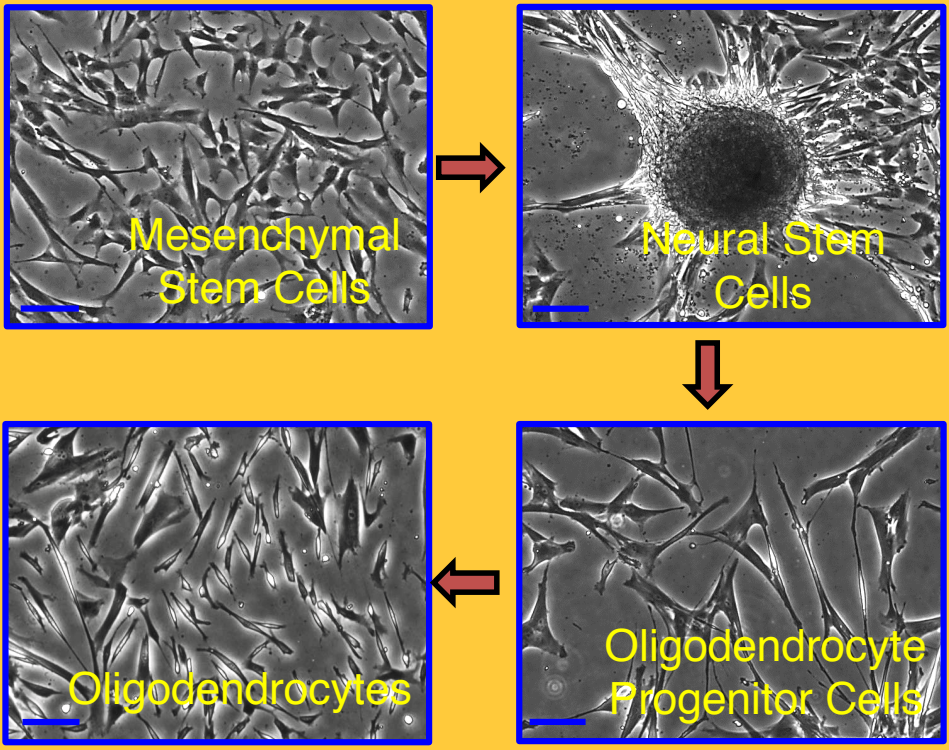
BIOLOGY RESEARCH DAY SYMPOSIUM



DEPARTMENT OF BIOLOGICAL SCIENCES | Oakland University

Image showing differentiation of MSCs to different lineages
— Courtesy Dr . Chaudhry's Lab

Sincere thanks to participating labs, presenters, Department of Biological Sciences' staff, faculty, and the Graduate and PhD Committees.

Sponsor: Department of Biological Sciences

Organizing Committee

Dr. Chhabi Govind govind@oakland.edu
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Schedule

	Research Lab	Time	Page #
Introductory Remarks		9:00 AM	
Rasul Chaudhry		9:15 AM	6
Jeff Medley	Dr. Song	9:30 AM	26
Aaron Scholl	Dr. Jiang	9:45 AM	3
Suraj Timilsina	Dr. Villa-Diaz	10:00 AM	23
Doug Wendell		10:15 AM	10
COFFEE BREAK 10:15 to 10:30			
Marisa Brake	Dr. Westrick	10:45 AM	16
Evan Hsueh	Dr. Blumer-Schutte	11:00 AM	12
Chhabi K. Govind		11:15 AM	27
Christopher Powell	Dr. Battistuzzi	11:30 AM	7
Dominic Mier	Dr. Lal	11:45 AM	9
Regan Miller	Dr. Madlambayan	12:00	19
Sang Rhee		12:15 PM	
LUNCH BREAK 12:30 to 01:55			
Introductory Remarks		2:00 PM	
Asma M. A. M. Khan	Dr. Blumer-Schutte	2:15 PM	21
Sara Blumer-Schuette		2:17 PM	4
Christina Brown	Dr. Chaudhry	2:19 PM	5
Cody Howe	Dr. Rhee	2:21 PM	8
Emily Biernat	Dr. Govind	2:23 PM	11
Jonathon Mitchell	Dr. Rhee	2:25 PM	13
Joseph DiPanni	Dr. Song	2:27 PM	14
Kaitlyn McCandliss	Dr. Villa Diaz	2:29 PM	15
Mena T Ibrahim	Dr. Blumer-Schutte	2:31 PM	17
Michael Powers	Dr. Liu	2:33 PM	18
Shahryar Raza	Dr. Chaudhry	2:35 PM	22
Walter Wolfsberger	Dr. Oleksyk	2:37 PM	24
Christian Rizza	Dr. Govind	2:39 PM	25

POSTER SESSION 3:00—5:00 PM

Mathematics and Science Center corridor spanning rooms 301 to 334

The novel gene apnoia regulates Drosophila tracheal tube size

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Distinct tube size is critical for the function of human tubular organs such as the lung, vascular system, and kidney. Aberrant tube sizes can lead to devastating human illnesses including polycystic kidney disease. The Drosophila trachea provides a premier genetic system to investigate the fundamental mechanisms that regulate tube size. Herein we describe the function of a novel gene, apnoia (apn), in tube-size regulation. Apn encodes an apical membrane protein, Apnoia (Apn), with three helical transmembrane domains. Overexpression of apn in Drosophila trachea leads to significant tube overexpansion. We analyzed the apical luminal matrix and cell polarity in these overexpanded tubes. Normal establishment of cell polarity was observed in apn overexpressing trachea whereas all luminal matrix components were significantly reduced. The significantly reduced luminal matrix likely contributes to the overelongation phenotype. In addition, we observed localization of luminal matrix components in cytoplasmic vesicles, indicating secretion defects. Furthermore, we observed co-localization of Apn protein and Rab proteins, which are regulators of vesicular trafficking. Taken together, these results strongly suggest the possibility that apn is directly or indirectly involved in the vesicular trafficking of luminal matrix components, which regulates tube size.

