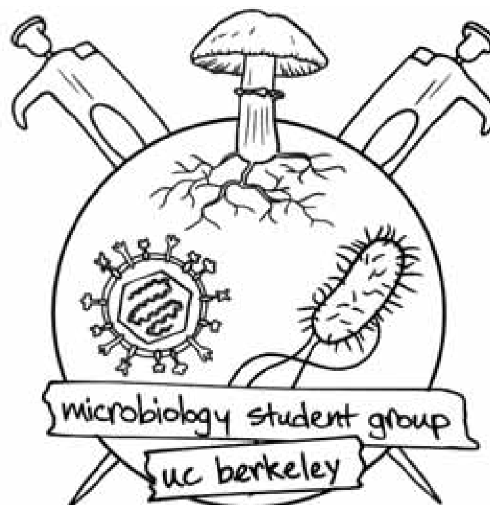
16th Annual Microbiology Student Symposium at UC Berkeley

April 25th, 2015 @ Li Ka Shing Building



Chair
Christopher Hann-Soden

MSG President
Susan Hepp

Funding Commitee
Amanda McRae
Darae Jun

Web Guru
Mikel Shybut

Vice Chair
Emma Abernathy

Publicity Officer
Han Teng Wong

Procurement Committee
Bret Peterson
Monika Fischer
Gabe Rosenfield

Hosted by the Microbiology Graduate Student Group at UC Berkeley.

The MSG logo was designed by Jeff Johnson.

MORNING SCHEDULE

8:30 AM	*Registration & Breakfast*
9:00 AM	**Welcome** **Christopher Hann-Soden**, Symposium Chair
9:05 AM	**Cat Adams, Bruns Lab, UC Berkeley** How to use social media to boost your academic career
9:25 AM	**Rachel Hood, Savage Lab, UC Berkeley** The stringent response regulates light/dark adaptation in cyanobacteria
9:45 AM	*Break*
10:00 AM	**Julie Choe, Welch Lab, UC Berkeley** *Rickettsia* species employ diverse actin assembly mechanisms for intracellular motility
10:20 AM	**Matt Gardner, Glaunsinger Lab, UC Berkeley** Manipulation of the ubiquitin-proteasome system by Kaposi's Sarcoma-Associated Herpesvirus
10:40 AM	**Keerthana Sekar*, Cline Lab, CSU Chico** Impact of H5N1 Influenza virus replication on macrophage function
11:00 AM	*Break*
11:15 AM	**Keynote Speaker: Jesse Bloom, Fred Hutchinson Cancer Research Center** Mapping constraints on the evolution of Influenza virus

AFTERNOON SCHEDULE

12:15 PM — *Lunch & Poster Session*

1:15 PM — **Onur Erbilgin, Kerfeld Lab, UC Berkeley**
Using Biolog PM Plates for Bacterial Organelle Function Discovery

1:35 PM — **Anna McGeachy, Ingolia Lab, JHU & UC Berkeley**
Bulk selection for diverse yeast expression libraries using large volume turbidostats

1:55 PM — **Christopher M. Jakobson, Tullman-Ercek Lab, UC Berkeley**
Promiscuous signal sequences: A common hydrophobic motif mediates localization to the Pdu bacterial microcompartment

2:15 PM — *Break*

2:30 PM — **Elias Valdivia, Tullman-Ercek Lab, UC Berkeley**
Mutating the type 3 secretion system in *Salmonella* to increase export of heterologous proteins

2:50 PM — **Yuanchun Wang*, Ma Lab, UC Riverside**
Three candidate effectors have been identified in USDA110

3:10 PM — **Matthew O'Neill*, Allen Lab, UC Riverside**
Does variation in biogeographical inoculum potential explain the invasiveness of an exotic annual?

3:30 PM — *Break – Vote for Best Student Speaker*

3:45 PM — **Keynote Speaker: Hazel Barton, University of Akron**
Cave microbes: Diversity in a microbial world

4:45 PM — **Awards & Closing Remarks**

5:00 PM — **Mixer**

* Biolog Travel Grant Award Winners

Presentation Abstracts

How to use social media to boost your academic career. Cat Adams. Dept. of Plant and Microbial Biol, University of California, Berkeley, Berkeley, CA.

As researchers it is now essential, and for some even compulsory, to communicate our research and knowledge of our subject to a wide audience. Though we may be effective communicators with other biologists and students, communicating our message to the general public requires different approaches, for which our abilities often fall short. The advent of online social media has produced novel ways to communicate to an extremely broad audience with very little time or effort. Though some academics criticize social media as an outreach-time-sink, using social media effectively can advance your academic career by broadening your professional network and increasing readership of your publications. Here, I will present on Twitter, the preferred social media platform of academics. I will familiarize the audience with the Twitter interface, provide statistics about how academics use Twitter, and share concrete tips for gaining followers and boosting your posts. Then, I will provide examples of how scientists have written for online magazines to defend and promote their research, and encourage you to do the same. Finally, I will show how social media can be harnessed to generate research funding through crowdsourcing. All the presentation slides are available online at www.ScienceIsMetal.com, and include additional resources on using social media effectively and tips for writing for general audiences.

***Rickettsia* species employ diverse actin assembly mechanisms for intracellular motility.** Julie E Choe, Matthew Welch. Dept. of Molecular & Cellular Biology, University of California, Berkeley, Berkeley, CA.

