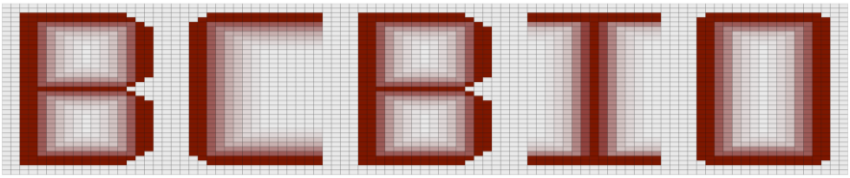

retreat 2017

August 21-22, 2017

The Connors Center
Dover, MA

Boston College Biology Department

Organizing Committee

Alex Auld
Beth Bearce
Burcu Erdogan
Elise Gray
Brigitte Lawhorn
Jaclyn Mallard
Amy Valera
Karen Zhu
Diane Butera
Dina Goodfriend
Charles Hoffman
Welkin Johnson

Welcome New Biology Ph.D. Students

Scott Daniska
Previous Institute: University of Connecticut
Structural Biology and Biophysics
Current Residence: Bethel, Connecticut
Research Interests: Molecular Biology and Genetics

Allison Drozda
Previous Institute: SUNY Geneseo
Current Residence: East Aurora, New York
Research Interests: Parasitology and Infectious Disease

Joseph Enders
Previous Institute: California Lutheran University
Current Residence: Big Timber Montana
Research Interests: Cellular, Molecular, and Neuroscience Biology

Suyen Espinoza Miranda
Previous Institute: John Brown University
Current Residence: Managua, Nicaragua
Research Interests: Infectious Diseases.

Catherine May
Previous Institute: Arizona State University
Current Residence: Brighton, Massachusetts
Research Interests: Evolution of developmental mechanisms in vertebrates

Stacy Nguyen
Previous Institute: CSU Dominguez Hills
Current Residence: Placentia, California
Research Interests: Neurobiology and Neurophysiology

2017 Biology Department Retreat
Day 1: Monday, August 21

8:45 am Continental Breakfast

9:15 am Opening Remarks

Welkin Johnson

9:30 am Guest Speaker

Ken Burch
Department of Physics
Boston College

10:15 am Break

Session I – Chair, Ben Fofana

10:30 am Doug Warner

11:00 am Jamie Henzy

11:15 am Amy Valera (Chiles Lab)

11:30 am Lunch

Session II – Chair, Rebecca Dunn

1:45 pm Clare O'Connor

2:30 pm Solar Eclipse

3:15 pm Guest Speaker

Vincent Racaniello
Department of Microbiology and Immunology
Columbia University

4:00 pm Group picture and break

4:30 pm Poster session and cocktail reception

6:30 pm Dinner

8:00 pm Pub Quiz

2017 Biology Department Retreat
Day 2: Monday, August 22

8:00 am Breakfast

Session III – Chair, Bret Judson

9:00 am Sarah McMenamin

9:30 am Paula Slater (Lowery Lab)

9:45 am Micaela Lasser (Lowery Lab)

10:00 am *Break*

10:15 am Babak Momeni

10:45 am Jaclyn Mallard (Williams Lab)

11:00 am Elise Gray (Meyer Lab)

11:15 am Awards

11:30 am Closing Remarks

Welkin Johnson

Session I

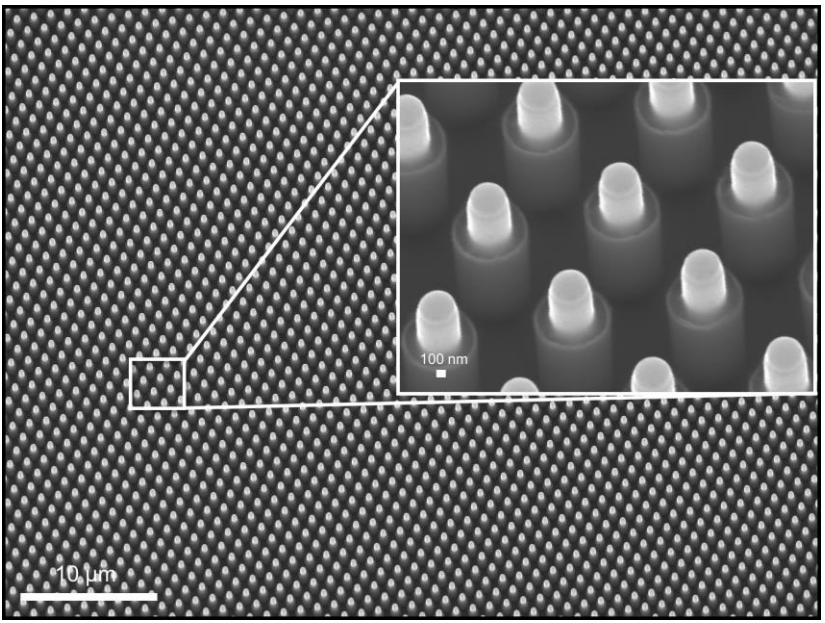

Image credit: Amy Valera. A scanning electron micrograph of an array of extended core coaxes magnified 2000x (inset magnified 30,000x).

Session Chair: Ben Fofana

The Structure, Science & Benefits of the Introductory Biology Laboratory

Doug Warner

Investigations in Molecular Cell Biology, BIOL2040, is the practical foundation in many undergraduate's research experience in the Biology Department. The course is also the most student-contact heavy experiences for graduate Teaching Assistants. In BIOL2040, students are taught basic molecular biology techniques all in the flow of a research project narrative involving yeast genetics. Seeking to give the students a baseline experience for laboratory research, the course includes data analysis, lab meetings, posters and manuscript writing. This presentation will explain the origins of the course, the scientific techniques that students are exposed to, and the current state of our "Pathways Over Time" research project.

Margaret Dayhoff: More than a flash in the PAM

Jamie Henzy

Most researchers are familiar with Margaret Dayhoff's name from the PAM series of amino acid scoring matrices. However, this contribution was only a small part of her work. From the one-letter amino acid code, to the first algorithms to assemble protein sequences from fragments, to the prototype of the first sequence database, her contributions were foundational to the field of bioinformatics. She was developing this groundbreaking work at a time when women scientists were rare, and some universities even had rules against promoting them to senior faculty positions. Although many institutional and societal barriers to women's advancement in the biological sciences have been removed, some biases still exist among critics who claim that Dayhoff's work with sequence collecting and computing were glorified clerical tasks. I'd like to present a fuller account of her contributions, particularly in terms of her vision regarding the potential utility of large collections of sequences and the biological principles these could reveal. In a sense, she helped to catalyze a metamorphosis of the comparative approach of organismal biology into similar methods applied at a molecular level, and at a wider, more productive scale.

A novel lab-on-a-chip immunosensor for point-of-care diagnostics

Amy Valera, Luke D'Imperio, Michael J. Naughton, Thomas C. Chiles

Several factors, including poor sanitation and a lack of public healthcare infrastructure contribute to the rapid spread of disease outbreaks in resource-limited areas around the globe. A diagnostic device that achieves point-of-care parameters, such as ease of use, low cost, portability, sensitivity and specificity, represents an urgent unmet need in disease epidemiology. We report here on the development of a novel lab-on-a-chip diagnostic technology for the detection of cholera toxin, based on a novel nanoarchitecture: the Extended Core Coax (ECC). The architecture represents a vertically oriented nanocoax comprised of a gold inner metal core that extends ~200 nm above a chrome outer metal shield, separated by a dielectric annulus. Each ECC chip contains 7 individual sensing arrays, 0.49 mm2 in size, containing ~35,000 nanoscale coaxes connected in parallel. We have demonstrated the ability to functionalize the ECC by forming a layer of poly-(2-cyanoethyl)pyrrole (PCEPy) on a chip surface via cyclic voltammetry (CV). A variety of macromolecules (e.g., antibodies) can be electrochemically tethered to the PCEPy layer and oriented away from the surface, allowing for maximal biomarker (cholera toxin) capture. Binding of antibodies to the ECC surface provides a platform for electrochemical ELISA-based detection, with a significantly decreased demand for reagents due to the small sensing region. Additionally, the close proximity of reagents to the sensing region will result in an increase in sensitivity beyond that of current state-of-the art technologies, such as optical-based ELISA and our previous non-ECC nanocoaxial architecture.

