminal cells of the prostate approximately 2-3 weeks post birth. No difference in gross size, structure, or lineage status was noted as analyzed by Immunohistochemistry by 2 months of age; additionally the mice were completely fertile.

The prostate is capable of involution and regeneration in response to fluctuating serum testosterone levels, presumably through the regenerative capacity of stem cells which have been demonstrated to be located in the basal cell layer. In order to test whether loss of Notch signaling attenuates prostate regeneration we castrated mice and evaluated whether they were able to regenerate efficiently upon replacement of androgen. Our results show that mutant mice and wild-type mice were equally competent in regeneration; however, compared to the wild-type littermates the mutant mice had significantly more cytokeratin 5 and p63 positive basal cells. Additionally there were more p63+Ki67+ cells in the mutant mice suggesting that more basal cells were undergoing proliferation. Additionally, using an in vitro prostate sphere assay we showed that attenuation of Notch signaling enhances the proliferation and inhibits differentiation of prostate basal cells.

The biological role of Notch is similar to what has been observed with Tgf-β signaling in the prostate. Indeed, we show that Notch signaling lies downstream of Tgf-β and is essential for the up-regulation of CDKIs to arrest the cell cycle. Furthermore, Notch is a key regulator of the expression of Tgf-β family members thus establishing a positive feedback loop reinforcing a cytostatic program on prostate basal cells.

Contributors: Valdez, Joseph; Zhang, Li; Xin, Li
MUSCARINIC M4 RECEPTORS MODULATES ANALGESIC RESPONSE AND ACOUSTIC STARTLE RESPONSE IN A MOUSE MODEL OF FRAGILE X SYNDROME

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The G-protein coupled muscarinic acetylcholine receptors, widely expressed in the CNS, have been implicated in Fragile X Syndrome (FXS). Recent studies have reported an overactive signaling through the muscarinic receptors in the Fmr1KO mouse model. Hence, it was hypothesized that reducing muscarinic signaling might modulate behavioral phenotypes in the Fmr1KO mice. Pharmacological studies from our lab have provided evidence for this hypothesis, with subtype-preferring muscarinic M1 and M4 receptor antagonists modulating select behaviors in the Fmr1KO mice. Since the pharmacological antagonists were not highly specific, we investigated the specific role of M4 receptors in the Fmr1KO mouse model, using a genetic approach. We created a double mutant heterozygous for the M4 receptor gene and hemizygous for the Fmr1 gene and examined the mutants on various behaviors. Each animal was tested on a behavior battery comprising of open-field activity (activity), light-dark (anxiety), marble burying (perseverative behavior), prepulse inhibition (sensorimotor gating), rotarod (motor coordination), passive avoidance (learning and memory) and hotplate (analgesia). Animals were also tested on the audiogenic seizure protocol and testis weights were measured. Reduction of M4 receptor expression in the heterozygotes completely rescued the analgesic response and partly rescued the acoustic startle response phenotype in the Fmr1KO mice. However, no modulation was observed in a number of behaviors including learning and memory, activity, perseverative behavior and audiogenic seizures. Reducing M4 receptor signaling altered only select behavioral phenotypes in the Fmr1KO mouse model, suggesting that other targets are involved in the modulation of fragile X behaviors.

Contributors: Veeraragavan, Surabi; Graham, Deanna; Bui, Nghiem; Paylor, Richard.
SHORT PROTEIN SEQUENCE TEMPLATES ACCURATELY PREDICT MOLECULAR FUNCTION

*Eric B Venner*

*Program in Structural and Computational Biology an Molecular Biophysics*

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Protein families diverge in function on evolutionary timescales, but retain evidence of their evolutionary history, which makes inferring function from sequence similarity alone difficult. Previously, we have exploited patterns of amino acid variation that correlate with the phylogenetic tree in order to detect clusters of evolutionarily important amino acids on a protein surface. Under the hypothesis that these clusters are likely to be important to protein function, we used them to predict enzymatic function by comparing them to similar clusters on the surface of known enzymes. While this method is very accurate, structures are available for only a small fraction of the proteins for which we wish to know the function. Here, instead of searching for clusters of likely functional significance on the protein structure, we search for runs of evolutionarily important amino acids in the protein sequence. We call these runs ‘sequence templates’. We then match the sequence templates against a database of proteins with known function and apply the GOtcha algorithm to generate molecular function Gene Ontology term predictions from these matches. In comparison to matches generated from a PSI-BLAST search and analyzed in the same way, sequence templates make many fewer predictions overall, but boost the precision of predictions by up to 51% over many levels of recall. In summary, we have increased the number of proteins for which we are able to make predictions by removing the requirement of a protein structure for making function predictions. Our approach ignores 95% or more of the protein sequence that we deem to be functionally irrelevant, improving precision over a method based on searching the entire sequence.

Contributors: Wilkins, Angela; Erdin, Serkan; Lichtarge, Olivier
RANTES INFLUENCES HEMATOPOIETIC STEM CELL SUBTYPES

Aysegul Verim  
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Hematopoietic stem cells (HSCs) undergo dramatic changes with aging. An increase in absolute numbers of HSCs along with a functional deficit in reconstitution potential and a shift toward production of myeloid cells are the hallmarks of murine hematopoietic aging. Here, we show that high levels of the inflammatory cytokine Rantes are found in the aging stem cell milieu. Forced over-production of Rantes resulted in a deficit of T-cell output, and brief ex-vivo exposure of HSCs to Rantes resulted in a decrease in T-cell progeny concomitant with an increase in myeloid progenitors. In contrast, Rantes knock-out animals exhibit a decrease in myeloid-biased HSCs and myeloid progenitors and an increase in T-cells and lymphoid-biased HSCs. In a heterochronic transplantation setting, we further show that aged HSCs placed in a young environment generate less myeloid cells. These data establish a critical role for environmental factors in the establishment of the aged-associated myeloid skewing phenotype, which may contribute to age-associated immune deficiency.

Contributors: Verim, Aysegul; Boles, Nathan C.; Goodell, Margaret A.
Background: Hedgehog (Hh) signaling is essential during embryogenesis and the adult organism. Aberrant Hh signaling is implicated in several cancers including skin, brain, prostate and breast. While the molecular underpinnings of Hh signaling are not fully understood, various studies have demonstrated that Smoothened (SMO) can function as a G-protein coupled receptor (GPCR) to activate heterotrimeric Gi proteins. Our laboratory demonstrated that activated SMO, a key effector in Hh signaling, leads to mammary hyperplasia, and is overexpressed in ductal carcinoma in situ and invasive breast cancers. Moreover, we have data suggesting that SMO functions as a GPCR in the mammary gland.

Experimental Design and Methods: Expression of Gi subunit(s) was tested by qRT-PCR on sorted mouse mammary epithelial cells (MECs) and whole mammary glands. Mammary gland Gi function was assessed by analyzing individual Gi subunit null mice for developmental abnormalities. Genetic interaction studies are underway in which SMO mice are crossed to Gi1, 2 & 3 null mice. To elucidate downstream signaling events triggered by SMO activation, we are treating SMO derived MECs cultured in a 3D matrix and whole gland organ cultures with inhibitors of candidate Gi effector molecules and assessing proliferation status.

Results: In vivo treatment of SMO hyperplasias with pertussis toxin (PTX), an inhibitor of the Gi family of G-proteins, significantly attenuates mammary gland hyperproliferation. qRT-PCR data reveal PTX-sensitive Gi subunits are expressed in the mammary gland. We have now found that Gi2 and Gi3 null mice display an increased number of terminal end buds and a more completely filled fat pad relative to WT control littermates. Transcriptional data indicates that Gi1 is the most abundant transcript in sorted MECs which overexpress SMO.

Conclusion: These data support the hypothesis that Smoothened functions as a GPCR in the mammary gland, interacting with one or more members of the Gi family. This work offers potential clinical implications with respect to breast cancer treatment as therapeutic agents targeting the Hh signaling pathway are in clinical trials that were developed by solely testing their ability to inhibit GLI1/2-mediated transcription. Our data identify targets for therapeutic intervention other than the GLI transcription factors.

Contributors: Villanueva, Hugo; Visbal, Adriana; Nicholas Plummer; Lutz Birnbaumer; Lewis, Michael
Polo-like kinase 2 (Plk2) was originally identified as an early growth response gene in mammalian cells critical for regulating proliferation. Plk2 is involved in cell cycle regulation, particularly S phase in which it is required for centriole duplication. We identified Plk2 in an RNAi tumor suppressor screen using human mammary epithelial cells. Loss of Plk2 led to the transformation of human mammary epithelial cells indicating a possible tumor suppressor role. To determine if loss of Plk2 occurs in human breast cancer, we used a publically available database, tumorscape, and found that Plk2 (chromosomal region 5q) was significantly focally deleted in many types of cancer, including breast. Furthermore, CGH analysis of breast cancers indicated that tumors with BRCA1 mutations had the 5q region deleted 86% more often than control tumors. Another study reported that Plk2 is frequently deleted in estrogen receptor (ER-) negative tumors. In addition, we found that low Plk2 expression in primary breast carcinomas correlated with poor survival. These studies have led to the hypothesis that Plk2 may be an important regulator of normal mammary gland development and a potential tumor suppressor in triple negative breast cancers, as well as a potential novel therapeutic target. To test this hypothesis the Plk2 germline knockout mouse was utilized. Initial characterization in Plk2-/- virgin mammary gland revealed a hyperbranching phenotype present as early as 8 weeks of age. Plk2 -/- glands from 12 week old mice showed epithelial cells positive for both BrdU and phospho-H3, an S-phase marker and mitotic marker, respectively, indicating a possible defect of a cell cycle checkpoint. Interestingly, hyperplastic lesions were present in Plk2-/- multiparous glands, further supporting a tumor suppressor role for Plk2. Accordingly a sensitized p53 mutant mouse model that carries a missense mutation (p53R172H), often observed in triple negative breast cancers will be employed to investigate the role of Plk2 in mammary tumor formation. Plk2 was deleted in the p53 sensitized background and Plk2-/- mammary epithelial cells were transplanted into 3 week old recipient mice. Tumor formation latency will be examined to determine if loss of Plk2 accelerates tumorigenesis. These studies will help to identify critical pathways that could serve as potential therapeutic targets and could aid in understanding the signaling networks involved in regulating triple negative breast cancer.