Rickettsia species are obligate intracellular bacterial pathogens that grow in the host cell cytosol and polymerize the host actin cytoskeleton to power intracellular actin-based motility and cell-to-cell spread during infection. Motility and virulence of Spotted Fever Group (SFG) species require the surface cell antigen 2 (Sca2), which directly polymerizes host actin by mimicking the host formin family of actin assembly proteins. However, Sca2 exhibits substantial sequence variability between Rickettsia species, leading us to question if and how Sca2 orthologs polymerize actin and drive motility. We sought to determine if Ancestral Group (AG) species R. belli Sca2 (RbSca2) is capable of assembling actin. RbSca2 lacks the formin-mimicking domain of SFG Sca2 but contains several putative WASP homology 2 (WH2) motifs, predicted to bind actin monomers. Biochemical assays using purified RbSca2 indicate that it binds to actin and accelerates actin filament polymerization. Mutagenesis and truncation experiments reveal the N-terminal WH2 motif is central to high-affinity actin interaction and actin assembly activity. Although nucleation by WH2 containing proteins typically requires 3 or more such motifs in tandem, our data suggest the alternate hypothesis that oligomerization is required for RbSca2 activity. Supporting this, an artificially dimerized construct exhibited enhanced polymerization activity. Additionally, host factors may play an important role in RbSca2 activity and bacterial motility. To identify host cytoskeletal factors that contribute to R. bellii motility, we utilized siRNA-mediated gene silencing. The host factors

profilin, plastin, cofilin, and capping protein were found to play an important role in motility of SFG species. However, these factors show less potent effects on AG Sca2 motility. Additionally, AG generates significantly fewer and shorter tails upon infection of insect cels, which is not the case with SFG Rickettsia, further supporting the hypothesis that the actin-based motility between species is dependent on different host factors. The role of additional host factors is currently being tested for AG-specific effects on motility. The diversity of both bacterial actin assembly mechanisms and host factor requirements highlight how species within the Rickettsia genus use distinct strategies to intercept actin pathways during infection. Since actin-based motility is an important virulence mechanism for many pathogens, understanding the details of these differences may underscore general mechanisms of pathogenicity.

Manipulation of the ubiquitin-proteasome system by Kaposi's Sarcoma-Associated Herpesvirus. Matt Gardner[1], Britt Glaunsinger[2]. [1] Dept. of Infectious Diseases and Immunity, University of California, Berkeley, Berkeley, CA.; [2] Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

The proteasome is a large ATP-dependent protease, responsible for the majority of non-lysosomal protein degradation in mammalian cells. Inhibition of the proteasome during Kaposi's Sarcoma-Associated Herpesvirus (KSHV) infection disrupts the replication cycle of the virus, preventing the production of infectious virions. The recently published KSHV interactome revealed several viral proteins that interact with host protein degradation pathways. These proteins were screened for alteration of proteasome-mediated degradation by a luciferase assay using a tetra-ubiquitin luciferase linear fusion. The proteins with the most drastic effects, ORF24 and ORF68, were chosen for further study. In cells expressing the KSHV protein ORF24, the half-life of a model substrate was reduced, indicating an increase in protein degradation. In cells expressing ORF68, the half-life of the model substrate was increased, indicating a decrease in protein degradation. During the viral replication cycle, these proteins are expressed with similar kinetics, but in different cellular compartments. Using a nuclear-restricted model substrate, we found that this differential localization is what allows two proteins with opposing activities to be expressed at the same time, in the same cell. Using a co-affinity purification approach, we found that both ORF24 and ORF68 co-purify with proteasomes from mammalian cells. ORF24 and ORF68 are both essential genes in KSHV, suggesting that the manipulation of the proteasome by these proteins is a required step in the KSHV replication cycle.

The stringent response regulates light/dark adaptation in cyanobacteria. Rachel Hood, Sean Higgins, Avi Flamholz, David Savage. Dept. of Molecular & Cellular Biology, University of California, Berkeley, Berkeley, CA.

Cyanobacteria have shaped the Earth's history by oxygenating the atmosphere, and continue to perform a significant amount of global photosynthesis. As photoautotrophs, they require light to grow and divide. Daily cycles of light and darkness, however, present

a challenge to these organisms. Circadian rhythm is a system by which cyanobacteria can anticipate light/dark cycles, but additional mechanisms regulate the physiological changes that occur in response to darkness. We hypothesized that the stringent response, a stress response pathway conserved in nearly all bacterial phyla, acts through the nucleotide second messenger ppGpp to regulate the light/dark response in cyanobacteria. We have used the model cyanobacterium Synechococcus elongatus PCC 7942 to investigate this question, by manipulating levels of ppGpp, testing growth under varying lighting conditions, and performing genome-wide transcriptional profiling to identify downstream targets of ppGpp in Synechococcus. A mutant that is unable to synthesize ppGpp exhibits a more pronounced growth defect in light/dark cycles than in constant light, demonstrating that ppGpp is important for fitness under oscillating environmental conditions. Using RNA-seq to compare gene expression levels among strains with varying ppGpp levels in light and dark, we have identified many ppGpp-regulated genes. We are following up on several of these targets, as they are regulators of core processes including central carbon and nitrogen metabolism and translation, in order to learn more about specific molecular mechanisms of light/dark adaptation. This study extends our understanding of how the stringent response has been adapted to suit cyanobacterial metabolism, a lifestyle very different than those of more well-studied bacteria like E. coli and B. subtilis. We address the fundamental biological question of how organisms sense and respond to their environment, in a member of the unique and fascinating cyanobacteria.

Promiscuous signal sequences: A common hydrophobic motif mediates localization to the Pdu bacterial microcompartment. Christopher Jakobson, Edward Kim, Mary Slininger, Alex Chien, and Danielle Tullman-Ercek. Dept. of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA.

Bacterial microcompartments, small protein-bound organelles used by bacteria to spatially organize their metabolism, are promising tools for the compartmentalization of heterologous enzymatic pathways in synthetic biology, as well as playing a role in the pathogenicity of Salmonella and other species. We find that the N-terminal localization sequences from diverse microcompartment systems direct proteins to the 1,2-propanediol utilization (Pdu) microcompartment of Salmonella, revealing an underlying hydrophobic motif mediating localization. Furthermore, the foreign signal sequences compete with the native Pdu signal sequences for localization, suggesting that they are encapsulated by the same interaction with the protein shell of the Pdu microcompartment. This promiscuous localization has implications both for the creation of novel suites of signal sequences for synthetic biology and for our understanding of the subcellular organization of infection-relevant metabolic pathways in Salmonella.

Bulk selection for diverse yeast expression libraries using large volume turbidostats.
Anna McGeachy, Nicholas Ingolia. Johns Hopkins University, Carnegie Institution, University of California, Berkeley, Berkeley, CA.

