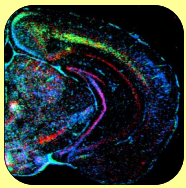
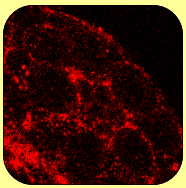
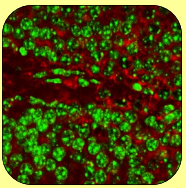
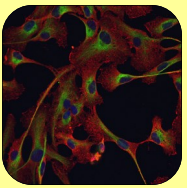
BIO-X

Undergraduate Research Program

Summer 2009

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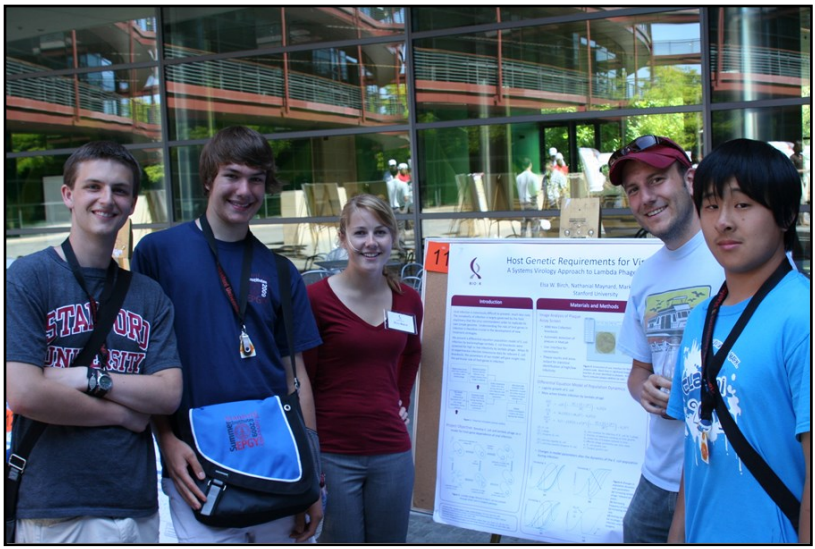
Bio-X Undergraduate Research Program

The Bio-X Summer Program funds research for undergraduates through an award designed to support interdisciplinary undergraduate projects. Awards are made through an application process available to any Bio-X affiliated faculty across campus. Students awarded a Bio-X Undergraduate Research Award receive a stipend equivalent for a 10 week period. To date 47 students have been awarded the opportunity to participate in this program.

To enrich the summer program and to encourage participating students to meet each other, we arrange weekly Faculty lunchtime talks. Each sponsoring Faculty member presents a talk about the work in their group. This is a unique opportunity for students to hear more about the broad range of research within the Bio-X Program, and the talks are also open to the Bio-X community. Each week, students learned about research from two or three faculty in new areas of research to which they may not otherwise be exposed. At the conclusion of the 10 week period, the students presented posters of their work.

The majority of funding for the support to our undergraduate summer research program was through the use of the Bio-X Director's discretionary funds. We also thank Pitch Johnson for his contribution to this program. In 2009, we had the largest pool of applicants for the program, and we supported the largest group of undergraduate students in the history of the program.



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Bio-X Undergraduate Research Program

The 2009 Bio-X Undergraduate Research Talks given by Stanford Faculty are listed below:

June 24
Zev Bryant "Engineering Molecular Motors"
Sarah Heilshorn "Designing New Medical Materials for Stem Cell Transplantation"
Dmitri Petrov "Studies of Molecular Adaptation"

July 1
Miriam Goodman "Using C. elegans to understand Pleasant and Painful Touch Sensation"
Geoff Gurtner "Understanding the Role of Progenitor Cell Mediated Repair Following Injury"
Cliff Wang "Evaluation of Combinatorial Gene Expression in Lymphocytes"

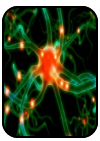
July 8
Carla Shatz "Brain Tuning"
Matthew Bogyo "Applications for Small Molecules in the Study of Protease Function"

July 15
Judith Frydman "Protein Folding and Misfolding in the Eukaryotic Cytosol"
Mike Longaker "Adipose-derived Cells for Skeletal Tissue Engineering"
Charles Taylor "Biomechanical Factors in Vascular Disease"

July 22
Kevan Yamahara "California Beach Sands - Reservoirs for Fecal Indicator Bacteria"
Margaret Fuller "Regulation of Self-renewal and Differentiation and in Adult Stem Cell Lineages"
Suchi Saria "Towards Holistic Diagnostic Models"

July 29
Helen Blau "Bioengineering Stem Cell Fate"
Jill Helms "Wnt-mediated Tissue Regeneration"
Steve Quake "Turning the Spotlight to Dark Matter in Biology"