Contributors: Villegas, Elizabeth; Westbrook, Thomas (Trey); Rosen, Jeffrey M.
Background: The aim of this analysis was to identify the proportion of coronary heart disease (CHD) patients achieving guideline-recommended low-density lipoprotein cholesterol (LDL-C) and non–high-density lipoprotein cholesterol (non-HDL-C) goals and to identify correlates of dual goal attainment.

Methods: We analyzed patient, provider, and facility characteristics for 21,801 CHD patients in one Veterans Affairs Hospitals Network.

Results: LDL-C goal attainment was 80%, but optional LDL-C goal attainment was 41%. Of patients with triglycerides (200 mg/dl, 51% attained both LDL-C and non-HDL-C goals. Correlates of higher dual goal attainment included older age (65–74 years: odds ratio [OR] 1.47, 95% confidence interval [CI] 1.28–1.69), diabetes (OR 1.33, 95% CI 1.16–1.53), obesity (OR 1.25, 95% CI 1.04–1.50), more primary care visits (OR 1.04, 95% CI 1.04–1.05), and mild increase in illness severity of patients in provider’s panel (OR 1.20, 95% CI 1.0008–1.46), whereas African American patients were less likely to achieve dual lipid goals (OR 0.63, 95% CI 0.48–0.82). Receipt of care from physician (vs. nonphysician) or specialist (vs. primary care) provider, number of patients in provider’s panel, and percentage of patients in provider’s panel with diagnosis of hyperlipidemia were not associated with dual goal attainment.

Conclusions: A large proportion of CHD patients attained LDL-C goal, but optional LDL-C goal attainment was low. Patients with elevated triglycerides had poor attainment of dual LDL-C and non-HDL-C goals, suggesting a treatment gap. Factors associated with dual goal attainment may identify interventions needed to improve future guideline adherence.

Contributors: Virani, Salim; Woodard, LeChauncy; Landrum, Cassie; Pietz, Kenneth; Wang, Degang; Ballantyne, Christie; Petersen, Laura.
Rotavirus is a large icosahedral virus, member of the family Reoviridae. Its genome consists of 11 segments of dsRNA encoding six structural and six nonstructural proteins. The genome is enclosed within a triple-layered capsid, consisting of: (i) an outer protein layer of VP7 from which spikes of VP4 project; (ii) a middle protein layer of VP6; and (iii) an inner protein layer of VP2 arranged as 60 asymmetric dimers forming a pentameric structure at the 5-fold axes. VP1, viral RdRP, and VP3, RNA capping enzyme, are tethered within the VP2 shell, near each fivefold axis. Using dsRNA genome segments as templates, VP1/VP3 transcription enzyme complex synthesizes capped, non-polyadenylated, +RNA molecules, which function as mRNAs for translation of viral proteins and, after association with VP1 and VP3 and incorporation into cores, as templates for replication and formation of genomic dsRNA. VP1, VP3, and mRNA form pre-core replication intermediates (RIs) that are inactive. These RIs must undergo a structural re-organization, triggered by binding to VP2 shell, leading to activation of RdRP and synthesis of dsRNA. Previous studies have suggested that this interaction is mediated by non-structural proteins NSP2 and NSP5. It is proposed that NSP2 acts as a motor which docks VP1/VP3/mRNA onto the VP2 pentamer during the genome packaging. However this role of NSP2 has not been studied extensively. NSP2 is known to interact with VP1 and with the RNA. Both interact with VP2 so it is possible that NSP2 also interacts with the VP2 shell during viral assembly. It is the aim of my project to structurally analyze protein-protein interactions during genome packaging and viral assembly. Both phage display and a peptide array identified VP2 as a possible ligand for NSP2. Pull-down assay and Isothermal Titration Calorimetry (ITC) were performed to demonstrate binding. Both assays demonstrated binding of the proteins, with KD~625nM. Co-crystallization trials of VP2 and NSP2 are currently underway.
CHARACTERIZATION OF THE NORWALK VIRUS MINOR CAPSID PROTEIN VP2

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*Advisor: Mary Estes, Ph.D.-Department of Molecular Virology & Microbiology*

Noroviruses are non-enveloped viruses with a single-stranded RNA genome of positive polarity and are the leading cause of gastroenteritis worldwide. They belong to the Caliciviridae family with Norwalk virus (NV) being the prototype strain. The inability to cultivate these viruses has required the use of biochemical techniques to understand viral and cellular proteins critical for regulating viral replication and assembly. Previous work showed a small highly basic protein encoded by ORF3 of the NV genome is incorporated into virus-like particles that self-assemble in insect cells when ORF2 and 3 are expressed from recombinant baculoviruses. Thus, this viral protein 2 (VP2) is a minor structural protein. VP2 also appeared to regulate the expression and stability of the major capsid protein VP1 in insect cells. The available crystallographic and electron cryomicroscopy structures of NV capsid have not revealed the location of VP2. Therefore how VP2 influences viral capsid formation and stability remains unclear. To better understand the function of VP2 for noroviruses, we expressed NV VP2 in mammalian cells, which produce a 23 kDa protein that fails to accumulate when overexpressed. Subsequent pulse-chase analysis and expression kinetics suggest a very short protein half-life. Sequence analysis predicts VP2 is conserved and it lacks any membrane-associated domains consistent with its diffuse cytoplasmic localization in transfected cells. Mammalian cell expression of both VP1 and VP2 yields virus-like particles (VLPs) that can be visualized by electron microscopy. The expression of VP2 is greatly enhanced in the presence of VP1 and vice versa, suggesting critical interactions between these two proteins. We therefore generated a collection of VP1 and VP2 truncation mutants and assessed their ability to interact by co-immunoprecipitation and by electron microscopy. The results indicate that the N-terminus of VP2 is required for efficient VP1 association during VLP assembly and that VP2 may bind to the interior of the viral capsid. Thus, these findings implicate a functional role for VP2 in regulating capsid assembly.

Contributors: Vongpunsawad, Sompong; Prasad, B. V. Venkataram; Estes, Mary K.
In the United States, heart attack and stroke are two major killers, which are the products of arterial thrombosis and thromboembolism. Platelets, blood cellular fragments that are key components in hemostasis, play a critical role in the thrombosis pathophysiology. Platelets adhere to the exposed subendothelium of ruptured atherosclerotic plaques, aggregate and thereby forming a life-threatening occlusive thrombus that blocks blood flow and kills the surrounding cardiac or neural tissue.

Platelet activation, which is obligatory to both hemostasis and thrombosis, catalyzes their granular contents, thereby propagating aggregation. Within this context, it is clear that a better understanding of the structural mechanisms underlying platelet activation will aid in the development of the appropriate interventions for thrombosis and bleeding disorders. However currently, the structural changes associated with platelet activation are poorly understood.

Although conventional electron microscopy reveals structural details of platelet, it fails to uncover platelet structure in its native solution state. Cryo-electron tomography (CET) is an emerging cellular imaging technique that offers some great advantages over other methods, particularly in the study of platelet structure. CET allows direct visualization of cellular structures at molecular resolution. Importantly, CET could deconvolute platelet structure in its native solution state without chemical embedding and fixation. CET and time-dependent rapid vitrification methods also permits kinetic analysis of activation according to changes in morphology. Unlike conventional methods that require chemical pretreatment that distort structure, CET reveals platelet morphology in a native physiological state. Cellular features such as lengths of cytoskeleton filaments, sizes and surface areas of secretory granules and organelles could be further quantified with specialized software packages.

Contributors: Wang, Rui; Chiu, Wah; Michael Schmid; Dong, Jing-Fei; Wensheng Sun; Khant, Htet
Human neurodegenerative diseases typically affect older people. Heritable forms of these diseases are associated with genetic defects, which provide an inroad to study the molecular mechanisms underlying the neurodegenerative phenotypes in model organisms. We have performed an ethylmethane sulfonate screen to induce mutations in essential genes located on the X-chromosome of Drosophila. By creating mosaic animals with mutant tissue in the eye and by using electroretinogram (ERG) recordings to screen for mutants, we have uncovered more than 50 different genes that affect neuronal function. Among them, we have isolated two mutant alleles of Vps26. Six hour old Vps26 mutant cells in the visual system show a normal response to light stimulation in ERG recording: the on- and off-signals and the amplitude are comparable to the isogenized control. However, in a regular light-dark cycle, the on- and off-signals are quickly lost and the amplitude is gradually decreased in a 3-week time scale. Indeed, electromicroscopy imaging shows that the rhabdomere structure of the lamina is intact in young mutant flies but severely disrupted in older flies. In addition, we observe a gradual accumulation of mitochondria in the surrounding cells in aged flies. Finally, we find numerous darkened/degenerative photoreceptor terminals in the lamina of aged mutant flies. These data suggest a role of Vps26 in neurodegeneration.

Vps26 is an evolutionarily conserved gene that encodes a 478 amino acid protein. The Vps26 protein is a component in the retromer complex, which regulates cargo transport from endosomes to the trans-Golgi network. It has been shown that depletion of Vps26 in mammalian cells affects the localization of secretase to early endosomes. Some studies suggest that there is a decrease in the retromer in patients with Alzheimer’s Disease.

I am currently generating genomic rescue and tagged constructs to characterize the subcellular localization of Vps26. In the future, I will be investigating the interactions between Vps26 and other genes that cause neurodegeneration.

Contributors: Wang, S; Jaiswal, MJ; Yamamoto, S; Xiong, B; Bayat, V; Zhang, K; Haueter, CM; Bellen, HJ
Fast developing next generation sequencing (NGS) technology is shifting the landscape of basic research as well as clinical practice. Recently there are many studies reported that utilizing NGS helps to identify disease variants and leads to clinical improvement for patients who have genetic disorders. To extend the NGS to clinic, the sequencing method should be cost effective, time saving, as well as accurate and robust. The recently released Personal Genome Machine (PGM) is aiming to achieve these goals. To systematically evaluate the potential utility of PGM for molecular diagnosis, we utilize PGM platform coupled with retinal capture penal to sequence hapmap sample NA11831 and two patients with retinitis pigmentosa (RP) from two Canadian families. Ion Torrent 314/316 chip and retinal capture panel including probes capturing 167 retinal genes are used. In sequencing the hapmap sample, we found that 77% of bases have coverage greater than 50, the accuracy is 99.59%, the sensitivity is 97.76%, and specificity is 99.67%. In sequencing the two RP patients, we reported the identification of two known compound heterozygous mutations for RP43 and two compound heterozygous mutations for RP510. We also found that 98.82% (335/339) of SNPs can be validated by Illumina data. These results demonstrate the clinical utility of PGM platform coupled with retina capture panel on disease variants discovery and clinical diagnosis for patients with genetic disorders.

