Segmenting continuity: how do film viewers perceive events?

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We perceive everyday activity – such as somebody preparing breakfast – as continuous. Our memory for such activity, however, seems to break this continuous stream into discrete units generally called events. Like every day activities, film narratives are continuous streams of information. Research shows that moviegoers “chunk up” the continuity of the film discourse when encoding it into memory. What drives viewers’ segmentation of film? Existing and future research suggest that there exist some kind of "boundaries" that lead people to rather have a non continuous perception of events. The most common may be the changes in space, time, and character, as well as culturally connoted images which may influence film segmentation. For this research, first some different movies were selected like: Inception (2010), The Hurt Locker (2008) and The Time that Remains (2009). Each movie was fragmented in scenes by making different cuts frame by frame. Later, a cut code was done where the cuts were classified depending on the angle in which the scene was taken. There were high camera angle, low camera angle and eye level.

In the first results, it was observed that the ones that were taken from a high camera angle were more repeated than the ones from a low camera angle. As previous research has indicated (Cutting, Swallow, Zacks, Magliano), people segment continuous streams of film. Moreover, agreement across viewers in segmentation is higher than 90%. Cultural sensitivity during film viewing is also supposed to be measured in future research.

Regulation of mitochondrial glutamin catabolism by the Rho GTPases

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Cerione Lab, Department of Molecular Medicine, College of Veterinary Medicine

An emerging hallmark of cancer is the reprogramming of cellular metabolism to supply the energetic and biosynthetic demands of continuous cellular proliferation. Many cancer cells are found to be addicted to glutamine, and overexpress glutaminase (Gls), the enzyme that breaks down glutamine to glutamate and ammonia. Glutamate can be further broken down to supply the citric acid cycle with α-ketoglutarate. Gls is the main isoenzyme of glutaminase in most cancer cells, and its inhibition prevents further growth of highly proliferating cells.

Glutaminase levels are increased in transformed cells by Rho GTPases. Here we elucidate the critical signaling pathway for this process, and identify c-Jun as a potential transcription factor for Gls. Glutaminase expression is increased after Jun overexpression, and this is reflected at the transcript level as demonstrated by the qPCR. Myc, another regulator of glutaminase was found to be consistent in both normal and jun transfected cells. Enzymes that break down substances to feed the citric acid cycle were also upregulated in highly proliferating cells, indicating the importance of the cycle in anabolism to provide biosynthetic precursors.

Investigating the roles of proteins that undergo a change in phosphorylation state during egg activation in Drosophila melanogaster

Hawra Al Lawati, Zijing Zhang, Mariana Wolfner

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Wolfner lab, Department of Molecular Biology & Genetics

Egg activation is the critical series of events that transform a mature oocyte to an embryo. Examples of the changes that happen during this process include hardening of the egg covering, resumption of meiosis, translation and modification of proteins. These changes are triggered by a rise in the intracellular concentration of free calcium in the oocyte. Two calcium-dependent factors CaMKII and calcineurin that transduce the rise in calcium are important regulators in egg activation in several organisms. Since both of these enzymes regulate protein phosphorylation state, and since egg activation is triggered post-
Al Lawati et al. contd.
transcriptionally, it is worthwhile to look at phosphoproteins to elucidate the pathways that regulate egg activation. A previous study identified 311 proteins that undergo changes in phosphorylation state during egg activation in Drosophila melanogaster, which may represent an enriched set candidates for regulators of egg activation or of initiation of early embryonic development (Krauchunas et al. 2012). This project aims to identify novel regulators of egg activation events by individually knocking down some of these phospho-regulated proteins in female germ cells and observing the impact on the fertility of the female flies. Knockdown was accomplished by generating female flies that carried the maternal-triple-driver-GAL4 and a GAL4-responsive transgene that expresses a double-stranded RNA for the gene being tested. Of 12 genes that were screened, 2 exhibited no effect on the fertility of the females when maternally knocked down. Thus, these two proteins are either redundant or play minor roles in egg activation. Knockdown of another 2 genes abolished egg production, indicating that these genes are critical for oogenesis. Knockdown of 7 additional genes reduced the hatchability of the eggs and knockdown of 1, αTubulin67C, eliminated hatchability. αTubulin67C is a structural constituent of the cytoskeleton. The phenotype shown by this RNAi line indicates that αTubulin67C might be important in egg activation or early embryogenesis. To determine which of these two processes the gene is specifically involved in, future work will include performing immunostaining on αTubulin67C knockdown flies’ eggs to determine the exact stage of arrest. 

SILS-Qatar Symposium, August 6th, Mann Library 100, 4:35 PM

Microscopic evidence for sporangiospore dimorphism in Rhizopus microspores
Colin Barber, Olga Lastovetsky and Teresa Pawlowska
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Despite recent advances in the study of the symbiosis between Rhizopus microsporus, known plant pathogen and an agent of mucormycosis in immunocompromised humans, and its bacterial endosymbiont, Burkholderia rhizoxinica, the growth and development of sporangio spores remains woefully undocumented. We examined two wildtype configurations of this system, R. microsporus ATCC 52314 and 52813 with their native endobacteria, as well as two configurations created in vitro by inoculating R. microsporus ATCC 52314 with bacteria native to R. microsporus ATCC 52813 and vice versa. These four isolates were observed under a fluorescence microscope in addition to a differential interference contrast microscope. We found that sporangiospore diameters are bimodally distributed in all isolates and two distinct spore colorations can be observed within the same isolate. In spite of those observations, the number of endobacteria per spore was found to be bimodally distributed in only some isolates. These findings are of interest because they may have implications for R. microsporus virulence in human patients and they open up new areas of research into molecular interactions between the fungus and its endobacterium. Warren 101; 10:30AM-10:42AM

Studies in mechanism of AMPA receptor activation using disulfide trapping
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Ionotropic glutamate receptors mediate the majority of excitatory synaptic transmission in the vertebrate central nervous system. One of the central issues in molecular biology is the mechanism of channel activation when an agonist binds to the bilobed ligand binding domain (LBD) of the AMPA receptor. One theory is that the LBD must be closed to activate the channel, yet crystal structures show only partial closure of the LBD when bound to partial agonists, which can lead to partial activation. This poses the question of whether all compounds that lead to channel activation are able to fully close the lobes of the LBD. Previous laboratory work has been conducted using LBD with dual cysteine point mutations, which allow the lobes to be in a fully closed conformation due to a disulfide bond that forms under oxidized conditions. Crystal structures and NMR spectroscopy showed that the disulfide bond can be formed when the LBD is bound to full agonists, such as glutamate, or to partial agonists, such as kainate and iodowillardine. Such findings suggest that a full closure of the LBD can be obtained with both full and partial agonists, which sheds some light on the channel activation mechanism of the AMPA receptors. In order to obtain further information regarding this topic, thermal denaturation and isothermal titration calorimetry experiments were performed using the cysteine mutant of LBD with a range of ligands, partial and full agonists and antagonists, including domoic acid and L-BMMA hydrochloride. These experiments demonstrate that there are differences in the association constants and thermal stability of the LBD between the oxidized and reduced conditions, indicating the structural changes that result from the formation of the disulfide bond. Such results are consistent with the previous results from the crystal structures and NMR spectroscopy. Taken together, the findings suggest that partial agonists in fact can produce a full lobe closure, and support the hypothesis that a fully closed conformation of the LBD is required to activate the channel, even with the partial agonists. 

SILS-Qatar Symposium, August 6th, Mann Library 100, 3:20 PM
Assessing antibiotic susceptibility of *L. monocytogenes* directed by alternative σ factor regulation

**Avery Becker**, Veronica Guariglia, Martin Wiedmann
Food Safety Lab, Department of Food Science

*Listeria monocytogenes* is a Gram-positive, foodborne bacterium that is ubiquitous in the environment and the causative agent of listeriosis. This illness most commonly affects persons with compromised immune systems resulting in meningitis, encephalitis, as well as septicemia, while causing stillbirths or life-threatening infections of newborns for pregnant women. It has been estimated that 1600 cases of listeriosis occur annually within the United States, leading to 400 to 500 deaths. Reasons for *L. monocytogenes* successful virulence include its ability to survive a wide range of temperatures, the presence or absence of oxygen, as well as numerous levels of osmotic or pH stress. An important mechanism for regulating the transcription of appropriate genes in response to dynamic environments is *L. monocytogenes*’s alternative sigma (σ) factor network. These four factors- σB, σC, σH, and σL- can each associate with the core RNA polymerase, allowing for the enzyme's recognition of specific DNA sequences and thus providing a regulated response.

To allow for the study of a single σ factor's ability to regulate responses, strains of *L. monocytogenes* were previously constructed with triple σ factor deletions. Along with the four triple mutants, a single quadruple mutant was created as a control. The general experimentation this summer involved the exposure of the five mutant strains as well as the wildtype strain to a variety of stresses to assess differences in susceptibility. The hypothesis is that the five mutant strains and the wildtype will each respond differently to various antibiotic agents as a result of changes in the organism’s regulatory network.

The primary method for comparing the strains was completed via disk diffusion assay. Brain-heart infusion agar plates were inoculated with the six *L. monocytogenes* strains to produce bacterial lawns. Filter disks loaded with antibacterial agents were set onto the surface of the plates. The antibiotics used were selected based on their class of action. These include: membrane affecting agents- Nisin, Bile salts, Polymyxin B, Bacitracin, and detergents including Triton, SDS, and Tween; cell wall affecting agents- Lysozyme, Fosfomycin, Penicillin G, and Ampicillin; protein synthesis affecting agents- Erthromycin and Tetracycline; as well as the DNA replication affecting agent- Ciprofloxacin. After a night of incubation, approximately 14-18 hours, the circular zones of growth inhibition were measured and compared across the strains.

The results thus far have shown a consistent trend of the quadruple deletion mutant being most susceptible. This is consistent with the current understanding that this strain lacks sophisticated regulatory mechanisms. Also, certain strains did interact with particular stresses differently and will be addressed with future, rigorous investigation. The majority of the agents did not show drastically different zones for the strains which has been speculatively attributed to the diffusion properties of the antibacterial agent being predominant rather than each strain’s particular susceptibility. **Warren 101; 3:14PM-3:26PM**

Protein isolation from a human placenta

**Fredrick Blaisdell**
O’Brien Lab, Department of Nutritional Sciences

The placenta is a key regulatory organ that is responsible for nourishing the fetus during development by establishing contact with the maternal blood circulation. Due to a neonate’s dependence on the placenta, the organ is of significant interest to researchers to try to understand placental physiology. Medical complications such as increased risk of developing cancers, autoimmune diseases or metabolic disorders can develop during the infant’s life, while increased risk of intrauterine growth restriction, premature delivery, low birth weight, postpartum depression and maternal morbidity are other factors that can develop during pregnancy due to nutrient deficiencies. Little is known about the physiological processes through which these complications arise and are thus the focus of many studies. The goal of this project was to explore the many techniques and methods that are used to investigate mechanisms at the placental level such as organ collection and processing, Bio-Rad modified Bradford protein assay, RNA extraction and western blot analyses. **Warren 101; 1:30PM-1:42PM**

Feasibility of utilizing IR sensors in order to detect gas respiration as a means of quantifying *Fusarium verticillioides* contamination of stored maize grain

**Dana Brems**, Tyr Wiesner-Hanks, Nick Morales, Laura Morales and Rebecca Nelson
Nelson Lab, Department of Plant Pathology / Plant Microbiology

Fumonisins is a mycotoxin that is produced by the fungal pathogen *Fusarium verticillioides*. Fumonisins disrupts sphingolipid pathways, which interferes with membrane binding proteins and causes damage to DNA. In humans, this can cause growth retardation in children (Kimanya et al. 2010), birth defects (Missmer et al. 2006,) and esophageal cancer (Wakhisi et al. 2005.) It can also cause heart failure in pigs (Colvin et al. 1992.) and necrosis of the brain in horses (Kellerman et al. 1990.)
**Dana Brems et al. contd.**

These toxins are present in many places maize is cultivated. In Kenya, one study suggested that between 37 and 39 percent of all the crop contained mycotoxin levels over the maximum tolerable limit (Mutiga et al., 2014.) Farmers who cultivate maize are not likely to have the time, expertise or money that is required to detect fumonisin contamination using current laboratory methods. For this reason, there is a need to develop methods of detection that are quick, easy and inexpensive.

One possible mechanism of detecting fungal contamination is measuring the amount that fungus respires carbon dioxide. Some studies have done large scale detecting of CO₂ for long term grain storage (Maier et al, 2011.) but there has not been a study demonstrating if CO₂ detection would work on a small scale, which would be more accessible to farmers.