August 5
Matthew Scott "Genetic Control in Development and Disease"
John Hugenard "Dissecting Neural Circuitry one Cell at a time"



August 12
Richard Zare "Cell, Cell, Cell! "
Michael Clarke "Molecular Regulation of Self Renewal"

August 19
Patrick Ng "Vaccines for the Treatment of Lymphomas"
Theo Palmer "Functional Roles for New Neurons in Old Neural Networks"
Raphael Guzman "Multimodality Imaging in Stroke Stem Cell Therapy"

August 26
Joachim Hallmayer "Genetics of Autism"
Kang Shen "Small Connections in Tiny Worms: Molecular Mechanisms of Synapse Formation"

Poster Presentations:

Quantification of Abdominal Aortic Aneurysms During Disease Progression Using Small Animal Magnetic Resonance Imaging

Kyla N. Barr[1], Craig J. Goergen[2,3], Maj Hedehus[3], Junya Azuma[4], Charles A. Taylor[2], Philip S. Tsao[4], Joan M. Greve[3]

[1]Mechanical Engineering, Stanford University, [2]Bioengineering, Stanford University, [3]Biomedical Imaging, Genentech, Inc., [4]Medicine, Stanford University Medical Center

Abdominal aortic aneurysm (AAA), a typically asymptomatic disease that is associated with significant morbidity, accounts for roughly 15,000 deaths per year in the United States. While the morbidities associated with aortic rupture are commonly understood, it is unknown how mechanical forces play a role in the early and middle stages of disease progression. In order to quantify aortic wall motion and aneurysm growth in commonly used angiotensin II and elastase AAA mouse models, time-of-flight gradient-echo (TOF-GRE) magnetic resonance angiography was used at six time points over a twenty-eight day period. Circumferential cyclic strain and changes in centroid position were calculated in both models using SimVascular. Three-dimensional segmentations of the AAA were also generated and compared to a theoretically healthy vessel based on the day zero images in each mouse. The abatement of cyclic strain and reduced centroid motion during disease progression in our results suggests a stiffening of the aortic wall during asymmetric aneurysm growth.

Identifying the Role of Land Use in Coastal Water Quality in Northern California

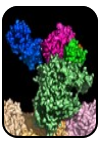
Debbie Lee, Sarah P. Walters, Alexandria B. Boehm
Environmental Engineering, Stanford University

The presence of fecal indicator bacteria (FIB) is used to evaluate water quality because epidemiological studies have shown a correlation between the prevalence of FIB and the incidence of waterborne disease among recreational users when FIB concentrations exceed threshold limits. This study aims to explore the relationship between numbers of the standard FIB, *Escherichia coli* and enterococci, the occurrence of *Bacteroidales* host-associated DNA fecal markers, physiochemical parameters and land use with the presence of *Salmonella* spp. Samples were collected from fourteen sites along the Northern California coast and categorized according to land use (rural, urban, or agricultural) and analyzed for FIB, *Bacteroidales* DNA markers, and *Salmonella*. This ongoing study contributes to growing research addressing the relationship between land use and water quality in order to elucidate the factors contributing to deteriorating coastal water quality in Northern California.

An ErbB ligand inhibits hippocampal neural progenitor cell differentiation

Rafael Wabl, Harish Babu, Theo Palmer
Neurosurgery , Stanford University

The sub-granular zone (SGZ) of the hippocampus and the sub-ventricular zone (SVZ) on the lateral wall of the lateral ventricle are two regions that continually generate neurons throughout an adult life. Neural stem cells take cues from their surrounding cellular environment to determine the nature and time of their differentiation. While several positive regulators of neural differentiation have been identified, no negative feedback regulators are known. These, however, are essential for the homeostatic balance of stem cell differentiation. Our work suggests that conditioned medium from mature hippocampal neurons can inhibit the neuronal differentiation of neural progenitor cells (NPCs). Thus, we hypothesize that mature neurons secrete a ligand that functions as an inhibitory homeostatic regulator of neuronal differentiation. This ligand likely stimulates EGFR/Erbb tyrosine-kinase receptor on the NPC surface. In future work, we will aim to show that the exact nature of this ligand is modulated by neuronal network activity.



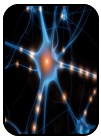
Improving the Efficiency of Cell Transplantation through Biomaterials Development

Brian Aguado[1], Sarah C. Heilshorn[2]
[1]Biomechanical Engineering, Stanford Univ., [2]Materials Science & Eng., Stanford Univ.