Contributors: Xia Wang, Donna Munzy, Hui Wang, Yuanqing, Irene, Christine, Robert Kenoekoop, Richard A. Gibbs, Rui Chen
Hematopoietic progenitor kinase 1 (HPK1) is a Ste20-like serine/threonine kinase that suppresses immune responses and autoimmunity. B-cell receptor (BCR) signaling activates HPK1 by inducing BLNK/HPK1 interaction. Whether HPK1 can regulate BLNK during BCR signaling is unknown. Here we show that HPK1 attenuates BCR-induced cell activation via inducing BLNK threonine152 phosphorylation, which mediates BLNK/14-3-3 binding. Furthermore, threonine152-phosphorylated BLNK is ubiquitinated, leading to attenuation of MAPK and IKK activation in B cells during BCR signaling. These results reveal a novel negative feedback regulation of BCR signaling by HPK1-mediated phosphorylation, ubiquitination, and subsequent degradation of the activated BLNK. Dysregulation of BLNK phosphorylation and ubiquitination may lead to autoimmunity.
MOLECULAR BASIS OF CELL-CELL RECOGNITION IN SOCIAL AMOEBA

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Dictyostelium, commonly known as social amoeba, live as single cells with nutrition. After nutrition is consumed, thousand of cells aggregate to form multicellular structures and develop into fruiting bodies. In the final fruiting body, there are two types of cells: 80% of them are spores in the sorus, and the remaining 20% become vacuolized and make up the stalk. Spores are the reproductive cells which can germinate when environment permits, while stalk cells eventually die. Therefore, it is crucial for Dictyostelium cells to be able to distinguish between self and non-self, kin and non-kin so that they can choose to associate with self or close relatives to pass their genes on. Our lab has identified TgrB1 and TgrC1 (tgr or tiger: Transmembrane, IPT, IG, E-set, Repeat protein) as two candidates mediating the recognition event between cells. We hypothesize that TgrB1 and TgrC1 interact with each other on the membrane and the interaction triggers downstream signaling pathway, which eventually leads to further development. This also puts the TgrB1-TgrC1 interaction as an essential checkpoint in normal developmental cycle, which is supported by Loomis et al that tgrC developmental defect cannot be circumvented by overexpressing PKA, the central regulator of Dictyostelium life cycle.

We have expressed TgrB1 and TgrC1 with epitope tags to study the biochemistry properties of the two proteins as well as to test the hypothesized interactions between the two proteins.

Contributors: Wang, Yue; Benabentos, Rocio; Hirose, Shigenori; Kuspa, Adam; Shaulsky, Gad
MECP2 FUNCTION IN MEDULLARY TH NEURONS IS REQUIRED FOR BREATHING RESPONSE TO ACUTE HYPOXIA

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Rett Syndrome (RTT) is a neurodevelopmental disorder caused by loss of function in the transcriptional regulator Methyl-CpG-Binding Protein 2 (MECP2). RTT is defined by developmental regression after the first year of life such that purposeful speech and hand skills are lost. Additionally, RTT also presents with abnormalities in the regulation of autonomic function and breathing. These deficits suggest a role for MECP2 within brainstem regions responsible for regulation of these functions. Furthermore, studies utilizing mice with a null allele of Mecp2 to model RTT have reproduced several of the phenotypes observed in human patients including deficits in control of breathing and cardiovascular function. Responses to environmental stresses such as hypoxia are also altered in these animals. The abnormal breathing response to acute hypoxia raises the question of whether loss of MeCP2 function leads to alterations in sensing, processing, or adapting the physiological responses to this stimulus. To investigate this we have made use of mice with conditional knock-out (CKO) and conditional rescue (CR) alleles of Mecp2 to target MeCP2 function in anatomical regions predicted to be important for the sensing, processing, and response to hypoxic challenge; additionally we examined patterns of neuronal activation following hypoxic challenge. CKO and CR studies suggest a requirement of MeCP2 function within neurons of the medulla defined by Tyrosine Hydroxylase expression, and is supported by a deficit in neuronal activation within the Nucleus of the Solitary Tract of the dorsal medulla following hypoxic challenge. These data suggest a requirement of MeCP2 within the circuits responsible for processing hypoxic stress.

Contributors: Huang, Teng-Wei; Samaco, Rodney; McGraw, Christopher; Zoghbi, Huda; Neul, Jeffrey
The vast majority of patients with localized “thin” melanoma will receive a favorable prognosis following excision of the primary lesion. However, 15% of these “metastasis-free” individuals will eventually succumb to metastatic disease. This common clinical experience of differing outcome among patients diagnosed with similar tumor burden suggests that tumors may be pre-ordained with metastatic events arising early in tumor evolution. To identify such deterministic factors in melanoma, our laboratory developed an integrative genomics strategy and high-throughput functional screen for drivers of metastasis. The top-scoring gene identified by this approach was HOXA1, a member of the homeobox transcription factor family. HOXA1 expression leads to potent oncogenic activity, greater than 10-fold invasion capacity and drives distal metastasis from primary tumors in vivo.

Transcriptome profiling and confirmatory cell-based studies indicate that HOXA1 markedly elevates TGFβ signaling activity. Importantly, TGFβ profiling assays indicate that HOXA1 globally up-regulates and/or hyperactivates numerous TGFβ-related receptors (e.g. TGFR13, TGFR1, CXCR4), ligands (e.g. IL6, BMP-7) and other molecules (e.g. SMAD3) positioned throughout the pathway.

In addition to these studies on full-length HOXA1, we recently uncovered a possible regulatory role for an expressed HOXA1 splice variant (VARB), which we posit is clinically relevant given its ability to bind full-length HOXA1 and negatively impact its activity.

Contributors: Wardwell-Ozgo, Joanna; Scott, Kenneth
Transcription has been associated with genomic instability, a phenomenon known as transcription-associated mutagenesis (TAM). One of the proposed molecular mechanisms behind TAM involves the interference between transcription and replication that may result from collisions between the transcription and replication machineries, leading to replication block/arrest. Arrested replication fork may collapse and subsequently rescued by recombination. Transcription-associated recombination (TAR) results in mutagenesis when recombination involves an ectopic homologous DNA sequence. Alternatively, when replication fork is arrested at damaged DNA templates, error-prone DNA polymerases may be recruited to release the replication block through translesion synthesis (TLS). It has been observed that replication block is enhanced when transcription occurs head-on to the direction of replication compared to co-directional transcription. The effect of this increased replication block on the mutational spectra of TAM, however, has not been widely studied. The goal of my project is to understand how transcription level and its directionality with respect to DNA replication influence mutagenesis in bacteria.

The model bacterium Bacillus subtilis has a very strong bias of transcription-replication co-orientation. While it is known that this general trend of coorientation (75%) promotes replication fork progression speed and coorientation of rRNA operons (100%) prevents disruption of replication created by head-on transcription, the evolutionary pressure behind additional enrichment of essential genes on the leading strand (94%) is not known. It is proposed that co-orientation bias at essential genes lowers the rate of their mutagenesis, offering a competitive advantage. Alternatively, head-on replication of essential genes disrupts their transcription, which could harm the cells if the product is not sufficiently produced, or if truncated mRNA is translated to produce toxic polypeptides.

I am developing a forward mutation assay in B. subtilis for two purposes: 1. to identify the consequence of head-on transcription on its rate of mutagenesis, and 2. understand the mechanisms of TAM in B. subtilis. In addition, the mutants obtained from these assays are also valuable as reporters for frameshift, deletion, duplication, and point mutations in reversion assays which can be used to understand the contributions of various genetic factors to various type of mutagenesis.

Contributors: Wastuwidyantyasa, Brigitta; Lewis, Sarah; Wang, Jue D.
GENOMIC ALTERATIONS IDENTIFIED IN ISOLATED AND NON-ISOLATED CONGENITAL DIAPHRAGMATIC HERNIA

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Many of the genetic factors that contribute to the development of congenital diaphragmatic hernia (CDH), a life threatening birth defect, remain unidentified. A cohort of 45 unrelated patients with CDH or diaphragmatic eventrations was screened for genomic alterations by array comparative genomic hybridisation or single nucleotide polymorphism based copy number analysis. Genomic alterations likely to have contributed to the development of CDH were identified in 8 patients. Inherited deletions of ZFPM2 were identified in 2 patients with isolated diaphragmatic defects and a large de novo 8q deletion overlapping the same gene was found in a patient with non-isolated CDH. A de novo microdeletion of chromosome 1q41q42 and two de novo microdeletions on chromosome 16p11.2 were identified in patients with non-isolated CDH. Duplications of distal 11q and proximal 13q were found in a patient with non-isolated CDH and a de novo single gene deletion of FZD2 was identified in a patient with a partial pentalogy of Cantrell phenotype. Haploinsufficiency of ZFPM2 can cause dominantly inherited isolated diaphragmatic defects with incomplete penetrance. These data define a new minimal deleted region for CDH on 1q41q42, provide evidence for the existence of CDH related genes on chromosomes 16p11.2, 11q23-24 and 13q12, and suggest a possible role for FZD2 and Wnt signalling in pentalogy of Cantrell phenotypes. These results demonstrate the clinical utility of screening for genomic alterations in individuals with both isolated and non-isolated diaphragmatic defects.

Contributors: Veenma D; Hogue J; Holder AM; Yu Z; Wat, J; Hanchard N; Shchelochkov OA; Fernandes CJ; Johnson A; Lally KP; Slavotinek A; Danhaive O; Schaible T; Cheung SW; Rauen KA; Tonk VS; Tibboel D; de Klein A; Scott DA
Background: Recent data suggest the existence of a unique subset of breast cancer cells (tumor-initiating cells or cancer stem cells) capable of initiating tumor growth and giving rise to all other cells characteristic of a given tumor. To enhance our ability to identify and localize such cells and their supporting niche cells, we have developed a series of signaling reporters interrogating the Wnt, Hedgehog, Notch, and STAT3-mediated pathways. The current study focuses on the STAT3 reporter.