Post-transcriptional regulation plays an important role in modulating gene expression in situations of stress, development, and even normal culture. Translation and RNA stability can be influenced greatly by the binding of effector proteins to target transcripts. Genome-wide studies have identified over 500 RNA binding proteins (RBPs); however, many of these genes lack functional annotation. Tethered function assays provide a method for characterizing the post-transcriptional role of proteins and protein domains. To extend the tethered function assay beyond candidate approaches into genome-scale screens, we have designed an approach to generate entire expression libraries of tethered protein and protein domains from randomly fragmented genomic DNA. One hurdle in making expression libraries is selecting productive translating fragments from the genomic pool. We overcome this by using a polycistronic selective marker downstream and in frame of the tether protein. Yeast are selected in a custom, large volume turbidostat. In contrast to previously published turbidostats, the larger volume growth chambers accommodate selection of thousands of unique expression products. Preliminary analysis shows that our system is capable of generating tens of thousands of unique, in-frame fragments genome-wide. Furthermore, yeast sorted for changes in reporter levels correspond to known post-transcriptional regulators.

Does variation in biogeographical inoculum potential explain the invasiveness of an exotic annual?.
Matthew R. O'Neill, Dariana Chow, Elizabeth Holmes and Michael F. Allen. Dept. of Biology. University of California, Riverside, Riverside, CA.

Exotic invasive plant species inflict substantial negative impacts on recipient biotic communities. In order to ameliorate these impacts it is necessary to understand the mechanisms that drive invader success. The majority of hypotheses proposed to explain invasive behavior have historically been biased toward aboveground interactions. While tests of these hypotheses have provided valuable information, general explanations within the context of these tests have remained frustrated by context dependencies. In contrast, data from the emergent field of plant-soil feedbacks has demonstrated consistent and predictable patterns. That is, invasive plants tend to perform better with invasive relative to native range soil biota. However, whether these positive plant-soil feedbacks are due to decreased pathogen loads or increased mutualist benefits remains unknown. As a first step in determining what biotic soil components drive these positive interactions, we surveyed the soil communities in association with the invasive grass Bromus rubens in its native and invaded ranges.

Roots from native range populations displayed a higher rate of colonization by different morphotypes of beneficial ($F = 28.84$, $p < 0.001$) and pathogenic fungi ($F = 7.43$, $p < 0.05$) relative to invasive populations. Preliminary data from molecular analyses supported this pattern. Roots from the native range harbored nearly twice as many pathogenic phylotypes as invaded range roots, and nearly 6 times as many mycorrhizal phylotypes.

However, the abundance of mycorrhizal spores was 48% greater in introduced relative to native soils ($F = 5.31$, $p < 0.05$). These data suggest that populations of Bromus rubens are interacting differently with soil biota among native and invaded ranges. Interestingly, these patterns suggest that the observed invasive behavior may be due to decreased interaction with both beneficial and pathogenic microbial species.

Impact of H5N1 Influenza virus replication on macrophage function. Keerthana Sekar, Troy Cline. Dept. of Biological and Biomedical Sciences, California State University, Chico, Chico, CA.

Highly pathogenic H5N1 avian influenza viruses pose a public health risk for their pandemic potential and infect humans with a 60% mortality rate. The mechanism of severe disease during H5N1 infection is only partially known. Despite being important for protection against infection with seasonal influenza viruses, macrophages are thought to contribute to severe disease caused by H5N1 viruses. Phagocytosis of influenza virus-infected cells by activated macrophages is an important antiviral defense and inhibition of phagocytosis leads to increased virus replication. Also, H5N1 virus infection is followed by an increase in levels of nitric oxide (NO), a potent inflammatory signaling molecule that contributes to viral pathogenesis. We demonstrated that H5N1 viruses are unique in their ability to replicate in macrophages in a hemagglutinin (HA)-dependent fashion. The objective of this project is to determine the impact of influenza virus replication on macrophage function. We hypothesize that H5N1 influenza virus replication in macrophages alters cellular functions in ways that contribute to increased disease severity. Macrophages infected with H5N1 were less efficient at phagocytosis compared to those infected with CA/09. Further, the H5N1 virus inhibited IFN-γ-mediated activation of macrophages in a replication-dependent manner, whereas cells infected with CA/09 were responsive to IFNγ-induced activation. Additionally, H5N1 influenza viruses induced greater NO production in macrophages than CA/09 and this required virus replication. Our data demonstrate that H5N1 influenza viruses decrease phagocytic capacity and IFN-γ responsiveness of macrophages and increase NO production following virus infection. Importantly, these changes in macrophage function are dependent on H5N1 virus replication, suggesting that macrophage dysfunction during H5 infection is an effect of the ability of these viruses to replicate in macrophages. This study will help to address the potential risk posed by H5N1.

Mutating the type 3 secretion system in *Salmonella* to increase export of heterologous proteins. Elias Valdivia[1], Kevin Metcalf[2], and Danielle Tullman-Ercek[2]. [1] Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA; [2] Dept. of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA.

We have engineered a protein pump in Salmonella to secrete proteins of interest that can be easily recovered from the media to avoid protein purification from cell lysate. In this work, we have made point mutations to the T3SS prgI in Salmonella enterica serovar

Typhimurium that increases the secretion of heterologous proteins into the media. To determine which mutation results with the highest protein secretion, we compared the secreted protein titer by western blotting. With genomic engineering methods, we express the T3SS prgI alleles from the genome with accurate spatial and temporal expression resulting in proper assembly of the protein pump, and we characterize mutants that result in increased titer of secreted protein. However, the secreted titers are low relative to total foreign protein produced, since most of the protein remains in the cytoplasm. This is not economical for large-scale production. Therefore, we are attempting to optimize the T3SS by making more modifications to the needle-like structure by modifying invJ and spaS.

Three candidate effectors have been identified in USDA110. Yuanchun Wang. Dept. of Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA.