Cell transplantation has demonstrated promising results in regenerating functionality after myocardial infarction and stroke. Recent studies have indicated that cell injection within hydrogels can improve cell viability; however, the mechanisms are unknown. We propose systematic studies of the biological and physical variables critical to cell transplantation via direct hydrogel injection. We hypothesize that cell delivery within a hydrogel cell carrier, using optimized injection parameters, will significantly improve cell viability. Alginate, a copolymer with easily tunable viscoelastic properties, is used as a model hydrogel for mechanistic studies. Ultimately, this research intends to translate effective transplantation therapies for clinical use.

Creating a Lentivirus Expressing USP16, a Possible Negative Player in Self-Renewal

Jonathan Noguchi, Maddalena Adorno, Ph.D., Michael F. Clarke, M.D.
Stanford University Stem Cell Biology and Regenerative Medicine Institute

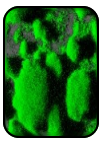


In assessing the fate of cancer, it has been proposed that a certain set of cancer stem cells plays a major role in the resurgent growth of tumors following chemotherapy treatment. Recently, the oncogene Bmi-1 was found to regulate the self-renewal of normal adult stem cells and was also linked to the self-renewal of colon cancer cells. The inferences made about Bmi-1s role in cancer stem cells prompted a race to find the so-called "anti-Bmi-1" gene to help reduce and even eliminate the resurgence of any human cancers. In this line of experiments, the gene USP16 was over-expressed in a colon tumor sample to see whether it played a role in countering the Bmi-1 oncogene and altering the cell cycle regulators downstream, thus eliminating any tumor growth. We constructed a lentivirus to infect the tumor cells because stem cells are non-cycling and notoriously difficult to infect, and used the EF1a promoter since it has been previously proven effective in normal adult stem cells.

The positive results gleaned from the over expression of USP16 have proven to reduce the effects of Bmi-1 downstream in colon cancer cells. The stem cells grown in vivo while over expressing USP16 ceased growth, inferring that USP16 is important in countering the effects of Bmi-1. The ramifications of these results could prove vitally significant in both the understanding of colon cancer stem cells as well as developing an effective treatment for colon cancer.

Toward Holistic Diagnostic Models: Time Series Modeling of Neonate Laboratory Tests

Andrew Duchi, Suchi Saria, Prof. Daphne Koller, Anna Penn M.D.
Computer Science, Stanford University

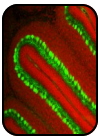


Premature babies often suffer from health problems early in life which can have severe impacts on their long-term well-being. As part of routine care, wide ranging data is already being recorded about a baby's physiological state. Our goal is to model this data to detect complications early and discover bio-markers that are indicative of these complications, thus enabling early treatment.

Modeling this data is fraught with technical challenges; it's a complex very high-dimensional non-linear system with several different sources of noise. We introduce a non-linear model based on the Switching Kalman Filter that helps address some of the technical challenges by incorporating knowledge from the doctors about physiologic development of these babies as priors and introducing factoring to reduce the dimensionality. This framework is used for predicting the onset of complications in a baby and to identify physiological signals indicating sickness.

Determining the roles of Aromatic and Hydrophobic Residues of an Interacting Amphipathic Caenorhabditis elegans MEC-6 Helix via Electrophysiological Expression in Xenopus laevis Oocytes

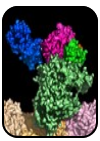
Don Vongviphut, Amy L. Eastwood, Valeria Vásquez, Miriam B. Goodman
Cellular Physiology, Stanford University



Homo sapien Paraoxonase-1 is a protein associated with High Density Lipoproteins and linked to atherosclerosis. The *Caenorhabditis elegans* MEC-6 protein, associated with the MEC-2 and MEC-4 surface channel complex, is hypothesized to be homologous in structure to HPON-1. HPON-1 is understood to interact with HDLs via two conserved helices: one trans-membrane and one amphipathic. This work investigates the MEC-6 homologue of the amphipathic helix and its role in association with the MEC-2 and MEC-4 channel complex. In a previous study, it is hypothesized that certain aromatic residues, all facing in the same direction, have important anchoring and other functions for the MEC-6 protein. This work studies the effects of certain aromatic residues, analogous and conserved in HPON-1, and also the effects of other surrounding hydrophobic amino acids within the same chain facing the same side. This was done by site-directed mutagenesis. The effects of the mutants, whose targeted amino acids were genetically changed to different types of amino acids (to either Leucine, Alanine, or Asparagine), were studied heterologously in *Xenopus laevis* oocytes. The mutations are inserted into the oocytes as mRNA, which is then translated by the oocytes. This was done alongside an insertion of MEC-2 and MEC-4 mRNA as well. The effects distinguished between non-insertion, wild-type insertion, and the three different mutational insertions were measured quantitatively via two-electrode voltage clamp recording (TEVC). The recording measures electrophysiological currents within the oocytes. Application of amilioride specifically blocks the MEC-2 and MEC-4 channels, thus the drop in current represents total MEC-2 and MEC-4 expression. Or data is consistent with the hypothesis that the mutations within the alpha helix changing the aromatic residues into non-aromatic residues inhibits MEC-6 function to an extent that indicates the possibility of another dominant-negative side-effect that is detrimental to the channel complex as a whole.