The STAT3 pathway is a critical regulator of the function of normal stem cells, and shows altered expression in human breast cancers. Moreover, IL-6, a STAT3 signaling agonist, is required for breast cancer stem cell function in human breast cancer cell lines. Recent reports also support an important role of Stat3 signaling in breast cancer stem cells. We hypothesize that STAT3 signaling is preferentially active in stem-like subpopulation and depend on surrounding non-stem cancer cells (niche cells) to maintain its activation.

Experimental design and methods: We have constructed a reporter for STAT3 signaling which contains four copies of consensus STAT3 binding sites upstream of enhanced Green Fluorescent Protein. This reporter system enables FACS-sorting of cells with active STAT3 signaling and in vivo/in situ localization of STAT3 responsive cells. The three main goals of the project are:

To test whether cells with activated Stat3 signaling are enriched for breast cancer stem cells or whether they may serve as niche cells.
To test whether antagonists of Stat3 signaling inhibit cancer stem cell function.
To identify novel targets of STAT3 signaling.

Results: GFP reporters for STAT3 signaling effectively report STAT3-mediated signaling activity in both patient xenografts and human breast cancer cell lines in vitro and in vivo, which enables effective separation of STAT3+/STAT3- cells for functional studies. MDA231 and SUM159 tumors carrying the STAT3 reporter have been established, with both models showing a distinct subpopulation of responsive cells. In MDA-231 cells, however, reporter activity does not select for tumor-initiating cell function based on our preliminary study.

Conclusion: Fluorescent STAT3 reporters function both in vitro and in vivo and should allow more meaningful evaluation of STAT3 inhibitors with respect to their ability to inhibit signaling, and allow analysis of the STAT3 responsive subpopulation of tumor cells.

Contributors: Zhang, Mei; Roarty, Kevin; Rosen, Jeffrey; Lewis, Michael
Nuclear distribution protein C (NudC) is a highly conserved protein that plays a role in mitosis and cytokinesis. We recently discovered that NudC is phosphorylated by the mitotic kinase Aurora B. Whether NudC phosphorylation by Aurora B plays a role in cytokinesis is not known. We found that NudC and Aurora B associate in the late stages of mitosis, from anaphase to cytokinesis. In vivo labeling showed that NudC is phosphorylated by Aurora B, as evidenced by reduced phosphorylation in the presence of the Aurora B inhibitor ZM447439. Using a series of GST-NudC truncation proteins in IP kinase assays, we mapped a site within the N-terminus of NudC at residue T40 as an Aurora B phosphorylation site. An anti-phospho-T40 (pT40) NudC peptide antibody was generated and used to show that pT40 NudC localizes to the midbody where Aurora B is localized during cytokinesis.

To determine the potential function of pT40 NudC in cytokinesis, we knocked down NudC in HeLa cells and rescued with wild-type NudC or NudC containing a T40A (phospho-defective) or T40D (phospho-mimetic) mutation. NudC knockdown increased the percentage of cells exhibiting elongated intercellular bridges up to 30 µm in length, demonstrating a lack of cell abscission and failure in cytokinesis. Furthermore, Aurora B was found to be mislocalized along the elongated bridges instead of being concentrated at the midbody. Both wild type and T40A NudC rescued the cytokinesis phenotype and Aurora B localization. In contrast, T40D NudC was unable to rescue the cytokinesis defect and Aurora B remained diffused along the elongated bridges, indicating that a function of pT40 NudC is to prevent the completion of cytokinesis. Our studies suggest that phosphorylation/dephosphorylation of NudC plays a role in cytokinesis regulation, and that dephosphorylation of pT40 NudC is required for timely cell abscission.

Contributors: Carol Chuang, Maria Fadri-Moskwik, Jing Pan, Sue-Hwa Lin and Li-yuan Yu-Lee
The correct functioning of the female reproductive system is critical for the generation of healthy offspring and for the survival of a species. In the nation today, 12% of women are clinically infertile and many others suffer from reproductive associated disease including endometrial cancer and endometriosis. A successful pregnancy requires proper attachment of the embryo to the uterine luminal epithelium and subsequent proliferation and differentiation of the underlying stroma to support further development. These significant processes are regulated by the steroid hormones estrogen and progesterone which bind to their subsequent receptors, the estrogen receptor and the progesterone receptor (PR). These receptors are located in the epithelial and stromal compartments and regulate the transcription of target genes as well as orchestrate paracrine cross talk between these two compartments. The PR is known to consist of two isoforms, PRA and PRB that differ only by 164 amino acids due to translation occurring at separate start sites. The two isoforms of PR provide increased specificity and variability to the molecular action of PR in the mammalian uterus. My research will focus on the elucidation of the role of the PR isoforms in epithelial and stromal crosstalk in the implantation process. Through the utilization of both ubiquitous PR knockout mouse models and cell specific epithelial knockout models, the molecular mode of action of PR in the uterine compartments can be investigated. Also, I will generate mouse models with deregulated expression of both PR isoforms to determine if alteration in the timing of these affects endometrial function and dysfunction. Since the expression pattern for the PR isoforms is under tight temporal and spatial control, it is my hypothesis that altered expression of these isoforms will negatively impact the process of embryo implantation. Furthermore, through the use of these new mouse models, the function of the specific PR isoforms can be evaluated through cistromic analysis. This deeper understanding of PR signaling in the uterine epithelium and stroma will aid in the development of new therapies to enhance fertility and to treat women’s reproductive associated disease.
Telomeres serve a crucial role in maintaining genomic stability. In most organisms studied, including humans, telomeres are maintained by the specialized reverse transcriptase telomerase. In *S. cerevisiae*, telomerase is composed of an RNA template, TLC1, and three protein subunits, Est1, Est2 and Est3. Although telomerase is active only during late S/G2, the catalytic component, Est2, is associated with telomeres throughout the cell cycle. Efficient Est2 recruitment to telomeres requires an interaction between TLC1 and Ku, a heterodimeric DNA end binding complex required for both nonhomologous end joining and normal telomere structure and function. When TLC1-Ku interaction is lost, Est2 is no longer detectable at telomeres in G1 and there is a 50% reduction in Est2 at telomeres in late S/G2. Notably, telomeres are stably short when TLC1-Ku interaction is disrupted and cells do not senesce. In addition to these findings, Ku is required for the accumulation of TLC1 in the nucleus raising the possibility that TLC1 localization might underlie Ku’s influence on telomere length. Thus, while there are clues, the principal means by which Ku influences telomere length is not clear.

Thus far, we have found an Exo1-dependent increase in telomeric single stranded DNA in strains where Ku cannot bind TLC1 indicating a role of Est2 recruitment by Ku in protecting telomeres from nucleolytic processing. However, this role is minor compared to the role of Ku alone in the protection of telomeric ends. We have also tested whether Ku’s ability to bind TLC1 influences Est2’s association with TLC1, and found that it does not. Future experiments, including co-immunoprecipitation and bimolecular fluorescence complementation, will test whether Ku and Est2, in fact, simultaneously bind TLC1, as often assumed by the field. We are currently testing the hypothesis that Ku’s role in telomere length regulation is to deliver sufficient amount of telomerase at telomeres in late S/G2. We have found that overexpression of Est1 or overexpression of Est1 and Est2 simultaneously in yeast lacking Ku-TLC1 interaction led to telomere elongation, whereas overexpression of Est2 alone did not, hinting to a complex set of interactions including Est1 that were not previously recognized. Future experiments include examining how Est1 versus Est1 and Est2 over expression influences Est2 and Est1 recruitment to telomeres across the cell cycle in wild type versus cells disrupted for Ku-TLC1 interaction. Because the human telomerase RNA subunit also interacts with Ku, these results may apply to human telomerase regulation as well.

Contributors: Williams Jaime, Alison Bertuch
A STRATEGY TO ARM AN ONCOlytic HERPES SIMPLEX VIRUS (HSV) TO SELECTIVELY INDUCE APOPTOSIS IN BYSTANDER TUMOR CELLS

Shana Marie Williamson  
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Oncolytic viral cancer therapies target tumor cells specifically, while sparing normal cells. Addition of therapeutic genes encoding secreted molecules that induce apoptosis in tumor cells surrounding infected cells would enhance the therapeutic quality of an oncolytic virus. Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) forms a transmembrane trimeric structure that activates the extrinsic apoptosis pathway upon binding death receptors (DRs) on cancer cells. We have designed a strategy to arm an oncolytic herpes simplex virus (HSV) with a secreted form of TRAIL to kill surrounding bystander tumor cells. The pivotal concept in our strategy is to fuse the TRAIL topological domain to a secretion signal and a multimerization domain from collagen that allows the synthesized chimeric molecule (91TRAIL-COL) to be trimerized and secreted for a maximal effect in inducing apoptosis in the surrounding tumor cells.

Western Blot shows that 91TRAIL-COL is secreted from transfected cells into the cell media and under non-reducing conditions 91TRAIL-COL is detected in multimerized form as either trimers or hexamers. Following 91TRAIL-COL transfection, caspase-8 and caspase-3 cleavage products are readily detectable, indicating apoptosis is activated in the transfected cells through the extrinsic pathway. Moreover, transfer of medium containing secreted 91TRAIL-COL to wells of human colorectal carcinoma HCT116 or esophageal carcinoma EC9706 tumor cells results in significant cell killing.

A mouse study is underway to test the effect of 91TRAIL-COL secretion from injected HCT116 cells in vivo. Also, the 91TRAIL-COL chimeric gene is currently being inserted into an oncolytic HSV under the control of a strict late viral promoter (UL38p) that will restrict 91TRAIL-COL expression to tumor cells. Once completed the potency of 91TRAIL-COL on bystander cells will be examined in the context of an oncolytic virus in vitro and in vivo.

Contributors: Williamson, Shana; Zhang, Shaun
Endocytic neoplastic tumor suppressor genes (nTSGs) restrict conserved signaling pathways to inhibit the growth of neoplastic tumors. Likely, mutant endocytic nTSGs cause the misregulation of signaling pathways. When endosomal protein sorting fails to function, components of signaling pathways become trapped in early endosomes and continue to signal when they otherwise normally would be degraded. Abnormal activity of these pathways leads tissues to fail to differentiate, overgrow, show disorganization, and invade other tissues. Significantly, mutations in some of these endocytic nTSGs have been linked to a variety of human cancers, including cervical, breast, prostate, and gastrointestinal (GI) cancers.