For this reason, I chose to see how accurately I could detect fumonisin and Fusarium contamination in small samples (10 grams) of ground maize using an IR CO₂ detector. I used the photosynq detector, which can measure multiple variables, including CO₂ ppm, percent humidity, temperature, and light intensity. Although it is only in the beta testing phase, it is accurate to a degree of one part per million, and has great potential to be mass-produced. I am still in the process of doing my experiment, but I have been able to draw some conclusions. I have found that additional bacterial and fungal contaminants affect the results, especially when grain is dry. It is difficult to detect accurate CO₂ change in dry maize, so water-mediated reactivation of the fungus in a small sample dramatically increases the ability to detect fungal respiration. I am currently looking at respiration of newly inoculated grain, and I should have results by the time I would present at this symposium. **Warren 101; 9:54AM- 10:06AM**

**Ribonucleotide reductase activity and uracil in DNA**

** Hilary Bright**, Martha S. Field, Yashira N. Abril, Patrick Stover and Robert Weiss

*Stover Lab, Department of Nutritional Sciences and Weiss Lab, Department of Veterinary Medicine*

Ribonucleotide reductase (RNR) is an enzyme that catalyzes the formation of deoxyribonucleotides (dNTPs) from ribonucleotides in DNA synthesis. Specifically, RNR catalyzes the formation of deoxyuridine (dUDP) from uridine diphosphate (UDP), a uracil derivative. This experiment attempts to identify the role of RNR in the excision repair process of DNA. We used a combination of cell culture and mouse model to test the efficiency of excision repair when this enzyme was either down or up-regulated in cells. We performed transfections of HeLa cells to quantify the percentage of uracil incorporation in the cell when RNR is down-regulated. Western blotting was used to confirm enzyme knockdown. Transgenic mice that overexpress both subunits of RNR were used to determine the effect of disrupting an enzyme involved in dNTP production. These mice showed several phenotypes: significant alterations in dNTP pool composition, hepatocellular swelling, pleural effusion, and kidney degeneration and proportionately larger heart sizes. We found a slight increase in uracil incorporation into DNA when RNR is up-regulated, but more data must be collected. **Warren 137; 9:54AM-10:06AM**

**Biological flow and surface topography support migration of bull sperm**

**Anna Paula Guerrero Castillo**, Florencia Ardona, Chih-kuan Tungb, Alyssa Fioreb, Lian Hua, Mingming Wub, Susan S.Suarez

*Medical Student, CienciAmerica/Mexico Program*

*aDepartment of Biomedical Sciences and bDepartment of Biological and Environmental Engineering*

To fertilize an egg, mammalian sperm need to travel quite a long distance through the female reproductive tract. Although the male inseminates millions of sperm, there is evidence that the female guides sperm and regulates their progress through the tract in order to ensure that sperm reach the site of fertilization in the oviduct just as oocytes are released into the oviduct from the ovary. In humans and cattle, sperm are deposited by the male into the vagina at the entrance to the cervix and must swim through the cervix to reach the uterus and, finally, the oviduct. In 1989, Mullins and Saacke [2] reported the presence of 10-20 μm wide microgrooves in the wall of the bovine cervix; these microgrooves ran through the length of the cervix to the uterus. Furthermore, they noted that many microgrooves were filled with sperm. They hypothesized that the microgrooves provide preferential passageways for sperm that facilitate their movement through the cervix. We were able to take advantage of current microfluidics technology to test this hypothesis on bull sperm. A microfluidics device was developed to replicate three main physical features of the cervix: the fluid flow within the main channel, the microgrooves in the wall of the cervix, and the viscoelastic mucus that is present in the cervix. The device contained channels that were 300 μm wide X 120 μm high; some of the channels contained 20 μm X 20 μm microgrooves on the upper walls. Fluid was pumped through the device to replicate the fluid flow that normally occurs from the uterus, through the cervix, and out into the vagina. Long chain polyacrylamide was added to the fluid to replicate the viscoelasticity of mucus in the cervix. When sperm were loaded into the device, they swam toward surfaces, and maintained mostly along a curved line with no preferred direction on a broad, flat surface of the larger channels. When sperm encountered sidewalls of the channels, they tended to align to the wall surface and persist in swimming along it. Sperm that encountered the microgrooves in the upper walls of the larger channels would immediately enter them, and persistently migrate along the grooves without orientation change. It was also observed that sperm responded to fluid flows of
Anna Paula Guerrero Castillo et al. contd.
greater than 1.13 µl/min by orienting into the flow (with an angle) and swimming upstream. The presence of gentle fluid flows creates an upstream bias outside the grooves, and the topography of the grooves ensures them to travel through. As the viscoelasticity in the media was increased by addition of long-chain polyacrylamide, sperm swim slower than their normal speed (13 ± 2 µm/sec), but followed walls and swam through microgrooves. It was concluded that microgrooves contribute to fertilization by providing a pathway that facilitates sperm movement through the cervix. Warren 101; 3:50PM-4:02PM

Interspecies variation in volumes of song-related brain areas between females
Pamela Cejudo, Roberto Llerenas and Timothy DeVoogd
DeVoogd Lab, Department of Psychology

Within songbirds, the brain nuclei HVC, RA and nXII are responsible for learning and producing song. In males, variation in the relative volumes of HVC and in HVC/RA predicts capacity for learning complex songs. No systematic study has looked at variation in these structures in females. We hypothesized that these areas would vary in relative volume between females in different species, and that the variation might be related to variation in the species’ social structure or the females’ capacity for singing. We used fixed brain slices stained by cresyl violet from multiple songbird species. HVC, RA and nXII nuclei were identified in the samples, drawn on paper, digitized and measured using ImageJ Software (NIH, 64b-1.48v).

Nucleus HVC was traced at 23X on every slide in which it was present. All reported values are from one side of the brain (typically the right except in cases where torn tissue or poor staining prevented measurements of that side). Nucleus RA was traced from one hemisphere in every section in which it appeared. Nucleus nXII was traced bilaterally at 81X in all the slices of each brain sampled. Nucleus volumes were computed by summing the areas and multiplying by the sampling interval. For nXII, the number of neurons in the syringeal part of the nucleus was estimated by counting neuronal nuclei at 20X from one third of the drawn samples and multiplying by 3.

Results: Substantial variation exists in females across species in the structure of these areas. HVC volume in females ranges from 0.072328 mm3 (Tawny pipit) (38% smaller than the male) to 0.096336 mm3 (skylark) 94.48 % smaller than male. RA volume varies by a factor of 10 between the species sampled. Nucleus nXIIIs in female brains ranged from 1512 (house martin) to 2046 (black redstart) neurons. We are now surveying the literature to see whether the anatomical variation correlates with variation in behavior across species. Warren 137; 1:06PM-1:18PM

A study of the MHC in the Qatari Arabian Oryx
Mhd Salama Chaker, Donald Miller and Douglas F. Antczak
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The Arabian Oryx, Oryx leucoryx, is an antelope species native to the Arabian Peninsula. The species was declared ‘Extinct in the Wild’ in 1972 due to poaching. Today, the Oryx is at the status of ‘Vulnerable’ and the wild population was reestablished from oryxes bred in captivity and reintroduced to the wild. It has been demonstrated that the Arabian Oryx suffers a genetic bottleneck. Unfortunately, the limited gene pool has given way to disease outbreaks in captive populations. Two Oryxes, Oryx 17 and Oryx 18, at the Al-Wabra Wildlife Preservation have been selected for breeding and genetic sequencing programs. We have investigated their genetic similarity through sequencing two Major Histocompatibility Complex loci. MHC loci are known to be highly polymorphic and representative of genetic variation in species. Amplicons of the second exon of the β subunit of the DR MHC Class II molecule, a highly polymorphic locus, were sequenced. Another locus investigated was the third exon in the α2 subunit of an MHC Class I molecule. The sequencing showed Oryx 17 to be heterozygous, possessing alleles 1 and 2, for DR-β whilst Oryx 18 to be homozygous, possessing allele 2 exclusively. As for the MHC Class I locus, both oryxes yielded identical sequences. Since Oryx 18 is homozygous for its DR-β locus, it is plausible that Oryx 18 is homozygous for other genetically linked MHC loci. As such, Oryx 18 may be indeed a good candidate for genetic sequencing for the MHC loci, a set of loci reputed to be difficult to sequence. On the other hand, little is known of the MHC Class I sequence investigated. It may well be that both Oryxes yielded identical sequences due to the fact that they are related and genetically similar, further verifying the genetic bottleneck effect in the Arabian Oryx. However, it may also be possible that the MHC Class I locus examined is a non-classical locus that is highly conserved and is identical in oryx individuals. Further research on the Oryx genome may identify the nature of this locus and possibly other loci. In addition, these results may indicate that Oryx 17 and Oryx 18 are relatively related since they share the same MHC Class I sequence as well as one allele at the DR-β locus. Applications of this research would be developing biochips to quickly and feasibly genotype Oryx individuals to assist breeders in safeguarding the future of the species. SILS-Qatar Symposium, August 6th, Mann Library 100, 3:50 PM
Microhemorrhages, or small bleeds, are common in the aging brain and contribute to cognitive decline and dementia. While taking aspirin decreases the risk of coronary artery occlusions and stroke, aspirin usage may also increase the incidence of hemorrhage and prolong bleeding. Because larger bleeds exacerbate damage to the surrounding tissue, we aimed to understand the influence of aspirin usage on the size of microhemorrhages in the brain. We used femtosecond laser ablation to rupture arterioles in the brains of both aged (18-24 month old) and young (2-4 month old) mice dosed on aspirin (0.4mg/mL) in their drinking water and measured the extent of penetration of both red blood cells and blood plasma into the surrounding tissue as compared to controls. In preliminary data, we find that aged mice dosed on aspirin have minimal difference in microhemorrhage size as compared to controls (diameter of red blood cell hematoma = 95 +/- 38 μm in controls and 112 +/- 29 μm in aspirin treated mice). There was also no difference in young animals (hematoma diameter = 110 +/- 32 μm in controls and 98 +/- 18 μm in aspirin treated mice). These data show that aspirin usage does not increase microhemorrhage size, thus supporting the safety of aspirin usage.

**Genetic engineering of Escherichia coli for heavy metal sequestration from contaminated waters**

**Jonlin Chen, Aaron Gittelman**

*Cornell iGEM Team, Dept. of Electrical and Computer Engineering*

Heavy metal contamination of water can be catastrophic to human health, aquatic life and the ecosystem. Industrial processes, manufacturing, and coal mining often release large quantities of hazardous metals into the environment. We are developing a biological sequestration and filtration system for such metals in hopes of reducing the risks of heavy metal pollution for the community and beyond.

We are engineering *Escherichia coli* to sequester the heavy metals nickel, mercury, and lead. To accomplish this, we are creating high copy bacterial vectors that contain genes for metallothioneins and heavy metal transport proteins. Metallothioneins are a class of low-molecular-weight proteins with high binding affinities for various heavy metals. Transport proteins specific to heavy metal ions can rapidly import those metals into the bacteria. We are incorporating the genes *nixA*, *merT* and *merP*, and *CBP4* that code for nickel, mercury, and lead transporters, respectively. Thus, heavy metal transport proteins and metallothioneins will be expressed simultaneously to create several sequestering strains of *E. coli*.

The engineered strains will be integrated into a hollow fiber reactor that will act as a continuous-flow sequestration system. As contaminated water flows past the cells, the heavy metal ions will be taken up by *E. coli* via their respective transport proteins and then bound intracellularly by metallothioneins. Metal detection systems and biological safety mechanisms will be incorporated downstream of the reactor unit to ultimately create an effective and environmentally friendly water filtration system.