Development of a Recombinase-Driven Mammalian DNA Oscillator

Kim Tran, Wes Overton, Cliff Wang
Chemical Engineering, Stanford University



The goal of this project is to study the effects of oscillating levels of gene expression and the implications this may have in the processes of tumor formation and aging. To this end, we have constructed a synthetic DNA oscillator in mammalian cells. The construct takes advantage of both the Cre-LoxP recombination system and the similar FLP-FRT system as well as several controllable elements. The oscillator will allow us to control the period and amplitude of the oscillations, permitting the quantitative study of the response of a cell population to oscillating levels of gene expression. Assays for senescence and apoptosis (cellular responses to different levels of the tumor suppressor gene p53) will be used to gather this data. Though the current model looks specifically at p53, the c onstruct could theoretically be used to study other genes as well.

Intradermal Scaffold Implantation Model For Improved Acellular Dermal Matrix Incorporation

Melanie Major, Michael Galvez BA, Victor Wong MD, Geoffrey C. Gurtner MD FACS
Surgery, Division of Plastic and Reconstructive Surgery, Stanford University

Numerous native and synthetic biomaterials have been engineered for dermal replacement, however; determining ideal scaffold properties remains difficult due to the lack of an appropriate intradermal model. Current strategies utilize either subcutaneous implantation or open excisional wounds, methods that are limited by implant encapsulation, extrusion, and infection. This study investigated the potential improvement of *in vivo* assessment of dermal matrices by evaluating the material in a dermal environment. A novel incisional implant model was developed whereby human acellular dermal matrix scaffolds were implanted within the mouse dermis using standard microsurgical techniques. Integration of intradermal scaffolds was compared *in vivo* with subcutaneously implanted scaffolds. Results of the fourteen-day study indicate that cellular infiltration into the scaffold increases four fold in the intradermal model compared to subcutaneous implantation (60.3 ± 4.5 cells vs. 14.5 ± 0.86 cells) and scaffold architecture was better evaluated with the intradermal model. This technique will provide tissue engineers with a more thorough method to evaluate dermal matrix incorporation, vascularization and biocompatibility for engineering an ideal dermal substitute.

Characterization of Skin Wound Healing in Axin2LacZ/+ Reporter Mice

Dani Zhao, Nick Evans, Zachary Stein, Alan Chen, Jill Helms
Plastic and Reconstructive Surgery, Stanford University

Wnt proteins are involved in stem cell self-renewal and proliferation in adult tissues. Wnt signaling is induced after skin wounding and is necessary for skin wound repair. This involvement suggests that the Wnt pathway may be a potential target for therapies that stimulate wound healing in the skin. Before we can characterize the effect of exogenous Wnt protein on wound healing, however, we must first characterize the natural healing process. To better understand skin wound repair, we created 8mm full thickness biopsies in Axin2LacZ/+ reporter mice and studied the healing process and mechanisms over a period of 10 days.

Our results show that re-epithelialization begins at the wound edges and migrates into the center of the wound. Complete re-epithelialization is observed 9 days post-surgery and the new epidermis is thicker than the pre-existing epidermis. Finally, we consistently observed punctate lacZ staining (indicating Wnt signaling) in the dermis directly beneath the nascent epithelial layer. These results indicate that Wnt signaling is active during would repair and provide a model for assessing the effects of Wnt protein as a therapeutic target for wound healing.

The association of polymorphims in circadian genes CLOCK and PERIOD3 and risk for developing pediatric bipolar disorder

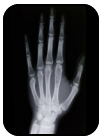
Arpine Davtyan, Hallmyer Joachim
Psychiatry and Behavioral Science, Stanford University