Study of nTSGs in Drosophila melanogaster has been greatly advanced by the use of the FLP/FRT system to generate mosaic tissues. Genetic screens employing this technique have isolated mutations in Endosomal Sorting Complexes Required for Transport (ESCRT) components, including mutations in vacuolar protein sorting 25 (vps25). Adults with eyes mosaic for vps25 are obviously overgrown. This overgrowth is caused by a unique mechanism: tissues mutant for vps25 undergo apoptosis but induce proliferation and cell death resistance in the surrounding non-mutant tissue. In addition, vps25 mutant tissue accumulates components of the Notch and Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) signaling pathways in abnormal endosomal compartments. Research indicates that upregulation of the Notch signaling pathway in the mutant tissue leads to secretion of the cytokine Unpaired (Upd), which increases proliferation in the neighboring tissues.

Our lab has focused on characterizing ESCRT-II components vps22 and vps25 as nTSGs. vps22 and vps25 mosaic imaginal discs show a disorganized, expanded, amorphic structure. Differentiation is reduced or completely absent in vps22 and vps25 mutant clones. Finally, both vps22 and vps25 mutant clones show high levels of a marker for basement membrane invasion. It is likely that apoptosis or the upregulation of signaling pathway components seen in vps22 and vps25 mutant clones contributes to the neoplastic phenotype. Our goal is to pinpoint how cell death, Notch signaling, and JAK/STAT signaling are involved in neoplastic tumorigenesis when they are upregulated in tissues in which endocytic trafficking is blocked. Our data indicates that although Notch and JAK/STAT signaling do not play a significant role in neoplastic transformation, elimination of vps22 and vps25 mutant cells via apoptosis functions as an innate tumor suppressor mechanism.

Contributors: Herz, Hans-Martin
Reciprocating exchange with other humans requires individuals to infer the intentions of their partners. Despite the importance of this ability in healthy cognition and its impact in disease, the dimensions employed and computations involved in such inferences are not clear. We used a computational theory-of-mind model to classify styles of interaction in 195 pairs of subjects playing a multi-round economic exchange game. This classification produces an estimate of a subject’s depth-of-thought in the game (low, medium, high), a parameter that governs the richness of the models they build of their partner. Subjects in each category showed distinct neural correlates of learning signals associated with different depths-of-thought. Playing an uncooperative partner induced subjects to play at the lowest depth-of-thought. The neural response categories identified by this computational characterization of theory-of-mind may yield objective biomarkers useful in the identification and characterization of pathologies that perturb the capacity to model and interact with other humans.


Contributors: Xiang, Ting; Ray, Debajyoti; Lohrenz, T; Dayan, P; Montague, P.R.
Crag, a GEF for Rab11, is required for the maintenance of Drosophila photoreceptors

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To identify mutations that cause neurodegenerative phenotypes, we screened the X chromosome for essential genes that cause physiological defects in eye clones. We isolated four mutants that affect the fly Crag gene. In newly eclosed flies, Crag mutant eye clones exhibit normal photoresponses. These responses decrease over time in flies which are exposed to light but not in flies which are kept in dark. Consistently, Crag mutant photoreceptor (PR) cells exhibit relatively normal rhabdomere morphology in newly eclosed animals, and upon extended light stimulation, the rhabdomere structures are severely disrupted. Since the degeneration is strictly light dependent, we hypothesized that Crag affects the phototransduction pathway. We therefore examined the localization of key players of the pathway and found that Rhodopsin is ectopically accumulated in the cytoplasm after prolonged light exposure in Crag PRs. In addition, we observed a massive vesicle accumulation in the cytoplasm and shrinkage of rhabdomere membranes in Crag PRs. Crag contains three DENN domains that may possess Rab guanine nucleotide exchange factor (GEF) activity. We therefore performed a screen of Drosophila Rab genes to identify its potential target. We found that overexpression of the Rab11 dominant negative isoform in the adult eye causes light-induced degeneration. Crag and Rab11 colocalize when they are both overexpressed in S2 cells and Crag is a binding partner of Rab11 based on immunoprecipitation assays. We mapped the Rab11 binding domain of Crag to the DENN domains which are the putative GEF activity domain. We also demonstrated that Crag exhibits GEF activity against Rab11 in an in vitro GEF assay and this activity could be enhanced by Calmodulin. A previous study has shown that Rab11 is required for the transport of Rhodopsin from the ER to the rhabdomeres. We propose that Crag is required for Rhodopsin recycling upon light stimulation via Rab11. Light stimulation causes a Calcium influx, which leads to binding of Calmodulin to Crag and Crag GEF activation. In Crag mutant cells, upon light stimulation, Metharhodopsin is endocytosed but newly synthesized Rhodopsin cannot be transported back to rhabdomeres. This causes an accumulation of vesicles and proteins in the cytoplasm, which in turn leads to a gradual PR degeneration.

Contributors: Xiong, Bo; Bayat, Vafa; Jaiswal, Manish; Zhang, Ke; Yamamoto, Shinya; Charng, Wu-lin; Haueter, Claire; Bellen, Hugo
Notch Signaling is an evolutionarily conserved signaling pathway that is required for various biological processes and involved in diverse human diseases such as CADASIL, Alagille Syndrome, Tetralogy of Fallot and various types of cancer. Notch encodes a large type-1 transmembrane protein, whose extracellular domain consists of 36 EGF repeats and 3 LNR repeats. Notch ligands can be classified into two distinct subfamilies: Delta/Dll and Serrate/Jagged family. EGF repeat 11 and 12 of Notch are absolutely necessary for these ligand-receptor interactions. In addition, Notch is known to be able to distinguish the two ligand subfamilies through sugar modification status of EGF repeats, but the exact domain involved in this molecular distinction is not clear.

Through a forward genetic screen in Drosophila, we generated 42 new mutations in the Notch gene. One mutation, which we named N jigsaw, display defects in the formation of the fly wing margin, but neurogenesis in the adult notal peripheral nervous system is not affected. We performed further phenotypic characterization and tissue culture experiments and revealed that the mutation affects Notch-Serrate signaling and binding without affecting Notch-Delta signaling and interaction. Sanger sequencing revealed a Valine-to-Methionine missense mutation in an evolutionally conserved residue in EGF repeat 8. These data suggest that this conserved Valine in EGF repeat 8 may also play a crucial role in the distinction between Dll and Jagged family ligands in mammalian Notch proteins, providing a putative drug target site to manipulate Notch signaling in a ligand specific manner.

Dyskeratosis congenita (DC) is a progressive and heterogeneous congenital disorder that affects multiple systems and characterized by bone marrow failure and a triad of abnormal skin pigmentation, nail dystrophy, and oral leukoplakia. One common feature for all DC patients is abnormally short telomeres and defects in telomere biology. Most of the known DC mutations have been found to affect core components of the telomerase holoenzyme. Recently, multiple mutations in the gene encoding the telomeric protein TIN2 have been identified in DC patients with intact telomerase genes, but the molecular mechanisms underlying TIN2 mutation mediated DC remain unknown. Here, we demonstrate that ectopic expression of TIN2 with DC missense mutations in human cells led to accelerated telomere shortening, similar to the telomere phenotypes found in DC patients. This telomere shortening, however, was not accompanied by changes in total telomerase activity, localization of TIN2, or telomere end protection status. Interestingly, we found TIN2 to participate in the TPP1-dependent recruitment of telomerase activity. Furthermore, DC mutations in TIN2 led to its decreased ability to associate with TERC and telomerase activity. Taken together, our data suggest that TIN2 mutations in DC may compromise the telomere recruitment of telomerase, leading to telomere shortening and the associated pathogenesis.
BACKGROUND: A surrogate for arterial stiffness, carotid arterial strain (CAS) measured with speckle tracking has been shown to be lower in diabetics. Its relation with hypertension has not been assessed. We examined circumferential systolic CAS measured by speckle tracking among diabetics, hypertensive non-diabetics and healthy controls.

METHODS: Bilateral ECG-gated ultrasonograms (US) (fps ~70-90) of the distal common carotid were performed using a 12 MHz vascular probe in 20 healthy volunteers, 11 hypertensives and 21 Type II diabetics. Using a cardiac strain analysis package (TomTec), peak CAS of the far wall segments (lateral, mid, and medial) of both carotids were measured with 2D speckle tracking (Table). Only far wall segments were analyzed due to potential lateral wall US beam dropout and anterior probe pressure. CAS derived from luminal diameters were obtained.

RESULTS: Baseline characteristics were similar among the 3 groups except for heart rate and hypertensive status (mean age 56.9 ± standard deviation 10.2 years, male 51.9%, 26.9% white, systolic blood pressure [BP] 126.7 ± 18.9 mm Hg, diastolic BP 76.6 ± 11.5 mm Hg, pulse pressure 50.1 ± 11.4 mm Hg). The mean far wall and global CAS were lower in diabetics (3.83 ± 1.20% and 3.98 ± 0.82%, respectively) and hypertensives (4.19 ± 1.56% and 4.30 ± 1.26%, respectively) than in controls (5.58 ± 1.28% and 5.48 ± 1.32%, respectively; p <0.05 for both comparisons), but not between diabetics and hypertensives (p >0.05). No difference was observed for luminal derived CSCAS. CAS differences persisted in only diabetic men (p <0.01) but were not significant in women and hypertensive men after accounting for pulse pressure. On regression analysis, hypertension but not diabetes remained a predictor of low mean global CAS (p=0.03), mean far wall CAS (p=0.04), and far wall lateral segment CAS (p <0.0001) with adjustment for age, sex, race, heart rate and pulse pressures, but not of far wall mid (p=0.55) or medial CAS (p=0.97).

CONCLUSION: Speckle tracking measured arterial strain is more sensitive to hypertension and diabetes than that with luminal diameters, especially in diabetic men. Lower arterial strain, or stiffer arteries, was more strongly associated with hypertension than with diabetes.

