# 2013 UC Berkeley Microbiology Student Symposium
# SPEAKER ABSTRACTS

**9:05 AM**


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## Evidence of Sex and Recombination in the Life History of the Choanoflagellate *Salpingoeca rosetta*


Tera Levin, Allie Greaney, Nicole King



Choanoflagellates are ubiquitous microeukaryotes that are key players in aquatic microbial food webs, linking bacterial primary producers to higher trophic levels. Choanoflagellates are also the closest living relatives of animals, and this important phylogenetic placement has made them an emerging model organism for studies of evolution and comparative genomics. As a new model organism, basic features of choanoflagellate biology, including their ploidy and mode of reproduction (sexual vs. asexual), have remained a mystery, but this information is necessary both for establishing genetic approaches in choanoflagellates and understanding their evolution. We report the discovery of a sexual phase in the life history of the choanoflagellate *Salpingoeca rosetta*. We find that *S. rosetta* can stably exist as either a haploid or diploid cell, and that cells of both ploidies are capable of asexual reproduction. The sexual phase is characterized by the appearance of new cell morphologies, cell-cell adhesion, and finally cell fusion. These morphological features accompany a population-wide switch from haploidy to diploidy, which can be readily induced under laboratory conditions. The discovery of sex in choanoflagellates is contributing to the development of genetic approaches and will facilitate our understanding of gene, genome, and organismal evolution in choanoflagellates.

**9:30 AM**


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# Organelles in Bacteria: Microcompartments Involved in Detoxification (and More!) in the Planctomycetes


The phylum Planctomycetes is one shrouded in mystery, and thus poised for new discoveries; many aspects of the physiology and cell biology of these organisms are rarely found in other phyla, and their evolutionary placement in the tree of life is contested. We have observed a set of genes related to Bacterial Microcompartments (BMCs) conserved in 8 out of the 10 sequenced Planctomycete genomes. BMCs are protein-bound organelles with functions in carbon fixation, carbon-source catabolism, and nitrogen-source utilization. BMCs are produced as polyhedral bodies and encapsulate functionally related enzymes, generally involved in oxygen-sensitive or toxic reactions. Initially thought to only be carbon-fixing organelles in Cyanobacteria, it was later discovered that BMCs are also used by enteric bacteria to degrade 1,2-propanediol and ethanolamine, and are proposed to be involved in virulence. To date, bioinformatic surveys of whole-genome sequences predict that there are 10 functionally distinct BMCs distributed throughout more than 20% of all sequenced bacterial genomes. Only 5 of these BMCs have been observed in electron micrographs, of which 3 have been genetically and biochemically characterized. We show that these genes are involved in the metabolism of not just one, but a number of carbon sources. Our data suggest that the BMC is used for harvesting energy from various sources present in the diverse ecological niches that the Planctomycetes inhabit.

**10:05 AM**


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# A Multi-regional Perspective on the Effect of Coffee Agriculture on Fungal Community Structure


The field of mycology still seeks to determine which factors, if any, structure fungal communities and limit dispersal. In this study we compared the diversity and structure of fungal communities in widely sourced green coffee beans to demonstrate how geography, farming practices, and commodity trade affect fungi. Using culture-based and culture-independent methods, we surveyed fungal communities in green coffee from Ethiopia, Costa Rica, Guatemala, and Papua New Guinea. The coffee was grown under different agricultural methods, enlisted different agroforestry practices, and was processed in different ways. In addition, we analyzed the population genetic structure of two mycotoxigenic species, *Aspergillus niger* and *Eurotium rubrum*, obtained by culture, to discern whether the trade of coffee has facilitated gene flow in these species. We found that fungal incidence rates varies as a factor of geographic origin, fungal community structure is significantly different in sun and shade-grown coffee beans, conventional coffee hosts greater fungal diversity than organic coffee, and sampling methods used to detect fungi in coffee significantly impact which species are recovered. Using a phylogenetic approach, we were also able to detect multiple species and distinct OTUs within the *A. niger* and *E. rubrum* clades, some with global populations, and others with distinct ranges. Thus with this data we have shown that the production and trade of commodity crops, such as coffee, affect the community and population structure of fungi by either facilitating or preventing dispersal.