Rhizobium who plays a crucial role in fixing the nitrogen could utilize the pathogenic-like T3SS (the type III secretion system) to influence symbiosis efficiency. T3SS in gram-negative bacteria is a specialized protein delivery system to inject protein effectors directly into the eukaryotic host cells. Once inside the host cells, these effectors can target specific host substrates and facilitate bacterial infection. In collaboration with bioinformatics, the Ma lab developed a novel bioinformatics prediction package to search for potential T3SS effectors(T3Ses) from the four rhizobial genome sequences. 57 proteins have been predicted to be potential effectors, 34 of which were candidate novel effectors. Consequently, the study mainly focused on identifying these candidate effectors from USDA110 based on different methods, including the N-terminal secretion signal, gene expression and effector secretion depended on genistein induction. RT-PCR results could indicate the expression of these three effectors could be regulated by genistein and this kind of regulation depended on transcriptional regulator TtsI (type III secretion), which is an important characteristic of T3Ses. Then HR (hypersensitive response) was employed to test the N-terminal secretional signal and expression of effector-Δ79AvrRpt2 fusion protein. That these three candidate effectors could induce HR exhibited they owned N-terminal secretional signal. Then protein secretion experiment was taken advantage to detect the effector secretion and the results showed the three ones could be induced by genistein and secreted into the supernatant of TY medium. Taken together, these findings revealed that three candidate effectors in USDA 110 have been determined to be T3Ses.

Poster Abstracts

Engineering high-titer heterologous protein secretion in Salmonella Enterica. Anum Azam, Kevin Metcalf and Danielle Tullman-Ercek. Dept. of Bioengineering, University of California, Berkeley, Berkeley, CA.

Secretion is emerging as a useful strategy for exporting proteins of biotechnological interest from bacteria. The type III secretion system (T3SS) in Salmonella enterica is an ideal path to protein export because it is non-essential for bacterial metabolism and allows for target proteins to cross both bacterial membranes in one step, via characteristic needle-like protein structures. We engineered a hyper-secreting strain of Salmonella for the high-titer production of a variety of biochemically challenging heterologous proteins, such as degradation-prone biopolymer proteins and toxic antimicrobial peptides. We achieve titers on the order of 100 mg/L for many proteins – a 100-fold improvement on wild type levels – at >90% purity. To design the hyper-secreting strain, we investigated a single T3SS protein, SipD. SipD is one of the first three proteins to be secreted through the assembled T3SS. These "translocon" proteins form a complex at the tip of the T3SS needle, instigating secretion of native proteins into mammalian cells in the first stage of an infection through a poorly understood signal transduction event. Our experiments suggest that SipD also acts as an intracellular regulator in a tightly controlled secretion hierarchy. Interestingly, these two roles have opposite effects on protein secretion, and occur on different sides of the cell envelope; the regulatory activity of SipD serves to inhibit secretion of heterologous proteins, while exogenous addition of SipD to T3SS-expressing cultures increases secretion. Furthermore, evidence suggests that different domains of the protein are involved in each function. We propose a physical and chemical mechanism for the dual role of SipD. This study serves as an example of how a complicated multi-protein apparatus can be rationally engineered to reveal how it functions as a beautiful natural system, while enabling progress in industrial protein production.

Agrobacterium-mediated Transformation of Setaria viridis as a Model for Assessing Genetic Engineering Strategies in Sorghum bicolor. Max Benjamin, Peggy Lemaux, Joyce van Eck, Nina Nim. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

Setaria viridis is a C4-photosynthesizing plant that is closely related to several major feed, fuel, and bioenergy crops. Its rapid life cycle, simple growth requirements, and well-documented genome make it an excellent model system for studying genetic engineering strategies in valuable cereal crops, such as sorghum and corn. Sorghum is an important cereal crop due to its current use in the U.S. as a bioenergy crop and its widespread cultivation and consumption in food-limited regions throughout the world. Sorghum serves as a valuable source of nutrition, and is often a staple food, in regions with unfavorable growth conditions due to its strong drought and flood tolerance. Unfortunately, sorghum is less digestible than other cereal crops, such as maize, rice, and wheat, especially after cooking. The decreased bioavailability of starch and protein in sorghum grains, compared to those nutrients in grains of other cereal crops, reduces its

overall nutritional value as a food. Manipulation of the NADPH-thioredoxin reductase (NTR)/thioredoxin (TRX) system in sorghum was found to increase seed size and digestibility in a limited number of engineered lines, providing a potential strategy to increase sorghum's nutritional value. Cells in immature sorghum embryos can be transformed to increase TRX expression via inoculation with Agrobacterium tumefaciens, a bacterium that is able to transfer genes to plant cells. This method of Agrobacterium-mediated transformation can also be used to transform embryogenic Setaria viridis callus tissue. Because Setaria can be transformed more rapidly than sorghum, this model system can be used to assess the outcome of a number of additional approaches to modifying TRX expression in sorghum. By increasing the TRX h expression in the Setaria, using an endosperm-specific promoter, we hope to demonstrate increased seed size in the grain, and therefore, provide proof-of-concept for increased grain yield via enhanced seed size in sorghum.

A secreted molecule represses virulence gene expression in Listeria monocytogenes. Sam Dubensky & Jonathan Portman. Dept. of Cellular and Molecular Biology, University of California, Berkeley, Berkeley, CA.

Septin Binding Factor Hsl1: Portion(s) of Hsl1 protein necessary and sufficient for bud-neck recruitment. Gregory Finnigan, Angela Duvalyan, Elizabeth Liao, Aspram Sargsyan, Jeremy Thorner. Dept. of Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA.

Septins are cytoskeletal elements found in all Eukarya, including humans. They are involved in numerous cell processes as organized, higher order structures that serve as (i) diffusion barriers, (ii) structural supports near cell membranes (including the division site between diving yeast cells), and (iii) acting as protein scaffolds for the recruitment of other enzymes. The yeast septins can integrate information across multiple signaling pathways. One such intersection involves the recruitment of the cell cycle checkpoint kinase Hsl1 and its associated partners, Hsl7 and Swe1. Once Hsl1 is recruited to the bud neck, it associates with its binding partner, Hsl7, and together they serve to inhibit Swe1. Negatively regulating Swe1 allows for progression through the G2/M cell cycle transition. We are interested in studying the molecular mechanism by which components of this pathway are recruited to and function at the bud neck.