Contributors: Brunner, Gerd; Dokainish, Hisham; Virani, Salim; Misra, Arunima; Lakkis, Nasser; McCulloch, Marti; Hartley, Craig; Morissett, Joel; Nagueh, Sherif; Ballantyne, Christie; Nambi, Vijay.
MECP2 duplication syndrome is a rare neurodevelopmental disorder caused by duplication of the X-linked gene methyl-CpG binding protein 2 (MECP2). MeCP2 binds to methyl-CpG dinucleotides and recruits chromatin-remolding proteins to regulate the expression of numerous genes. Unlike patients with MECP2 deficiency, patients with MECP2 duplication have recurrent respiratory tract infections, suggesting that MeCP2 overexpression results in immunodeficiency. To test this, we examined mice with constitutive MeCP2 overexpression. These mice exhibited significantly enhanced susceptibility to Leishmania major cutaneous infection and reduced production of IFN-\(-\)secreting T helper type 1 (Th1) cells, suggesting that MeCP2 overexpression results in deficient Th1 cell commitment in vivo. MeCP2 overexpressing T cells further failed to differentiate into the Th1 lineage in vitro. On the other hand, MeCP2 overexpressing mice demonstrated normal Th2 cell development and allergic responses, suggesting that MeCP2 selectively affects Th1 differentiation. We isolated peripheral blood mononuclear cells (PBMCs) of patients with MeCP2 duplication and discovered that CD4+ T cells from PBMC with MECP2 duplication exhibit identically impaired Th1 differentiation and dysregulated proliferation in vitro. We are performing analyses of the effect of MeCP2 overexpression on epigenetic modifications of transcription factors and cytokines during Th1 differentiation to understand how MeCP2 influences Th1 lineage commitment. In addition to the marked neurodevelopmental anomalies that MeCP2 overexpression is known to induce, these findings are the first to established that MeCP2 is also an important regulator in Th1 lineage commitment and host defense against intracellular pathogens.

Contributors: Yang Tianshu1, Lu Wen2, Kheradmand Farrah1,5,Zogbi Huda Y.3, Ramocki Melissa B.4 and Corry David B.1,5.
Neural activity in the primary visual cortex (V1) has been studied extensively in the search for general principles of cortical computation. The functional properties of individual neurons have been classically characterized by correlating their evoked activity to features of the visual stimulus. However, even in the absence of external stimulus drive, populations of V1 neurons remain spontaneously active. The patterns of spontaneous activations of groups of cells have been hypothesized to reflect the functional connectivity of the feedforward and recurrent circuits feeding into the neuronal populations. Spontaneous spike correlations as well as noise correlations during evoked activity are often analyzed as parameters determining the information-carrying capacity of the network. The correlation structure of spontaneous activity is not static: shifts in the global cortical state or the behavioral state of the animal have been shown to alter it. Particularly well recognized is the distinction between activated cortical states and endogenously generated slow-wave activity (SWA) characteristic of some stages of sleep and anesthesia. In SWA, the brain is thought to be less attuned to afferent inputs than in information-processing activated states. We asked the specific question how the onset of SWA would affect the structure of spontaneous population activity in the primary visual cortex and how this change would account for the change in the population response of visually driven cells to external stimulation.

To address this question, we monitored the global brain state of adult mice under urethane anesthesia using local field potential recordings. Population activity of layer 2/3 neurons of the primary visual cortex was recorded using two-photon calcium fluorescence signals. Visual stimuli consisting of drifting gratings were used to measure the orientation selectivity of cells. In seven of the 11 mice analyzed, the cortical state alternated periodically between SWA and activated states with the period ranging between 1.5 and 6 minutes across mice while remaining stable across multiple recordings in the same animal. Responses to visual stimuli were significantly more reliable in activated states than in SWA partially due to lower baseline firing rates. The correlation structure of neuronal activity was significantly different between the two states: cortical activation triggered a significant decrease in pairwise correlations. Changes in the correlation structure were not accompanied by significant shifts in the tuning properties of cells. Additionally, visual stimulation decreased the pairwise correlations in both activated and slow-wave states. These observations are consistent with a model in which SWA is generated by endogenously in recurrent circuits with limited direct contribution to the receptive field properties of cells, that this activity is dissipated by both external stimuli and cortical activation, and that its input could be recognized and isolated by its multineuronal correlation structure.

Contributors: Yatsenko, Dimitri; Froudarakis, Emmanouil; Tolias, Andreas
Despite significant advances in our understanding of Alzheimer’s disease (AD) at the genetic and biochemical levels, little is known about how the amyloid plaques, neurofibrillary tangles, and neurodegeneration that characterize AD lead to the hallmark neurological conditions. The progression of any nervous system disorder is intimately linked to the specific neural circuits that are affected. In order to understand how circuit malfunction and destruction by molecular lesions in AD leads to progressive decline in memory, cognition, and affect, we need to understand the specific function of the brain regions hit by the disease. The entorhinal cortex is the first structure in human brains to show tau tangles and neuronal loss. Its suspected role as a key hub in the episodic learning and memory circuitry complements the observation that AD manifests itself in early stages as an inability to remember recent autobiographical events.

The entorhinal cortex sits at a crossroads between sensory cortical areas and the hippocampus. It has two primary inputs to the hippocampus, the perforant and temporoammonic paths, which arise from layer 2 and 3 neurons respectively. Transgenic mouse technology has allowed direct examination of the temporoammonic path and each relay in the hippocampal trisynaptic loop. However, the exact role of the perforant path in this entorhinal-hippocampal circuitry has remained elusive. We have developed a system in which layer 2 medial entorhinal cortex neurons can be silenced in vivo through the systemic application of a common antiparasitic drug, ivermectin. Treatment with ivermectin activates a transgenically expressed chloride channel, lowering the cell’s membrane potential and preventing them from firing action potentials. These silencer mice will allow us to determine the specific contributions of the medial perforant path to episodic learning and memory, adult neurogenesis in the dentate gyrus, and spatial tuning in hippocampal neurons, all of which are thought to be impaired in Alzheimer’s disease.

Contributors: Yetman, Michael; Lee, Tang-Cheng; Jankowsky, Joanna
Female fertility in mammals depends upon the highly organized growth and death of the oocyte bearing follicles of the ovary. Follicular development is dependent on many factors; the proper segregation of oocytes into primordial follicles, cell signals generated by the granulosa, theca, and oocyte cells in activated and growing follicles, as well as an intricate cycle of hormonal messages that exists between the ovary, the pituitary, the hypothalamus and the adrenal gland. A precise and coordinated balance between all of these elements is critical in all mammals for ovarian health and reproduction. Perturbation of any stage of folliculogenesis, whether by internal or external defect, can lead to infertility, and of especial concern in human fertility are clinical conditions such as polycystic ovarian syndrome (PCOS), premature ovarian failure, anovulation, and ovarian cancers. In folliculogenesis the primordial follicles proceed through an ordered transformation to primary, pre-antral, antral, and then Graafian follicles. Of the thousands of primordial follicles present in the developing mouse ovary, greater than ninety percent will disappear before they reach maturity through the process of controlled follicular death, called atresia, that continues throughout the life of the organism, but which is especially critical in the developing ovary where it serves in part to break up the clusters of oocytes known as oocyte nests so that they can be individually sequestered within their primordial follicles. We have discovered that the loss of growth arrest specific 2 (Gas2) perturbs folliculogenesis, resulting in subfertile female mice. Gas2 was initially discovered in mouse fibroblasts where increased expression of Gas2 was associated with cell cycle arrest, and the protein was later found to have roles to play in both apoptosis and cytoskeletal rearrangements. Gas2 expression was found in developing mouse embryos, with a pattern of expression suggesting roles in differentiation, proliferation, and the apoptosis of inter-digital tissues in the developing embryo. Follicular development in Gas2 -/- mice is disrupted, with reduced numbers of both mature follicles and corpora lutea found in the mutant mice; however Gas2 does not appear to be expressed in the adult ovary, despite an apparent increase in follicular atresia present in the mutant. Gas2 expression is present in both the adrenal gland and the pituitary, and the observed defect in folliculogenesis is consistent with a disruption of the ovarian-pituitary-hypothalamic axis; however, the presence of a large number of multiple oocyte bearing immature follicles in the adult and the persistence of unsegregated oocyte nests after birth indicates that it is a disruption of early ovarian development that leads to the dramatic subfertility observed in the mutant adults. Current work is therefore focused on this early stage of ovarian development and upon determining whether it is the apoptotic or cytoskeletal organizational functions of Gas2 that are critical for the sequestration of individual oocytes into primordial follicles.
ALTERATIONS IN THE GUT MICROBIOME ASSOCIATED WITH TRAVELERS’ DIARRHEA

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The human body is home to a diverse community of bacteria, a majority of which is found in the gastrointestinal (GI) tract. These microbiota perform functions that host cells cannot, such as metabolism of complex polysaccharides and biosynthesis of vitamins. Alterations in the GI microbiota have been correlated to diseases such as obesity and inflammatory bowel disease. Secretory diarrhea is another example exhibiting alterations of the GI community. Up to 60% of individuals traveling from industrialized countries to developing countries acquire a form of secretory diarrhea known as Travelers’ Diarrhea (TD). Enterotoxigenic Escherichia coli (ETEC) is the leading cause of bacterial TD. It expresses two toxins, heat labile toxin (LT) and heat stable toxin (ST), which ultimately lead to secretory diarrhea. It is thought that ETEC-TD would cause a dramatic decrease in the GI community, however the specific effect of ETEC and its toxins on the GI microbiota has not been studied. 16S metagenomic sequencing has been used to examine the GI microbiome of four groups of individuals: healthy; TD negative travelers; ETEC positive TD; and pathogen negative TD controls. The differences in the GI populations between these three groups of samples have been examined to determine if LT and ST have similar effects on the GI community. Compared to healthy controls, ETEC TD samples showed a significant increase in bacterial species belonging to the phyla Firmicutes and Actinobacteria and a significant decrease in the phylum Bacteroidetes. Compared to TD negative travelers, ETEC TD samples showed a significant increase in Actinobacteria and a significant decrease in unclassified bacteria. Overall, there were no significant differences between the ETEC samples and the pathogen negative TD samples or between the toxin producing groups at the phylum level. These data show how the GI microbiota is altered during ETEC infection. This research will provide a comprehensive understanding of the alterations caused by TD-ETEC and may aid in development of preventative treatments for TD.