Based on family and twin studies, the heritability of bipolar disorder has been estimated to be in the range of 40 to 70%. However, there has been no single gene definitively linked to bipolar disorder development. Rather, it is likely that many genetic polymorphisms confer varying levels of risk. Abnormalities in circadian rhythm are thought to be involved in the pathophysiology of bipolar disorder. Positive associations between polymorphisms in genes regulating circadian rhythm and bipolar disorder have been reported in adult patients but their role in pediatric bipolar disorder is unknown. Our sample of 138 families(488 individuals) with one or more parent with bipolar disorder is uniquely suited to test to what degree the risk of bipolar disorder in adults and pediatric bipolar disorder is influenced by the same genetic polymorphisms. We investigated polymorphisms in CLOCK and PERIOD3 which are genes that regulate circadian rhythm. More specifically, we tested the hypothesis that polymorphisms in CLOCK and PERIOD3 are associated with both the presence of sleep abnormalities and with subsyndromal presentations of bipolar disorder (mood lability, episodic irritability, depression, inattention) in probands with or at risk for pediatric bipolar disorder. Polymorphisms were amplified using standard PCR conditions and analyzed according to standard RFLP analysis. Family based association tests (TDT) and linkage analysis was conducted to test for association and linkage.

The Taming of the Ion

Simon H. Ye, Griffin K. Barbula, Matthew D. Robbins, Richard N. Zare
Chemistry, Stanford University

In a mass spectrometer, collisional cooling radio frequency (RF) multipole ion guides are used for the efficient transport of ions from a high pressure ionization source to a low pressure mass analyzer. These RF guides have a mass-to-charge (m/z) transmission range with a well-characterized and sharply defined boundary for low m/z values. However, high m/z values have a soft decline in transmission efficiency that is poorly understood. A possible explanation for the limitation on ions with high m/z is that RF multipoles create a radial stratification of ions as a function of mass to charge ratio, although it has never been experimentally proven. Using a custom 3D imaging multiplexing time-of-flight mass spectrometer which can measure both the x-y positions and flight times of ions, we were able to apply the technique to a variety of small organic molecules and supercharged proteins to quantitatively measure the effects of radial stratification within an RF quadrupole.



Synthesis and Evaluation of Matriptase-Selective Activity-Based Probes

Thinh Nguyen Duc[1,2], Margot Paulick[3], and Matthew Bogyo[3,4] –
[1]Biological Science and [2]Chemistry, Stanford University, [3]Pathology, Stanford University, [4]Microbiology and Immunology, Stanford University

Matriptase is a member of the type II trans-membrane serine protease that is highly expressed in breast, prostate, ovarian and colorectal cancers. The protease has also been implicated in tumor growth and metastasis in mouse models of prostate cancer. *We hypothesize that matriptase's enzymatic activity correlates with tumor phenotype and that this enzyme could serve as a useful biomarker for cancer cells*. To test our hypothesis, we are developing matriptase-selective activity-based probes (ABPs) to functionally characterize this protease. An ABP is a small chemical probe that covalently binds to the active site of its enzyme target. A typical ABP contains (1): a reactive moiety, also termed a warhead, that binds to an enzyme in an activity-dependent manner, (2): a peptidyl linker that confers specificity to the target enzyme, and (3): a tag, such as biotin or a fluorophore, for the visualization and identification of the labeled enzyme. We have synthesized a small library of eight different matriptase-selective ABPs. These probes all contain a diphenyl phosphonate ester warhead, but differ in their peptide specificity linkers. The synthesis of these probes involved solution phase synthesis of the phosphonate warhead and solid phase synthesis of the peptide-tag moiety. To generate the complete ABP, the phosphonate warhead was coupled to the peptide-tag moiety in solution. We then evaluated the ability of these probes to label recombinant matriptase. The ABPs were allowed to react with recombinant enzyme, and labeled matriptase was visualized by Western blot analysis. Our preliminary results showed that, of the four probes tested thus far, all label matriptase *in vitro*. Future experiments will evaluate the binding of the other four ABPs to recombinant matriptase and the selectivity of these probes to matriptase in complex proteomes (i.e., in live cells expressing matriptase). Ultimately, we hope to use our matriptase-selective ABPs as non-invasive imaging agents for human cancer diagnosis.

Protein Interactions with MHC Class I at the Mouse CNS Synapse

Xuchen Zhang, Barbara K. Brott, Carla Shatz
Biology and Neurosciences, Stanford University

MHC class I complexes play a key role in regulating synaptic plasticity and neural development. However, their mechanism of action is not yet understood. To identify proteins that interact with MHC, I performed immunoprecipitations of mouse brain lysates for the MHC light chain, β-2-microglobulin, and probed for candidate proteins by Western blotting. The scaffold protein PSD95 may associate with MHC. Probing for the complement factor C1q and the MHC heavy chain H2-Kb yielded inconclusive results. Further work is needed to optimize immunoprecipitation conditions and antibody efficacy.