Session II

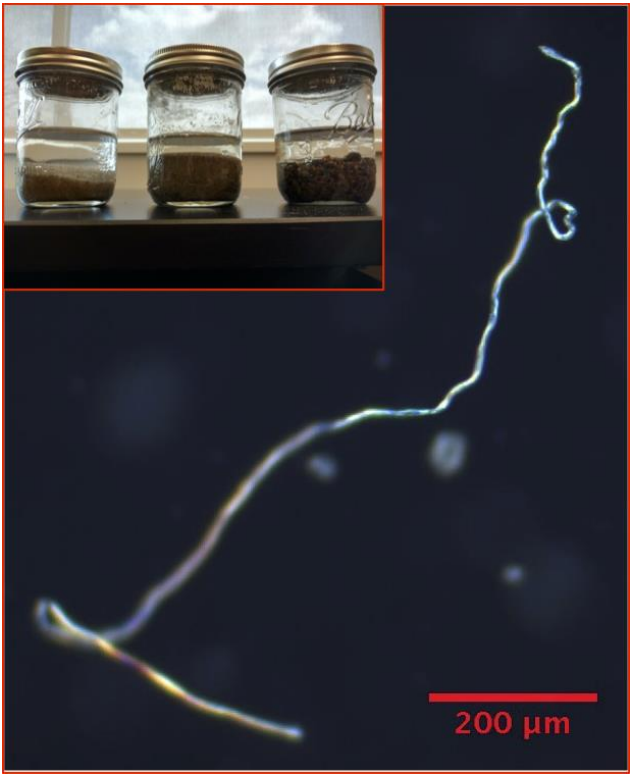

Image credit: Amanda Rickert and Bret Judson. A microfiber found in a sand sample from Skaket beach (inset shows collection methods). Scale bar 200 µm.

Session Chair: Rebecca Dunn

Serendipity - in retrospect

Clare M. O'Connor

Scientific careers rarely take a straight path to a predetermined goal. I'll talk about the many twists and turns in my own scientific career, spanning a time in which the biological sciences underwent transformative changes. A career in science is also a personal journey, and I'll discuss the delicate balancing act between my own professional and family/community lives. In many respects, my career is the product of serendipity - recognizing and embracing the opportunities that arise.

Session III

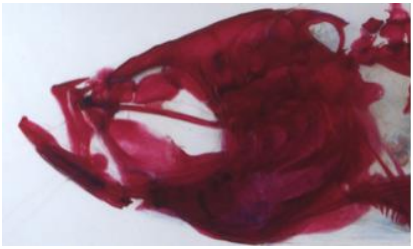
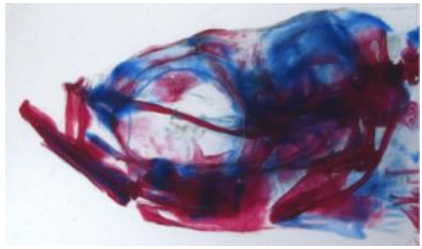

Image credit: Sarah McMenamin. Left, a wild-type adult zebrafish skull showing nearly complete ossification (red); Right, a hypothyroid adult zebrafish skull showing extensive ossification failure (blue).

Session Chair: Bret Judson

Roles of thyroid hormone in sculpting the craniofacial skeleton

Sarah McMenamin

Improper coordination of post-embryonic development can lead to debilitating diseases, including craniosynostosis. Our lab is interested in the factors that control developmental coordination in vertebrates, particularly as coordination affects development of the craniofacial skeleton. Using a transgenic conditional thyroid ablation line and a hyperthyroid mutant zebrafish, we have shown that thyroid hormone is essential in the proper regulation of post-embryonic development. Moreover, zebrafish with abnormal levels of thyroid hormone show severe skull malformations. Our work aims to understand the pathways through which thyroid hormone functions to coordinate skull morphogenesis and ossification, and skeletal development overall.

XMAP215 contributes to the spatiotemporal guidance of microtubules and the morphology of Xenopus laevis growth cones

Paula Slater, Annika Samuelson, Alexandra Magee, Laura Anne Lowery

During neuronal development, neurons migrate towards their final destination and elongate their axons to form new connections. The growth cone is a dynamic structure located at the tip of the axon that senses and interprets cues in the embryonic terrain, driving axon outgrowth. The coordination between the actin and microtubule (MT) cytoskeletons underlies the growth cone shape and is crucial for generating an axonal morphological response. The family of plus-end tracking proteins (+TIPs) binds to and regulates MT behaviors, and couples MTs to F-actin dynamics. Our lab has demonstrated that the +TIP and MT polymerase, XMAP215, participates in MT features that are dependent on F-actin regulation, such as MT translocation rates and MT trajectory collinearity. Our goal is to determine the XMAP215 contribution to growth cone morphology and to MT/F-actin coupling. For that purpose, we use Xenopus laevis spinal cord explants, which is an ideal model system for examining growth cone cytoskeletal dynamics. We first analyzed the growth cone morphology after genetic manipulation by using spinning disc confocal microscopy. We found that the XMAP215 KD growth cones are larger and have longer filopodia, while the number of filopodia and F-actin distribution are not altered. By analyzing the MT morphology and distribution using super resolution microscopy, we found that for XMAP215 KD: 1) there is an increase in the looped morphology of MT; 2) MTs are more spread throughout the entire growth cone; and 3) there is an increase of dynamic MTs in the growth cone. Additionally,

the number of MTs that are coupled to F-actin bundles in the peripheral zone of the XMAP215 KD growth cone is not altered, but there was an increase in the number and orientations of MTs advancing into the periphery that are not coupled to F-actin bundles. These results show that XMAP215 could have a role in the regulation of spatiotemporal guidance of MTs, a function that is attributed to the actin cytoskeleton. Thus, XMAP215 could be a participant in MT/F-actin coupling.

Using Xenopus laevis as a model for studying genes associated with intellectual disabilities

Micaela Lasser, Connor Monohan, Jessica Tiber, Sangmook Lee, Aleks Ostojic, Santhosh Girirajan, Lucilla Pizzo, Laura Anne Lowery

Intellectual disabilities are complex neurodevelopmental disorders that affect a significant portion of the general population and can be an immense health issue. Genetic mutations caused by rare copy number variants (CNVs) have been shown to play a role in intellectual disabilities or severe developmental delays. It has been previously shown that one such CNV results in a deletion within chromosome 16 in a subset of children clinically diagnosed with an intellectual disability. In addition to having intellectual disabilities, individuals with this deletion, located at 16p12.1, also have severe craniofacial abnormalities, cardiac defects, seizures, and growth retardation. This deletion encompasses six genes, many of which have not yet been studied in relation to neurodevelopment. In order to elucidate the role of these six genes during development, we have created antisense morpholino oligonucleotides to knock down gene function and observe phenotypic differences in Xenopus laevis. Importantly, each gene is highly conserved between humans and Xenopus laevis, making it an ideal model organism to understand the developmental mechanisms of this genetic deletion. Here, we show phenotypic differences of the 16p12.1 deletion by quantifying changes in craniofacial morphology, brain morphology, and axon outgrowth patterns of each individual gene knock down. We have found that reduction of three of these genes, POLR3E, UQCRC2, and C16orf52 leads to severe craniofacial defects. Additionally, we have found that reduction of C16orf52 also decreases axon length, and that knockdown of POLR3E causes smaller

brain size. Further, we have also performed in situ hybridization to show the localization of each gene during different developmental stages. Together, our results suggest that several of the 16p12.1 genes do play an important role during neurodevelopment and future studies will focus on the effect of combinatorial knockdown of these genes, as well as the mechanisms by which they modulate aspects of craniofacial morphology, brain morphology, and axon outgrowth patterns.