Cellulose Binding Tāpirins: Allies of the Type IV Pilus in Caldicellulosiruptor bescii Cell Binding and Efficient Utilization of Plant Biomass.

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Thermostable, non-catalytic proteins that promote attachment to plant biomass are proposed to enhance the efficiency of plant biomass degradation, giving C. bescii a competitive edge over other heterotrophs that share its nutrient limited habitat. Previously we have identified a functional role for the C. bescii major pilin (PilA) in attachment to plant biomass-related polysaccharides. In addition, encoded directly downstream of the T4P locus are two classes of cellulose-binding tāpirins. Prior proteomics data indicated that both tāpirins were enriched in the cellulose bound protein fraction harvested from strongly cellulolytic Caldicellulosiruptor species. In this study, we tested if C. bescii tāpirins mediate adhesion of cells to cellulose via the T4P. Recombinant C. bescii tāpirins used in competitive cell binding assays resulted in an inhibition of C. bescii cell attachment to cellulose. This effect was greater than the effect previously observed when using PilA. Using surface plasmon resonance, we measured the affinities of tāpirin1 and tāpirin2 for PilA with a KD of 2.9 μM and 2.1 μM, respectively. The observed dissociation constants are significant, as they are in the same range as those of cellulosomal carbohydrate binding modules for cellulose. We also observed that newly sequenced C. changbaiensis solubilizes cellulose to a lesser extent than C. bescii, even though C. changbaiensis encodes for the same key cellulase orthologues as C. bescii ('ACE' cellulases). The most striking difference between these two strongly cellulolytic species is the atypical tāpirin proteins encoded for by C. changbaiensis, which have yet to be described in a strongly cellulolytic Caldicellulosiruptor. Based on these results we propose that C. bescii tāpirins are incorporated into the T4P in order to mediate cell attachment to cellulose and enhance efficiency of cellulose degrading enzymes.

Treatment of multiple sclerosis using mesenchymal stem cells

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Multiple sclerosis (MS) is a debilitating disease of the central nervous system (CNS). MS is caused by demyelination of neurons due to an inflammatory response against oligodendrocytes. There is no cure for MS. We hypothesize that cell therapy could be a viable option to reverse and/or halt the neural damage responsible for MS. In the current study, we developed a mouse model of MS by inducing experimental autoimmune encephalomyelitis (EAE) using MOG immunization. EAE mice were then transplanted with PKH26-labeled mesenchymal stem cells (MSCs) and neural stem cells (NSCs) and monitored for EAE symptoms using a neurobehavioral assay. EAE symptoms improved following cell transplantation at disease stage 1 while the EAE continued to progress in untreated EAE mice. Animals treated with NSCs exhibited greater improvement than MSCs. Post-transplantation histochemical analysis after euthanizing the animals showed presence of labeled cells in the spleen. There was a significant reduction in the cell infiltrates in the brain and spinal cord of EAE mice transplanted with NSCs followed by MSCs. Furthermore, levels of Treg and Th17 cells of blood and spleen were increased to near wild-type levels. However, NSCs had a greater effect than MSCs on restoring the T-cells in blood. Transplantation of NSCs upregulated BDNF but downregulated inflammatory markers (TNFα, IL-2 and IL-17), in the spinal cord. The results of this study could provide basis for the development of strategies using MSCs and derivatives to treat not only MS but also other neurodegenerative diseases.

Efficacy of retinal progenitors to treat retinal degenerative disease using an animal model

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Loss of vision caused by degeneration of the retina is one of the most debilitating age-related diseases. There is no cure for common retinal degenerative diseases (RDD) such as macular degeneration and retinitis pigmentosa. The goal of this study is to develop a cell therapy to treat RDD. To achieve this, we differentiated human mesenchymal stem cells (MSCs) into retinal progenitor cells (RPCs) using a defined differentiation medium. MSCs and RPCs were labeled with PKH26 and transplanted into the sub-retinal space of rd12 mice. The transplanted animals were monitored and subjected to behavioral assays on a daily basis. The results showed significant improvement in the vision of animals transplanted with both MSCs and RPCs. However, the vision improvement was greater in the case of RPCs than MSCs. There were striking differences in the thickness of various retinal layers of animals treated with MSCs and RPCs compared to the untreated mice, which were parallel to the improvement in the vison acuity. While the transplanted MSCs homed predominantly to the RPE layer, the RPCs were localized in the outer nuclear layer. Furthermore, significant upregulation of human RPE (RPE65 and LHX2) and photoreceptor (CRX, SIX3, OTX2, and RCVRN) markers was noticed in animals transplanted with MSCs and RPCs, respectively. Neuroprotective genes such as BDNF and GDNF were highly expressed in RPCs treated animals. These findings demonstrated that RPCs were more efficacious than MSCs to improve vision and retinal structure. This study provides basis for developing strategies to treat RDD using cell therapy.

Quantifying the error of secondary calibrations in a simulated environment

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The use of calibrations to estimate absolute ages in a phylogeny is a known source of controversy for two primary reasons: first, few are available from independent sources (e.g., fossil record) and second, their boundaries (and internal distribution between these boundaries) are heavily debated. Despite these issues, molecular clock analyses cannot avoid using them if absolute time estimates are the final goal. Our study was designed to evaluate the validity and accuracy of secondary calibrations, which are estimates derived from previous analyses We utilized a phylogeny of 248 species and divided it into two nested subtrees (A and B) giving us the scaffolding to test the accuracy of secondary calibrations. RelTime was used to estimate times for tree A with three primary calibration nodes. The estimated time of the common node in tree A and B was then used as a secondary calibration allowing node estimates from tree B to be compared to the true times. We found that gene length is positively correlated with the accuracy of time estimates, this also holds true for gene concatenations. Looking at the accuracy of secondary calibrations, we find that, on average, secondary-derived time estimates are within 23% of the true time and that >95%of all true times fall within the estimated confidence intervals. However, on average, the confidence interval estimates from the secondary calibrations are 3 times larger than those from primary calibrations. Overall, time estimates based on secondary calibration are accurate but imprecise, although imprecisions is improved by longer alignments.