BMP Induced Healing of Calvarial Defects in the Athymic Nude Mouse Model

Ankur Gupta, Nicholas Panetta MD, Deepak Gupta MD, Michael Longaker MD MBA FACS

Calvarial bone defects are a common clinical scenario in craniofacial surgery. Numerous approaches are used to reconstruct skull defects, and each possesses its own inherent disadvantages. This fact underscores the opportunity to develop a novel method to repair osseous defects in craniofacial surgery. Recent literature strongly suggests that cell-based therapies in the form of regenerative medicine may be a developing paradigm in reconstructive surgery. Although numerous studies have probed osteoprogenitor cells in the presence of bone morphogenetic protein (BMP), few have explored the biology of human adipose-derived mesenchymal stem cells (hASC) in with BMP in a murine model. This study proposes an athymic, immunocompromised nude mouse model of critical-sized calvarial defects to study the in vivo biology of human osteoprogenitor cells. Critical-sized 4.0-mm calvarial defects were created in nude mice (n = 6) with a custom trephine drill bit outfitted to a dental drill handpiece. During the craniotomy, the dura mater was spared from injury. Radiographic analysis by micro-computed tomography was performed at 2, 4, and 8 weeks postoperatively. Based on preliminary results, the combination of hASC treated with BMP on polylactic-co-glycolic acid scaffolds show significant closure of the initial defect, 28, 51, and 74% respectively. Data for control groups showed no healing, therefore we propose that this preliminary combination study shows promise as a therapeutic modality. Future work will include optimizing BMP concentration, scaffold materials, and clonal purification of more osteogenic cell populations within hASC.

Thalamocortical Oscillations in the 4th Dimension: Calcium Imaging of an Epileptic Network

Max Kleiman-Weiner, Mark P. Beenhakker, John R. Huguenard
Neurology & Neurological Sciences, Stanford University

In the thalamic network each reticular nucleus (RT) neuron receives converging inputs from multiple relay neurons and produces divergent output onto multiple relay neurons including long range “tickler” connections. However, the spatio-temporal dynamics of these oscillations have not been explored due to lack of fine spatial-temporal resolution. We used high speed simultaneous calcium imaging of multiple cells to elucidate the dynamics of these oscillations in an in vitro brain slice containing the thalamus. Using intracellular recordings we first demonstrated that the T-type calcium channel bursts generate a large calcium transient and that we are capable of recording high frequency bursting activity. We then bulk loaded thalamic slices with Indo-1-AM or Oregon-Green-Bapta-AM. Electrical stimulation of the internal capsule in the presence of BMI yielded a robust long lasting oscillation detected by an extracellular field electrode and in the imaging data. These experiments, while preliminary, demonstrate that simultaneous imaging of multiple neurons in the thalamus is possible and can be used to measure the spatial dynamics and synchrony of the network. Future work will investigate how normal and epileptic oscillatory dynamics differ and may elucidate the network mechanisms underlying absence epilepsy.

Matrix Rigidity Regulates Skeletal Muscle Stem Cell Self Renewal in Culture

Penney M. Gilbert, Karen Havenstrite, Alessandra Sacco, Nora Leonardi, Nghi Nguyen, Peggy Kraft, Matthias P Lutolf, Helen M. Blau

Baxter Lab. in Genetic Pharmacology, Microbiology and Immunology, Stem Cell Institute

Procedures for isolating enriched populations of muscle stem cells (MuSCs) were recently developed by us and others. Transplantation studies revealed an extraordinary potential of MuSCs to contribute to muscle fibers, and to access and replenish the satellite cell compartment. However, MuSCs are a relatively rare cell type and their stem cell properties are rapidly lost once plated in culture limiting their clinical utility. We hypothesized that the long term culture of MuSCs on surfaces mimicking key features of the *in vivo* microenvironment would be able to increase their viability, promote division, and maintain their *in vivo*function. To this end, we employed a novel bioengineered hydrogel culture platform and assayed the *in vitro* and *in vivo* behavior of MuSCs cultured on hydrogels with different biophysical and biochemical microenvironmental cues. We found that culture upon compliant hydrogel increased MuSC viability compared to rigid plastic. Further, transplantation studies revealed that MuSCs exposed to a compliant hydrogel surface with mechanical properties similar to native skeletal muscle (~12KPa) retain similar regenerative potential to freshly isolated MuSCs and could demonstrate self renewal capacity. Our results establish parameters for the long term culture and self renewal of MuSCs *in vitro* that maintain MuSC function *in vivo*. Importantly, these studies could potentiate translation of MuSC biology to the clinic for treatment of human muscle degenerative disorders.