## 10:30 AM


## Bubba Brooks , University of California, Berkeley

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# Microbes in the Neonatal Intensive Care Unit are also Found in the Gut of Premature Infants


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**Background:** The source inoculum of gastrointestinal tract (GIT) microbes is largely influenced by delivery mode in full-term infants, but these influences are often decoupled in very low birth weight (VLBW, <1,500 g) neonates through conventional broad-spectrum antibiotic treatment. We hypothesize the built environment (BE), specifically room surfaces frequently touched by humans, is a predominant source of colonizing microbes in the GIT of premature VLBW infants. Here we present the first matched fecal-BE time series analysis of two pre-term VLBW neonates housed in a neonatal intensive care unit (NICU) over the first month of life.

**Results:** Fresh fecal samples were collected every three days and metagenomes sequenced on an Illumina HiSeq2000. For each fecal sample, approximately 33 swabs were collected from each NICU room from five specified areas: sink, feeding and intubation tubing, hands of healthcare providers and parents, general surfaces, and nurse station electronics (keyboard, mouse, and cell phone). Swabs were processed using a recently developed PCR-EMIRGE pipeline in which full-length 16S rRNA amplicons were sheared and sequenced using an Illumina platform, and short reads assembled with the EMIRGE software. Nearly 40,000 full-length 16S rRNA sequences were produced, generating an average of approximately 16,500 OTUs (clustered at 97% nucleotide identity) per room-infant pair. Dominant gut taxa, including *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Bacteroides fragilis,* and *Escherichia coli*, were widely distributed throughout the room environment with upwards of 78% of gut microbes found in all room samples. Additionally, reconstructed genomes from gut colonizers revealed a suite of antibiotic resistance genes, such as tetracycline, fluoroquinolone, and aminoglycoside resistance genes, which likely offer a competitive advantage both inside and out of the gut.

**Conclusion:** We have developed a high-throughput culture-independent approach that integrates room surveys based on full-length 16S rRNA gene sequences with metagenomic analysis of fecal samples collected from infants in the room. The approach enabled identification of discrete ICU reservoirs of microbes that also colonized the infant gut and provided evidence for the presence of certain organisms in the room prior to their detection in the gut. The methods are suitable for application to samples collected with higher time resolution, and will enable better and more comprehensive resolution of the direction and/or bi-directionality of the room-infant relationship.

**1:30 PM**


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# Deciphering the Transcriptional Landscape of *Caulobacter crescentus*

A key to a systems-level understanding of the complex regulatory circuitry that guides the development of a single cell is to be able to see when, where, and how is genomic information extracted through the process of transcription as well as which genomic information is absolutely essential for cellular development. We have taken advantage of high-throughput sequencing, high-density microarrays, and hyper-saturated transposon mutagenesis to map, at the resolution of single base pairs, all sites of transcriptional initiation as a function of the Caulobacter cell cycle as well as all essential elements of its genome including 480 essential genes, 8 small RNAs, 402 regulatory regions, and 90 intergenic regions.

Our study has shown that the transcriptional landscape of Caulobacter is much more complex than previously thought. We have discovered many novel transcriptional elements including 343 open reading frames (ORFs) with internal transcriptional initiation, 23 small non-coding RNAs, 159 ORFs with multiple promoters, as well as 229 ORFs with antisense transcription; one of which is the cell cycle master regulator dnaA. We have also made the surprising discovery that the transcription o fgenes within operons can be dynamic and cell cycle regulated. Furthermore, our study has also enhanced the resolution of the cell-cycle expression of genes down to the level of individual promoters, a drastic improvement over standard microarrays, which shows gene expression as the result of multiple regulatory phenomena. Analysis of all transcription initiation sites during the cell cycle also allowed us to search upstream of these sites for regulatory inputs associated with co-expressed promoters. Finally, integrating our datasets on the Caulobacter transcriptome, essential genes and regulatory regions, as well as their associated regulatory inputs enables us to build the essential transcriptional network that drive the development of Caulobacter.