**Janus-faced microglia: stiffness dependent activity of murine microglia**

**Hee Jin Cheon, Erika Gruber, Justin Im, Jody Lopez and Cynthia Leifer**

*Leifer Lab, Department of Microbiology and Immunology*

Alzheimer’s Disease (AD) is a progressive neurodegenerative disease that affects millions of Americans and has no cure. AD develops due to inflammatory responses from mishandled proteins such as amyloid beta and tau protein. Accumulation of these proteins elicits a local inflammatory response and tissue damage. Immune responses in the central nervous system are driven by resident immune cells called microglia; the microglial cells typically engulf cellular debris and amyloid beta peptides, secrete growth factors and anti-inflammatory cytokines, and monitor neuronal synaptic connections. However, by as yet unclear mechanisms, microglial cells in aged patients undergo morphological and functional change and produce significantly more TNF-α and other pro-inflammatory cytokines. The increased inflammatory response leads to accumulation of debris and amyloid beta and tau protein. How the microglia transform into activated, pro-inflammatory cells is unknown but likely involves local tissue environmental cues. Magnetic resonance elastography in vivo has demonstrated that the physical stiffness of brain is lower in patients with AD and other neurodegenerative diseases. Our previous studies have shown that the physical stiffness of growth substrates regulates proinflammatory and antimicrobial function of macrophages, analogous cells to microglial cells. Therefore, we hypothesize that reduced brain stiffness signals microglia to adopt a pro-neurodegenerative profile. I developed highly quantitative mechanisms to measure cytokine production and cellular morphology in order to evaluate microglial cell function. Using tunable acrylamide gels of various stiffness mimicking normal tissues or reduced stiffness brain, I used these quantitative assays to evaluate pro-neurodegenerative functions on different stiffness growth substrates. My studies show that microglial cells grown on less stiff substrates adhere similarly but produce more pro-inflammatory and anti-inflammatory cytokines, and have a more amoeboid morphology. These data suggest that the reduced stiffness observed in AD brains may be a contributing factor to perpetuate inflammatory pathology in the brain. Future studies...
Hee Jin Cheon et al. contd.
will address phagocytosis of amyloid beta peptides on different stiffness substrates and molecular mechanisms regulating the ability of microglial cells to sense growth substrate stiffness. These studies will identify targets for new therapeutic development to slow or reverse the inflammatory damage of neurodegenerative diseases. Warren 101; 2:30PM-2:42PM

Fabrication of alginate hydrogel microtubules with entrapped microbubbles for enhanced cell encapsulation-based drug delivery
Mark Colasurdo, Duo An and Minglin Ma
Ma Lab, Department of Biological and Environmental Engineering

Cells that produce certain therapeutic factors such as genes, proteins, or other molecules, can be encapsulated in natural or synthetic polymer scaffolds and implanted in the body. Once in the body, the cells interact with their biological surroundings by diffusing the therapeutic agents across the scaffold, while also having nutrients and oxygen diffuse into the scaffold for the encapsulated cells. In this case, a novel hydrogel scaffold has been developed for the encapsulation of such cells. The device is a solid, alginate matrix tube that also has microbubbles of air entrapped in it. It was fabricated using a coaxial nozzle with crosslinking alginate flowing in the outer annular stream, while air bubbles form in the inner stream and become encased in the surrounding alginate matrix. Many studies have shown that encapsulating microbubbles into drug delivery devices can enhance drug delivery when subjected to ultrasound radiation. The ultrasound causes the microbubbles to oscillate in volume, a process known as cavitation, which creates microstreams of fluid that enable greater mass transfer across the encapsulating device. Thus, this technique allows these devices, once implanted in vivo, to have a controlled release of drugs. However, ultrasound-mediated drug delivery has only ever been tested directly on drugs and not with encapsulated cells. It is hypothesized that the cavitation of the microbubbles in the alginate microtube may cause sufficient mechanical stimulation and hyperthermia to the encapsulated cells to potentially increase their metabolism and in return produce more drugs and uptake more oxygen and nutrients, thus increasing their survivorship and efficacy as drug delivery devices. Warren 101; 9:30AM-9:42AM

Deer browse impacts on herbaceous vegetation in Beebe Lake
Mariana C. Robles Cruz, Todd Bittner, Robert Wesley and Hanna Rosner-Katz
Cornell Plantations

According to the Cornell University Integrated Deer Management Program, since 2009 the deer population has been increasing slightly and has stabilized, even with the attempts at reducing it. That is, until 2014, when the deer population seemed to be reduced considerably. It is known that deer prefer to browse on certain native herbaceous plant species located in and around the campus and it is hypothesized that these populations can be affected by the overabundant deer population. To test this, exclosures were erected in three different locations in order to look for differences between a “not impacted area” and a normally deer visited area; Beebe Lake was also studied because of its importance in the campus natural areas. Plots of one square meter were placed both in and outside these exclosures, where the number of individuals of deer-preferred species were counted, the height of each individual measured, and the amount of browsing observed. Even if large amounts of deer browse are not observed during the growing season, effects were seen on plant height and reproductive status. Warren 137; 2:30PM-2:42PM

Procoagulant activity of monocyte subpopulations in the horse
Nicole DeAngelis, Jorge Adarraga, Wee Ming Yeo and Tracy Stokol
Stokol Lab, Population Medicine and Diagnostic Sciences, College of Veterinary Medicine

Equine herpesvirus type-1 (EHV-1) causes abortion and a neurologic syndrome, equine herpes myeloencephalopathy (EHM). These clinical syndromes are associated with thrombosis of vessels supplying the placenta and spinal cord. After inhalation, EHV-1 establishes a peripheral blood mononuclear cell-associated viremia, with monocytes being a target of infection. In diseased states, monocytes are the main source of tissue factor (TF), a transmembrane glycoprotein that initiates coagulation. Recently, it has been shown that monocytes are phenotypically and functionally heterogeneous. These different subsets of monocytes are likely important for facilitation of innate and adaptive immune responses to infectious agents, such as EHV-1. We have found that two monocyte subsets can be identified in horses with the surface markers CD14 (lipopolysaccharide [LPS] receptor) and CD163 (hemoglobin-haptoglobin receptor): CD163+14+ and CD163+14+. These subsets correspond to anti-inflammatory and pro-inflammatory populations in humans, respectively. We hypothesized that these monocyte subpopulations would have a differential procoagulant response to EHV-1 infection, in vitro.

We separated the two subpopulations, CD163+14+ and CD163+14+, by immunomagnetic bead sorting. The sorted subsets were infected with EHV-1 at a multiplicity of infection of 1 and then performed a procoagulant assay, which measures TF activity. Our initial experiments did not demonstrate differences in virus-induced TF activity in the two subsets. We did, however, see higher TF activity induced by LPS, a pro-inflammatory positive control for TF stimulation, in the CD163+14+ subset,
suggested that there are functional differences between these two monocyte populations. In future studies we will examine the mRNA expression of anti- and pro-inflammatory cytokines, such as interleukin (IL)-10, IL-6, TNF-α, in response to viral infection in the two monocyte subsets. Warren 101; 3:38PM-3:50PM

Neural mechanisms of self-evaluation in singing birds
Alexander Farhang, Vikram Gadagkar and Jesse Goldberg
Goldberg Lab, Neurobiology and Behavior

Many of our behaviors like speaking, writing, or even throwing a ball are not innate but are learned through years of practice involving trial and error. We are all familiar with the babbling of an infant developing into the structured speech of adults, but the neural mechanisms underlying such learning are not fully understood. Like humans, zebra finch chicks start out with highly variable babbling that turns into remarkably stereotyped song as adults, making them an ideal model system to study the trial and error learning of complex behavior. Additionally, the zebra finch brain has an intrinsically connected network of neurons (a “circuit”) that is dedicated to song learning. To learn the song, the baby bird listens to its father’s song, and spends his early life trying to reproduce it. The hypothesis for the learning mechanism is that the baby memorizes the father’s song and stores it as a template. The bird then sings in an attempt to copy the song and compares what he hears (the song he just sang) to this template. If the song is better than expected, the bird keeps that version of the song; if the song is worse than expected, that version is discarded. We hypothesize that dopamine neurons in the ventral tegmental area (VTA) transmit the quality of the song to the song circuit. This means that neurons in the VTA should fire differently when the song is “worse than expected” compared to when it’s “better than expected.” We expect these dopamine neurons to decrease their firing rate when the song is worse than expected. To test this, we record from this region while the bird sings and play white noise over certain syllables. We then determine how this distorted song affects the firing pattern of the dopamine neurons. In order to record from the VTA, we create microdrives from scratch. These microdrives have an electrode array and a motor that allows us to move the electrodes in the brain using a computer. Our preliminary data suggest dopamine neurons in the VTA seem to encode the quality of the song in an expectation violation signal. Warren 137; 1:18PM-1:30PM

Characterization of Epulopiscium sp. isolates using Multilocus Sequence Typing
Zanah Francis, Francine Arroyo and Esther Angert
Angert Lab, Department of Microbiology

Epulopiscium sp. are large bacterial symbionts found in the intestines of some tropical marine surgeonfish. The largest morphotypes have been shown to grow a million times larger than Escherichia coli or Bacillus subtilis. While most bacteria and certain Epulopiscium morphotypes rely on binary fission to reproduce, many Epulopiscium morphotypes propagate by viviparity and form intracellular offspring. Here, we focus on the viviparous B morphotype that grows up to 100 to 300µm long. Characterizing different populations can help determine what genetic modifications in Epulopiscium allow for its larger cell size and unique reproduction methods. Single cell genome amplification was used to obtain a reasonable quantity of DNA for analysis. We then used multilocus sequence typing (MLST) to examine the clonal relationships and genetic relatedness of Epulopiscium sp. isolates by indexing gene sequence variation in seven housekeeping gene loci: recA, dnaC, ftsZ, mreB, radA, rpoB and secA. MLST identification of different Epulopiscium populations allows for further investigation of the genotypic variation among the same Epulopiscium morphotypes. It is hypothesized that there will be greater genetic similarity among isolates from the same population and there will be increased genetic variation among isolates from different populations. We also examined the temporal relationship of genetic variation in Epulopiscium populations over 22 years so it is predicted that there will be greater variation between the older and more recently collected samples. Further MLST studies of different Epulopiscium isolates will provide insight into the unique genetic modifications that allow for intracellular offspring development in these large bacteria. Warren 101; 10:18AM-10:30AM

Towards a functional assay for maize endophytes: seedling studies
Diana Aguilar Gómez, Jenny B. Cornell, Nick Morales, Alice C.L. Churchill, Rebecca J. Nelson
Rebecca J. Nelson Lab, Maize Disease Resistance Lab, Department of Plant Science

It is known that endophytes can be beneficial for a plant or act as pathogens. Their effect on the plants can depend on the specific conditions of each plant and its environment. The objective of this work is to see how endophytes might affect seedling vigor. To quantify seedling vigor, we measured growth rate (Δweight) and respiration (CO2) of sets of maize seedlings grown in plastic pouches. If measuring CO2 helps knowing the seedlings vigor it may be an inexpensive way to test them. In previous experiments in the lab, it was found that seeds from North Carolina contained more endophytes than seeds from New York. In this study, three experiments are being performed to test seedling from different sources and endophytes previously isolated from kernels. The first experiment involved seeds from the same line (CML322) and different sources (FL and NY),
Aguilar Gómez et al. contd.
grown in plastic pouches in the incubator. Their CO₂ production was measured, but the results did not show the anticipated correlation. The NY seedlings had a better growth rate. This may be due to the fact that New York seeds were from 2008 and Florida’s from 2005, and/or other differences between the seeds, including potential differences in endophytes. For the second experiment, based on growth rate (Δweight), the best seedlings from NY and the worst seedlings from FL were grown with buffer to yield a solution intended to contain endophytes that are potentially affecting each seedling’s vigor. This solution was used for three different purposes: (a) Plate solution in petri dishes to culture the endophytes from the seedlings of the first experiment; (b) DNA extraction and (c) Dip seeds and grow them again in plastic pouches to measure their growth rate. If endophytes contribute to the differential growth rate, we expect that the endophytes from FL worst seedlings and NY best seedlings will affect growth rates of the inoculated seedlings. We believe the results from the first experiment may be affected by some factors, such as different initial weight of seeds and inefficient CO₂ measurements. Improvements were made for the second experiment such as selecting all the seeds from similar sizes/weights and changing the technique to measure CO₂. In a third experiment, CML322 seeds from NY were dipped on putative Fusarium-type fungal endophytes isolated from Mo17 maize kernels to test how they affect their growth and general vigor, indirectly measured using respiration (CO₂).

Understanding the epithelial-to-mesenchymal transition (EMT) to improve the isolation of circulating tumor cells.
Conor Gruber, Fredrik Thege, Marie Godla and Brian Kirby
Kirby Research Group, Department of Biomedical Engineering

The isolation of rare carcinoma cells found in blood, known as circulating tumor cells (CTCs), serves as an important tool for both the treatment and study of cancer metastasis. As a noninvasive tissue sample, CTCs have proven insightful for early detection, prognosis and personalization of therapy for cancer. Immunocapture, a promising method of CTC isolation, relies on targeting specific cell surface markers with antibody-functionalized devices. However, CTC surface expression is highly variable and inadequately understood. Despite growing evidence that epithelial markers are downregulated during tumor cell dissemination, immunocapture devices typically target an epithelial phenotype, thus limiting CTC detection. This technology is further hindered by the identification of CTCs by their presence of epithelial cytokeratins. Yet, this dedifferentiation away from epithelia, termed the Epithelial-to-Mesenchymal Transition (EMT), remains poorly characterized. The discrepancies between EMT theory and anti-epithelia immunocapture approaches necessitate an improved understanding of phenotypic changes experienced during EMT in order to improve CTC isolation.