Contributors: Youmans, Bonnie; Ross, Matthew; Ayvaz, Tulin; Muzny, Donna; Qin, Xiang; Gibbs, Richard; DuPont, Herbert; Petrosino, Joseph and Highlander, Sarah
RAS proteins are thought to engage growth factor receptors at the plasma membrane (PM) to mediate cell growth and tumorigenesis. How RAS travels to the PM is still largely unclear, therefore, we conducted a genetic screen using the fission yeast, Schizosaccharomyces pombe, to address this. S. pombe has a single Ras ortholog, Ras1p, which controls mating from the PM and morphogenesis from the endomembrane. We mutagenized cells carrying GFP-Ras1 and then sought sterile colonies by iodine staining. Isolated sterile mutants were then screened microscopically to seek mutants with the normal elongated cell morphology and diminished levels of GFP-Ras1 on the PM. Five mutants were isolated whose GFP-Ras1 was detectably mislocalized in logarithmically growing cells, and complementation analyses show that these 5 mutants carry recessive mutations that affect the same gene (rms1). We screened a low copy genomic library and isolated a clone that fully rescued the phenotype of the rms1-1 mutant. Genetic data indicate that rms1 is the same gene as mug142, which encodes one of five DHHC-domain proteins in S. pombe and is a predicted ortholog of ERF2p, a budding yeast Ras-specific palmitoyltransferase. Sequence analysis has revealed candidates of human orthologs. We tested the top human candidate and indeed, it can functionally rescue the mislocalization of GFP-Ras1 and growth defects of mug142( cells. In conclusion, the identification of a single gene from this screen suggests that palmitoylation is the pivotal event in Ras trafficking to the plasma membrane at steady state and validates that Ras localization is dependent on a specific DHHC palmitoyltransferase. Ongoing study focuses on whether the human functional ortholog is responsible for a similar Ras-specific role in mammalian cells.
SYNERGISTIC ACTIVATION OF COMPLEMENT AND TLR PATHWAY IN THE PATHOGENESIS OF EMPHYSEMA

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Smoking-induced chronic obstructive pulmonary disease (COPD), which encompasses chronic bronchitis and emphysema, is a progressive inflammatory lung disease with no known effective treatment. An important hallmark of emphysema is the irreversible degradation of lung matrix proteins, especially elastin, by protease such as neutrophil elastase, MMP9 and MMP12. Our studies have shown a link between adaptive immunity against elastin in emphysema pathogenesis. However, the mechanism of how cigarette triggered innate immunity eventually lead to adaptive immunity remains unclear. Complement proteins and their signaling receptors bridge innate and adaptive immunity, thus making their role as pathogenic mediators in emphysema, quite plausible. Prior work in our lab has found C3 fragments deposition on lung parenchyma of smokers with emphysema, but not healthy controls. C3d, the degradation product of C3, is known to act as a molecular adjuvant by signaling through complement receptor (CR)2, to lower the threshold of immune activation. Similarly, C3b conjugated with antigen can enhance the function of antigen presenting cells (APCs) via complex with CR1; together these findings provide the basis for their role in auto-immune mediated inflammation is emphysema. Further, structural damage to the lung, as seen in heavy smokers, is associated with chronic lung infection and bacterial colonization, and activation of Toll-like receptors (TLRs) signaling synergistic activation of CRs and TLRs may promote autoimmune inflammation in the lungs of chronic smokers.

We hypothesized that activation of CRs and TLRs converge to initiate and or propagate auto-immune inflammation in human emphysema and in animal model of this disease. We will determine if C3 fragments conjugate with lung elastin and/or CRs in human emphysema, and whether their synergistic action with TLRs in human lung APCs and in mice exposed to cigarette smoke accelerates disease. We will measure the expression profile of TLR2, 4 and 9 and C3aR and C5aR on different subsets of APCs in human lung. To measure systemic activation of complement pathway, we will measure C3 and C5 level in human plasma. In the context of localized complement activation, C3a and C5a level will be determined by western blotting in human lung tissues. C3-/-, C3aR-/-, C5aR-/- and MyD88-/-/Trif-/- mice strains will also be employed in the mouse model of smoke-induced emphysema to test our hypothesis. Our findings will help to clarify the immunological mechanisms underlying emphysema pathogenesis and may be promising for new treatment options.

Contributors: Yuan, Xiaoyi; Cheng, Han-Fang; Kheradmand, Farrah
In this study, we investigate the physiological roles of the RFWD3 (Ring Finger and WD domain 3) ubiquitin ligase by using conditional knockout mouse strategy. RFWD3, also known as FLJ10520 and RNF201, was identified as one of the putative ATM substrates and plays a role in maintenance of the G1/S checkpoint. Our biochemical studies indicate that RFWD3 is required for stability of both p53 and MDM2 and it modulates MDM2-dependent ubiquitination of p53, rendering p53 resistant to 26S proteosome degradation in late response to DNA damage (PNAS, 2010, Fu and Yucer et al).

According to Mouse GNF (Genomic Institution of the Novartis Research Foundation) Atlas, Rfwd3 is mainly expressed during embryonic development. In fact, we show that RNAi knockdown of Rfwd3 in embryonic stem (ES) cell causes differentiation; demonstrating that Rfwd3 promotes ES cell self-renewal and suggesting that full-body Rfwd3 knockout may lead to embryonic lethality. In order to elucidate its physiological function, we created uterus- and liver-specific Rfwd3 knockout mice. Uterus-specific Rfwd3 knockout mice displayed significant decrease in organ size, suggesting that Rfwd3 is involved in organ development. We are currently characterizing the mechanisms underlying this phenotype.

To investigate the Rfwd3 function in liver, we performed DNA affinity pull-down / mass spectrometry to profile changes in transcription factor (TF) levels, followed by WB analyses of selected TFs in wild type and Rfwd3 knockout liver. As a result of our first screen, we determined that Rxr- (Retinoid X Receptor-alpha) also known as Nr2b1 (Nuclear Receptor Subfamily 2) is significantly reduced in Rfwd3 knockout liver. We confirmed this trend with knockdown of RFWD3 in human cells. Our goals in this project are to further characterize the Rfwd3 function in regulation of Rxr- and elucidate its role in regulation of metabolism, where Rxr-a play a key role.

Contributors: Malovannaya,Anna; Chan, Doug; Li, Chunshu; Jung, Sung Yun; Cho, Sung-Nam, Jeong, Jaewook; Zhang, Pumin; Wang, Yi; and Qin, Jun
ANALYSIS OF MODULATION OF FXTAS rCGG-MEDIATED NEURODEGENERATION BY CUGBP1 IN MUS MUSCULUS.

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Fragile X-associated Tremor/Ataxia Syndrome, or FXTAS, has been identified as a late-onset neurodegenerative disorder in Fmr1 premutation carriers, principally in older males. The neuropathological hallmarks of FXTAS include ubiquitin-positive intranuclear inclusion bodies throughout the brain and marked dropout of cerebellar Purkinje neurons. We hypothesize that FXTAS arises through an RNA-mediated gain-of-function toxicity model. In this model, the ribo-CGG (rCGG) repeat acts to functionally limit rCGG repeat-binding proteins. Previous work in Drosophila demonstrated the singular sufficiency of rCGG to exert a toxic gain-of-function. The Drosophila ortholog of hnRNP A2/B1 was found to be capable of suppressing phenotype and binding rCGG. A subsequent genetic screen identified CUGBP1 as a specific interacting partner of hnRNP A2/B1, and also as a modifier of rCGG-mediated toxicity. We report our recent work in furthering these earlier results using mouse models. We previously reported two Purkinje neuron-specific transgenic models in which a CGG premutation is fused to a reporter (FMR1 or EGFP), and are also making use of the “knock-in” model developed by the Oostra group, which replaces the endogenous 5 CGG repeats with a >100 CGG-repeat fragment within the Fmr1 locus. We predict that reduced levels of CUGBP1 will exacerbate rCGG phenotypes and that CUGBP1 overexpression will rescue the FXTAS phenotype. To this end, we have developed two mouse models to examine the role of Cugbp1 in FXTAS pathogenesis: a gene trap loss-of-function, in which vector pGT1Lxf has been inserted into the second intron within the coding sequence of Cugbp1, and mice with tetracycline-controlled overexpression of Cugbp1 in Purkinje neurons. We have crossed these mice to our FXTAS mouse models and are in the process of determining the resulting phenotypes. These results should significantly advance our understanding of FXTAS pathogenesis, and provide potential targets for therapeutic intervention.

Terminal and interstitial deletions of 1p36 are a common cause of cognitive and developmental delay with an incidence of 1 in 5000 newborns. Although 28% of individuals with 1p36 deletions also have sensorineural hearing loss, the gene(s) responsible for this phenotype have yet to be identified. The arginine-glutamic acid dipeptide repeats gene (RERE), located in the proximal region of 1p36, encodes a nuclear receptor coregulator that is required for normal embryogenesis, and is highly conserved from Drosophila to humans. In zebrafish, reduced expression of the RERE homolog Rerea leads to inner ear anomalies—fused otoliths and abnormal semicircular canals—and diminished microphonic potentials. We hypothesized that RERE plays a similar role in the development of the inner ear in mammals. To test this hypothesis, we used immunohistochemistry to show that RERE is expressed in critical regions of the mouse inner ear including the inner and outer hair cells and the marginal and basal cell layers of the stria vascularis. However, further studies of the function of RERE in the inner ear were hampered by the early lethality seen in Rere null mice (Rere-/-), which die in utero at E9.5 due to cardiac failure. To overcome this, we generated an allelic series of RERE-deficient mice bearing different combinations of a Rere null allele and a hypomorphic allele (V193A) we identified in an ENU mutagenesis screen. Rere-/-V193A mice have diminished startle responses to a 108 dB burst at 19.9 kHz emitted from a click box—a common preliminary test for severe hearing loss. Distortion product otoacoustic emission (DPOAE) and auditory brainstem evoked response (ABER) testing at p21 revealed that Rere-/- V193A and Rere V193A / V193A mice have early onset hearing loss. Rere-/- V193A mice also fared poorly in the dowel test which requires normal balance and coordination. Histological analyses and 3D micro-CT reconstructions of the inner ear showed that Rere-/- V193A mice have normal cochlear morphology at p21 but have increased cross-sectional diameter of their semi-circular canals when compared to their wild type littermates. We conclude that RERE is required for normal hearing and vestibular function and that Rere-/- V193A and Rere V193A / V193A mice represent useful models for studying the molecular mechanisms by which RERE-deficiency contributes to the hearing loss in individuals with 1p36 proximal deletions.