Coexistence of interacting microbes: It's about how to treat each other

Babak Momeni, Minghua Liu, Lori Niehaus, Kevin Chen, David Fu, and Sandra Dedrick

Background and motivation: Microbial activities, often taking place within assemblies of different microbial species (called "microbial communities"), affect us and the environment around us. Some microbes harm us by causing persistent infections. Others benefit us as our resident microbiota by blocking infections. Having an expanded repertoire of genetic capabilities, a community of microbes can perform functions (such as degradation of complex compounds) that individual species cannot achieve efficiently. We aim to discover how interactions among members of a community can lead to species coexistence.

Methods: Communities are usually modeled as a network of ecological interactions between pairs of species. Such pairwise modeling has a long history, supported by empirical success of Lotka-Volterra models of prey-predation and popularized by Robert May's seminal work on community stability. However, we have recently shown that such models poorly represent microbial communities where interactions often occur through interactions with diverse molecular mechanisms. Here, we build an experimentally-inspired mechanistic model of microbial communities by explicitly incorporating the chemicals (such as metabolites, toxins, or waste products) that mediate microbial interactions. Using mechanistic models, we computationally screen for network configurations that can lead to coexistence. Simulating the process of enrichment, we compile an ensemble of communities and search for commonalities in their interaction networks that allow coexistence.

Results: Our screen revealed certain network features that correlate with coexistence. For instance, we have identified facilitation (e.g. producing beneficial metabolites) and self-inhibition (e.g. producing compounds that are inhibitory to the producer) to commonly occur among coexisting species. Interactions that inhibited other species were found to be detrimental to coexistence. Through “knock-out” experiments, we confirmed that such interactions causally contribute to coexistence in communities. We will further explore the role of interaction mechanisms on these findings. These results expand our understanding of interaction networks that can promote coexistence.

Conclusions: Using a mechanistic model of microbial interactions, we computationally search for network configurations that support coexistence. Facilitation and self-inhibition interactions are found to support coexistence, whereas inhibition of other members disrupts coexistence. Through a better understanding of coexistence mechanisms, we aim to discover strategies to maintain communities beneficial to us or to disrupt the ones that cause us harm.

A Method for Obtaining Simian Immunodeficiency Virus RNA Sequences from Laser Capture Microdissected and Immune Selected CD68+ and CD163+ Macrophages from Frozen Tissue Sections of Bone Marrow and Brain

Jaclyn Mallard, Emily Papazian, Caroline Soulas, David J Nolan, Marco Salemi, Kenneth C Williams

Laser Capture Microdissection (LCM) is used to extract cells or tissue regions for analysis of RNA, DNA or protein. Several methods of LCM are established for different applications, but a protocol for consistently obtaining lentiviral RNA from LCM selected immune cell populations is not described. Obtaining optimal viral RNA for analysis of viral genes from immune-captured cells using immunohistochemistry (IHC) and LCM is challenging. IHC protocols have long antibody incubation times that increase the risk of RNA degradation. Also, often only a fraction of captured cells are productively lentivirally infected and viral copy numbers are low. In this study we sought to obtain simian immunodeficiency virus (SIV) RNA from SIV gp120+ and CD68+ monocyte/macrophages in bone marrow (BM) and CD163+ perivascular macrophages in brain of SIV-infected rhesus macaques. Here, we report an IHC protocol with RNase inhibitors that consistently results in optimal quantity and yield of lentiviral RNA from LCM-selected immune cells.

The rplU-ysxB-rpmA operon in Bacillus subtilis is auto-regulated by ribosomal protein L21 via an RNA cis-regulatory element.

Elise Gray, Arianne Babina, Carolyn Larkins, Michelle Meyer

Ribosomes perform an essential cellular function and are structurally complex with numerous RNA and protein components. In addition, the rate of ribosome production is growth limiting in many bacterial species. Thus to facilitate proper ribosome assembly and make the best use of cellular resources, ribosome building blocks must be produced in precise stoichiometric ratios. To control the production of ribosome components many bacterial ribosomal protein operons are regulated by cis-regulatory RNAs that occur in 5' untranslated regions. These cis-regulatory RNAs, or leaders, form secondary structures that bind a ribosomal protein from the same operon, leading to repression of translation, or premature transcription termination. This phenomenon is well established in E. coli, a gram-negative bacterium, but little work has been done to assess how stoichiometry is maintained in other bacteria. In the model gram-positive organism B. subtilis several potential mRNA leaders have been identified computationally by comparative genomics, but few have verified regulatory action. One such leader precedes the rplU-yskB-rpmA operon encoding ribosomal proteins L21 and L27 as well as a protease involved in the maturation of L27. The mRNA leader preceding L21 was identified in organisms across the phylum Firmicutes and has a well-defined and conserved structure. Using beta-galactosidase reporter assays and qPCR, over-expression of L21 was found to repress reporter gene expression 2-fold, but have minimal impact on mRNA levels, suggesting translational regulation. In vitro studies

confirm binding of the L21 protein to the L21 leader RNA, supporting the in vivo evidence that the rplU-yskB-rpmA operon is cis-regulated by the L21 protein. Together, these studies provide the first experimental evidence for cis-regulation of the L21 operon in Gram-negative species.

2017 Biology Department Retreat
Poster Presentations

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Brett Bukowski	Characterization of the *Pseudomonas aeruginosa* ExoY virulence factor using genetic and chemical genetic screens in *S. pombe*	2
Mary Ann Collins	Nucleus-nucleus interactions are regulated by two distinct genes linked to Emery-Dreifuss Muscular Dystrophy and Centronuclear Myopath	3
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2017 Biology Department Retreat
Poster Abstracts

1. Aplip1 (Drosophila JIP1) regulates myonuclear positioning and muscle stability

Alexander Auld, Ciaran Murphy, Jaclyn Camuglia, Eric Folker

During muscle development myonuclei undergo a complex set of movements that result in evenly spaced nuclei throughout the muscle cell. In Drosophila two separate pools of Kinesin and Dynein govern this process. Although these two pools of motors work in synchrony, how these two pools are specified is not known. Here, we investigate the role of Aplip1 (a JIP1 homolog), a known regulator of both Kinesin and Dynein, in myonuclear positioning. Aplip1 localizes to myotendinous junction where it can extend from the muscle pole. Additionally, Aplip1 has independent roles in myonuclear positioning and muscle stability. In Aplip1 mutant embryos there was an increase in the percentage of embryos that had both missing and collapsed muscles. Via a separate mechanism, we demonstrate that Aplip1 is required to position and dynamically move nuclei within muscle. Through genetic interaction experiments, we saw that Aplip1 interacted with the Dynein anchor, Raps, and Kinesin to correctly position nuclei within muscle cells. We propose that Aplip1 is important for normal myonuclear movement via the regulation of Dynein at the cell cortex and the regulation of Kinesin activity at the nuclear envelope.