Here we developed a 4-color immunofluorescence assay to mark the EMT status of a cell based on known epithelial and mesenchymal markers. Additionally, we induced EMT in vitro in model cell lines as confirmed by the developed assay. Together, these techniques allow for modeling of CTC formation and will provide insight into limitations of modern immunocapture platforms. Ultimately, these model CTCs can be used to design immunocapture platforms that recognize EMT and capture more robust, heterogeneous CTC populations.

Role of ER associated degradation in nephrogenic diabetes insipidus
Robert Guber, Guojun Shi, Diane Somlo, Ling Qi
Qi Lab, Division of Nutritional Sciences

Water homeostasis is a critical component of survival and nephrogenic diabetes insipidus (NDI), a rare disorder, is a disorder where the body is unable to concentrate urine. Patient’s suffering from NDI have dysfunctional collecting ducts and patients can inherit NDI via an X-linked or autosomal mutation, or it can be induced by other mechanisms that destroy the collecting duct cells. X-linked mutation result in defective vasopressin 2 receptor, and autosomal result in mutations in Aquaporin 2(AQP2) water channel. Endoplasmic Reticulum associated degradation (ERAD), is an important regulatory system that targets and degrades misfolded proteins within the ER. AQP2 is a responsible for water reabsorption in the collecting ducts, and proper folding of AQP2 is essential to translocation to the apical membrane. However, the exact mechanism that regulates AQP2 is unknown and it is thought that both autophagy and ubiquitin-proteasome system play a role in AQP2 internalization, although the exact mechanism is unknown. A subset of patients that have autosomal mutations in AQP2 have functional water channels, but the AQP2 is retained within the ER. They are hypothesized to be retained in the ER due to the monomers are unable to form their final confirmation, a tetramer, and they need to be degraded within the ER. Therefore we hypothesized that ERAD, is responsible for targeting and degrading misfolded AQP2 in the ER. More specifically the E3 ligase, Hrd1, in conjunction with the ER adaptor protein suppressor enhancer Lin12 1 like (Sel1L), which stabilizes Hrd3, is able to recognize and degrade AQP2. Through inducible knock out Sel1L (IKO) we discovered that our mice suffered from polydipsia and polyuria, two clinical manifestations of NDI. Additionally to broaden the clinical relevance mouse models of type 1 and type 2 diabetes were also used, because these animals and patients can also suffer from kidney disease, related to water reabsorption. To further understand these interactions and phenotypes observed in mice, human embryonic kidney cells (HEK) were used to examine the expression and interaction of Sel1L with AQP2 and ER retained AQP2 mutants. In the absence of Sel1L levels of
**Guber et al. contd.**

AQP2 remained constant however when Sel1L was added to KO cells, levels of AQP2 decreased. Additionally in the animals the localization of the AQP2 was not on the apical membrane indicating a dysfunction in the translocation of AQP2. Further research into the interaction will provide us with a greater understanding of AQP2 regulation and degradation, and will hopefully provide insight into potential avenues of treatment for people suffering from water imbalances. **Warren 101; 2:18PM-2:30PM**

**Durability of engineered resistance to Xanthomonas oryzae pv. oryzicola in rice**

**Margaret L. Harvey, A. C. Read, K. Wilkins and A. J. Bogdanove**

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*Xanthomonas oryzae* pv. *oryzicola* (Xoc) is a plant pathogen that causes bacterial leaf streak [BLS] in rice. A critical component of pathogenesis is the injection by Xoc of transcription activator-like [TAL] effectors into host cells. TAL effectors are DNA binding proteins that can function as virulence factors by activating host genes called susceptibility (S) genes that are important for pathogenesis. Most TAL effectors are composed of an N-terminus required for secretion, a C-terminus containing nuclear localization signals and an acidic activation domain, and a central domain containing a variable number of 33-34 amino acid repeats. DNA-binding specificity is derived from the repeat variable diresidue [RVD] found at positions 12 and 13 in each repeat. Each RVD specifies a single binding-site nucleotide, in a linear fashion.

Dr. Bogdanove’s laboratory showed that Tal2g from Xoc strain BLS256 activates a sulfate transporter gene [SULTR], which acts as an S gene in rice cultivar Nipponbare. One strategy for developing disease resistant plants is to breed or engineer S gene alleles that cannot be bound and activated by the corresponding TAL effector due to sequence differences in the gene promoter. But targeting of an S gene by multiple TAL effectors, or the presence of other genes in the host genome that could substitute for the S gene if targeted, could make this approach difficult or ineffective. During my time in Dr. Bogdanove’s lab, I have worked on two projects with the same overall goal of identifying how easily Xoc might overcome a promoter mutation that prevents activation of the sulfate transporter S gene. My first objective was to determine whether any of the six other closely related SULTR genes in rice can act as an S gene in place of the originally identified one. This would suggest that Xoc could overcome the knockout of the previously identified SULTR by acquiring a TAL effector that targeted one of the six substitutable SULTR genes. My second objective was to predict whether multiple TAL effectors from the pathogen are targeting the same S gene. This would suggest that Xoc is redundantly targeting S genes and that it is necessary take this into account when designing resistant rice varieties. **Warren 101; 10:42AM-10:54AM**

**Structural studies of the Pannexin 1 C-terminal domain**

**Anahi Higuera, Toshimitsu Kawate**

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Pannexin 1 (Panx1) is a plasma membrane ion channel that plays important roles including ATP release. Ubiquitously expressed throughout the human body, Panx1 has been a drug target for treating devastating conditions such as arthritis and memory loss from stroke. The C-terminal domain (CTD) of Panx1 has been proposed to control its gating, however, the underlying mechanism remains unclear. To understand how the Panx1 CTD functions, we sat out to elucidate the three-dimensional structure of the protein using crystallography. To evaluate the behaviors of the human Panx1 full length and a deletion of the frog-Panx1, we expressed these constructs in BL21 bacterial cells, purified them using affinity chromatography, and assess the stability and monodispersity using biochemical techniques. While SDS-PAGE and Western blot suggested that we successfully obtained some Panx1-CTD, size-exclusion chromatography (SEC) showed signs of aggregation and a low expression. We tried to stabilize the Panx1-CTD by reducing agents and glycerol, however, almost all the protein remained aggregated. These results suggest that the human Panx1-CTD and frog Panx1-CTD are prone to aggregation upon purification. Nonetheless, if we overcome this issue, we might be able to obtain enough amount of proteins for crystallization trials. **Warren 137; 10:18AM-10:30AM**

**Effects of endoplasmic reticulum associated degradation dysfunction using CRISPR genome editing**

**Merry Huang**

*Qi Lab, Department of Nutritional Sciences*

The endoplasmic reticulum (ER) is a membrane bound organelle inside of the cell. Secretory proteins initiate translation in the cytosol before being translocated into the lumen of the ER. Once in the luminal side, nascent proteins undergo proper folding into their functional conformation before exiting the ER and moving to the golgi apparatus for further processing. Protein folding is critical for protein function and misfolded proteins can form detrimental aggregates. To combat the deleterious effects of protein misfolding, cells have ER homeostatic systems including Endoplasmic Reticulum Associated Degradation (ERAD) and Unfolded Protein Response (UPR). In ERAD, misfolded proteins are recognized by adaptor protein Sel1L, which brings them to the ER membrane protein Hrd1. This E3 ligase ubiquitinates and translocates the misfolded proteins to the
cytosol for degradation by the 26S proteasome. Os9 is a lectin protein that is also involved in the process of ERAD and identifies misfolded proteins. UPR consists of three ER transmembrane proteins including PERK, Ire1α, and ATF6. The downstream pathways of these proteins act to halt protein translation, increase the production of chaperone proteins, and induce cell apoptosis during unsalvageable ER stress conditions.

ERAD and UPR act together to manage ER stress and to facilitate the removal of misfolded proteins. Dysfunctional components in ERAD have been shown to increase UPR activation. This is explained by increased ER stress during the ablation of the ERAD function of translocating misfolded proteins to the cytosol for degradation. Therefore, defects in ERAD may have an impact on UPR.

CRISPR (clustered regularly interspaced short palindromic repeats) genomic editing technology uses the bacterial natural defense system of silencing foreign genetic material through cleavage of gene sequences by endonuclease Cas9. A recombinant plasmid containing sequences for Cas9, its required guide RNA, and the targeted sequence for deletion can be transfected into mammalian cell lines and used to silence specific genes. Therefore, CRISPR technology has clinical applications in gene therapy. CRISPR genomic editing was used to create components of ERAD knockouts in HEK 293 FT cells and the effects on UPR were analyzed.

**Development of enzyme-linked Immunosorbent Assay (ELISA) for diagnosing Theiler’s Disease Associated Virus (TDAV) infected horses**

**Mu Ji Hwang**, Weiwei Yan, Yung-fu Chang  
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*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine*

Theiler’s disease is a viral hepatitis that affects horses and is often associated with serum sickness. Although the condition has been documented for over a century, the etiological agent for the disease, Theiler’s Disease Associated Virus (TDAV), has only recently been found through deep sequencing. Due to decreased risk of mortality with early treatment, developing an efficient diagnostic tool is of critical importance to increase the odds of survival for horse livestock. Currently, however, no such serological diagnostic tool is available. In this study, we developed an Enzyme-Linked Immunosorbent Assay (ELISA) using TDAV NS5A recombinant protein as the antigen.

The ELISA technique is an ideal diagnostic tool due to its high sensitivity to antibodies in infected animals, relative promptness leading to results within a day, and the non-invasive nature of the test utilizing serum routinely drawn from animals. To develop the technique, several conditions of the indirect ELISA assay was optimized, which included: the blocking buffer utilized, concentration of antigen plated, and dilution of primary and secondary antibody. Thereafter, the optimized indirect ELISA was used to evaluate 300 horse serum samples to determine the cut-off value for immune horses.

In addition, four horses, previously thought to be uninfected, were challenged with TDAV. Subsequently, every week following the challenge, for a period of three months, serum was drawn and an indirect ELISA assay was performed. Results indicated that a period of 3 months was insufficient for significant antibody production as three of the four horses showed no statistically significant changes in antibody titer. One of the horses, however, consistently showed a titer indicating previous exposure to TDAV.

**Translocator protein/peripheral benzodiazepine receptor deficiency exacerbates injury in a mouse model of acute colitis**

**Isabel A. Jimenez**, Allison P. Stilin, Mahmoud Hassan, Kanako Morohaku and Vimal Selvaraj  
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Ulcerative colitis is a form of inflammatory bowel disease of unknown etiology that causes continuous superficial ulceration of the distal colonic mucosa. In both human and animal models of ulcerative colitis, the translocator protein (TSPO), a highly conserved mitochondrial membrane protein, is significantly upregulated in the colonic epithelium. Although its function remains unclear, TSPO expression has been associated with cell proliferation and apoptosis. In this study, we used TSPO gene-deleted (TSPO<sup>−/−</sup>) mice to examine the role of TSPO in the onset and progress of acute colonic inflammatory pathology. Acute ulcerative colitis was induced in TSPO<sup>−/−</sup> and TSPO floxed (TSPO<sup>fl/fl</sup>) cohorts by providing 2.5% dextran sodium sulfate in drinking water, *ad libitum*, for 7 days. TSPO<sup>−/−</sup> mice showed exacerbated clinical signs of acute colitis compared to TSPO<sup>fl/fl</sup> mice, exhibiting severe rectal bleeding, hunched posturing, limited movement and body weight loss. Gross pathological examination showed significant shortening of the colon in TSPO<sup>−/−</sup> compared to TSPO<sup>fl/fl</sup> mice. Histopathological examination of the colonic epithelium showed increased ulceration, crypt and goblet cell loss, and immune infiltration in TSPO<sup>−/−</sup> compared to TSPO<sup>fl/fl</sup> mice. These results suggest that TSPO expression limits epithelial damage and decreases inflammation in acute ulcerative colitis.
In vitro assembly of Rous Sarcoma Virus Gag AMBD protein

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A retrovirus is an enveloped virus that replicates in a host cell as an obligate parasite, through the process of reverse transcription. Rous Sarcoma virus (RSV) is a well-studied chicken retrovirus that has become a model for the study of the human immunodeficiency virus (HIV). Research in our lab focuses mainly on the function of Gag, the multidomain structural protein of RSV, including the way it interacts with the plasma membrane and its contribution to retroviral assembly. After virus particles budding and pinching off, maturation follows, during which the Gag protein is cleaved into three domains: MA, which is the membrane binding domain; CA, which forms the protein capsid of RSV; and NC, which binds to the retroviral genome. Assembly of purified Gag protein into virus like particles (VLPs) can occur in vitro under specific buffer conditions.