Differential expression of tumor-associated genes and altered gut microbiome with decreased Akkermansia muciniphila confer a tumor preventive microenvironment in intestinal epithelial Pten-deficient mice

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Phosphatase and tensin homolog (Pten) has many functions, however, it is mostly known for being an antagonist for PI3K-Akt signaling. Because of this, Pten is considered to be a tumor suppressor gene, but this remains to have direct evidence, specifically with colon cancer. To study this correlation, we examined if Pten deficiency in intestinal epithelial cells (IECs) induces tumorigenesis. Pten mRNA was analyzed by quantitative PCR from mucosal biopsies of human colon cancer and of normal colon. Using IEC specific Pten knockout mice (PtenΔIEC/ΔIEC), we examined the mitotic activity of IECs; and we created PtenΔIEC/ΔIEC; Apcmin/+ mice by combining PtenΔIEC/ΔIEC with Apcmin/+ mice. We evaluated the expression of tumor-associated genes and fecal microbiome of the mice. We found Pten mRNA level was reduced in human colon cancer compared to normal tissues. Augmented chromatids, increased Ki-67 and PCNA expression, and enhanced Akt activation were identified in IECs of PtenΔIEC/ΔIEC mice compared to Pten +/+ littermate. Combining PtenΔIEC/ΔIEC with Apcmin/+ mice caused rapid and aggressive intestinal tumorigenesis, however, PtenΔIEC/ΔIEC mice did not develop any tumors. the expression of tumor-promoting and tumor-suppressing genes was decreased and increased, respectively, in the intestine of PtenΔIEC/ΔIEC mice compared to controls. The abundance of Akkermansia muciniphila, capable of inducing chronic intestinal inflammation, was also diminished in PtenΔIEC/ΔIEC mice compared to controls. These findings indicate that IEC-Pten deficiency alone did not induce tumorigenesis in mice, while still maintaining the tumor-driving potential, altered tumor-associated gene expression and changed gut microbiota, which shape a tumor-preventative microenvironment in PtenΔIEC/ΔIEC mice.

Maize and Human RNA Binding Motif Protein 48 Have an Evolutionarily Conserved Essential Role in U12-Dependent Intron Splicing

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The removal of introns from precursor-mRNA is elemental to eukaryotic gene expression. A vast majority of eukaryotes, in addition to major U2, also contain a minor U12-type intron, spliced by a distinct minor spliceosome. Comprising less than 0.5% of all introns, minor introns and the genes in which they reside play essential roles in growth and development. Recently, we reported a maize RNA Binding Motif Protein 48 (RBM48) is required for U12-type intron splicing. Maize rbm48 mutants with abnormal endosperm cell differentiation and proliferation display genome-wide aberration of primarily U12-type intron splicing. A CRISPR/Cas9-mediated RBM48 functional knockout (RBM48 FunKO) was subsequently generated to investigate if RBM48's role in U12 splicing is conserved between maize and human. Using RNA-seq transcriptome profiling we demonstrate that splicing of several minor intron containing genes (MIGs) with conserved roles between maize rbm48 mutants and human RBM48 FunKO cells was affected, pointing to potentially similar roles in normal growth and development. In addition, in vitro protein pull-down assays indicate the interaction of RBM48 with an Armadillo Repeat Containing protein, ARMC7 is conserved between humans and maize (Hart et al., 2015) (Bai et al., 2019). However, in vivo localization assays show RBM48 localizes within the cell to the nuclear speckles, a region which houses transcription factors, while ARMC7 localizes within the cytoplasm of human cells. Here, we report in vivo protein interaction analysis between maize RBM48 and ARMC7 through transient expression of their fluorescent-tagged constructs in tobacco leaves, leading to further understanding of their interaction in U12 splicing.

My Experience Developing a Genetics Laboratory Kit With Carolina Biological Supply

D. Wendell

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I will give an overview of my experience working with Carolina Biological Supply to make an educational kit out of something I developed for a lab course I teach her at OU. The kit "Identifying the Mutation in Non-Purple Stem Wisconsin Fast Plants" is a combination of Mendelian and molecular genetics targeted to high school and college labs. Wisconsin Fast Plants are a highly popular model used for teaching a variety of topics in biology. One of the activities using Fast Plants for genetics is a simple Mendelian trait of presence or absence of anthocyanin pigments. This trait is easily phenotyped as purple or nonpurple stems, but the mutation responsible was not known. In my research lab, my students and I genetically mapped the anthocyaninless gene. We then identified a transposon insertion mutation in the gene for dihydroflavonol 4-reductase (DFR) as being responsible for the non-purple trait. I subsequently developed a lab activity involving mendelian and molecular genetics based on this gene. Over a period of a few years I established a relationship with product developers at Carolina Biological Supply and this has ultimately led to a finished kit which is now on the market.

Genome-wide analyses of the RSC complex and histone acetyltransferases on chromatin organization and transcription in Saccharomyces cerevisiae

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Eukaryotic DNA is packaged into chromatin by wrapping ~147 bp of DNA around a histone octamer (two copies of H2A, H2B, H3 and H4) to form a nucleosome, which is a major impediment to DNA dependent processes, including transcription of protein coding genes by RNA polymerase II (Pol II). This impediment is relieved by sliding or evicting nucleosomes in an ATP-dependent manner by chromatin remodelers, such as the Remodel the Structure of Chromatin (RSC) complex. RSC is the only essential remodeler in S. cerevisiae and contains seven bromodomains (BDs) which recognize acetylated histones. The Gcn5-containing SAGA and Esa1-containing NuA4 histone acetyltransferase (HAT) complexes acetylate H3 and H4, respectively. To examine the extent to which the catalytic subunit of the RSC complex, Sth1 bromodomain cooperates with H3 and H4 HATs in regulating transcription, we determined H3 and Pol II occupancies by ChIPseq in WT, sth1BD, gcn5, esa1ts, gcn5/esa1ts, sth1BD/esa1ts, sth1BD/gcn5 cells. Both sth1BD and HAT mutants elicited higher H3 occupancies in gene-promoters genome-wide. Deleting the bromodomain in the HAT mutants showed histone occupancies similar to those of the single HAT mutants. Interestingly, however, the double mutants exhibited greater increases in the open reading frames (ORFs). Consistent with this, we observe greater recruitment of RSC in coding sequences. Furthermore, increased histone occupancies in the mutants were accompanied by reduced Pol II occupancies, especially in the highly transcribed ORFs. Altogether, these results indicate that RSC functions with major HAT complexes to promote Pol II elongation by evicting histones from transcribed coding regions.