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2. Characterization of the *Pseudomonas aeruginosa* ExoY virulence factor using genetic and chemical genetic screens in *S. pombe*

Brett Bukowski, Olivia Duddy, and Charles S. Hoffman

The *Pseudomonas aeruginosa* ExoY protein is a promiscuous cyclase that acts as a virulence factor and requires unknown mammalian cofactors in order to be active. However, it is a highly active cyclase that produces cGMP, cAMP, cUMP, and cCMP when expressed in the fission yeast *Schizosaccharomyces pombe*, indicating that *S. pombe* produces the homologous cofactor or cofactors required for ExoY activation. To identify these cofactors, we carried out a plasmid-based screen for hypomorphic alleles of exoY. This collection of mutant alleles suggests that there are two distinct surfaces of the protein, and thus possibly two cofactors, required for activation. In addition, we are purifying a 6his-tagged form of ExoY in an effort to identify co-purifying proteins. Finally, there are currently no known ExoY inhibitors with which to study the functional role of ExoY in P. aeruginosa virulence. We developed a *S. pombe* screening assay using a PKA-repressed fbp1-GFP reporter whose expression reflects intracellular cyclic nucleotide levels and have carried out a high throughput screen on approximately 100,000 compounds to identify ExoY inhibitors. We validated some of the candidate hit compounds by directly measuring cGMP and cAMP levels in compound-treated cells via mass spectrometry. In addition, we identified a compound that fluoresces upon binding lysed cells and have used this to demonstrate the ability of other compounds to reduce the ExoY-induced loss

of viability in stationary phase that is characteristic of strains with unregulated PKA activity. This work demonstrates the utility of *S. pombe* for both genetic and chemical genetic studies of proteins involved in cyclic nucleotide metabolism.

3. Nucleus-nucleus interactions are regulated by two distinct genes linked to Emery-Dreifuss Muscular Dystrophy and Centronuclear Myopathy.

Mary Ann Collins, Torrey R. Mandigo, Jaclyn M. Camuglia, and Eric S. Folker

Syncitia, cells with multiple nuclei, are less prevalent than their mononucleated counterparts. However, there are many examples of syncytia in biology. The developing Drosophila blastoderm and skeletal muscle are commonly studied examples as are less frequently studied examples such as placenta and osteoclasts. Little is known about how the presence of many nuclei in each of these cells impacts their specific biology. Fundamental to understanding the biology of syncytia is understanding how and when each nucleus interacts with the other nuclei. In muscle, the rapid movement of nuclei to the cell center to join already incorporated nuclei suggests attractive interactions. Conversely, there are repulsive interactions between nuclei later in muscle development which are utilized to keep nuclei spaced distant from one another. Because muscle exhibits both attractive and repulsive interactions that are separated in developmental time, it is an ideal syncytia to identify the mechanisms and functions of nucleus-nucleus interactions. In Drosophila, movement of nuclei in muscle occurs in three distinct stages. After the completion of fusion, nuclei separate into two clusters, one positioned dorsally and the other positioned ventrally. Each cluster then moves directionally toward its respective pole with fewer than 2%

of nuclei change directions. During directional movement, the nuclei remain in tightly associated clusters. Finally, after nuclei reach the muscle end they then move back into the cell center and maximize the distance between adjacent nuclei. Disruption of two different genes, bocksbeutel (dEmerin) and klarsicht (dNesprin), blocked initial separation of nuclei into two distinct clusters. Live-embryo time-lapse microscopy demonstrated occasionally a nucleus could escape the cluster. These nuclei moved directionally as in controls and moved more quickly than controls indicating that the force generating machinery and the spatial cues were functional. Thus, bocks and klar were necessary to regulate the necessary repulsive interactions between nuclei. Conversely, disruption of Amphiphysin, inhibited the attractive interactions between nuclei. This was evident by the regular dissociation of nuclei from clusters and the presence of these nuclei in the center of the muscle. Together these data indicate that nuclei do indeed have attractive and repulsive interactions in skeletal muscle and that these interactions are regulated by distinct proteins at specific developmental times.

2017 Biology Department Retreat
Poster Abstracts

4. Double, Double, Toil, and Trouble

Matthew Crum, Nikhil Ram Mohan, Michelle M. Meyer

Riboswitches are ligand binding structures, called aptamers, within an mRNA that affect the expression of downstream genes. They are found widely spread throughout bacterial species and come in many varieties, each of which binds a distinct ligand. The glycine riboswitch is unique. First, it can be found as a single structure or as a tandem doublet structure, which appears to be the result of a duplication event. Second, it frequently acts as both an "on" switch (*Bacillus subtilis*) and an "off" switch (*Streptococcus pneumoniae*). These divergences in structure and regulatory action have led us to question how singlets have diverged to form doublet riboswitches and whether the duplication event occurred multiple times. To address these questions, we utilized a covariance model to identify over 1,000 putative glycine riboswitches within the RefSeq database. We find that the aptamers tend to cluster so that singlets and doublets each grouped together. Moreover, not only do the identified singlet and doublet riboswitches differ significantly in their conformation to the covariance model, but the individual aptamers of doublet riboswitches differ from each other as well. We are currently in the process of generating a covariance model that better identifies individual doublet aptamers or doublet riboswitches as a unit. Having such models will allow for more readily identified doublet riboswitches enabling further investigation into regulatory and structural differences between singlet and doublet riboswitches.

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5. Interactions in a human-associated microbial community: picking apart the nasal microbiome

Sandra Dedrick, Babak Momeni

The nasopharynx is a reservoir to a diverse range of microbial phyla that includes commensal and pathogenic bacteria. In this host environment, interspecies interactions play a significant role in shaping the composition of the microbial community. More specifically, previous research has demonstrated that certain commensal bacteria of the nasal microbiome produce compounds that inhibit the growth of opportunistic pathogens such as Staphylococcus aureus and Streptococcus pneumoniae. In our research, we aim to elucidate the mechanisms by which: 1) commensals interact with pathogenic bacteria and, 2) commensals interact with one another. To achieve this, we are employing a number of in vitro experimental methodologies to test all pair-wise interactions between isolated species and establish a steady-state multi-species community. From this, we aim to create a computational model describing the observed interactions of simple pair-wise interactions with the goal of expanding these data to a more complex multi-species (3+) community. Through initial microbial-supernatant exposure experiments, we have obtained phenotypic data on individual species in various cell-free supernatants. The growth rate and population carrying capacity data thus far suggests that factors influencing growth inhibition and/or enhancement are produced by a number of species in the nasal microbiome. By using both a 'bottom-up' and 'top-down' approaches, we hope to converge upon a clear understanding of what a steady-state nasal microbiome community looks like along with what mechanisms drive the interactions observed within the community.

6. Modeling bacterial contact-dependent growth inhibition

Samantha Dyckman, Babak Momeni

Bacteria within communities have complex interactions. Contact-dependent growth inhibition (CDI) is an example of a negative cell-to-cell interaction that can strongly impact the dynamics of a community. The CDI cells will inhibit target cells, and this interaction will only occur if direct contact is made between the cells. CDI positive cells also produce an immunity protein to neutralize the CDI toxin, preventing self-harm or harm to other closely related members of the community. However, there is not a model for describing this negative inhibition on target cells.
Here, we are using CDI to better understand negative interactions within communities. Modeling CDI dynamics will elucidate negative interactions as a whole and their role in community stability. For this approach, we use two-species communities comprised of an *Escherichia coli* (*E. coli*) strain that exerts CDI and a target *E. coli* K12 strain. The two species are labeled with different fluorescent markers. This allows us to quantitatively measure how the community's populations change over time. The fluorescence intensity at varying ratios and density of the populations are measured. Then using this data, we quantify the impact of the CDI on the target cells. We construct a model to describe the impact of this negative interaction in a community. This will facilitate future efforts in microbial community modeling.