By co-expressing mCherry tagged septin along with GFP tagged Hsl1 constructs, we analyzed the localization patterns found through a series of deletions and mutations. From this extensive analysis we found that, together, two Hsl1 segments, residues 611-950 and residues 1315-1518, were necessary and sufficient for bud neck localization. Specifically, when tagged with GFP, residues 1315-1518 alone localized to the plasma membrane; however, its fusion with the Hsl1 central region, residues 611-950, was able to redirected localization of the resulting chimeric protein to the bud neck, whereas expression of Hsl1(611-950) alone was unable to decorate the bud neck. These findings suggest that association with the plasma membrane is required before

recruitment to the septin collar can occur. Indeed, we have shown that replacement of residues 1315-1518 with a totally unrelated membrane-binding element (C2 domain from mammalian lactadherin) also permits residues 611-950 of Hsl1 to target the bud neck.

The current efforts of the Thorner Lab in characterizing this pathway in vivo in the model organism S. cerevisiae have led to the identification of protein domains within Hsl1 that are both necessary and sufficient to recruit this critical kinase to the septin structure at the bud neck. Our research plan includes extending this analysis to the entire checkpoint kinase signaling pathway (septins, Hsl1, Hsl7, and Swe1). Understanding the mechanism by which these cellular components coordinate information exchange between the cytoskeleton and cell cycle signaling pathways can serve as a model for how other recruited factor(s) can associate, modify, and function in both yeast as well as other Eukaryotes.

Characterizing the germling fusion pathway in Neurospora crassa. Monika Fischer, Wilfried Jonkers, N. Louise Glass. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

Establishment of a robust colony is dependent on cell fusion between genetically identical germinating conidia (germlings) in Neurospora crassa. Two germlings in close proximity to each other will engage in a molecular conversation that facilitates chemotropic growth, membrane fusion, and cytoplasmic mixing. The conversation is initiated by an unknown factor that leads to activation of a conserved MAP-kinase cascade. Once activated, the MAP-kinase, MAK-2 activates the transcription factor PP-1, which regulates expression of many genes required for homing and fusion. Several genes have been identified as being necessary for germling communication and/or fusion, but are largely characterized and both the receptor(s) and signal(s) remain elusive. Orthologs of the MAK-2 cascade function downstream of the pheromone responsive G-Protein Coupled Receptors (GPCR). Surprisingly, all known or predicted GPCR's, G-proteins, 7-transmembrane-domain proteins, and pheromones are not required for germling communication and fusion. Even double knock-out mutants of candidate GPCR genes continue to communicate and fuse like WT. We utilized a communication-activated Luciferase reporter and assessed phosphorylation/activation of MAK-2 in each of the known fusion mutants to broadly organize uncharacterized fusion genes and specifically identify genes that function upstream of the MAK-2 cascade. Our data indicates three general groups of genes required for germling fusion; genes that function downstream of the MAK-2 MAPK cascade, genes that function upstream of the MAK-2 MAPK cascade, and genes that are involved in a potential positive feedback loop. Notably we've identified two more transcription factors (ADV-1 and ADA-3) in addition to PP-1 that are required for germling fusion and expression of communication genes. Future work will focus on characterizing candidate upstream transmembrane-domain proteins in addition to RNAseq on Δadv-1, Δada-3, Δpp-1, and WT.

Genetic Basis of Adaptation to Deuterated Media in Escherichia coli. G.L. Fisher[2], N.B. Justice[1], M. Price[1], J. Khuel[1], K. Wetmore[1], A. Deutschbauer[1], A.P. Arkin[1,2]; 1) Lawrence Berkeley National Lab, Berkeley, CA; 2) University of California, Berkeley, Berkeley, CA.

Deuterium (2H) has been extensively used in the elucidation of reaction mechanisms and biochemical pathways. In the study of biological systems, the utility of deuterium is due to its increased atomic mass relative to hydrogen. This increased mass leads to altered reaction rates, a phenomenon known as the kinetic isotope effect. One of the most widely studied deuterated compounds, deuterated water (D2O) can also fundamentally alter the structure and bonding properties of cellular components in living systems—this is known as the solvent isotope effect. Despite contending with both kinetic isotope and solvent isotope effects, many bacteria have been successfully cultivated on nearly 100% deuterated media. Although growth is initially impaired, spontaneous mutants have been shown to arise that are adapted to the fully deuterated media. However, the nature of these mutations is unknown. In order to better understand the genetic determinants of fitness in an environment wherein all molecular hydrogen reactions have been altered, we used two complementary genetic-based approaches on E. coli (BW25113) cultivated in M9 minimal media with D2O (99.0%) and deuterated glucose (1,2,3,4,5,6,6-D7, 97-98%). The first approach employed a genome-wide transposon mutant library in a competitive fitness assay. This approach uncovered 130 genes important for fitness of E. coli in deuterated environments, including multiple genes in a membrane-associated branched-chain amino-acid transporter that are deleterious (livFGHMJ), as well as genes involved in cell wall maintenance (ldcA) and thiamine synthesis and tRNA modification (thiI). Our second approach was an adaptive laboratory evolution experiment that ran for more than 460 generations. Compared to the wildtype, the adapted strains displayed an average 20.1 ± 4.2% improvement in growth yield, a 28.3 ± 15.0% increase in growth rate, and a -74.0 ± 1.8% reduction in lag phase duration when cultivated in deuterated media. Genome sequencing of our evolved isolates is underway and we hope to identify the genetic basis of these phenotypic improvements. Data from these two approaches will provide a greater understanding of cellular physiology useful not only in the manufacture of deuterated compounds but also in a systems-level elucidation of how organisms interact with and transform water and hydrogen.

Proteasomal Inhibition by KSHV protein ORF68. Apurva Govande, Matthew Gardner, Britt Glaunsinger. Dept. of Cellular and Molecular Biology, University of California, Berkeley, Berkeley, CA.