Contributors: Kim, Bum-Jun; Pereira, Fred.A; Groves, Andrew.K; Oghalai, John; Justice, Monica; Lee, Brendan; Scott, Daryl.A.
MicroRNAs are negative regulators for target genes and are involved in various biological processes. However, there are few reports regarding microRNA interactions in osteoblast differentiation and only one study has in vivo mouse model. To identify more microRNAs affecting osteoblast differentiation, we induced osteogenesis in C2C12 cells with BMP2 treatment and performed a microRNA microarray. From the array experiment, 14 microRNAs were significantly downregulated and 20 microRNAs were significantly upregulated in differentiated C2C12 cells compared to undifferentiated cells. Among these microRNAs, miR-23a cluster were upregulated and this cluster has several potential targets which are important for osteoblast differentiation according to Targetscan database.

To study the function of miR-23a cluster in bone development in vivo, we made a transgenic mice overexpressing miR-23a cluster using the osteoblast-specific Col1a1-2.3kb promoter. The mice showed growth retardation in transgenic mouse group compared to wild type littermates and it became more significant around weaning. To understand the miR-23a cluster function during bone development, we analyzed bone density of spinal bone from 7-week old female mice by micro-computed tomography (μCT). In transgenic mice group, the spines exhibited a low bone mass phenotype. Both trabecular bone density and thickness decreased significantly while the trabecular space was larger in the transgenic group. To further understand the molecular mechanism of low bone mass in miR-23a cluster transgenic mouse line, we evaluated the several osteoblast markers expression by qRT-PCR using RNA isolated from calvaria. Osterix, an essential transcription factor, and Osteocalcin, the late osteoblast maturation marker, were decreased significantly in the transgenic groups. These data suggest that miR-23a cluster may suppress terminal maturation of osteoblasts.

Contributors: Zeng, Huan-Chang; Bae, Yangjin; Campeau, Phillippe; Dawson, Brian; Chen, Yuqing; Bertin, Terry; Lee, Brendan
To identify genes that cause neurodegeneration, we performed a large chemical mutagenesis screen on the fly X-chromosome in Drosophila. One of the complementation groups, XE07, consists of 6 alleles. Flies bearing mutant clones in the visual system exhibit defective electroretinograms (ERGs) that worsen with age. Transmission electron microscopy (TEM) shows that the morphology of mutant retinas and laminas deteriorate with age and that they contain expanded mitochondria with dissociated cristae. Homozygous mutant larvae have an extremely long lifespan but the mutants eventually die as pupae. The gene was mapped to CG15738 and encodes an evolutionarily conserved protein with unknown function. Its human homolog, C8orf38, has been linked to a mitochondrial complex I deficiency and Leigh syndrome. Our mutants exhibit decreased enzymatic activity of complex I and various subunits of complex I are severely reduced in mitochondria. However, proteins of other complexes are not affected. We therefore named the gene “sicily”, for severe impairment of complex I with lengthened youth. We also observed that sicily mutants display key features of oxidative stress and a mitochondrial unfolded protein response, a common feature associated with complex I deficiency.

To determine where the protein is localized, we tagged a genomic construct and discovered that the protein is associated with the cytoplasmic phase of mitochondria. To isolate protein partners, we performed a yeast two hybrid screen and identified a key complex I subunit, ND42. We confirmed that ND42 interacts with Sicily by co-immunoprecipitation (coIP). Interestingly, Sicily and ND42 preferentially interact in the cytoplasm. In addition, ND42 is severely down-regulated in sicily mutants and loss of ND42 leads to complex I deficiency and retinal degeneration in flies, very similar to what we observe in sicily mutants. These data indicate that loss of sicily and ND42 display very similar phenotypes.

To determine how Sicily affects ND42, we performed coIP with lysate from adult flies expressing Sicily-mCherry and subjected the IP complexes to mass spec analysis. We identified Hsp83 as an interactor of Sicily and confirmed that Hsp83 interacts with the Sicily-ND42 complex in cultured cells. Hsp83 is a chaperone required for the stability of many cytoplasmic proteins and has been shown to be involved in mitochondrial import. Moreover, a specific inhibitor of Hsp83, geldanamycin, affects Sicily levels and causes ND42 to aggregate, indicating that Hsp83 is required for Sicily and ND42 stabilization in the cytoplasm and subsequent mitochondrial import.
PREVENTING CONFLICT BETWEEN DNA REPLICATION AND TRANSCRIPTION MACHINERIES

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DNA replication and transcription are essential processes sharing the same DNA template. The transcription complexes can cause 'roadblocks' that inhibit the progression of replication forks. This replication-transcription conflict leads to lesions in the DNA templates and threatens genome integrity. We recently found that in the model bacterium E. coli, nutrient starvation elevates the conflict between replication and transcription, resulting in replication arrest and DNA damage. DksA, a functional analog of the mammalian TFIIS transcription factor, prevents the conflict between replication and transcription machineries. To understand how DksA prevents the replication-transcription conflict, we applied ChIP-chip (Chromatin Immuno-Precipitation with Microarray) method to analyze the association of RNA polymerase (RNAP) and Sigma70 factor to the E. coli chromosome. Comparing with wild type cells, we found significantly decreasing RNAP association through transcription elongation of genes in dksA deletion cells, which can be explained by stalled transcription elongation complexes (TECs) that can hinder the replication fork progression. The nature of replication-transcription conflict is different from the known effect of rRNA transcription on replication, as inversion of rRNAs head on to replication doesn’t result in significant growth defect in dksA deletion cells. The R-loop structure, proposed to be a physical replication barrier caused by rRNA transcription, is not the major barrier removed by DksA. Based on these observations, we hypothesize that DksA prevents the conflict between replication and transcription machineries, by inhibiting the formation of stalled TECs, which is a formidable barrier to DNA replication.

Contributors: Yan Zhang, Jeff Grass, Rachel A. Mooney, Robert Landick and Jue D. Wang
Telomere dysfunction has been implicated in cancer and aging. In mammalian cells, the telomeres are bound by protein complexes formed by DNA binding proteins TRF1, TRF2 and POT1, and their associated proteins RAP1, TIN2 and TPP. These six core telomeric proteins in turn associate with a collection of molecules that regulate diverse signaling cascades necessary for telomere maintenance. However, the mechanisms by which human telomeric proteins communicate with different signaling pathways remain largely unknown. Our previous study has demonstrated that TIN2-TPP1 interaction is critical for telomeric protein complex formation, and spatial regulation of TIN2 and TPP1 localization plays an important role in telomere maintenance. In addition to their nuclear localization, TIN2 and TPP1 are also localized and interact in the cytoplasm. TIN2 stabilizes TPP1 nuclear localization, which is necessary for telomere length regulation and end protection. The non-nuclear localization of TIN2 and TPP1 also suggests extra-telomeric activities of these proteins. Here we report an unexpected role of TIN2 in the mitochondria. The mitochondrial localization of TIN2 is mediated by its N-terminal targeting sequences. TIN2-TPP1 interaction mediates nuclear localization and inhibits mitochondrial localization of TIN2. Overexpression of mitochondrial TIN2 led to mitochondrial structural change and dysfunction. In addition, TIN2 regulates ATP synthesis. Our findings indicate that TIN2 is a multi-function protein, linking telomere regulation to metabolic control.

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ROLE OF DAPK-1 IN GROWTH REGULATION OF ER-NEGATIVE BREAST CANCER

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Breast cancer is known as the second leading cause of cancer-related death in women. According to estrogen expression status, breast cancer can be subgrouped into ER-positive and ER-negative breast cancer. Clinical data has shown that ER-negative breast cancers are more aggressive and have a poor prognosis; however, there are few effective targeted treatments for these cancers. A recent human kinome study conducted by our laboratory has identified specific kinases highly expressed in ER-negative breast tumors. We conducted a siRNA screen to knockdown each of these kinases that are highly expressed in ER-negative breast tumors in order to determine their roles in regulating breast cancer cell growth. Of these, DAPK-1 was selected for further study. Death-associated protein kinase-1 (DAPK-1) is a member of death-related kinase family. DAPK-1 is a stress-regulated protein kinase that mediates signal induced cell death through p53 dependent or independent pathways. Recent studies have identified a diverse role of DAPK-1 in growth factor signaling and apoptosis. DAPK-1 expression is suppressed in many types of solid cancers due to hypermethylation at the promoter region. However, the expression of DAPK-1 is 2 fold higher in ER-negative breast tumors compared to ER-positive breast tumors, indicating an important role of DAPK-1 in the mitogenic pathways of ER-negative breast cancers. The hypothesis is that DAPK-1 and its related pathway are important regulators of ER-negative breast cancer growth. We are currently investigating the role of DAPK-1 in regulating proliferation and death of ER-negative breast cancer cells as well as the signaling pathways utilized by DAPK-1 in ER-negative breast cancer growth regulation. This study aims to identify potential kinase targets for the prevention and treatment of ER-negative breast cancer. The mechanisms of how these kinases regulate the proliferation of breast cancer can provide insights into the pathways that are important for the survival of breast cancer. Thus, these kinases and related pathways may serve as potential therapeutic targets.

Contributors: Zhao, Jing; Zhang, Yun; Hill, Jamal; Speers, Corey; Mazumdar, Abhijit; Brown, Powel
My goal is to understand how endocytosis influences cell surface remodeling during cell shape change. Endocytosis accompanies cell shape changes including cytokinesis, wound healing and cell motility, though its function and regulation in these processes remains unclear. Our lab uses Drosophila cellularization as a model for cell shape change. Cellularization is a modified cytokinesis whereby furrows ingress to package ~6000 nuclei of the syncytial embryo into individual cells. Due to the simple morphology of cellularizing embryos, their suitability for live imaging and the dramatic growth of the plasma membrane, cellularization provides a very good system to address questions about cell surface remodeling. We previously reported that unregulated endocytosis compromises the structural integrity of cellularization furrows and causes them to regress. Thus, by screening for mutants with furrow regression phenotypes, we have now identified two novel regulators of endocytosis, Serendipity- (Sry-) and its homolog Spitting Image (Spt). According to the Protein Homology/analogy Recognition Engine (Phyre), these proteins belong to the Vinculin Family whose members are actin binding proteins. In vitro, we have confirmed that both Sry- and Spt bind actin filaments directly using an actin co-sedimentation assay. In vivo, we find that sry- null mutant embryos have significantly reduced levels of cortical actin. In addition, both Sry- and Spt proteins are expressed during cellularization and localize to the furrow tips where actin concentrates and endocytosis occurs. Thus, we conclude that Sry- and Spt can regulate endocytosis at cellularization furrows via their direct interaction with the actin cortex. Experiments are ongoing to understand the mechanism underlying this regulation, and to examine whether Sry- and Spt act redundantly, or have their own distinct functions.

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