Acidophilic communities are a source of novel sulfite oxidizing enzymes

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Sulfite oxidizing enzymes are encoded for by many species across all three domains of life, where they function to detoxify sulfite. Mutations in the sulfite oxidase (SOx) locus are often fatal in the Metazoa, due to the buildup of sulfite after cysteine and methionine degradation resulting in unfavorable by-products and sulfitolysis, where protein disulfide bonds are disrupted. Among the prokaryotes, functionally similar enzymes have been characterized as sulfite dehydrogenases (SDH), which require a separate cytochrome for activity. Genomes of metal-oxidizing, acidophilic bacteria often encode for both iron- and sulfur oxidation enzymes. However, the systems used by these microorganisms to oxidize iron are better characterized, as sulfur oxidation pathways are more complex. In this study, we used genome mining approaches to identify microbial sulfite oxidase enzymes that use molecular oxygen, rather than a heme cytochrome, as an electron acceptor for sulfite oxidation. In this case, sulfate and hydrogen peroxide are generated. Seventeen coding sequences identified as putative true SOs were synthesized for expression in Escherichia coli. We initially assayed for sulfite oxidation using ferricyanide as a terminal electron acceptor, however this method was unsuccessful when screening purified putative SO enzymes. For identification of true SOs that use oxygen as a terminal electron acceptor, I used a commercially-available quantitative peroxide assay kit. Using this technique, I have identified three enzymes as being capable of reducing oxygen to hydrogen peroxide in the presence of sulfite. Furthermore, I have determined their temperature and pH optima for downstream enzyme kinetics analysis. These enzymes are the first described true sulfite oxidases from a microbial source, and will expand our understanding of microbial sulfur cycling.

Inflammation and altered neural signaling of the hippocampus in microbiota-induced colitis

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Recent years have seen an explosion of evidence suggesting that the gastrointestinal (GI) tract's microbiota regulate brain function with landmark studies demonstrating that commensals modulate hypothalamic-pituitary-adrenal (HPA) axis responsivity, GABAergic and serotonergic signaling, and expression of multiple synaptic plasticity-related genes among a number of other effects. The possible role of gut commensals in central nervous system (CNS) dysfunction has garnered interest due to its translational potential in pathologies as diverse as autism spectrum disorder (ASD) and inflammatory bowel diseases (IBD). However, with a few exceptions most landmark studies have identified microbiota-related effects using germ-free models in which the microbiota is absent, or other similar models which do not represent the pathologies in question. Furthermore, these studies have not provided direct evidence of altered neural activity, nor have they elucidated a clear mechanism. Using a dextran sulfate sodium (DSS) model of colitis, which is dependent upon the microbiota and closely resembles human ulcerative colitis (UC), we demonstrate that mice suffering from chronic colitis experience bacterial translocation to the brain, which does not occur in water-treated controls. These DSS-treated mice have increased hippocampal expression of the pro-inflammatory cytokines interleukin (IL) -1β, IL-6, and tumor necrosis factor (TNF) -α and demonstrate altered hippocampal neurophysiology, revealed by manganese-enhanced magnetic resonance imaging (ME-MRI) which indicates altered patterns of hippocampal neuronal calcium uptake compared to controls. Together, our results demonstrate a clear role of microbiota-induced colitis in the development of hippocampal inflammation and altered hippocampal neurophysiology, likely mediated in part by bacterial translocation to the brain.

KEN-Box Mediated Proteolysis of SAS-7 Regulates Centrosome Assembly

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Centrosomes are nonmembraneous and evolutionarily conserved organelles that function as the primary microtubule organizing center (MTOC) in animal cells, playing a key role in cell polarity and bipolar spindle assembly. Centrosomes duplicate exactly once per cell cycle, and deregulation of centrosome assembly leads to genomic instability that is often associated with human cancers and developmental defects. Maintaining proper levels of centrosome regulators, through protein degradation, promotes normal centrosome number and function. The Anaphase Promoting Complex/Cyclosome (APC/C) is an E3 ubiquitin ligase that functions as a part of the proteasome pathway. The APC/C and cofactor FZR-1 targets SAS-5, a centrosome protein, in a KEN-Box-dependent manner, however, our preliminary data indicates additional targets of the APC/CFZR-1 acting in the centrosome assembly. Recently another centrosome protein, SAS-7, was identified that functions in the earliest step of the centrosome assembly pathway. Intriguingly, SAS-7 also contains five putative KEN-Box motifs, which led us to hypothesize that SAS-7 might be another substrate targeted by APC/CFZR-1 in centrosome assembly. Using the CRISPR/Cas9 method, we mutated each KEN-Box motif to prevent SAS-7 targeting by APC/CFZR-1. If the putative KEN-Boxes are targets of APC/CFZR-1, mutating them will prevent recognition of the motif and lead to an increase SAS-7 stability and accumulation and could also increase downstream factors involved in centrosome assembly. We have begun to examine how each KEN-Box mutation affects SAS-7 levels and centrosome assembly in each SAS-7 KEN-Box mutant. Excitingly, our preliminary data shows that a subset of the SAS-7 KEN-Box mutations restores embryonic viability to zyg-1(it25) mutants, suggesting that SAS-7 might be targeted by APC/CFZR-1 via recognition of multiple KEN-Box degron motifs. We are continuing to investigate how the APC/CFZR-1 affects SAS-7 stability and function by using genetics and biochemical approaches.