Analyzing the Efficacy of Protein-Engineered Vaccines Against B-Cell Lymphoma

Alejandro Virrueta, Patrick Ng

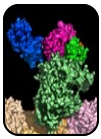
Levy Laboratory, Division of Oncology, Stanford University

B-cell lymphoma is a common type of non-Hodgkin cancer, afflicting approximately 60,000 people in the United States annually. Our project, based on a variation of monoclonal antibody treatment and immunotherapy, aims to directly stimulate anti-idiotype (Id) B cells with a diabody vaccine. The diabody consists of a tumor Id single-chain variable fragment (scFv) and a scFv targeting the CD19 B-cell co-stimulatory receptor complex. These fusion molecules should cross-link the Id-specific B cell receptor and the co-stimulatory complex, initiating a strong activation signal to the targeted B cells. To date, the vaccine has been synthesized in a transgenic bacterial system and has undergone preliminarily testing in mouse models with 38C13 lymphoma. During the last ten weeks, we have continued testing the diabody vaccine, and have begun examining the efficiency of two other protein variations: a GM-CSF/diabody fusion protein, and an experimental viral adjuvant vaccine from the Dow Chemical Company.

Intraarterial transplantation results in superior delivery of neural stem cells to the ischemic brain in contrast to intravenous infusion

Arjun V Pendharkar, Xavier Gaeta, Josh Y Chua, Nancy Wang, Hui Wang, Abhijit De, Raymond Choi, Robert H. Andres, Shawn Chen, Brian Rutt, Sanjiv S Gambhir, Raphael Guzman

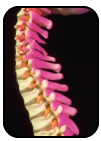
Neurosurgery, Stanford University



Stem cell transplantation represents a promising experimental therapeutic avenue for stroke. Furthermore, emerging minimally invasive intravascular transplantation techniques have begun to bridge the gap between the laboratory and clinic. Intravenous (IV) infusion is an attractive candidate based on ease of administration and clinical precedent. However, recent studies have reported poor cell delivery to the brain and cell entrapment in peripheral organs. Intraarterial (IA) delivery may overcome limitations of IV by utilizing a more direct route to the central nervous system. For both intravascular techniques, in depth assessment of biodistribution must be conducted before stem cell based therapies can come to fruition. Here, we utilize a multi-modality imaging approach to explore the biodistribution of transplanted neural stem cells (NSCs) in a mouse model of hypoxic-ischemia. Mouse NSCs were transduced with a triple-fusion reporter gene harboring a monomeric RFP, firefly luciferase, and truncated thymidine kinase. HI was induced in adult mice and NSCs were transplanted IA or IV at 24 hours after stroke. *In vivo* bioluminescence imaging (BLI) was used to track transplanted cells. At one and two weeks, animals were sacrificed and whole organ homogenates were further analyzed for luciferase activity *ex vivo*. Immediately after transplant, BLI revealed significantly higher luciferase activity in the brain in IA groups ($p<0.001$). In stark contrast, IV transplant groups showed marked increased luciferase activity in the torso of the mice ($p=0.014$). One week following transplant, luciferase signal disappeared in the torso of both groups but remained significantly higher in the brain of IA transplanted mice ($p=0.025$). *Ex vivo* whole organ assays revealed that IA animals had 69% of the total signal from the brain. In contrast, IV animals had 27% luciferase activity in the brain. At two weeks, in IA transplanted animals, signal from the brain was significantly higher than the rest of the organs at 1 week ($*p<0.0001$) and 2 weeks ($*p=0.007$) but not in IV transplanted animals ($p>0.05$) Thus, we demonstrate that intraarterial transplantation results in superior delivery of NSCs to the ischemic mouse brain in comparison to intravenous infusion.

Single Cell Genomics: Shining Light on Microbial 'Dark Matter'

Geoff Schiebinger, Paul Blainey, Stephen Quake



The vast majority of biological diversity lies in the microbial branches of the tree of life. In light of this fact, it is shocking that more than 99% of the known microbial species are uncultivable and so cannot be studied by the standard techniques of molecular biology. But recently Quake et al. broke this technological barrier and demonstrated that near complete genome sequences can be retrieved from single cells—without culture. The technique exploits power of multiple displacement amplification (MDA) for whole genome amplification in a microfluidic environment. The purified, high molecular-weight product is then recovered from the chip and sequenced. Here I report on several improvements to the pipeline: real time monitoring of the MDA reaction through SYBR Green fluorescence; computer automation of the cell sorting process; and an analysis of the amplification bias introduced by MDA.