7. The microtubule plus-end-tracking protein TACC3 promotes persistent axon outgrowth and mediates responses to axon guidance signals during development

Burcu Erdogan, Garrett Cammarata, Eric Lee, Ben Pratt, Erin Rutherford, Riley St. Clair, Bryan Ballif, Laura Anne Lowery

Precise neuronal connection requires proper axon guidance. Microtubules (MTs) of the growth cone are the driving force to navigate the growing ends of axons. Pioneering microtubules and their plus-end tracking proteins (+TIPs) play integrative roles during this navigation. Recently, we introduced the protein TACC3 as a member of the +TIP family regulating microtubule dynamics in Xenopus laevis growth cones and show that manipulation of TACC3 levels affects axon outgrowth by regulating axon outgrowth velocity and the frequency of axon retraction. Additionally, we show that over-expressing TACC3 mitigates nocodazole-induced reduction in MT dynamics parameters suggesting that TACC3 could play a protective role against nocodazole induced MT depolymerization. Moreover, we find that TACC3 and its partner XMAP215, a well-characterized MT polymerase, cooperate to promote axon outgrowth and rescue axon growth defects. Finally, we show that reduction of TACC3 levels causes pathfinding defects in axons of developing spinal cord motor neurons in Xenopus laevis in vivo and increased TACC3 levels interfere with the growth cone response to the axon guidance cue Slit2. Currently we are investigating whether TACC3 could be a potential target

of Abelson kinase downstream of the Slit2 guidance cue. Together, our results suggest that by regulating MT behavior, the +TIP TACC3 is involved in axon outgrowth and pathfinding decisions of neurons during embryonic development and TACC3 phosphorylation events, which remains to be elucidated, could be important regulator of this involvement.

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Poster Abstracts

8. Expression and Characterization of Ancient Retrovirus Envelope Genes

Kate Halm, William Diehl, Jamie Henzy, Welkin Johnson

Endogenous retroviruses (ERVs) make up a significant portion of vertebrate genomes; for example, approximately 8% of the human genome is composed of ancient retroviral sequences. Although most ERV loci are ancient and non-functional due to the accumulation of substitutions and insertions/deletions, some ERVs retain one or more open reading frames (ORFs), possibly reflecting exaptation by the host lineage. It has been reported that envelope genes (env) of the human ERV-Fc1 (HERV-Fc1) locus and an ERV-Fc locus in the baboon genome (ERV-Fc-Bab) retain intact ORFs. We have identified ERV-Fc-related env sequences with intact ORFs in seven additional mammalian species: chimpanzee, aardvark, grey mouse lemur (n=2), squirrel monkey, marmoset, dog, and panda. The dog and panda env ORFs are the result of a recombination even, having significant similarity to Envs of viruses in the RDR super group. This group of viruses use ASCT2 as a receptor, and include both exogenous (MPMV, SMRV, REV-A) and endogenous (RD-114, HERV-W) retroviruses. Investigation of the ERV-Fc ORFs has the potential to reveal information about the process of exaptation, and to give insight into the evolutionary history of the ERV-Fc viruses. Search through EST databases turned up evidence that the human, baboon and marmoset ORFs are transcribed. We were able to express all of the full-length Env proteins except one from the lemur genome (lemur #1) as codon-optimized synthetic genes. Unexpectedly, we found that

most of the Env precursors were not cleaved to form the surface (SU) and transmembrane (TM) subunits, even when a canonical furin cleavage site was present. This suggests that loss-of-cleavage mutants were selected for during exaptation by the host lineage, and may have helped in maintaining the env ORFs.
ERV-Fc-Bab lacks a canonical cleavage site, but when we created one by site-directed mutagenesis (I-Q-K-Q to R-Q-K-R), the Env protein was processed into SU and TM subunits. Furthermore, removal of 22 residues from the C-terminus of the cytoplasmic tail of ERV-Fc-Bab enhanced syncytia formation (in cell-cell fusion assays) and the ability of ERV-Fc-Bab pseudotyped virions (MLV-Bab) to infect 293T cells, suggesting the presence of an R-peptide cleavage site like that of murine leukemia virus (MLV). A survey of a small panel of cells revealed that only human cell lines were infected by MLV-Bab, whereas cells of old world monkey, canine, feline and chicken origin were not susceptible to infection. This indicates that a receptor for ERV-Fc-Bab is expressed on human cells. Ectopic expression of ERV-Fc-Bab Env can also inhibit infection by MLV-Bab, raising the possibility that the endogenous glycoprotein encoded in the baboon genome could function as a viral entry inhibitor. The other ERV-Fc env ORFs were not able to block infection by MLV-Bab this may be due to the Envs being non-functional or using a different receptor.

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9. Boston College Imaging Facility

Bret Judson

Facility description: The shared Boston College imaging facility has 712 ft2 of space within Higgins Hall (room 525), the Physics and Biology building completed in 2002 on the main campus of Boston College. The facility has 5 individual rooms for microscopes and space for the facility manager (Bret Judson). Bret has over 15 years of experience running core facilities, and is available for all issues concerning imaging. The facility also houses several workstations for advanced image analysis. Bench space is available for set up of experiments and a fume hood is present. Use of the equipment is for all members of Boston College after receiving training. There are no fees for services. In addition, an advanced imaging course (BI 545) is offered every Fall/Spring by the facility manager for advanced undergraduate and graduate students.

10. PercomORF: a 135-million year old viral gene with a function in modern fish?

Andrea Kirmaier, Isabel Garcia, Natalie Curtis, Welkin E Johnson

Endogenous viral elements are ubiquitous in vertebrate genomes but the majority is heavily mutated and no longer expressed. Long-term maintenance of endogenized viral genes as open reading frames (ORF) suggests co-option by the host for a novel function. Henzy et al. discovered an intact ORF of retroviral origin in over 20 species of fish in the Percomorpha lineage. The gene, dubbed "PercomORF", is closely related to the envelope genes of extant gamma-type retroviruses. Phylogenetic analysis suggests that the integration event dates back ~135 million years, making PercomORF the oldest known intact vertebrate gene of viral origin. Henzy et al. also found PercomORF transcripts in at least one Percomorpha fish species, consistent with the hypothesis that PercomORF is a functional gene in modern fish.

Typical gammaretroviral envelope proteins are translated as a precursor that is cleaved into a surface subunit (SU) and a transmembrane domain (TM) by a host protease. Env proteins are also glycosylated post-translationally and form covalently attached homo-trimers (3x SU/TM) on the cell surface. Only fully processed trimers can induce membrane fusion. Other co-opted retroviral envelope genes are known to be involved in tissue fusion (e.g. fusion of embryo and placenta mediated by syncytin) or to perform antiviral functions.

The PercomORF gene is not located near any known or

suspected promoter elements, raising the question of how expression is driven. We are currently investigating PercomORF transcripts in multiple fish species in order to define the gene. PercomORF can be expressed from synthetic constructs in human cells and appears to be cleaved into SU and TM but with low efficiency, and we are currently asking whether PercomORF is glycosylated and forms trimers. This would suggest that PercomORF may still be fusogenic. Analysis of publicly available RNAseq data will shed light on whether PercomORF is ubiquitously expressed or limited to certain tissues/organs or developmental stages. The contribution of viral genetic elements to the evolution of all life forms is an emerging aspect of virology, and we hypothesize that PercomORF has been co-opted in the Percomorpha fish and performs a novel function.

Henzy JE, Gifford RJ, Kenaly CP, Johnson WE. An Intact Retroviral Gene Conserved in Spiny-Rayed Fishes for over 100 My. Mol. Biol. Evol. 2016.

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11. Coordination of nuclear positioning by bocksbeutel (Drosophila emerin)

Torrey Mandigo, Michael R. Hussey

Muscle cells are a syncytial cell type conserved from Drosophila to humans. In these cells, the many nuclei are positioned at the cell periphery to maximize the distance between adjacent nuclei. The importance of this feature of muscle cells is highlighted by the correlation between mispositioned nuclei and many distinct muscle disorders. However, it remains unclear why nuclei are mispositioned and whether nuclear positioning contributes to muscle weakness and wasting. Mispositioned nuclei are a hallmark of Emery-Dreifuss Muscular Dystrophy (EDMD) which has been linked to mutations in a range of genes families, all of which have gene products that localize to the nuclear envelope. We have begun to investigate whether these various genes lead to mispositioned nuclei through a common mechanism. We have examined three gene families linked to EDMD, Nesprin (Klarsicht and Msp300), SUN (Klaroid), Emerin (Otefin and Bocksbeutel) and whether they genetically interact. We found that during larval stages all nuclei were mispositioned except in Msp300 larva. We found that among the genes tested, all shared a common genetic interaction with Bocksbeutel. There was also a genetic interaction between Otefin and MSP300. These data suggest that the EDMD-linked genes tested all play a role in nuclear positioning but there may be multiple related mechanisms that drives the mispositioning of nuclei. Although not all the genes tested interact, it appears that the pathways these gene products function in all converge on a shared member, Bocksbeutel. This suggests that Bocksbeutel plays an essential role in the positioning of nuclei and may regulate multiple pathways involved in nuclear positioning.