Fabrication of synthetic substrates for the culture of human pluripotent stem cells in xenogeneic-free, feeder-free conditions

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Tissue culture polystyrene (TCPS) dishes grafted with poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH) provide multiple benefits to the growth of human pluripotent stem cells (PSCs) - including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). PMEDSAH serves as a matrix for maintaining PSCs in an undifferentiated state and supporting their long-term growth. PMEDSAH-coated culture dishes do not require animal proteins to adhere PSCs to dishes and are chemically defined, feeder-free, reproducible to fabricate, inexpensive, and highly manipulable. UV-treated TCPS dishes, monomer (MEDSAH, Sigma-Aldrich), and 20% ethanol solution are stirred and heated in a reaction vessel at 80C for 2.5 hours. The dishes are then submerged in 1% (wt/vol) NaCl solution, undergo ultrasonicaton, and are incubated under UV light. For verification of the grafting of PMEDSAH, H1 and H9 human ESCs are grown in chemically defined medium (StemFlex Basal Medium, Gibco), passaged, and characterized for PSC-associated transcription factors (Oct4, Sox2, and Klf4, Nanog) using immunochemistry. To date, hESCs have been grown for two passages, demonstrate attachment and normal morphology, and express Oct4, Sox2, Nanog and Klf4. Future plans include extending the culture, inducing differentiation, performing genomic stability analysis, and verifying the structure of the PMEDSAH-coated culture dishes using Nuclear Magnetic Resonance spectroscopy. PMEDSAH was successfully grafted onto TCPS dishes. Human PSCs were cultured with the support of a chemically defined and xeno-free medium. This indicates a chemically defined microenvironment to expand clinically relevant PSCs.

Investigating the Complex Genetics of Blood Coagulation in Mice

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Factor V Leiden (F5L) is an incompletely penetrant thrombosis susceptibility variant common in humans. To identify F5L modifiers, we performed a sensitized mouse ENU mutagenesis screen for mutations suppressing the F5L/L Tfpi+/- perinatal lethal thrombotic phenotype. We identified thrombosuppressor mutations in two of 22 MF5L mouse lines, the others are still unknown. The highly penetrant (77.2%) MF5L16 line produced a large multigenerational pedigree containing 136 F5L/L Tfpi+/- mice. The in vitro Prothrombin Time (PT) assay revealed a potent antithrombotic effect in MF5L16 mice compared to F5+/L Tfpi+/- and Tfpi+/- controls ($q<0.01$). To identify the suppressor, four F5L/L Tfpi+/- mice were analyzed by whole genome sequencing; 44 candidate ENU mutations were identified. Sanger resequencing analysis determined 22 were false-positives. Seven were introduced into MF5L16 by F5L/L breeders. Three arose on the Tfpi- background. The remaining 12 candidates were in repetitive/low complexity regions and could not be analyzed. Due to the lack of ENU-induced mutations in MF5L16, the seven mutations introduced from the F5L/L breeders were analyzed in all MF5L16 mice. An intergenic mutation on Chromosome 18 was significantly associated with survival of F5L/L Tfpi+/- mice ($p=0.0031$). Two intronic mutations on Chromosome 9 and 13 were significantly associated with survival when co-inherited with the Chromosome 18 mutation ($p=0.000113$). These results suggest additional suppressor mutants arising from selective pressure in F5L/L breeders were superimposed upon the ENU screen. This illuminates the complexity arising during analysis of genetically sensitized ENU screens. The genetic interactions between these mutations could explain the complexity of human thrombosis.

Cellular Adhesion of Gram-Positive Bacterium to Plant Biomass and Polysaccharides

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The aim of my research is to characterize the functions of orthologous type IV pilus-related proteins encoded by the thermophilic, anaerobic, plant biomass degrading bacteria Caldicellulosiruptor bescii and Caldicellulosiruptor changbaiensis. Previously, cellulose adhesins (tāpirins) were identified, however a xylan adhesin has yet to be identified from the Caldicellulosiruptor. Based on its collocation with the tāpirin genes, and larger molecular size of the largest minor pilin encoded for by C. bescii (Athe_1872), we suspect that it may function as the xylan adhesin, and that the ortholog in C. changbaiensis (ELD05_09500) will not, since C. changbaiensis does not adhere to xylan. After producing recombinant forms of these putative pilin proteins, we will test them for their affinity to xylan. We have also tested both tāpirins and PilA for affinity to insoluble xylan and cellulose, using polysaccharide pull-down assays. Surprisingly, the tāpirins appeared to solubilize xylan at elevated temperatures. I further tested this phenotype using a colorimetric assay that measures the release of reducing sugars, and determined that while the tāpirins bind to cellulose, they appear to have some catalytic activity against xylan. Lastly, I am comparing the ability of C. bescii and C. changbaiensis to grow on representative polysaccharides that are present in plant pectin, in order to define how C. changbaiensis can metabolize pectin without encoding for any identifiable pectin lyases. Taken together, my project has expanded the functional role of the tāpirins, and will add to our understanding of the functional role for type IV pili produced by Gram-positive bacteria.

Role of biometal membrane transporter ZIP8 in alcohol responses

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Biometals and minerals, such as Zn, Se, Mn, play important roles in alcohol metabolism and overall alcohol responses. Our preliminary results indicate that in mouse model with alcohol treatment, retention of Zn, Se and Mn was altered in various tissues including liver. The most significant changes in liver were observed in mitochondria, where Zn, Se and Mn were all elevated following alcohol treatment. This indicates alcohol may alter specific biometal membrane transporters. As alternation of biometal homeostasis is a trait of alcohol exposure, and their cellular access is controlled by respective membrane transporters, in this study, we investigate role of ZIP8 (encoded by Slc39a8), a multi-functional membrane transporter that influxes essential metal cations Zn2+, Mn2+, Fe2+ and non-metal inorganic selenite (HSeO3–), in regulating alcohol metabolism and responses, using ZIP8-gain and loss cells and animals. In cultured hepatocytes, we found that loss of ZIP8 induced alcohol sensitivity while overexpression of ZIP8 increases alcohol tolerance. Consistent with this observation, overexpress of ZIP8 in liver can protect alcohol induced liver injury while hepatic ZIP8 knockout have higher ALT after chronic and binge ethanol feeding. As ZIP8 was found to regulate liver Zn and Se, Mn level, and affect overall mitochondrial function, we predict that ZIP8 regulates the expression and activities of Cu/Zn-SOD1, GPX1/2 in cytoplasm and MnSOD2 in mitochondrial, thus overall redox status during alcohol metabolism. Future work will focus on the mechanisms involved in ZIP8 regulated alcohol metabolism and identify the molecules that link ZIP8 function to alcohol metabolism in liver.