Characterizing the Rwandan HIV Epidemic in 1990-1993 Through Sequencing Analysis of Archived Plasma Specimens and Insights on Mother-to-Child Transmission

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Although HIV has been the subject of extensive research because of the scale of its global human impact, little investigation has been directed towards the study of HIV phylogenetics in mother-to-child transmission (MTCT), and even less towards the associations between the virus's within-host evolutionary characteristics and the risk of perinatal HIV transmission. To investigate the molecular evolution of HIV in the context of MTCT, we are studying reverse transcriptase sequences from mothers (transmitter, non-transmitter, and late-transmitter) from Butare, Rwanda (1990-1993). Early analyses of sequences from this cohort of mothers have revealed important characteristic features of the Rwandan HIV epidemic of the early 1990's, including the subtype and phylogenetic relation of these viruses with other African and non-African strains, which we present here. In addition, these genotypic data provide insight into the role that diversity and strength of selection may play in affecting the risk of MTCT.

Characterization of the Role of the Heparosulfate Proteoglycans Dally-like and Syndecan in Drosophila Germline Stem Cells

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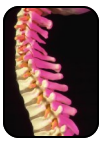
Adult stem cells maintain tissue homeostasis by replenishing the highly differentiated but short-lived tissue cells. Stem cells can adopt two alternate fates – they can self-renew to maintain their numbers or differentiate to restore tissue cells. In vivo analysis of adult stem cells show that they reside in a specialized microenvironment called a niche that provides signals to regulate stem cell self-renewal and differentiation. Stem cell–niche cell adhesion helps regulate stem cell fate by keeping stem cells close enough to receive short-range signals from the niche. Stem cells can attach to and orient towards the niche to achieve an asymmetric outcome of stem cell division. We examined the role of factors that regulate stem cell-niche adhesion using the *Drosophila* male germline as a model system. The receptor tyrosine phosphatase LAR is required for stem cell maintenance by promoting adhesion between the stem cells and the niche. Since LAR is a receptor protein, we hypothesized that ligands of LAR are expressed in the germline and regulate its function. There are two known ligands of LAR – the heparosulfate proteoglycans Dally-like (Dlp) and Syndecan (Sdc). We have shown that both Dlp and Sdc mRNAs are expressed in the male germline and that the Dlp protein localizes to the hub-GSC interface. Loss of function of *Dlp* and *Sdc* result in stem cell loss suggesting that these heparan sulfate proteoglycans are required for stem cell maintenance.



Toward high-throughput analysis of processive stepping by engineered myosin motors

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In order to accelerate a design cycle for myosin engineering, we are developing high-throughput microscopy assays for characterizing myosin motors. Some natural and engineered myosin variants are able to "walk" processively along actin filaments. This motion can be directly observed using single fluorophore tracking. We are working to implement this single molecule assay using automated total internal reflection fluorescence microscopy in a multiwell format that should allow rapid data acquisition on large numbers of engineered myosin variants. To optimize cost and processing time for fluorescent labeling of myosins, we have been exploring the use of next-generation fluorescent proteins as a replacement for organic dyes. The blinking behavior of previously characterized fluorescent proteins precludes their effective use in processive stepping assays. We successfully created an insect cell expression plasmid containing a new fluorescent protein, and constructed several myosin fusion constructs. We are currently expressing and purifying the new myosin constructs and characterizing the properties of the fluorescent label in single fluorophore tracking assays.

Identification of Endogenous Substrates of the Group II Chaperonin Mm-cpn from the Archaeal Methanogen *Methanococcus maripaludis* using computational and biochemical approaches

Anthony Tuan Nguyen[1], Lukasz Joachimiak[1], Jeremy Dodsworth[2], Murray Hackett[3] and Judith Frydman[1]
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The eukaryotic chaperonin TRiC (TCP-1 ring complex) is a principal chaperone that binds and folds cytosolic proteins. Investigation of the mechanisms of TRiC folding is complicated by its hetero-oligomeric composition. As a model for TRiC, we adopt the homo-oligomeric chaperonin Mm-cpn from *Methanococcus maripaludis*, an archaeal methanogen. However, our understanding of Mm-cpn function is hindered by the lack of known endogenous substrates. With the aim of elucidating substrate recognition and the evolutionary pathway of substrate specificity, we seek to identify natural substrates of Mm-cpn. First, using a bioinformatics approach, we compared the distribution of assigned protein folds between known TRiC and bacterial chaperonin (GroEL) substrates and proteins in the *M. maripaludis* genome. From genomic analysis, methyl-coenzyme reductase adopts a triose-phosphate isomerase barrel motif that is also folded by TRiC and GroEL. Second, using a proteomic screen by affinity purification and mass spectroscopy, we have identified five candidate substrates that interact with Mm-cpn: ribonucleotide-triphosphate reductase, methyl-coenzyme reductase, heterodisulfide reductase, nitrogenase related protein and formylmethanofuran dehydrogenase. We can use these substrates and adapt existing assays to study the folding mechanisms of Mm-cpn in its physiological context. Similarly, these enzymes are involved in important metabolic processes such as methanogenesis and may have important implications in biofuel production.

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