In a predicted model of Gag-Gag interaction, a short stretch of Gag protein between CA and NC called the SP assembly domain interacts intermolecularly to form a hexameric bundle. The goal of my project is to provide more evidence for the six-helix bundle model. Results from recent studies show that in vitro assembly of retrovirus-like particles requires nucleic acid oligos, and that the role of nucleic acid is probably to help induce Gag dimerization. Nucleic acid-independent retroviral assembly can also be driven by other conditions that trigger dimerization. I am planning on expressing a series of Gag proteins, which include the C terminal part of MA, the whole CA, and SP which extends into the NC domain (ΔMBD-Gag-SP), with mutations that may lead to dimerization. SP is predicted to form a helix that stretches six to eight amino acids into NC. It is predicted that the fifth and the sixth amino acid, which are R and E respectively, have the ability to form an intermolecular salt bridge, and that the following two residues R and D may stabilize the six-helix bundle. I have cloned and purified versions of ΔMBD-Gag-SP that extend through 6 or 8 residues of NC. I have also purified protein with cysteine mutations at amino acid positions 5 and 6 of SP to test the salt bridge model by cysteine crosslinking. Purified protein will be dialyzed in buffers that differ in pH and salt to induce virus-like particle formation. Formation of VLPs will be screened for using electron microscopy.

Using the CRISPR/Cas9 system to study Cdk9 at Drosophila Hsp70

Lydia Lam, Martin S. Buckley and John T. Lis
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Within the past few years, advancements have been made to adapt a bacterial immune response that utilizes clustered regularly interspaced short palindromic repeats and the associated protein 9 (CRISPR/Cas9) for RNA-guided genome editing and mutation. This system can be further modified to study DNA-protein interactions at specific loci by mutating Cas9 and removing its nuclease activity (dCas9). The two part RNA-protein system utilizes RNA-DNA interactions to achieve its sequence specific targeting abilities.

We are currently using the CRISPR/Cas9 system to observe the function of Cdk9 as a transcriptional activator on Drosophila Hsp70. We want to know whether it is possible to activate the Hsp70 gene solely with P-TEFb recruitment or if additional factors are required. To address these questions, our overall approach is to employ the RNA-guided recruitment of dCas9 fusion proteins (dCas9 alone, dCas9:GFP, and dCas9:Cdk9) to specific locations of Hsp70. We are using both fly cell lines and transgenic flies to preform co-transfections and crosses respectively. The lone dCas9 and dCas9:GFP constructs will serve as negative controls for the system. Additionally, dCas9:GFP will be used to visualize the localization of the RNA:dCas9 complex within the cell or fly. The dCas9:Cdk9 fusion will be our positive control where successful expression and recruitment to Hsp70 should result in transcriptional activity even under non-heat shock conditions. Cdk9’s influence on transcription will be tested by assaying Hsp70 mRNA using qRT-PCR. In addition, dCas9:GFP will be used to examine the localization of the fusion protein on polytene chromosomes. We will be optimizing the guide-RNA portion of the two part RNA:protein system to increase efficiency of expression.

Structural study of Pannexin 3 C-terminal domain

Maria Lammoglia
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Pannexins (Panxs) are emerging potential pharmaceutical targets for alleviating arthritis, epileptic seizures, memory loss from stroke, and proinflammatory response in cancer. Panxs are membrane channels that regulate intracellular ATP/cAMP concentrations by controlling the release of ATP. Previous studies suggest that this ATP releasing activity may be controlled by the C-terminal domain (CTD). However, little is known about the mechanism of how the CTD regulates the channel activity. To gain structural and mechanistic insights into the CTD function, we sat out to solve the atomic resolution structure of this domain using X-ray crystallography. In these experiments, we decided to work with different constructs of human and zebra fish Panx3 (hPanx3 and zfPanx3). We transformed and induced the protein production in Escherichia coli BL21 under
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optimized conditions, sonicated the cells, and purified them using a Strep-resin elution column. SDS-PAGE, Western blot, and size-exclusion chromatography were used to assess the stability and monodispersity of our target proteins before proceeding to crystallization. An alternative hPanx3 construct covalently fused to a green fluorescence protein (GFP) was also screened with fluorescence-detection size-exclusion chromatography (FSEC) to evaluate its monodispersity, expression level, and approximate molecular size. Both hPanx3 and zfPanx3 showed single and symmetrical Gaussian peaks on SEC traces, which suggest these proteins are stable in solution. However, the yield and quality of the purified Panx3 turned out to be poor, as indicated by the degraded bands on the Western blot and polydisperse elution pattern on SEC. With the information acquired, we consider hPanx3 and zfPanx3 to be good candidates for structural studies because of their monodispersity, but further experiments are needed to establish the conditions that prevent aggregation. Once this is achieved, the proteins would be ready to undergo crystallization, which may in turn provide the first atomic resolution structures through X-Ray crystallography.

Warren 137; 10:30AM-10:42AM

Characterization of the role of Tec Kinase Itk and Btk in eosinophil function
Karen Larios, Kindra Stokes and Avery August
August Lab, Department of Microbiology and Immunology

Asthma is a chronic inflammatory condition of the airways characterized by mucus secretion, bronchoconstriction and remodeling. The cells that orchestrate this process include eosinophils, mast cells and lymphocytes. Formerly, eosinophils were considered terminals effector cells in airway allergic diseases, however more recent findings suggest a broader view of the function of these cells. Eosinophils are leukocytes that are produced in the bone marrow from CD34+ progenitor cells. They are involved in the defense against parasites, but are best known for their role in allergic asthma by modulating T cell recruitment and remodeling in the lung, in part by their production of Th2 cytokines (Interleukin (IL) -4, -5 and -13). These mediators orchestrate the recruitment of inflammatory cells to the lung, and subsequent development of inflammatory allergic asthma. However, the signals regulating the activation of these cells are not well known. In this study we examined the role of two Tec kinases, Interleukin-2 inducible T cell kinase (Itk) and Bruton’s tyrosine kinase (Btk), which regulate T cell and B cell activation, differentiation and survival, respectively. The aim of this study is to characterize the role of Itk and Btk in eosinophil cytokine and chemokine signaling. We found that there was no significant difference in development of eosinophils or expression of surface markers between WT, Itk-/-, and Itk/Btk-/- eosinophils. Thus, Itk and Btk are not required for eosinophil development. Experiments comparing the ability of WT eosinophils, Itk-/-, and Itk/Btk-/- eosinophils to migrate will also be discussed. Warren 137; 10:42AM; 10:54AM

Investigating the role of Sel1L in brown adipose tissue
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Qi Lab, Division of Nutritional Sciences

Brown adipocytes in brown adipose tissue (BAT) are packed with mitochondria and contain uncoupling protein-1 (UCP1) that short circuits the electro chemical gradient in mitochondria and enables rapid heat production (thermogenesis) by combustion of available substrate. Recent studies suggest BAT as a desirable site to treat obesity and metabolic diseases. However, the regulation of thermogenesis and metabolism in brown adipose tissue is not fully understood. We recently discovered an indispensable role of Sel1L for lipid storage in white adipocytes by regulating lipoprotein lipase (LPL) secretion. Here we further investigate the function of Sel1L in brown adipocytes. Interestingly, Sel1L deficient brown adipose tissue demonstrated lighter color than the brown color of BAT in wild-type (WT) mice. Indeed, brown adipocytes with Sel1L deficiency contain larger lipid droplets than WT ones, suggesting less efficiency of lipid burning in the absence of Sel1L. TEM analysis further indicated aberrant mitochondria dynamics in Sel1L-knockout brown adipocytes. It has been shown that hormone-induced mitochondrial fission is an amplification pathway for energy expenditure in brown adipocytes. However, Sel1L-deficient brown adipocytes demonstrated increased mitochondria fusion and distorted cristae, where the UCP1 protein locates. Moreover, key genes responsible for BAT function including PGC1α and UCP1 were greatly reduced in Sel1L-knockout BAT. These findings suggest that Sel1L may play an important role in regulating BAT function by controlling mitochondrial dynamics and thermogenetic efficiency. Further studies are required to elucidate the underlying mechanisms, which may lead to a discovery allowing stimulation of BAT to treat obesity and metabolic syndromes. SILS-Qatar Symposium, August 6th, Mann Library 100, 4:05 PM
ARID1A: A potential driver in breast cancer tumorigenesis in the Chaos3 mouse model

Caroline Maskin, Nithya Kartha

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ARID1A is a gene that encodes a protein in the SWI/SFI chromatin-remodeling complex and is commonly found mutated in a variety of cancers. In other studies, ARID1A has been identified as a bona fide tumor suppressor; however, the molecular mechanisms by which it works are not yet known. Importantly, ARID1A appears to exhibit tissue-specific behavior, and has not yet been studied extensively in the context of breast cancer. I am studying the tumor suppressive role of ARID1A, and the specific ways in which it contributes to tumorigenesis in both the Chaos3 mouse model and in human immortalized cell lines.

A stable sh-RNA knockdown of ARID1A in human immortalized mammary epithelial cells was generated. From this I was able to examine gene expression changes of some of the targets of ARID1A. Additionally, gene expression levels were examined in cell lines derived from mouse mammary tumors from our C3H-Chaos3 mouse mammary tumors, known to be deficient for ARID1A, in order to compare the two systems. The Chaos3 mouse model has a very high incidence of mammary tumors, and in a prior experiment, I verified through q-RT PCR that these mice contain a heterozygous deletion in the portion of the genome containing ARID1A. From these gene expression assays, several genes (CDKN1A, SMAD3, TRIM8, and p53) have been picked for further analysis in the future. We also were able to overexpress ARID1A into previously ARID1A-deficient mouse tumor cell lines following lentiviral transduction. After verifying the level of ARID1A mRNA in this line, a cell proliferation assay was performed using BrdU incorporation to compare the number of cells in S-phase between an untreated cell line and the transduced cell lines. From this, we have shown that cell lines that overexpress ARID1A grow more slowly than untreated tumor cells. Finally, an ongoing study has been to explore the epigenetic changes that may be occurring within our tumor cells to cause silencing of the remaining ARID1A allele. Prior studies examining ARID1A gene expression have shown that promoter hyper-methylation often contributes to decreased gene expression levels. I am currently using bisulfite conversion to characterize the methylation status of the ARID1A promoter in tumor cells bearing one mutated allele of the gene. In the future, ARID1A has the potential to be a target for gene therapy. ARID1A-deficient breast tumors tend to convey poor prognosis, and so developing therapies that target ARID1A may help improve patient life expectancy. Warren 137; 3:02PM-3:14PM

Exploration of a dominant-negative single nucleotide polymorphism in the human Toll-like receptor 5 gene

Cameron McConkey, Cynthia Leifer and Ruth E. Ley

Ley Lab, Department of Molecular Biology and Genetics and Department of Microbiology, Leifer Lab, Department of Microbiology and Immunology

Innate immune receptors, such as Toll-like receptors (TLRs), play a key role in recognizing microbial infection; however, more recently they have been implicated in intestinal homeostasis. TLR5 is the receptor for bacterial flagellin and is involved in the production of anti-flagellin antibodies. Moreover, recent studies have shown that mice deficient in TLR5 develop metabolic syndrome that may be linked to their lower than normal amount of anti-flagellin antibody and alterations in the microbiome. In humans, there is a frequently observed single nucleotide polymorphism (SNP) that introduces a premature stop-codon near the 3’-end of the flagellin-recognition domain, resulting in a truncated protein lacking the signaling domain. Individuals heterozygous for this TLR5 SNP have reduced response to flagellin, low levels of anti-flagellin antibody, and may be more likely to have metabolic disease. It is assumed that a single copy of the SNP generates a TLR5-null-like phenotype because TLR5 binds flagellin as a dimer and introduction of one non-signaling copy of the protein into the dimer will allow flagellin binding, but eliminate signaling potential. However, the assumption that the SNP results in a dominant-negative phenotype has not been directly tested. Therefore, we hypothesize that expression of the SNP-mutant version of TLR5 will associate with the wild-type protein and reduce signaling in response to flagellin. We observed that wild-type TLR5 transplanted into human embryonic kidney cells, which do not express TLR5, endowed the cells with the ability to respond to Salmonella typhimurium flagellin. Furthermore, co-expression of the SNP-mutant TLR5 reduced response by wild-type TLR5. Future studies will use epitope-tagged receptors to test co-localization of the mutant TLR5 with wild-type TLR5, and investigate the ability of the mutant receptor to directly associate with wild-type TLR5. By studying the mechanistic consequences of genetic variability in the human immune system, I hope to contribute to a broader understanding of the evolution of the host-microbial interface. Warren 101; 3:02PM-3:14PM