The proteasome is an ATP-dependent multi-subunit protein complex responsible for protein degradation in eukaryotic cells. The 26S eukaryotic proteasome consists of the 20S core particle and the 19S regulatory particle. Protein substrates modified with a poly-ubiquitin tag are targeted to the proteasome for degradation. The 19S regulatory particle is responsible for de-ubiquitination and substrate unfolding, and is the gatekeeper to the core particle. Protein degradation, which occurs in the core particle, is an important part

of regulating cellular processes and is required for the generation of MHC I peptides. Kaposi's sarcoma-associated herpesvirus (KSHV) protein ORF68 inhibits the proteasomal degradation of a model substrate, reducing its rate of degradation in cells expressing the viral protein. ORF68 has been found to have two forms, the full-length protein (ORF68), and a shorter isoform (ORF68.1). ORF68.1 is translated from a secondary start codon and is 78 amino acids shorter than ORF68. Co-purification of the proteasome with fragments of ORF68 expressed in HEK293T cells reveal a strong interaction within the N-terminal region of ORF68.1, described as ORF68-F2. This isolated region of the protein is sufficient to replicate the proteasome-inhibition and reduced rate of degradation phenotype observed with the larger protein. As the region for inhibiting degradation and interacting with the proteasome are the same, this suggests that a physical interaction is responsible for the proteasome inhibition.

A putative, novel interaction surface on ATR1. Derrick Grunwald, Adam Steinbrenner, Sandra Goritschnig, Brian Staskawicz. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

The oomycete plant pathogen Hyaloperonospora arabidopsidis employs effector proteins to evade a host immune response. In turn, plants have adapted an effector-triggered immune response to respond to pathogen invasion, the hallmark of which is localized cell death—the hypersensitive response (HR). The robustness of this immune response, however, is not homogeneous; rather, it is contingent upon the alleles of both the oomycete's effector protein and the plant's resistance protein. To characterize the allele dependency on HR induction, we used the effector protein ATR1 and the resistance protein RPP1 as our model both because of the presence of a crystal structure (of ATR1) and the presence of many differentially recognized alleles (of ATR1). Through site directed mutagenesis of an unrecognized ATR1, we were able to create a mutant that was recognized when competed against the resistance protein that previously did not recognize it. The residues inducing this change constitute a heretofore-uncharacterized surface on ATR1, which we speculate to be a putative interaction surface with the particular allele of the resistance protein.

ORF24-ORF34 interaction mediates KSHV late gene expression and progeny virion production. Charles Hesser, Zoe Davis, Britt Glaunsinger. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

KSHV (Kaposi's sarcoma-associated herpesvirus) is a γ-herpesvirus that is the causative agent of several B cell lymphomas, as well as the AIDS-associated neoplasm Kaposi's Sarcoma. KSHV genes are expressed in an ordered cascade, starting with immediate early (IE) genes, followed by delayed early (DE) and finally late genes. While the mechanisms governing IE and DE gene expression have been extensively studied, late gene regulation remains poorly understood. Our lab has previously shown that late gene expression in KSHV is mediated by a viral protein termed ORF24, which displaces the cellular TATA-box binding protein (TBP) and recruits RNA polymerase II (Pol II) to

late gene promoters (3). However, ORF24 requires the presence of other viral transcription factors (vTFs) to activate late gene expression. As ORF34 had been shown to be necessary for late gene transcription in the related γ-herpesvirus, MHV-68 (2), we postulated that interaction between ORF24 and ORF34 may be critical for late gene expression in KSHV. Indeed, not only did we confirm that ORF24 and ORF34 interact, but that a functional ORF34 protein is required for late gene expression and production of infectious virions. Furthermore, we mapped the region of ORF24 responsible for the interaction with ORF34, and generated a viral mutant which did not interact with ORF34 but retained interaction with Pol II. As seen with the ORF34.Stop virus, the interaction mutant also lacked late gene expression and infectious virion production. Finally, in agreement with data recently reported in the related γ-herpesvirus Epstein Barr Virus (EBV) (1), we found that six KSHV vTFs are necessary and sufficient for activation of a minimal KSHV late gene promoter. This activation is abrogated by substitution of the interaction mutant for wild-type KSHV ORF24. Thus, specific interaction between ORF24 and ORF34 is responsible for nucleating the assembly of a viral transcription pre-initiation complex (vPIC), facilitating late gene expression and subsequent virion production.

Dissecting the genetics of cellulase hyper-secretion in Neurospora crassa. Jason Liu & N. Louise Glass. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

One major expense that governs the price of cellulosic fuels is the cost of the enzymes used to convert lignocellulose into soluble sugars for fermentation. The current industrial cellulase producing host, Trichoderma reesei, is able to produce >100g/L of protein, however, the dearth of genetic tools has hindered the identification of synergistic genetic interactions. Utilizing Neurospora crassa as a cellulolytic, genetically amendable filamentous fungus, we can dissect the necessary genetic interactions by generating a hyper-secreting strain through forward mutagenesis and identifying the required mutations. Using a fluorescently labeled cellulase, we are able to visualize cellulolytic induction and sort using fluorescently activated cell sorting (FACS) to isolate mutant strains up regulated in cellulase expression. Subsequently, we use high throughput plate based cellulolytic assays and candidate based approaches to identify strains increased in cellulolytic activity. Through iterative mutagenesis, we have currently isolated two strains that are >8 fold increased in total cellulolytic activity over WT strains. Through iterative backcrossing and bulk segregation analysis, we hope to dissect the genetic interactions necessary for synergistic hyper-secretion of cellulases. With a better understanding of the mutations necessary to form a cellulase hyper-secreting strain in a filamentous fungus, we can apply this knowledge broadly to industrial systems.

Escape From KSHV-induced mRNA Decay. Oliver Marigold, Mandy Muller, Britt Glaunsinger. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

KSHV, or Kaposi's sarcoma-associated herpesvirus, is the leading cause of cancer in untreated AIDS patients. KSHV acts through an aggressive lytic phase that causes widespread host cell mRNA degradation via the viral endoribonuclease, SOX. Despite such vast mRNA degradation, it has been found that the pro-inflammatory cytokine, human interleukin-6 (IL-6), has been able to avoid the effects of SOX. A portion of the IL-6 3' UTR, known as the SOX Resistant Element (SRE), has been shown to bind cellular proteins, in particular nucleolin (NCL), which lead to its ability to resist degradation. Through the construction of various NCL mutants, we have shown through qPCR data that NCL is indeed a protein which is vital to the success of the SRE. In further studies, we also observed the effects of NCL binding to GADD45A, a gene we believe is similar to IL-6.