**1:55 PM**


## Kyle Frischkorn, Univ. of Washington/NOAA/UC Berkeley

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# *Vibrio parahaemolyticus* Type IV pili Mediate Interactions with Diatom-derived Chitin and Point to an Unexplored Mechanism of Environmental Persistence


Kyle Frischkorn, Asta Stojanovski and Rohinee Paranjpye



*Vibrio parahaemolyticus* is a naturally occurring bacterium common in coastal waters where it concentrates in shellfish through filter feeding. The bacterium is a human pathogen and the leading cause of seafood-borne gastroenteritis. Presently there is little information regarding mechanisms of environmental persistence of *V. parahaemolyticus* or an accurate early warning system for outbreak prediction. Vibrios have been shown to adhere to several substrates in the environment, including chitin, one of the most abundant polymers in the ocean. Diatoms are abundant in estuarine waters and some species produce chitin as a component of the silica cell wall or as extracellular fibrils. We examined the role of specific surface structures on the bacterium, the type IV pilins PilA and MshA, in adherence to diatom-derived chitin. Biofilm formation and adherence of *V. parahaemolyticus* to chitin is mediated by the ability of the bacterium to express functional type IV pili. The amount of adherence to diatom-derived chitin is controlled by increased chitin production that occurs in later stages of diatom growth. The data presented here suggest late-stage diatom blooms may harbour high concentrations of *V. parahaemolyticus* and could serve as the foundation for a more accurate early warning system for outbreaks of this human pathogen.

## 2:30 PM


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# Structural Basis for the MukB-topoisomerase IV Interaction and Its Implications *in vivo*


Seychelle M. Vos, Nichole Stewart, Martha G. Oakley, and James M. Berger



Chromosome partitioning in Escherichia coli is assisted by two interacting proteins, topoisomerase (topo) IV and MukB. The physical interaction between topo IV and MukB stimulates the ability of topo IV to relax negatively-supercoiled DNA. To better understand the interplay between topo IV and MukB, we determined the crystal structure of a minimal MukB-topo IV complex to 2.3Å resolution. The structure shows that a region of MukB known as the hinge forms a heterotetrameric complex with a C-terminal DNA binding domain (CTD) of topo IV's ParC subunit. The configuration of the complex sterically prohibits intradimeric interactions indicating that MukB and topo IV are capable of forming oligomeric arrays with one another. Biochemical studies show that the MukB hinge, like full-length MukB, is sufficient to stimulate topo IV relaxation activity on negatively-supercoiled DNA by competing for a site on the CTD that normally represses topo IV activity on these substrates; complementation assays further show that the cellular requirement for topo IV derives from two independent needs for both its strand passage activity and its association with MukB. Together these data suggest a framework by which MukB and topo IV may collaborate to disentangle newly replicated daughter chromosomes.



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**2:55 PM**


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# Exoribonuclease XRN2 as a Novel Antiviral Factor against Hepatitis C Virus


The nuclear 5'-3' exoribonuclease XRN2 plays important roles in RNA metabolism as it was recently found to be responsible for the degradation of mature miRNAs in *C. elegans*. In an effort to examine whether XRN2 is involved in miRNA turnover in mammalian cells, XRN2 was depleted by siRNAs in Huh7 liver cells. We found that XRN2 depletion had no effect on miRNA turnover in both uninfected cells and HCV-infected cells. However, depletion of XRN2 caused a significant increase in HCV RNA and protein abundances. Sucrose gradient analysis showed that viral RNA isolated from XRN2-depleted, HCV-infected cells was associated with translating polysomes at an enhanced level as compared to viral RNA from control samples. Curiously, after labeling newly synthesized HCV RNA with 4-thiouridine, we determined that XRN2 did not have an effect on viral RNA replication rates. However, when HCV-infected cells were treated with the polymerase inhibitor MK-0608 and viral RNA decay was measured, XRN2 depletion resulted in slower rate of viral RNA decay. While the subcellular localization of XRN2 does not change during HCV infection, its steady state abundance dramatically increases during infection. Overall, these findings support a novel role for XRN2 in antiviral response by destabilizing translating viral RNA.