Combating the Chemoprotective Effects of Endothelial Cells in AML Through IL-8 Blockade

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Even with recent advances in the treatment of acute myeloid leukemia (AML), mortality rates have remained relatively unchanged for the past 30 years. A major factor contributing to the lack of efficient treatments is the intricate relationship between endothelial cells and AML. Previous in vitro studies mimicking the vascular niche have shown a positive feedback loop in which AML promotes endothelial cell activation, resulting in an upregulation of adhesion molecules and secretion of cytokines, such as interleukin (IL)-8. IL-8 is a pro-inflammatory factor known to promote proliferation and chemoresistance. Based on this knowledge, we tested the hypothesis that blockade of endothelial cell produced IL-8 could decrease the circulating leukemic burden and result in increased cell death following chemotherapy. Through small molecule screening, a novel compound was found that inhibited the binding of IL-8 to its receptor, CXCR2. A co-culture system was used to recapitulate the intercellular relationships and to determine the effects of decreased IL-8/CXCR2 signaling. IL-8 inhibition resulted in significantly lower cell proliferation and enhanced response to cytarabine resulting in an overall decrease in viable AML cell numbers. Molecular analysis showed involvement of the Akt pathway in these responses. These studies provide another potential route for combating the AML supportive effects of endothelial cells. Ongoing studies will develop this treatment for clinical applications in AML.

Autotaxin-mediated immunity

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Autotaxin (Atx) converts lysophosphatidylcholine (LPC) and sphingosyl-phosphorylcholine into lysophosphatidic acid and sphingosine 1-phosphate, respectively. Given the high affinity of LPC to cholesterol and the enrichment of cholesterol and sphingolipids in the membrane lipid rafts wherein LPS sensor Toll like receptor 4 (Tlr4) and its co-receptor Cd14 reside, we hypothesized that Atx deficiency inhibits Tlr4-mediated immune responses. To study this, we examined macrophage-specific Atx-knock (ko) mice (Atx-del/del) and found disrupted lipid rafts in AtxAtx-del/del macrophages. These cells had the inhibition of the receptor complex formation between Tlr4 and Cd14 and the suppression of Tlr4 endocytosis in response to LPS. Accordingly, Tlr4 cannot recruit TIR-domain-containing adaptors; consequently, LPS-stimulated intracellular signaling and cytokine production were inhibited in AtxAtx-del/del macrophages compared to Atx+/+ cells. iNOS and MHC-II expression were diminished in AtxAtx-del/del macrophages compared to controls. Cd4+ effector T cell responses were disrupted in the intestinal lamina propria of AtxAtx-del/del mice. Accordingly, AtxAtx-del/del mice exhibited higher bacterial prevalence in the intestinal mucosa than Atx+/+ mice. Notably, Atx protein levels were substantially lower in the patients' serum of inflammatory bowel diseases than in normal subjects. Indeed, a combination of AtxAtx-del/del with Il10-/- mice (AtxAtx-del/del;Il10-/-) did accelerate spontaneous colitis development compared to littermate Atx+/+;Il10-/- mice. These findings demonstrate that Atx deficiency results in compromised immune response, causing microbe-associated inflammation in the gut.

Stuck on you: Caldicellulosiruptor changbaiensis uses alternate cellulose attachment mechanisms

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Past pangenome analyses of the genus Caldicellulosiruptor have highlighted the vast biodiversity of this genus, and the unique mechanisms used to attach to and degrade plant biomass. Caldicellulosiruptor changbaiensis, representing the 14th genome in the Caldicellulosiruptor pangenome, is a strongly cellulolytic species isolated from China. The C. changbaiensis genome was assembled as a single contig from both long- and short sequence reads. In comparison to other available Caldicellulosiruptor genome sequences, the size of the C. changbaiensis genome (2.91 Mb) is larger than the average (2.69 Mb), and encodes for 120 genes not observed in prior pangenome analyses. These unique genes were annotated as hypothetical proteins, underscoring the "known unknowns" of the genus Caldicellulosiruptor that require further study, others were annotated as ABC transporter loci, response regulators, transcriptional regulators and metabolism-related genes. Interestingly, notable physiological differences were observed between C. changbaiensis and C. bescii, the benchmark plant biomass degrading species. While C. changbaiensis possesses a glucan degradation locus (GDL) similar to C. bescii, it does not grow as fast on microcrystalline cellulose, nor solubilizes it to the extent that C. bescii can. Furthermore, C. changbaiensis is able to metabolize pectin and glucomannan more efficiently than C. bescii. Most striking was the absence of classical tāpirin genes, and instead atypical tāpirins are encoded for in the genome of C. changbaiensis. C. changbaiensis is capable of attaching to cellulose, and may represent a new alternative to non-cellulosomal polysaccharide attachment.