Heterologous proteins refold after secretion from a type III secretion system. Kevin James Metcalf, Sandy Rosales, Elias Valdivia, and Danielle Tullman-Ercek. Dept. of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA.

Heterologous protein production in bacteria is a batch process, where the cells are lysed and the protein of interest is purified from the cellular milieu. We are interested in engineering protein secretion in bacteria to simplify protein purification and enable continuous processing. We have engineered the type III secretion system (T3SS) of Salmonella enterica to secrete heterologous proteins at titers exceeding mg/L. In this work, we characterize the refolding of the secreted protein product. Recent structural data shows that the protein is unfolded and linearized as it is secreted through the secretion "tube", which is approximately 2 nm in diameter and 50 nm long. We use enzymatic activity as a proxy for protein folding, and demonstrate enzyme activity in the extracellular space, indicating that secreted proteins indeed refold after secretion. Genetic and chemical methods are used to probe the folded state of the model enzymes beta-lactamase and alkaline phosphatase. The ability to recover folded heterologous protein in the extracellular space demonstrates a strength of the use of a protein secretion chassis.

Detection of Beta-Lactamase in Staphylococcus Isolates Potential Impact on Human Health. Rozina Omidi & Harriet Wilson. Sierra Student Chapter, American Society for Microbiology.

Bacteria isolated from healthy humans, domestic animals and abiotic surfaces were investigated to determine their identity. Cultures were maintained on Tryptic Soy Agar (TSA) plates where colony morphology was determined through direct observation. Cellular morphology was investigated through microscopic examination of nigrosin indirect stains; Gram stains and KOH tests were used to determine cell wall composition. Isolates were tentatively identified through 16S ribosomal-RNA nucleotide sequencing, and were then subjected to a series of enzymatic tests for verification. The presence of catalase, urease, cytochrome c oxidase, coagulase, and hemolysin proteins was determined. Carbohydrate slants were used to detect aerobic acid production from arabinose, glucose,

inositol, lactose, mannitol, raffinose, rhamnose, sorbitol and sucrose. Methyl-red and Voges-Proskauer (MR-VP) tests were completed to determine fermentation capability with the production of acid and acetoin. The bacterial isolates were genetically most similar to Staphylococcus aureus, S. auricularis, S. capitis, S. epidermidis, S. equorum, S. felis, S. haemolyticus,S. hominis, S. pasteuri, S. pseudointermedius, S. saprophyticus, S. sciuri, S. simulans, S. stephanovicii, and S. warneri. Results of enzymatic testing generally confirmed these identities. Because some of the isolates were recognized as potential human pathogens, or as emerging pathogens in domestic animals, 30 isolates were tested for the presence of β-lactamase using Nitrocefin, out of which 47% were strongly positive, 30% were weakly positive, and 23% were negative.

Increased Secretion of Heterologous Proteins via Elimination of Flagellar and Type 3 Secretion System Virulence genes in Salmonella. Michelle Reid, K. Metcalf, E. Valdivia, D. Tullman-Ercek. Dept. of Molecular and Cellular Biology, University of California, Berkeley, Berkeley, CA.

The production of biomaterials, analysis of protein structure and function, and protein therapeutics require large quantities of purified protein that can be synthesized in a host such as E. coli. The overexpression of heterologous proteins and purification processes pose an array of issues such as degradation by proteases, contamination by endotoxins, and the successive loss of protein product due to the many necessary purification steps (2). The type III secretion system (T3SS) pathogenicity island 1 (SPI-1) present in Gram negative bacteria like Salmonella enterica, plays an important role in pathogenicity because it can efficiently secrete proteins into a host cell that initiate virulence, known as virulence effectors. We propose that engineering the T3SS and eliminating genes that encode natively secreted proteins (structural flagellar proteins and T3SS virulence effectors) in Salmonella enterica can circumvent the issues associated with obtaining highly expressed and secreted heterologous protein product. Previous work demonstrates that S. enterica T3SS SPI-1 could be engineered to export heterologous POI such as spider silk monomers (3). Currently, secretion levels are a fraction of the industry standard and secretion samples often contain a variety of natively secreted proteins which serve as contaminants in our secreted POI samples. Komoriya et al. found that the deletion of structural flagellar genes could increase secretion levels of virulence effectors, this is an important finding because we use the T3SS's native transcription factors and promoters to express the POI and thus we expect this effect to be conserved. I hypothesize that by deleting native flagellar and T3SS virulence effector genes, we can further increase secretion levels of the desired protein while simultaneously minimizing secreted flagellar proteins and virulence effectors into the supernatant. I will use recombineering methods to eliminate the genes that encode secreted flagellar proteins and T3SS virulence effector proteins. After the progressive elimination of genes coding for flagellar and T3SS virulence effector , secretion and expression levels will be quantified by western blot and contaminant levels will be visualized by coomassie staining. We expect these results to further our lab's efforts to create a strain for highly

specific export of heterologous proteins into the growth media at levels meeting industrial demand.

High-throughput quantification of folded, secreted protein titer by the Type III Secretion system in Salmonella. Sandy Rosales, Kevin Metcalf, Elias Valdivia and Danielle Tullman-Ercek. Dept. of Nurtitional Science and Toxicology, University of California, Berkeley, Berkeley, CA.