Measuring the brightness of the abyss: developing a local information depth algorithm

Rodrigo A. Morales Mendoza, Kedarnath P. Vilankar, James R. Golden, Damon M. Chandler and David J. Field

Fields Lab, Department of Psychology

This study explores the relationship between the statistical structure of our visual environment and the properties of neurons in the visual pathway. In the last 30 years, a wide range of insights into visual coding have developed from our understanding of the scenes we typically encounter (natural scenes). Here we investigate the statistical properties of occluding edges (edges formed when one region of an object occludes another object). The results here provide important insights into how the
Investigating the expression of serotonin receptor 5HT1A on V2a interneurons and motor neurons before and after spinal cord injury in the upper lumbar mouse spinal cord

Natalia Mesa
Harris-Warrick Lab, Department of Neurobiology and Behavior

In vertebrates, coordinated rhythmic movements, such as flying, walking, chewing, and breathing are generated by complex neural networks known as Central Pattern Generators (CPGs). The mammalian hindlimb central pattern generator, in particular, is located in the upper lumbar region of the spinal cord and is involved in generating locomotion. The CPG generates rhythmicity, alternating limb extension and flexion, and right-left alternation of the limbs even in the absence of descending input from the brain, or sensory input. Though the CPG can generate a rhythmic output in the absence of descending input, it is still essential for its proper function. After spinal cord injury (SCI), much of this neuromodulatory input disappears, and the neurons of the CPG make compensatory changes, both in modulator sensitivity and structural organization. In particular, a class of interneurons known as V2a interneurons, which are involved in alternation of the limbs at high speeds, become approximately a thousand-fold more sensitive to the neuromodulator serotonin after SCI. Motor neurons also become hypersensitive to serotonin after SCI. Our lab has been further studying one of the potential causes of these changes: the effect of spinal cord injury on a specific inhibitory serotonin receptor type, the 5-HT1A receptor. Double immunohistochemistry was performed on mouse spinal cord segments isolated from the upper lumbar region in an effort to visualize and quantify the expression of this receptor on V2a interneurons and motor neurons. Thus far, evidence shows that the receptor is downregulated on the V2a interneurons, and the number of 5-HT1A positive cells decreases after SCI. However, the amount of 5-HT1A throughout the spinal cord doesn’t change, indicating that plastic changes after SCI may vary by cell type.

Foraging behavior of Manduca sexta on three wild tobaccos
José Manuel Sevenello Montagner, Rainee Kaczorowski and Robert A. Raguso
Raguso Lab, Department of Neurobiology and Behavior

Olfactory and visual floral cues are important for the foraging choices of pollinators such as hawkmoths. In particular, Manduca sexta, a large, nocturnal hawkmoth species, exhibits preferences for flowers with certain visual traits, such as white or pale color, long, narrow corolla tubes, wider corolla limbs and upright orientation. They can be found feeding on Nicotiana plants throughout the Americas. In this work, we were interested in M. sexta foraging behavior on two wild Nicotiana species; Nicotiana alata (white flowers with long corolla tubes), N. forgetiana (red flowers with short corolla tubes), and a horticultural hybrid N. x sanderae (red flowers with long corolla tubes). We analyzed the different phenotypic aspects of flower morphology, nectar, smell and color from these species and found that, in each of the phenotypic aspects, the three species are different.

In order to know how the different Nicotiana species influence the foraging behavior of the pollinator we set-up an experiment in which each trial involved the use of one naïve moth placed inside a large cage, a Nicotiana plant was placed in the center of the cage and the moth was allowed to fly freely and to feed from the flowers. Each trial was recorded and used to analyze the response rate, number of probes, success rate, latency (time elapsed from initiating flight to first probe) and the time from...
Sevenello Montagner et al. contd.

initial probing of a flower to the entry of the proboscis into the nectar tube (discovery). We chose these variables because they are indicative of how attractive the plant is and whether moths could access the nectar of the different flower morphs. We found that moths were more attracted to *N. alata* than the other 2 species, because they showed the highest response rate and number of visits, and the lowest latency time. Furthermore, the highest success rate was registered for *N. alata* which indicates greater nectar accessibility; however, when moths have learned how to access the nectar, the discovery time does not differ between *N. alata* and *N. x sanderae*. Together, these results show that *M. sexta* is more attracted to the phenotypic characters of *N. alata* than the other two species, suggesting that those floral characters have evolved to large moth pollination. Warren 137; 1:30PM-1:42PM

**Efficacy of an intelligent tutoring system on nutrition and fitness enhanced by Fuzzy-Trace Theory**

Kate Morant  
Reyna Lab, Department of Human Development

Obesity is a major problem in the United States, with more than half of adults overweight or obese in 2014 and increasing numbers of both adults and children for the past decade. We created an Intelligent Tutoring System (ITS) using Sharable Knowledge Objects (SKO) to teach women about obesity prevention and tested it in a randomized controlled experiment.

SKO is a computer-based system using artificial intelligence techniques to mimic one-on-one human tutoring. A talking avatar presents information orally and in text, graphics and video. The avatar also converses with people, responding to what they type and processes users’ verbal input using latent semantic analysis (LSA).

Fuzzy-Trace Theory is a theory of memory and decision making that has been extended to interventions to increase extraction of important information and long-term retention of bottom-line meaning. FTT was extended to nutrition and fitness by enhancing a tutorial called Eatfit to emphasize the gist of information (as opposed to verbatim). Gist and verbatim representations are formed in parallel during information acquisition, but when making decisions, people prefer to reason with the most vague gist that can be used to decide among options. This preference actually increases with expertise and experience, suggesting that providing the details about nutrition and exercise are not enough to change thought processes and, ultimately, behavior.

Female college students were randomly assigned to gist-enhanced EatFit or a control tutorial. Each curriculum took about 1.5 hour followed by a 1.5-hour survey. The survey included measures of knowledge, gist principles, gist comprehension, attitudes, and intentions for healthy behavior about nutrition and fitness. Results showed that subjects engaged in the tutorial scored significantly better on knowledge, gist comprehension, and gist principles measures than those who failed to engage. Subjects assigned to the gist-enhanced Eatfit condition outperformed control on gist knowledge about nutrition and verbatim knowledge. They also increased endorsement of healthy gist principles about nutrition, fitness, and lifestyle, in addition to attitudes about fitness and intentions about nutrition.

These results are consistent with both the efficacy of the SKO and with Fuzzy-Trace Theory. Intelligent tutors, such as Eatfit, are scalable, cost-effective methods of helping people understand complicated topics and improve health-related decision-making. Warren 101; 1:18PM-1:30PM

**Ribosomal decoding and reading frame maintenance**

Basem Oraby, Xiangwei Gao, Ji Wan and Shu-Bing Qian  
Student of Weill Cornell Medical College in Qatar  
Qian Lab, Division of Nutritional Sciences

In the past, research has been done on the importance of the ribosomal P-site in the maintenance of the proper reading frame required for the production of the desired protein. We hypothesize that 5’ end codons of ribosome-protected fragments are also crucial in preventing frameshifting. In order to test this hypothesis, we engineered plasmids containing *Renilla* and firefly luciferases. The firefly luciferase was inserted into the plasmids as an internal control in order to normalize the *Renilla* signal against the firefly signal, correcting for differences in transfection efficiency. We inserted various sequences upstream of the *Renilla* luciferase, at different reading frames (frame 0, 1 and 2). We hypothesize that different sequences make the ribosome more or less susceptible to frameshifting. We transfected mammalian cells with the engineered plasmids and are currently conducting dual luciferase assays. We will be looking at the *Renilla*/firefly ratios to determine whether the different inserted sequences affect the fidelities and frames of the *Renilla* luciferase. The study will help us elucidate the mechanisms underlying the ribosome decoding process. SILS-Qatar Symposium, August 6th, Mann Library 100, 4:20 PM
Isolation and measurement of the procoagulant activity of tissue factor-expressing exosomes and microparticles from a breast cancer cell line

Jeannie Yoojin Park, Sara Che and Tracy Stokol

Stokol Lab, Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine

Tissue factor, overexpressed in breast cancer samples and cell lines derived from breast tumors, is associated with tumor progression and cancer metastasis. By binding to and activating its enzymatic partner factor VII, tissue factor can initiate coagulation and signal through surface receptors. We are interested in tumor-derived microvesicles, which express surface proteins characteristic of the cell they originate from. Two previously identified types of microvesicles that differ in size and biogenesis are microparticles (100nm-1µm) and exosomes (<100nm). Our studies focused on a tissue factor-expressing cancer cell line that releases microvesicles expressing tissue factor into the culture supernatant. Our goal was to develop a method to separate the two types of microvesicles and characterize their procoagulant potential.

We obtained tumor-conditioned media (TCM) from MDA-MB-231, a breast cancer cell line with high tissue factor expression. We used two different methods to separate the microparticles and the exosomes from the TCM: centrifugation and filtration. We characterized the size of the isolated microvesicle samples using dynamic light scattering (DLS) and tested the tissue factor activity of these microvesicles by measuring factor X (FX) activation (in presence of activated factor VII) and thrombin generation (in full plasma).

The DLS results indicated that the TCM contained both microparticles and exosomes. Based on the DLS data, centrifugation isolated microparticles better than filtration, with the exosome fraction from filtration being more contaminated with microparticles than the centrifugation fraction. For both methods, the exosome samples generated more activated FX and thrombin than the microparticles.

Currently, centrifugation seems like a more promising technique to separate the microvesicles. In future studies, we will use this method to isolate microparticles and exosomes to investigate how these cancer-derived particles affect endothelial cell function and behavior.

Unraveling complexity in plant immune signaling networks

Phoebe Parrish, Suma Chakravarthy, and Alan Collmer

Collmer Lab, Department of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science

Over the course of millions of years, plants and their bacterial pathogens have evolved complex repertoires of interacting defense and attack molecules. Plants recognize microbe-associated molecular patterns (MAMPs), which are conserved microbial features, and mount pattern-triggered immune responses, which are effective against most microbes. Major microbial virulence factors include effector proteins that bacteria inject into plant cells to defeat defenses and promote growth. Plants detect effectors with resistance proteins, leading to strong effector-triggered immune (ETI) responses that often lead to hypersensitive death, or HR.

A model system for plant-microbe interactions involves a disease caused by Pseudomonas syringae pv. tomato DC3000 in tomato plants. An important P. syringae effector is HopM1, which is widespread among P. syringae pathovars and is believed to be a conserved, ancient effector. Its plant target is MIN7, a protein that regulates vesicle trafficking, a crucial part of the immune response. However, destabilization of MIN7 alone does not lead to ETI, and there is no known R gene to detect HopM1.

Another effector, which is variable and not as widespread among P. syringae pathovars is AvrPto, which targets MAMP perception. Plant resistance proteins recognize such variable effectors and elicit ETI.

Past research in the Collmer Lab and elsewhere has led scientists to hypothesize that cell death caused by HopM1 is fundamentally different from ETI elicited by variable effectors. In the case of HopM1, ETI is triggered by damage to a process, rather than a specific protein. This suggests that plants may default to HR when crucial immune proteins and processes are disrupted. However, it has been hypothesized that plants require a second signal (such as MAMP perception) to reduce spurious cell death in the event of blocked vesicle trafficking.

To test these hypotheses, we silenced the MIN7 gene in Nicotiana benthamiana, mimicking the action of HopM1. We challenge-inoculated the MIN7-knockdown plants with DC3000D29E, a functionally effectorless strain of the bacterium that still contains flagellin, a major MAMP. We also inoculated with D29E ΔfliC, which lacks flagellin. Our results suggest that
**Ring and limited in capacity. However, recent research suggests increasing attention to one task enhances performance in a second, unrelated task. In the attentional boost effect, participants are asked to perform two tasks at once. For one task they memorize a series of pictures for a later memory test. For the other task they monitor a series of unrelated but concurrently presented stimuli for occasional targets (e.g., a low tone rather than a high tone). Despite the fact that targets require more attention than distractors, pictures that are presented at the same time as a**
target are better remembered than those presented with distractors. In this study we investigate whether this enhancement also occurs for rewarding stimuli. We ran participants through 3 different tasks. In the first task, participants learned to associate positive and negative monetary rewards with different colors. In the second task participants performed a detection task on colored shapes as they also encoded a series of faces into memory. Critically, half the shapes were presented in a rewarding color, and half were presented in a neutral color. The third task measured memory for faces presented during the target detection task. Despite limitations in attention, memory for faces presented with targets is enhanced.