Differentiation of mesenchymal stem cells into dopaminergic neurons

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Parkinson's disease (PD) is a neurodegenerative disease that affects muscle movement and coordination. PD is caused by the deterioration of dopaminergic (DA) neurons in a specific area of the brain called the substantia nigra. When DA neurons degenerate during PD, the level of dopamine is insufficient for proper muscle contractions, which leads to the disease associated symptoms, such as tremors, impaired balance, and changes in speech. Currently there is no cure for PD. In the current study, we focused on developing a cell-based therapy to treat PD. As a first step, we investigated the potential of highly proliferative mesenchymal stem cells (MSCs) isolated from perinatal tissues to differentiate into DA neurons. We used a defined differentiation medium containing growth factors including SHH and FGF-8. The differentiated cells were characterized using morphological, immunocytochemical and, qRT-PCR analyses. These cells displayed distinct morphology with neural filaments, a characteristic of neural cells. They also expressed neural and DA-specific proteins (MAP2, PITX3, and TH). Based on quantitative immunocytochemical analysis the differentiation rate was determined to be >50%. Further studies are focused on transcriptional analysis to confirm that the differentiated cells are DA neurons. The findings of this experiment could help to find a more effective cell-based treatment for PD.

Identification of biomarkers indicative of functional skeletal stem cells

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Skeletal stem cells (SSCs) are characterized by expression of cell surface biomarkers and their ability to differentiate into bone, cartilage and fat. However, the current biomarkers used to identify these cell populations are not cell-type-specific or indicative of the differentiation status of these cells and are therefore unreliable. Our objective was to identify alternative cell surface biomarkers and transcription factors shared between SSCs isolated from the bone marrow (BM) and those derived from pluripotent stem cells (PSC). Human PSCs were induced into SSCs. FACS and qRT-PCR were used to determine differences in expression of cell surface biomarkers and transcription factors between SSCs derived from PSCs and isolated from BM, in differentiating cells, in cells from early and late passage, and in fibroblasts. A significant reduction in proliferation and capacity of SSCs to differentiate into adipocytes and osteoblasts was observed after 3 passages. Protein and mRNA analysis indicated that commonly used biomarkers remain highly expressed in cells that lost capacity for differentiation. However, integrin α6 (CD49f) and transcription factors GATA6, PRDM16, SIM2 and SOX11 were significantly upregulated in SSCs compared to fibroblasts. In early stages of adipogenic and osteogenic differentiation, the expression of CD49f, GATA6 and SIM2 was reduced in later passage cells, which have limited proliferation and differentiation capabilities. Our results suggest that CD49f and transcription factors GATA6 and SIM2 identify functional SSCs.

Using genomics to decipher the history of Puerto Rican Horses

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Domestication of the horse, with its impact on transportation and agriculture was an important breakthrough in the history of humankind. Over the centuries, different horse breeds have been established by selective breeding for specific traits. In this study we use genetic methods to investigate and describe the origins and genetic diversity among horses of Puerto Rico check the genetic markers that are reported to be responsible for the prized "gaitedness" trait present in Puerto Rican Paso Fino(PRPF) breed. We sequenced a 668bp fragment of the mitochondrial DNA D-loop (HVR1) in 199 horses: 38 PRPF and 161 Non Pure-Bred (NPB). Additionally, 24(21 NPB and 3 PRPF) samples were genotyped with Illumina Neogen Equine Community genome-wide array. Both PRPF and NPB populations show a relatively high haplotype diversity, which coincides with the Iberian origin. In case of the DMRT3_Ser301STOP mutation, associated with "gaitedness", all 100% of the PRPF horses possess the mutation. However, 99.3% of the NPB also show its presence. This is a high frequency when compared to other "non-pure bred" horses in the Americas, and points to the horses as the possible ancestral stock for the selective breeding of the prized Puerto Rican Paso Fino. This conclusion is supported by various population genetic analyses of PRPF and its historically ancestral breeds. Resulting data adds valuable information that can be of use for maintaining and even increasing genetic diversity among Paso Fino horses due to constant interbreeding and give extra value for the on growing non-purebred population.

The Genome-Wide Organization of Chromatin in FACT complex (Facilitates Chromatin Transcription Complex) Mutants

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Transcription in eukaryotes is a complex and dynamic process. Within the nucleus of a cell, DNA is condensed to a fraction of its original length. This is accomplished by spooling DNA around a histone octamer to form a structural unit known as a nucleosome, which is a barrier to all DNA-dependent processes. During transcription nucleosomes are both disassembled and reassembled, resulting in changes to chromatin structure. A multitude of protein complexes contribute to regulating chromatin structure. The Facilitates Chromatin Transcription (FACT) complex, a histone chaperone, participates in restoring chromatin structure by reassembling displaced histones H2A and H2B. FACT is a heterodimeric protein composed of two subunits Spt16 and Pob3. Mutations to the FACT complex have resulted in decreased binding affinity for histones in vitro. We postulated that defects in Spt16 or Pob3 would result in altered chromatin structure in vivo. In order to test this, MNase-Seq (micrococcal nuclease sequencing) was performed using FACT C-terminal mutants, which show reduced histone interactions in vitro. Overall, we report that nucleosome occupancies were altered genome-wide in the FACT mutants. However, this was complicated by the observation that decreased nucleosomal density resulted in hypersensitivity of DNA to MNase nuclease activity. DNA fragments which matched nucleosomal length showed a reduction in +1 nucleosome occupancy (the first nucleosome downstream of the transcription start site) for all mutant phenotypes as well as increases in occupancy downstream of the +1 position. In support of previous findings, we conclude FACT is important for maintenance of chromatin structure genome-wide.

APC/C Negatively Regulates ZYG-1 via D-Box-Mediated Proteolysis to Influence Centrosome Assembly in Caenorhabdits elegans embryos.