Current yields of heterologous proteins from E. coli are drastically reduced during purification and isolation steps for converting cytoplasmic aggregates into functionally active proteins. Secretion into the extracellular space by the Type III Secretion System (T3SS) in Salmonella may achieve higher industrial-level productions of recombinant protein, compared to conventional methods using E. coli, by promoting proper protein folding and preventing aggregation. Previously, we observed activity of the secreted enzyme, beta-lactamase (Bla), after being translocated through the narrow opening of the T3SS indicating that proteins refold into native confirmations post- secretion. With active secreted Bla we can quantify concentrations of folded, secreted protein by obtaining the kinetic parameters Vmax, the maximum reaction rate, and kcat , which represents the turnover rate of a single enzyme. Quantifying secreted proteins with conventional western blotting techniques requires a large amount of time to screen mutants that improve secretion; however, through application of the Michaelis-Menten rate equation, we determined a more robust analysis of secreted enzyme titer. Indeed, a higher reaction rate indicates a higher concentration of secreted protein, and a lower reaction rate indicates a lower concentration of secreted protein. This enzyme assay is a higher-throughput method for calculating the total concentration of protein because of it allows for a quick screen that can detect smaller quantities of secreted enzyme from various mutants as compared to quantitative western blotting.

Phylogenomics of Xanthomonas field strains infecting pepper and tomato reveals diversity in effector repertoires and identifies determinants of host specificity. Allison R. Schwartz, Neha Potnis, Rebecca S. Bart, João C. Setubal, Jeffery B. Jones and Brian J. Staskawicz. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

Bacterial spot disease of pepper and tomato is caused by four distinct Xanthomonas species and is a severely limiting factor on fruit yield in these crops. The genetic diversity and the type III effector repertoires of a large sampling of field strains for this disease have yet to be explored on a genomic scale, limiting our understanding of pathogen evolution in an agricultural setting. Genomes of sixty-seven Xanthomonas euvesicatoria (Xe), Xanthomonas perforans (Xp), and Xanthomonas gardneri (Xg) strains isolated from diseased pepper and tomato fields in the southeastern and midwestern United States were sequenced in order to determine the genetic diversity in field strains. Type III effector repertoires were computationally predicted for each strain, and multiple methods of constructing phylogenies were employed to understand better the genetic relationship of

strains in the collection. A division in the Xp population was detected based on core genome phylogeny, supporting a model whereby the host-range expansion of Xp field strains on pepper is due, in part, to a loss of the effector AvrBsT. Xp-host compatibility was further studied with the observation that a double deletion of AvrBsT and XopQ allows a host range expansion for Nicotiana benthamiana. Extensive sampling of field strains and an improved understanding of effector content will aid in efforts to design disease resistance strategies targeted against highly conserved core effectors.

Phage Selection by Plant Root Soil Systems. Jessica Trinh[1] and Evan Starr[2]. [1] Dept. of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA; [2] Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

The rhizosphere, or the narrow region of soil that is directly influenced by a plant root system, comprises of dynamic interactions between plant roots and the surrounding microbial community. Roots produce important carbon sources which select for bacteria that can quickly use them to their advantage. As the plant grows and dies, it causes blooms of bacteria that can adapt to the changing conditions, showing that the plant is a major factor in selecting for these types of bacteria. Bacteriophages are key regulators of bacterial populations in non-soil environments, such as marine systems. However, little is known about the roles that they play in soil. We would like to discover how phages factor into the interactions and selection processes with the microbial communities of the rhizosphere. To do this we will compare the ability of phages isolated from the rhizosphere to infect bacteria from the rhizosphere and bulk soil, soil that is not influenced by a root. Plaque assays will be performed on both types of isolated bacteria. We believe that the existence of a root causes a chain reaction which selects for certain types of bacteria, thus allowing corresponding phages to infect and multiply. Therefore, the root environment selects for particular phages in this network of interactions. Molecular and sequencing techniques can then be used to identify the bacteria and their corresponding bacteriophage. This information can then be used in further metagenomics studies to see what other roles these phages have in the rhizosphere. By understanding these roles, we can better improve our understanding of plant-microbe interactions.

Characterization of KSHV mutant P176S. Aprilgate Wang, Emma Abernathy, Sarah Gilbertson, Britt Glaunsinger. Dept. of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA.

Infection by KSHV progresses immediately into the latency stage where the viral protein SOX induces widespread global mRNA degradation by incising mRNA and inducing the cellular 5'->3' exonuclease XRN1 to complete full decay. This pathway and the interactions between the host and virus has been closely studied in the KSVH mouse homolog, MHV68, but has yet to be studied in humans. We have created a mutant through bac mutagenesis in the 176 position of the SOX protein from proline to serine. Through characterization we hope to see deficiency in mRNA degradation. SOX also

causes hyperadenylation, an abnormally long poly-A tail on the mRNA due to the relocalization of PABPC, a cytoplasmic protein that normally binds and stabilizes the poly-A tail. It's relocalization into the nucleus decreases mRNA protection and allows for an easy target. Deficiency of a working SOX should render the buildup of PABPC in the cytoplasm.

On the flip side, we are also looking at XRN1 localization in sites known as P Bodies in the cytoplasm where it has been found that mRNA degradation occurs. Although we have confirmed XRN1 resides in these sites, it is unknown whether other proteins localize. We are targeting proteins such as DCP2, an exonuclease, GW182, a scaffolding protein, and Tia-1, stress granules, in order to better characterize the P bodies.

Travel Grants

As part of the Microbiology Student Group's efforts to promote microbiology in California, we are proud to award Travel Grants, generously sponsored by Biolog, to the following distinguished speakers from around California:

Matthew O'Neill
Department of Biology
University of California, Riverside

Keerthana Sekar
Department of Biological and Biomedical Sciences
California State University, Chico

Yuanchun Wang
Department of Plant Pathology and Microbiology
University of California, Riverside

The Microbiology Student Group at UC Berkeley would like to thank the following companies and organizations for their kind support in making the 16th Annual Microbiology Student Symposium a success.

Biolog, Inc.

PLANT & MICROBIAL BIOLOGY

COLLEGE OF NATURAL RESOURCES
UNIVERSITY OF CALIFORNIA, BERKELEY

The Department of Plant and Microbial Biology

Molecular & Cell Biology

The Department of Cellular and Molecular Biology

The GraduateAssembly

The Graduate Assembly of UC Berkeley

AMERICAN SOCIETY FOR MICROBIOLOGY

The American Society for Microbiology