12. Etiology of the progression of Periodontitis: A meta survey of the genes and putative regulatory mechanisms driving disease

Nikhil Ram Mohan and Michelle M. Meyer

Periodontitis is a common inflammatory disease that severely deteriorates the bone supporting teeth. Pathogens underlying the disease have been classified into Red, Orange, Purple, Blue, Green, and Yellow complexes based on their roles in the periodontal pocket and the healthy gingival sulcus. Earlier metatranscriptomics based findings suggest a common shift in metabolic signatures in disease. In this study, we employed a meta approach to screen for common genes and regulatory small non-coding RNAs (ncRNAs) that form the core etiology of periodontitis. RNA-seq data from three previous studies summing 49 healthy and 48 periodontal samples were accumulated and assembled into transcripts de novo. Analyses revealed 859 differentially expressed (DE) transcripts, 675 up and 174 down regulated. Interestingly, only ~20% of the DE transcripts originate from organisms in the red/orange complexes, and ~50% originate from unaffiliated organisms. Comparison of expression profiles between samples revealed variations among disease samples, greater correlation among samples collected from a single study than between studies. A survey of the DE transcripts for known ncRNAs in the Rfam database identified a large number of tRNAs and tmRNAs as well as riboswitches like the FMN, glysine, lysine, and SAM in the up regulated transcripts and the cobalamin riboswitch in both up and down regulated transcripts. De novo in silico discovery also identified many putative novel ncRNAs in

highly differentially expressed transcripts. While our results reflect those of the previous individual studies, our meta-analyses reveal the common DE genes and ncRNAs, a shift in metabolic signatures, in progression of periodontitis.

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13. Adjacent diffuse flow hydrothermal vents reveal distinct transcriptomic profiles of microbial activity

Heather Olins

Despite years of research into microbial activity at diffuse flow hydrothermal vents, the extent of microbial niche diversity in these settings is not known. To better understand the relationship between microbial activity and the associated physical and geochemical conditions, we obtained co-registered metatranscriptomic and geochemical data from a variety of different fluid regimes within the ASHES vent field on the Juan de Fuca Ridge. Microbial activity in the majority of the cool and warm fluids sampled was dominated by a population of *Gammaproteobacteria* (likely sulfur oxidizers) that appear to thrive in a variety of chemically distinct fluids. Only the warmest, most hydrothermally-influenced flows were dominated by active populations of canonically vent-endemic *Epsilonproteobacteria*. These data suggest that the *Gammaproteobacteria* collected during this study may be generalists, capable of thriving over a broader range of geochemical conditions than the *Epsilonproteobacteria*. Notably, the apparent metabolic activity of the *Gammaproteobacteria*—particularly carbon fixation—in the seawater found between discrete fluid flows (the intra-field water) suggests that this area within the Axial caldera is a highly productive, and previously overlooked, habitat. By extension, our findings suggest that analogous, diffuse flow fields may be similarly productive and thus constitute a very important and underappreciated aspect of deep-sea biogeochemical cycling that is occurring at the global scale.

14. Microbial Ecology at Hydrothermal Vents: the importance of low temperature habitat in a high temperature ecosystem

Heather Olins

Hydrothermal vent ecosystems are defined by steep thermal and chemical gradients. Chemosynthetic microorganisms are the primary producers in these systems, utilizing the available chemical energy to support substantial animal biomass. The variety of chemical substrates provided by hydrothermal fluid and surrounding seawater enables a metabolically diverse community of microbes. However, our understanding of how abiotic factors such as temperature, geochemistry, and mineral substrate influence the activity of these microbes is limited. The overarching goal my research has been to examine the influence of these abiotic factors on free-living microbial community composition, structure, and function. A combination of metabolic rate measurements, metatranscriptomics, and colonization experiments, all with co-registered geochemistry, underscore the substantial heterogeneity of these systems and offer insights into the relative influences of the abiotic forces that help to govern these ecosystems.

15. Mapping the Genetic Basis for *Toxoplasma gondii* Virulence Traits Through Adaptive Laboratory Evolution (ALE)

Vincent Primo, Andrew Farrell, Gabor Marth, Marc-Jan Gubbels

Having a genetic difference of only 0.002%, RH and GT1 (both of Type I) show remarkable phenotypic differences in vitro, including plaque size and extracellular survival. Since RH has been in culture for ~40 years, Adaptive Laboratory Evolution (ALE) is likely the root of these differences. Replaying the evolutionary changes that occur throughout ALE of *T. gondii* may help to identify alleles, expression patterns, and epistatic relationships associated with virulence traits. Here we have subjected GT1, a non-lab-adapted strain, to ALE for 130 passages (~342 generations, ~13 months) and observe the evolution of several phenotypes over time. Upon serial passaging of GT1, we observed a steady increase in plaque size (1.67 fold; $P < 0.05$), an increase in reinvasion efficiency (2.63 fold change; $P < 0.05$), and an increase in extracellular survival (2.15 fold change; $P < 0.05$). Comparative genomics using Whole Genome Sequencing (WGS) over the course of ALE (5 time points) shows an accumulation of mutations over time, suggesting these as the genetic factors responsible for the observed lab adaptive traits. To better pinpoint genetic factors specific to extracellular survival, GT1 was subject to several extracellular survival bottlenecks during ALE; by passage 50, this specific selection had enriched for parasite populations with extracellular survival equivalent to RH. Subsequent WGS of this enriched population identified

candidate genotypes correlating with the enhanced extracellular survival phenotype. ALE of the non-lab-adapted GT1 strain holds promise for identifying the genetic basis for host-independent virulence traits. We are currently in progress to correlate phenotype and genotype development with changes in gene expression profiles as well.

16. Microplastics on Cape Cod

Amanda Rickert, Bret Judson

Microplastics, or small pieces of plastic less than 5mm in length, have been increasing in prevalence in the oceans since the late 1900s and have been found on beaches all across the world. Microplastics have been shown to absorb toxins present in seawater or inherently contain endocrine disruptors, and their small size microplastics allows them to be easily taken up by marine organisms at the bottom of the food chain. Current studies are examining the possible ecosystem dynamics effects of microplastics, and their possible effect on human health through seafood consumption. This study aims to examine the characteristics of microplastics on Cape Cod, from their spatial distribution and abundance to their physical characteristics. Sand samples were collected from three beaches on Cape Cod, and microplastic particles were separated using a saline solution. Microscopy revealed microplastic particles in all samples collected, with a variety of sizes and shapes. The characteristics of these particles can be used to elucidate their origin and their implications for marine life. Further studies will expand the locations studied and a refining of techniques for plastic identification.

17. Predicting species-wide virulence for *Streptococcus pneumoniae*; a bacterial pathogen with a large pan-genome

Federico Rosconi, Defne Surujon, Eva Pauli, Matt Phillippo and Tim van Opijnen

Streptococcus pneumoniae is a natural inhabitant of the human nasopharynx, but it can trigger severe disease (>1 million fatalities/year) when it disseminates to other sites like the lungs or blood. Its success in colonizing these different compartments is often thought to be primarily dependent on the capsule. However, besides variability in available capsules, *S. pneumoniae* has a large pan-genome, where two random strains may differ by more than 200 genes (>10%). We hypothesize that non-capsule related genetic determinants, as well as the host immune system play an equally important role. To elucidate this complexity, we are performing a comprehensive functional study of S. pneumoniae-host interaction in a number of strains representing a large part of the pan-genome. In that order, we carefully selected 33 strains that cover ~70% of the pan-genome and 15 different capsule serotypes. We have set-out to characterize these 33 isolates by determining the genetic elements important for colonization of the nasopharynx, induction of pneumonia and sepsis, and by studying the host immune response to infection. To achieve this we apply cutting-edge tools including transposon insertion sequencing (Tn-Seq) and Luminex xMap® technology. Our latest results show that our isolates have: 1) widely different virulence levels; 2) different lungs or blood colonization abilities, and 3) different induction-levels of the host immune response.