Results indicated that participants learned the colors that were rewarding. The data also replicated the attentional boost effect: Faces that were presented with a target were better remembered than those presented with a distractor. However, there was no evidence that memory for faces presented with rewarding colors was enhanced relative to those presented with a neutral color. This was true when the rewarding color was in a target shape or a distractor shape. Combined with earlier studies that used explicit monetary reward, these data suggest that the value of an item is likely to have a limited effect on memory for concurrent pictures.

Gutiérrez Gómez Rueda et al. contd.

Understanding the development and organization of the lymphatic system in the midgut

**Abigail Shilvock**, Aparna Mahadevan and Natasza Kurpios

*Kurpios Lab, Department of Molecular Medicine*

The lymphatic vascular network of the intestines plays a critical role in regulating fluid homeostasis, immune surveillance, and absorption of digested fat, through specialized lymphatic vessels known as lacteals. Furthermore, this dense lymphatic network serves as a primary route for metastasis of colorectal cancer. Despite the paramount importance of the lymphatic vasculature, very little is known about it due to the technical difficulties associated. While, significant advancements have been made in the past decade in identifying markers that are expressed on lymphatic vessels, such as Prox1, VegfR3, Lyve1 and Podoplanin amongst others, the origin of intestinal lymphatic network and the mechanisms underlying its development are yet to be elucidated.

Preliminary research in the lab has shown that arterial development is first initiated in the left side of the dorsal mesentery (DM), a structure that supports the intestines and serves as the conduit for all vascular supply to and drainage from the intestines. This process is regulated by the Cxcl12-Cxcr4 chemotactic axis downstream of Pitx2, a key transcription factor involved in left-right organogenesis. Interestingly, the lab also showed that lymphatic development is also initiated in the left DM dependent on and secondary to the formation of arterial vasculature.

The goal of my project is 2-fold; Firstly, I will be developing methods which will enable us to visualize the lymphatic endothelial cells with greater cellular resolution. To that end, I have been performing RNA in-situ hybridization on sectioned chicken embryonic tissue with lymphatic markers, and optimizing this technique. In addition, I have also been doing immunohistochemistry on sectioned mice embryonic tissue with various lymphatic markers such as Prox1, Podoplanin, Lyve1, and VegfR3. Secondly, I would like to understand lymphatic defects in mice deficient for Cxcr4, a key player in establishing arterial vasculature in the intestines. Since, lymphatic development has been shown to be dependent on arterial development, I would like to further understand how lymphangiogenesis is affected in the Cxcr4-/- mice. We have recently obtained these Cxcr4 deficient embryos, and I have devised a strategy to genotype the embryos to distinguish between the wild type (Cxcr4+/+), heterozygous (Cxcr4+/-) and null (Cxcr4-/-) embryos, which will be important for characterizing the lymphatic phenotype.

Collectively, my aim is to characterize lymphatic development in the intestines with a high spatio-temporal and cellular resolution, which will help us in understanding the origin of lymphatic vasculature of the intestine.

Identifying interaction partners among membrane-associated proteins of the BMP signaling pathway in *Caenorhabditis elegans*

**Neta Shwartz**1, Herong Shi2 and Jun Kelly Liu2

1Department of Biological Sciences, Towson University
2Liu Lab, Department of Molecular Biology and Genetics

We wish to understand the levels of interaction among membrane-associated proteins in the bone morphogenetic protein (BMP) signaling pathway. BMPs belong to the transforming growth factor β (TGFβ) superfamily, and the BMP pathway regulates developmental, homeostatic, and physiological processes in multicellular organisms. Malfunction in the pathway causes somatic and hereditary disorders in humans, such as cardiovascular disease and cancer. The BMP-like Sma/Mab (DBL-1) signaling pathway regulates body size, male tail patterning, and mesoderm development in *Caenorhabditis elegans*. Using
Shwartz et al. contd.

_C. elegans_ as a model organism, previous studies have identified a number of evolutionarily conserved membrane-associated factors that modulate BMP signaling at the ligand-receptor level. We aim to learn the extent to which these proteins interact with each other by utilizing a mating-based split-ubiquitin system (mbSUS) in yeast.

The mbSUS system adapts the principle of split ubiquitin, where the N-terminal ubiquitin domain Nub and the C-terminal ubiquitin domain Cub can reconstitute a full-length ubiquitin protein only when brought into proximity via interacting proteins. The reconstituted ubiquitin in the mbSUS system is then recognized by ubiquitin-specific proteases (USP) to release the artificial transcription factor PLV (proteinA-LexA-VP16) fused to the C-terminus of Cub, which then goes into the nucleus and activates reporter gene expression.

During the summer, we have successfully generated, and confirmed by sequencing, corresponding Cub and Nub fusions for a number of membrane-associated proteins, including TSP-12, TSP-14, TSP-21 and DRAG-1. To determine whether these proteins interact with each other and with themselves, we have performed systematic pair-wise mating between the various Nub and Cub fusion constructs.

In the future, this work will be extended to test all possible interactions among the proteins of interest. Results from these studies will allow us to better understand how the different proteins modulate the BMP signaling pathway. Warren 137; 9:30AM-9:42AM

**Understanding cell fate specification of egg-laying musculature in *Caenorhabditis elegans***

Seul-E Son
Liu Lab, Department of Molecular and Cell Biology

We would like to understand the underlying mechanisms behind cell fate specification in the egg-laying muscles of _Caenorhabditis elegans_. The _C. elegans_ egg-laying apparatus is composed of three structures: the vulva, non-striated egg laying muscles, and motor neurons. The egg-laying musculature is composed of 16 cells and arises from the sex myoblast cells (SMs) of the postembryonic mesoderm M lineage. The SMs produce two distinct types of vulval muscle cells (4 VM1s and 4 VM2s) and two types of uterine muscle cells (4 UM1s and 4 UM2s). These 16 cells are non-striated or smooth muscle like cells, but they exhibit different morphology, location and function. The process of egg laying occurs due to the contractions caused when vulval muscle 2 (VM2) cells are innervated directly by motor neurons and the signal is conducted to the surrounding uterine muscle and VM1 cells through gap junctions. Very little is known about the molecular mechanisms underlying the specification of these different types of egg laying muscles. The Liu lab has previously conducted an RNAi screen and identified a number of transcription factors or chromatin remodeling factors that when knocked down, cause defects in the development of these egg-laying muscles. We aim to determine how these factors function to regulate proper development of the egg-laying muscles.

To begin these studies, we first attempted to generate _C. elegans_ strains where different types of egg-laying muscles are simultaneously labeled with different colored fluorescent protein or fluorescent proteins with different subcellular localization. These strains will allow us to observe the development of the four distinct types of non-striated muscle structures in the same animal and assess possible fate transformations among them in different mutant background. During the summer, we have successfully generated several new strains of _C. elegans_ that express specific combinations of GFP (green fluorescent protein) and RFP (red fluorescent protein) reporters via genetic crosses. We hope to use these strains to dissect the functions of the factors that we previously identified in egg-laying muscle development. Warren 137; 9:42AM-9:54AM

**Investigating the effects of phosphorylation on the Rab GTPase, Ypt7p***

Olya Spassibojko, Dante Lepore, and Ruth Collins

Collins Lab, Department of Molecular Medicine

A critical aspect required for normal cellular functioning is the highly organized and well-regulated movement of vesicles within cells. Members of the Rab protein family are involved in these mechanisms of cell growth and membrane trafficking, and play especially important roles in the process of endocytosis and secretory pathways. These proteins cycle between GTP- and GDP-bound states in order to carry out their cellular functions. Interestingly, the proteins exhibit a number of different modifications, one of which is the attachment of a phosphate group to particular amino acid residues. Bioinformatic analysis reveals a number of possible phosphorylation sites in Rab proteins, including a site in their GTP-binding domains that is highly conserved between Rabs of both yeast and humans.
**Spassibojko et al. contd.**

The *Saccharomyces cerevisiae* Rab GTPase Ypt7p has been shown to be phosphorylated in vivo, based on mass spectrometry analysis, and was chosen for study as a model for this modification. Ypt7p is involved in trafficking to the vacuolar/lysosomal system. Absence of Ypt7p results in vacuolar fragmentation, an abnormal phenotype that is a clear sign of defects in membrane trafficking. This research aims to uncover the details of the Ypt7p pathway and how phosphorylation may influence its activity. Preliminary results suggested that phosphorylated Ypt7p is not able to associate with vacuolar membranes and impacts vacuolar morphology. To understand the molecular details of this defect, Ypt7p as well as its mutant versions will be purified, and a biochemical test will be conducted to determine whether the modification interferes with the ability of the protein to bind and/or hydrolyze guanine nucleotides.

Elucidating the role of Ypt7 in yeast is also important as a model for its mammalian ortholog, Rab7. The neurological disease Charcot-Marie-Tooth Type 2B Neuropathy is associated with mutations of Rab7. Due to the conserved nature of membrane trafficking between yeast and humans, in addition to sequence conservation of the Ypt7p orthologs, we expect that elucidations of the pathway(s) will translate to a better understanding of mammalian membrane trafficking. **Warren 137; 10:54AM-11:06AM**

**Understanding cell fate specification of egg-laying musculature in Caenorhabditis elegans**

**Seul-E Son**  
*Liu Lab, Department of Molecular and Cell Biology*

We would like to understand the underlying mechanisms behind cell fate specification in the egg-laying muscles of *Caenorhabditis elegans*. The *C. elegans* egg-laying apparatus is composed of three structures: the vulva, non-striated egg laying muscles, and motor neurons. The egg-laying musculature is composed of 16 cells and arises from the sex myoblast cells (SMs) of the postembryonic mesoderm M lineage. The SMs produce two distinct types of vulval muscle cells (4 VM1s and 4 VM2s) and two types of uterine muscle cells (4 UM1s and 4 UM2s). These 16 cells are non-striated or smooth muscle like cells, but they exhibit different morphology, location and function. The process of egg laying occurs due to the contractions caused when vulval muscle 2 (VM2) cells are innervated directly by motor neurons and the signal is conducted to the surrounding uterine muscle and VM1 cells through gap junctions. Very little is known about the molecular mechanisms underlying the specification of these different types of egg laying muscles. The Liu lab has previously conducted an RNAi screen and identified a number of transcription factors or chromatin remodeling factors that when knocked down, cause defects in the development of these egg-laying muscles. We aim to determine how these factors function to regulate proper development of the egg-laying muscles.

To begin these studies, we first attempted to generate *C. elegans* strains where different types of egg-laying muscles are simultaneously labeled with different colored fluorescent protein or fluorescent proteins with different subcellular localization. These strains will allow us to observe the development of the four distinct types of non-striated muscle structures in the same animal and assess possible fate transformations among them in different mutant background. During the summer, we have successfully generated several new strains of *C. elegans* that express specific combinations of GFP (green fluorescent protein) and RFP (red fluorescent protein) reporters via genetic crosses. We hope to use these strains to dissect the functions of the factors that we previously identified in egg-laying muscle development. **Warren 137; 9:42AM-9:54AM**

**PfeT is a putative iron efflux pump under possible control by the Fe(II)-dependent peroxide stress sensor PerR**

**Tina Su, Ahmed Gaballa, Azul Pinochet Barros, John Helmann**  
*Helmann Lab, Microbiology Department*

Metal ion regulation pathways are important for cell survival, since metals such as Fe and Mn are required as enzyme cofactors and structural components of proteins. Regulation is complex and can involve cross talk between metals, such as iron, manganese, and potentially cobalt in *Bacillus subtilis*, a model Gram-positive soil bacterium. Metal homeostasis in the cell is achieved through tight regulation of import and efflux pumps, which are often under the control of repressors such as Fur, a putative peroxide induced iron efflux transporter that contains Fur and PerR binding boxes, but the exact regulation under different metal conditions is currently unknown. PfeTlacZ fusions were used in WT, Fur, PerR, and FurPerR null backgrounds grown in LB and minimal media with different metal concentrations (Fe, Mn, Co) to compare induction of PfeT. Preliminary beta-Gal data indicates that PfeT is not regulated by Fur but is possibly regulated by PerR with iron, not cobalt, induction. **Warren 101; 11:42AM-11:54AM**
Estrogen/estrogen receptor alpha (ERα) and bone morphogenetic protein (BMP) signaling pathways interact to regulate bone mass in vivo

Gina Surita1, Katherine M. Melville1, Scott Pearsall2, John Schimenti1, F. Patrick Ross3, Marjolein C. H. van der Meulen1,3
1Cornell University; 2Acceleron Pharma Inc; 3Hospital for Special Surgery
van der Meulen Lab, Mechanical/Biomedical Engineering

Osteoporosis is a prevalent bone disease characterized by decreased bone mass and increased risk of fracture. Serious health and financial implications are associated with osteoporosis. Approximately one in two women and up to one in four men over age fifty will suffer an osteoporotic fracture. Due to the increasingly aging population, projections for the year 2025 estimate that osteoporosis will be the cause of three million fractures and will total $25.3 billion per year in related costs.