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As microtubule-organizing centers, centrosomes must duplicate once per cell cycle to establish spindle bipolarity. Errors in centrosome assembly lead to chromosome missegregation and genomic instability and the process of centrosome duplication must be tightly controlled. Proteolysis of centrosome duplication factors plays a key role to maintain the proper level of centrosome factors, leading to normal centrosome number. Our recent study showed that an E3 ubiquitin ligase, the Anaphase Promoting Complex/Cyclosome (APC/C) and its co-activator FZR-1/Cdh1, regulates centrosome duplication in C. elegans embryos. APC/CFZR-1 recognizes proteins containing KEN-box or Destruction box (D-Box) motifs for proteasomal degradation. While we showed that APC/CFZR-1 regulates SAS-5 stability via a KEN-box motif, our data suggest that that APC/CFZR-1 targets additional factors to regulate centrosome duplication. As we observed that loss of APC/CFZR-1 leads to increased ZYG-1 levels at centrosomes, here we focus on ZYG-1 as a potential substrate of APC/CFZR-1. To examine if ZYG-1 is directly targeted by APC/CFZR-1, we mutated four putative degron motifs, (D-Boxes), within ZYG-1 by the CRISPR/Cas9 method. Our preliminary data suggest that mutation of a degron motif, D-Box (AA:349-352), blocks APC/CFZR-1 from recognizing ZYG-1 target, protecting ZYG-1 from proteasomal degradation. These results are consistent with our hypothesis that APC/CFZR-1 controls ZYG-1 stability through D-Box motif to regulate centrosome assembly. We are continuing to investigate how APC/CFZR-1 influences ZYG-1 stability and centrosome duplication through quantitative confocal imaging, genetic and biochemical approaches.

Genome-wide analysis of nucleosome remodeling by the RSC complex in Saccharomyces cerevisiae

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Dynamic changes to chromatin, essential for regulating gene expression, are mediated by many factors including remodelers. RSC (Remodels the Structure of Chromatin), a conserved ATP-dependent remodeling complex, regulates many biological processes, including transcription by Pol II. Mutations in this complex is linked to many human diseases, including cancer. RSC uses its ATPase subunit Sth1 to slide or eject nucleosome, unit of chromatin, which is formed by 147 bp DNA wrapped around a histone octamer (2 copies H3, H4, H2A and H2B). Micrococcal nuclease digestion of chromatin followed by DNA sequencing (MNase-seq) is used to map nucleosomes genome-wide. We performed MNase ChIPseq to determine which nucleosomes are bound by RSC in vivo. We found RSC enrichment in the ORFs of highly transcribed genes, suggesting a role for RSC in Pol II elongation. Since nucleosomes impede transcription, nucleosomal DNA must be accessible to Pol II during transcription, and in this case the DNA around histone octamer should be prone to MNase digestion. In agreement with this idea, we found shorter nucleosomal DNA fragments (<130 bp) in transcribed ORFs. Interestingly, RSC-bound nucleosomes displayed greatly heterogenous DNA fragment length, indicating that RSC unwraps nucleosomal DNA in ORFs. Furthermore, our data suggested RSC might remodel only those nucleosomes within ORFs which are being transcribed by Pol II. Not surprisingly, we find that Pol II-interacting nucleosomes display a very similar fragment-length profile as those which are RSC-bound. Overall, we show remodeling of ORF nucleosomes by RSC in vivo using a novel strategy.

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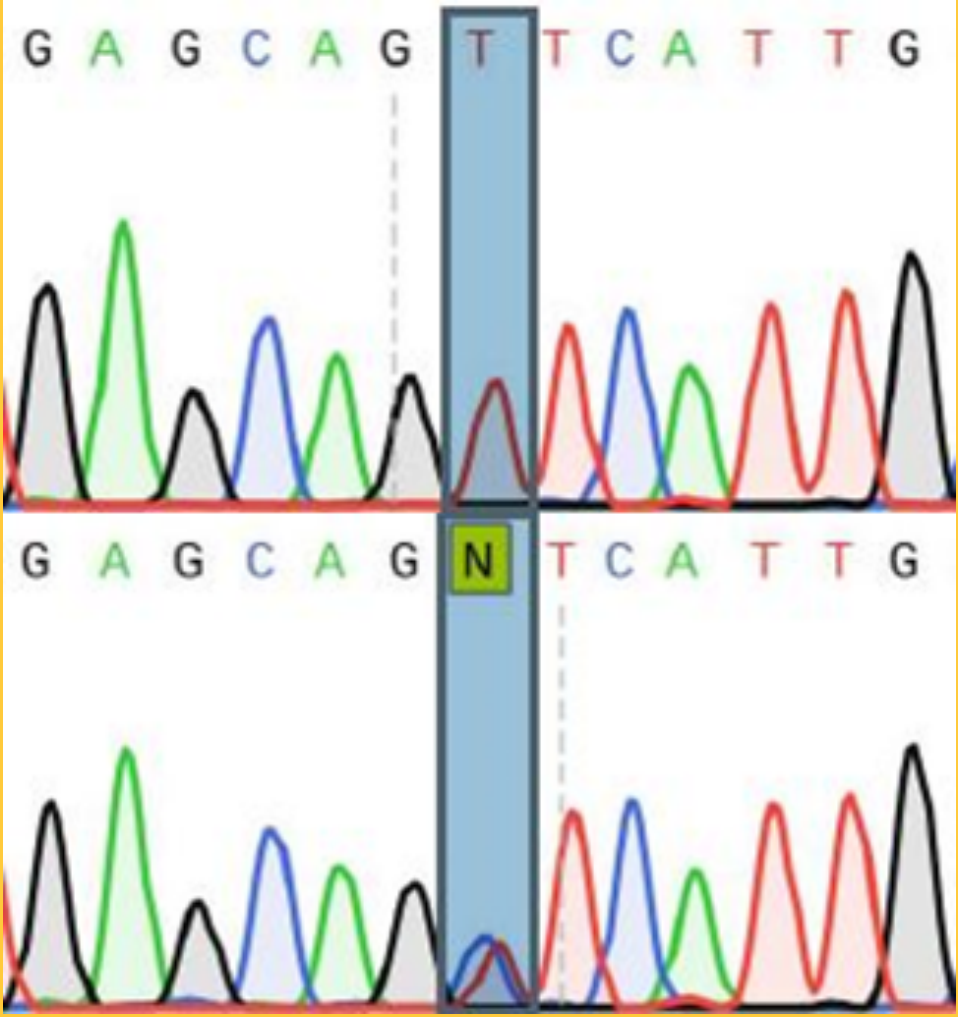

Image showing mutation in the DNA sequence
— Courtesy Dr . Westrick's Lab