Our final aim is to collect and integrate all the genetic and phenotypic data to create a *S. pneumoniae* species-wide model that is able to predict the main phenotypes for new or non-characterized isolates.

18. Simian betaretrovirus envelope glycoprotein is a tetherin/BST2 antagonist

Anindita Sinha, Marielle Melconian, Ruth Serra-Moreno, Andrea Kirmaier,
Welkin E. Johnson

Tetherin (BST2/CD317), is a lipid raft associated interferon-inducible host restriction factor (RF) that acts as a potential block to the replication of numerous enveloped viruses including HIV-1. The unusual membrane topology of tetherin allows it to engage enveloped virions and inhibit their release as they bud from the plasma membrane of the infected cells. The tethered virions are eventually endocytosed and degraded. Unlike most RFs, tetherin's mode of restriction is non-specific as restriction does not require the recognition of any virally encoded structure. Consequently, viruses have adapted to actively evade restriction by tetherin (by down-modulating tetherin) or passively evade tetherin (by egressing from tetherin-devoid membrane micro-domains). Most complex retroviruses like HIV and SIV encode accessory proteins like Vpu and Nef respectively to counteract tetherin-mediated restriction however simple exogenous retroviruses do not. We established that Mason-Pfizer monkey virus (M-PMV; also called SRV-3), a prototypical type-D betaretrovirus of rhesus macaques, is resistant to restriction by rhesus macaque tetherin. However the virus is sensitive to restriction by human tetherin. Differential sensitivity to tetherin orthologues suggested that M-PMV is adapted to evade the tetherin ortholog of its native host, and may have mechanisms to actively evade restriction in a species-specific

manner. By performing single-cycle virion release assay, we identified the M-PMV envelope glycoprotein as the key determinant for the differences observed in the pattern of restriction by human and non-human primate tetherin orthologues. When expressed in-trans-, M-PMV envelope was also able to rescue a heterologous virus (SIVΔEnvΔNef) from rhesus tetherin but not from human tetherin. We also found that the envelope glycoprotein from another related virus (SRV-4), isolated from Asian macaques exhibited similar patterns of tetherin-antagonism in vivo. We tested a series of cell lines stably expressing rhesus-human tetherin chimeras and found that the specificity of M-PMV envelope mapped to the cytoplasmic tail of rhesus tetherin. Overall our data substantiates that simian betaretroviral envelope glycoprotein is a potent BST2 antagonist and exhibits characteristics similar to some of the well-known tetherin antagonists of complex retroviruses such as HIV-1 Vpu and SIV Nef. Such finding is very significant as it elucidates the evolutionary importance of the viral env gene as an adaptation in type-D betaretroviruses to overcome inhibition by tetherin.

19. A Comparative Genomics Toolkit Tailored to Explore the Core and Pan-Genome of *Streptococcus pneumoniae*

Defne Surujon, Alexander Farrell, Federico Rosconi, Tim van Opijnen

Streptococcus pneumoniae is a human nasopharyngeal commensal and respiratory pathogen. It triggers pneumococcal pneumonia, meningitis, and septicemia, causing ~1 million deaths annually making it one of the most devastating pathogens worldwide. With an average genome size of 2Mbp, any given *S. pneumoniae* strain has about 2100 genes. Due to its receptiveness to take up DNA, only a fraction of these genes are shared across all strains, leading to a core genome (genes shared amongst all strains) of ~1200 genes and a pan-genome between 3500 and 7000 genes. The isolation of novel clinical isolates and the increase in sequencing data have resulted in a wealth of information on *S. pneumoniae*. However, while it is easy to generate draft genomes comprised of unannotated contiguous sequences in arbitrary order and orientation, it is not straightforward to obtain closed, fully–assembled genomes. Importantly, such files are invaluable for functional genomics studies such as RNA-Seq or Tn-Seq. Here we present a suite of tools for the assembly, organization and summary of new *S. pneumoniae* genomes that are annotated and formatted for easy integration into genomic analysis pipelines. We validated the performance of our assembly by comparing genomes assembled from Illumina reads to the single assembled contigs obtained from PacBio sequencing. Further, we present a web-based easy-to-use graphical interface for the

simultaneous comparison of many *S. pneumoniae* isolates, construction of phylogenetic trees based on gene content, and retrieval of a gene cross-reference table for the identification of genes in strains that have not been extensively studied.

20. Deciphering the events and mechanism mediated by *Toxoplasma gondii* Ca2+ sensors in protein secretion

Daniel Tagoe, Bradley Coleman, Marc-Jan Gubbels

Toxoplasma gondii, the causative agent of toxoplasmosis is a protozoan parasite that infects most species of warm-blooded animals, including humans. The pathological stage of *T. gondii*, which is driven from the lytic cycle, is made up of replication, egress, motility and invasion. The central role of Ca2+ in driving the lytic cycle by triggering the release of secretory proteins from the apical microneme organelles is unequivocal. Microneme proteins are essential for invasion and egress from a host cell by this obligate intracellular parasite. Double C2-domains (DOC2) are known Ca2+-sensors mediating protein secretion. Of the five known DOC2 protein families, only two are present in the Toxoplasma genome: 3 ferlin genes (FER1-3) and one unconventional protein we named TgDOC2. We are dissecting the function of these proteins using the genetic and cell biological toolbox available for Toxoplasma.

Through the use of a temperature sensitive allele of TgDOC2 we know that it is required for all microneme secretion, and its phenotype is an abrogation of all egress and invasion. By using a dominant negative overexpression approach of FER1 without its transmembrane domain, we found that it is required for responding to high Ca2+ fluxes leading to reduce growth rate whilst inhibiting microneme secretion; absence of FER1 still sustains constitutive microneme secretion mediated by intermediate Ca2+-levels. Knocking

down FER2 expression surprisingly did not affect microneme protein secretion. Consistent with this, egress was normal in this mutant, but invasion was inhibited. Besides micronemes, the rhoptry organelles are also released during invasion. Upon FER2 depletion, rhoptries did not secrete anymore, which demonstrates for the first time that secretion of this organelle, which is not well understood, is mediated by Ca2+. Finally, we were able to generate a complete gene knock-out of FER3, which did not affect viability of the acute life stage. FER3 is very degenerated compared to the ferlins and is not conserved well among the Apicomplexa, and it may therefore have acquired a new function in Toxoplasma.

Taken together, we identify a very narrow Ca2+ sensor repertoire in Toxoplasma acting on the secretion dynamics of (at least) two different secretory organelles. Our work also lifts the veil on the mechanism of rhoptry secretion, which cannot be triggered in vitro and requires recognition of a host cell.