At the cellular level, a diverse assembly of local and systemic signaling pathways interact to regulate bone remodeling. In the healthy skeleton, these integrated pathways work to build and maintain bone mass in response to mechanical stimuli. Osteoporosis is ultimately a result of deleterious interactions among multiple signaling pathways active in bone and their failure to respond appropriately to the loads experienced.

The estrogen/estrogen receptor alpha (ERα), the bone morphogenetic protein (BMP), and the canonical Wnt/β-catenin signaling pathways are among the most important regulators of bone mass. All have been implicated in the skeletal response to mechanical loading and have been shown to interact to regulate bone homeostasis in vitro. The exact mechanisms by which these pathways interact in vivo have yet to be firmly established.

Our lab has generated osteoblast-specific ERα knockout mice (pOC-ERαKO) to study the effects of estrogen signaling in bone cells on bone mass and in vivo mechanical loading-based adaptation. Using these mice, we have demonstrated that sex-specific differences exist in the estrogen/ERα signaling pathway’s regulation of bone mass and mediation of the response to mechanical loading in growing male and female mice. We have also shown that the compromised cortical and cancellous bone mass and architecture present in female pOC-ERαKO mice is improved by treatment with RAP-661, a soluble BMP inhibitor drug that blocks BMP signaling.

We now plan to perform β-catenin immunohistochemistry on tibia from these pOC-ERαKO experiments to examine Wnt pathway activity in response to mechanical loading and the Wnt/β-catenin pathway’s interactions with the ERα and BMP pathways. Once the β-catenin immunohistochemical staining protocol is optimized for bone, the data collected will provide valuable insight into the mechanisms involved in the cellular regulation of bone homeostasis and could have important future pharmaceutical applications.

Developing an efficient procedure to generate asymmetric vesicles

Thomas Torng
Feigenson Lab, Department of Molecular Biology and Genetics

Studies have shown phase co-existence on model lipid membranes as a function of membrane composition. However, these studies have primarily been done on model symmetric bilayers. Real physiological membranes are unlikely to have symmetric bilayers; the inner leaflet of the bilayer often has a composition distinct from the outer leaflet. While these studies have produced much insight to membrane behavior, we must now study model asymmetric bilayers as well in order to reveal more of the phase behavior of physiological membranes.

Experimentally, we can use cyclodextrin to exclusively effect lipid exchange of the outer leaflet of a vesicle to generate asymmetric vesicles. Cyclodextrin is a ring of sugars with a polar exterior and a non-polar interior cavity that is able to solubilize lipids. To catalyze lipid exchange of the outer leaflet with fine control, I am attempting to develop a procedure to prepare a donor solution of cyclodextrin loaded with lipids that when mixed with a suspension of vesicles will transform the vesicles into asymmetric ones of a desired composition.

Monte Carlo simulations can model the phase behavior of cell membranes

Thomas Torng
Feigenson Lab, Department of Molecular Biology and Genetics

Lipid membrane phase behavior can be explained by a competing-interactions model. Monte Carlo simulations have shown that line tension tends to produce distinct co-existing phases, while membrane curvature and electrostatic interactions tend to break up phases, thus producing a uniform phase morphology.
Torg et al. contd.
However, the full parameter space of this simulation has not been explored. My project focuses on trends in membrane phase behavior as parameters are changed. Data from this exploration will then ideally be matched to experimental data.

There are various phenomena that must be considered when interpreting simulations, including renormalization. Due to how the membrane is modeled in a simulation, parameters such as line tension do not scale simply as coarse-graining is applied. Renormalization is required in order to account for this consequence. Warren 137; 11:05AM-11:30AM

Pre- and post-wean early life social environments influence OTR density in male prairie voles
Jennifer J Trejo, George S Prounis, Lauren Foley, Asad Rehman and Alexander G Ophir
Ophir Lab, Department of Psychology

The quality and composition of the social environment during development can impact how the brain is organized, which can influence the developmental trajectories of animals. Parent-offspring interactions provide the principle social experiences for developing young. Social experiences during adolescent development may also profoundly influence the brain and behavior. Studies on prairie voles (Microtus ochrogaster) have shown that social and spatial memory, predisposition for affiliation, and parental care all appear to influence reproductive decisions of adults. These behaviors are largely mediated by the mammalian neuropeptides: Arginine Vasopressin (AVP) and Oxytocin (OXT), and their receptors (V1aR and OTR). Our research is focused on a possible neurobehavioral phenotype shaped by the socio-environmental influences during pre- and post-weaning. We are interested in knowing if early life experiences alter OTR receptor expression in the male prairie vole brain. We manipulated the social context at two stages of development. First, we altered the early-life social context by either removing or leaving the father in the nest from birth to weaning. Next we altered the post-wean social experience by either housing animals alone (single) or with a sibling (group). Once the animals reached adulthood, we compared the OTR expression throughout the forebrain for males that were in each of these four groups. Although several areas of the brain showed no differences across treatment groups, many others consistently demonstrated that post-wean development (i.e., social experience during adolescence) altered OTR expression patterns, while pre-wean development (i.e., social experience with parents during childhood) did not. The altered neural profiles indicate that adolescent development is particularly important in shaping the social brain, which may have long term consequences on adult behavior. These results improve our understanding about the importance of social stimuli and bi-parental care and how their influences can affect brain and behavior in males. Warren 137; 11:30AM-11:42AM

Functional Characterization of CG14509 in Drosophila Melanogaster
Nahel Tunio
Student of Weill Cornell Medical College in Qatar
Deitcher lab, Department of Neurobiology and Behavior

Around 50 million people worldwide suffer from epilepsy. It is characterized by recurrent seizures; a result of uncontrolled neuronal activity. Seizures are brief episodes of involuntary shaking that involve parts of the body or the entire body. Recent studies in humans have revealed that susceptibility to seizures is influenced by genetic factors. The class of bang-sensitive Drosophila melanogaster mutants reproduces many aspects of the human disease and can be used to study the role of certain genes involved in epilepsy. Ectopic gene expression in D. melanogaster through GAL4 system can be used to determine the significance of a particular gene. GAL4 system allows selective expression or knockdown of a gene in specific cells or tissues. CG14509 is one of the genes expressed in the nervous system of D. melanogaster; however its gene function is unknown. In order to identify the effect of this protein on seizures we used specific drivers to induce its knockdown pathway by RNA interference. Results indicated that gene knockdown in the nervous system made them bang-sensitive, while knockdown in glia showed no effect. CG14509 was also silenced in cholinergic and GABAergic neurons.
To further identify the role of the gene during development of D. melanogaster, protein expression was altered using specific drivers at different stages of the development. Results indicated the two critical periods during which changes in gene expression can greatly influence the bang-sensitivity of flies. Seizures were also timed to quantify the severity of seizures. SILS-Qatar Symposium, August 6th, Mann Library 100, 4:50 PM

Astrocytes found further from spinal cord injury could be reactive astrocytes
Giselle Ventura
Harris-Warrick Lab, Department of Neurobiology and Behavior

Central pattern generators in the spinal cord are neurons whose interactions produce the alternating movement in the hind limbs in locomotion. Though central pattern generators receive input from the brain for initiation of locomotion, properties such as timing and phasing pertain to the interactions of the neurons. Our lab works to investigate what plastic changes occur in the different neurons and other cells after spinal cord injury (SCI). Our model organisms are mice with spinal cord injury in the thoracic region. SCI stops descending input to the central pattern generator and causes an abundance of homeostatic changes.
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We study changes that occur specifically in the lumbar region. One particular and very important change is the activity of astrocytes. Astrocytes are a type of glial cell, abundant in the brain and spinal cord. They provide structure, nutrients, maintain a suitable environment for neuron communication, and produce semaphorins 3A, ephrins and slit which are molecules that prevent the regrowth of axons in brain damage and more. Previous work in our lab determined that an increase in astrocyte expression is caused by proliferation of astrocyte cell bodies, thriving mostly around the injury but also further down. The present study inquires as to whether the astrocytes found further from the spinal cord injury are reactive. Reactive astrocytes are astrocytes that go under changes in molecular expression and morphology, as a response to injuries to the central nervous system. This allows them to respond to injury by hypertrophy, proliferation or scarring. Therefore, using immunohistochemistry, the purpose of this study is to find out if there are reactive astrocytes in locations other than the spinal cord injury site along the lumbar region; as well as seek how far from the injury site they are found and their purpose. In the future, the study will use double immunohistochemistry with GFAP (glial fibrillary acidic protein) antibodies to stain for astrocytes, and bixin antibodies to stain for reactive astrocytes. Using a confocal microscope the spinal cords will be imaged and analyzed with Image J to investigate whether the converging antibodies stain for a reactive astrocyte. **Warren 101; 11:30AM-11:42AM**

**Investigating the effect of trypsin on PAR1 and PAR2 expression on human endothelial cells**

**Linda Wang**, Sara Che and Tracy Stokol  
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Protease-activated receptors (PAR) 1 and PAR2 are G-protein coupled receptors that are expressed by human endothelial cells. Activating PAR1 and PAR2 on endothelial cells leads to diverse downstream effects, such as increased vascular permeability and upregulation of surface adhesion molecules. These effects can potentially promote cancer metastasis by enabling circulating tumor cells in the blood stream to adhere to the blood vessel wall and extravasate into a distant organ to form metastases. PAR1 and PAR2 can be activated by different proteases biologically; we are interested in their activation by the coagulation factors, tissue factor (TF), factor VIIa (FVIIa; a denoting activation) and factor Xa (FXa). TF is overexpressed in epithelial tumors and its expression is associated with tumor progression and metastasis. TF initiates coagulation by binding to and activating its enzymatic partner, FVII, which then activates FX. By activating FVII and FX, TF can also signal through PAR1 and PAR2.

Trypsin, commonly used to dissociate cells during cell culture, activates both PAR1 and PAR2, which may affect their expression in our experimental procedures. Using flow cytometry and immunofluorescent (IF) microscopy, we wanted to determine whether the use of trypsin in cell culture could affect PAR expression on endothelial cells. We dissociated human umbilical vein endothelial cells (HUVECs) grown on tissue culture dishes using trypsin or an enzyme-free dissociation solution over various passages and compared the PAR1 and PAR2 expression on the cells. Using flow cytometry, we found that trypsin decreased both PAR1 and PAR2 expression on HUVECs immediately after treatment. However, trypsin only substantially affected PAR2, not PAR1, expression 48 hours post-treatment (observed with IF microscopy). Moreover, PAR2 expression did not recover within 2 passages of cells that had been dissociated with trypsin. We will dissociate cells using only enzyme-free dissociation solution in future experiments studying PARs to minimize confounding activation by trypsin. **Warren 137; 3:14PM-3:26PM**

**Identifying novel proteins in microRNA biogenesis and regulation**

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MicroRNAs (miRNAs) are small non-coding RNAs, ~22 nucleotide long, that play important roles in post-transcriptional regulation of gene expression in animals, plants and some viruses. MiRNAs target mRNAs for degradation or translational repression via base-pairing with complementary sequences within the 3' UTR of the mRNA. Loss or amplification of miRNA genes has been reported in a variety of cancers, and altered patterns of miRNA expression may affect cell cycle and survival programs. Understanding how miRNAs are regulated will help us better understand post-transcriptional gene regulation. Using a genome-wide screening method, the Grimson Lab has identified candidate proteins that may be involved in the miRNA-mediated gene expression pathway. If the method can be applied widely, a new way to screen miRNA-related protein will be established. In this project, we examined two of the candidates, ZCCHC8 and eIF4A1, together with eIF4A2, a homolog of eIF4A1, which has recently been implicated as participating in the miRNA pathway. We used luciferase reporter assays to monitor the activity of let-7, a well-known human miRNA. The 3' UTR of HMGA2, which contains let-7 target sites, was inserted into a luciferase vector to regulate its expression. When let-7 is synthesized and binds to the 3' UTR of HMGA2, it will reduce luciferase activity. By knocking down the candidate genes, we expect to see a significant increase of luciferase activity compared to negative controls w. We also included Pasha and Drosha genes, which are known to be required for miRNA
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biogenesis, as positive controls. Preliminary data suggested an increase of luciferase activity in positive controls and a slight increase in candidate constructs. The data show the potential that the candidates are involved in miRNA regulation. In future studies, we will measure the knockdown efficiency through qPCR assay and do double knockdowns of the candidate genes to see the effects. We will also do the screening using different 3’UTRs, to examine whether the role of candidate genes is specific to let-7 or the HMG2 3’UTR. Warren 137; 3:50PM-4:02PM