21. dTn-Seq: Combining droplet-based microfluidics with Tn-Seq to uncover novel bacterial genetic elements involved in overcoming antibiotic and host-induced stress

Derek Thibault, Stephen Wood, Tim van Opijnen

Pathogenic bacteria have evolved mechanisms to overcome a wide variety of environmental disturbances including starvation, the host immune system and antibiotics. We lack a comprehensive understanding of how pathogens are able to survive such diverse stresses partially due to the complexity of uncovering the genetic origin of a phenotype. Transposon insertion sequencing (Tn-Seq), a technique developed by our lab, utilizes pooled libraries of transposon insertion mutants to untangle the genetic complexity of a phenotype by accurately calculating mutant growth rates on a genome-wide scale. The pooling and batch culture of insertion mutants is one of the characteristics that makes Tn-Seq so powerful, however this also comes with a major drawback: It remains unclear how bacterial mechanisms such as cheating, cooperation, and complementation affect the phenotype of individual mutants growing in these pooled mutant libraries. To overcome this challenge we have combined Tn-Seq with droplet-based microfluidics (dTn-Seq) thereby encapsulating and culturing single mutants in individual environments, but still on a genome-wide scale. Furthermore, by optimizing our library preparation method for Illumina sequencing we have successfully created a high-throughput procedure that allows us to probe the individual behavior of millions of mutants simultaneously. As a result dTn-Seq is helping us to

disentangle complex phenotypes in pathogenic bacteria such as *Streptococcus pneumoniae*, that include scavenging for host glycoproteins and overcoming stress induced by antibiotics, and host serine proteases.

22. Identification of vancomycin-responsive RNA regulators in *Streptococcus pneumoniae* using Term-seq

Indu Warrier, Nikhil Ram-Mohan, Jon S Anthony, Tim van Opijnen, Michelle M. Meyer

Riboswitches and other cis-regulatory RNA elements are found in the 5' untranslated region (5'UTR) and play key roles in primary metabolism and virulence. They control gene expression in several ways including the formation of metabolite-mediated premature transcription termination. Recent studies have shown that several of these RNA regulators can respond to the presence of an antibiotic by alleviating premature transcription termination and expressing antibiotic resistance genes. To identify such ribo-regulators in *Streptococcus pneumoniae*, a causative agent of invasive pneumococcal disease and meningitis and the leading cause of antibiotic-resistant pneumonia, we employed a high-throughput RNA-sequencing technique called term-seq that enables genome-wide discovery of conditional, regulated premature transcription termination events in bacteria. In this study, term-seq was applied to *S. pneumoniae* following exposure to sublethal dose of vancomycin, a common clinically used drug to treat pneumococcal infections. The transcriptome is currently under investigation to identify ribo-regulators that specifically activate gene expression in response to vancomycin exposure. With antibiotic resistance becoming a growing public health concern, an understanding of the mechanism of resistance would prove invaluable. This also paves the path for discovery of compounds that target ribo-regulators that could mitigate antibiotic resistance.

23. Assessment of the genome-wide phenotypic response of *S. pneumoniae strains* to various antibiotics by Tn-Seq

Stephen Wood, Tim van Opijnen

The bacterial genes and pathways involved in responding to antibiotic stress includes those directly targeted by the antibiotic, as well as a system-wide response involving genes not targeted by the antibiotic. The selective pressure on both direct-target and off-target genes can drive the development of antimicrobial resistance. A detailed understanding of the genes and pathways involved in responding to antibiotic stress is not well established. It is also not clear if these genes and pathways are conserved in strains of differing genetic backgrounds. In order to address these questions, the phenotypic response of *Streptococcus pneumoniae* strains exposed to sub-inhibitory concentrations of a panel of antibiotics was assessed by utilizing the high-throughput technique transposon insertion sequencing (Tn-Seq). Mutant transposon libraries of strains, Tigr4, Taiwan-19F, and 22F, were grown in the presence of 20 different antibiotics at sub-inhibitory concentrations. By creating gene-antibiotic interaction maps for each strain, shared and unique genes involved in responding to different antibiotic classes and individual antibiotics are highlighted. Our preliminary data demonstrates that the genes involved in responding to the same antibiotic can differ amongst strains. Additionally, our data can aid in identifying putative functions for unannotated genes as well as provide a way to predict which combination of antibiotics may act synergistically. Tn-Seq shows that the response to antibiotic stress is not conserved across strains and suggests that strain-specific differences can affect the development of resistance, especially in pathogenic bacteria with a large pan-genome such as *S. pneumoniae*.

24. Engineering an applicable bacterial bioremediator through Artificial Selection.

Marco Zaccaria, Babak Momeni

One of the most stimulating aspects of microbiology is to employ bacterial genetic potential for our contingent needs. *Rhodococcus erythropolis* is a bacterial strain that harbors a wide enzymatic inventory and an ample catabolic potential. Aflatoxin B1 (AFB1) is a stable fungal secondary metabolite (a mycotoxin) that contaminates about 50% of crops around the world. It is the most carcinogenic metabolite in nature. We intend to exploit *R. erythropolis* potential to engineer an efficient and applicable AFB1 bioremediator through the process of Artificial Selection; our final objective is identification of essential variables and modelization of general principles to inform future works aimed at applications in bioremediation.
Developing an effective bioremediator compels us to a deep understanding of the mechanics underlying the detoxification process. We shed light on such mechanics trying to answer essential, single-instance questions. We use the information to set up proper selection schemes for optimizing bioremediation efficiency, and finally we formulate theoretical predictions about how selection, in each of these schemes, would influence the trajectory of evolution. Through adaptation experiments, we will isolate clones from evolving populations at different times. To identify what genes or regulatory mechanisms might be involved in improving the bioremediation efficiency, we intend to compare phenotypic and genotypic changes across different populations during the course of adaptation (using

the ancestral strain as the reference). Correlating this information with phenotypic data of degradation efficiency for the same clones, we will establish what genetic changes impact the bioremediation potential and, possibly, how to induce them.

25. Genome-wide transcriptional and phenotypic stress responses are separated but linked in a stress-dependent manner in pathogenic bacteria.

Karen Zhu, Paul Jensen, Tim van Opijnen

Bacterial genes that change in expression upon an environmental perturbation are commonly assumed to contribute to the fitness of an organism. However, several recent studies have suggested that differentially expressed genes are somehow rarely phenotypically important. Here we apply an integrative approach of transcriptomics, phenotypic screening, metabolic modeling and network analyses to interrogate the links between transcriptional and phenotypic stress responses in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. We demonstrate that for both Gram-positive and Gram-negative bacteria, these two separate gene sets - phenotypically important genes (PIGs) and transcriptionally important genes (TIGs), can be coordinated through a topological network analysis in genome-scale metabolic models. However, such coordination turns out to be stress-dependent: while nutrient depletion triggers a well-coordinated response between PIGs and TIGs; antibiotics trigger an erratic response, in which PIGs and TIGs are neither coordinated in their network distance nor in their magnitude. Moreover, a gene expression meta-analysis reveals that independent of the stress-type or species, the partition of PIGs and TIGs is a genome-wide phenomenon. Our work demonstrates that transcriptional regulation and phenotypic importance of bacterial genes can be better understood through integration into network models. By uncovering genes of transcriptional and

phenotypic importance, our integrative approach could aid in predicting how a bacterium's gene network evolves under a particular stress, e.g. exposure to antibiotics, and leads to resistance. Finally, our observations raise considerations for studies (e.g. drug target prediction) that assign importance to genes solely based on transcriptomic data.

NOTES

The Biology Department would like to thank our sponsor who helped make this retreat possible

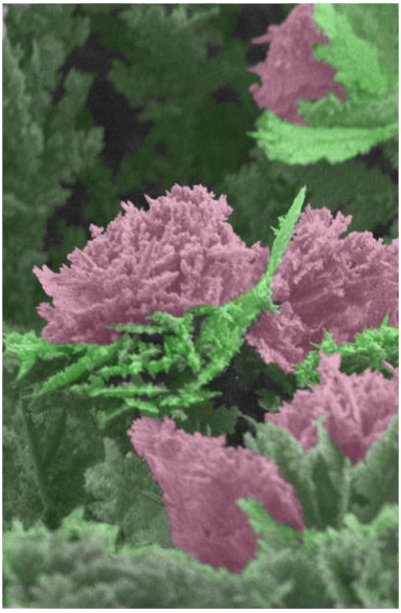

Image Credit: Amy Valera. Dendritic gold grown via
Directed Electrochemical Nanowire Assembly (DENA) on a planar gold electrode surface, false colored to resemble roses.

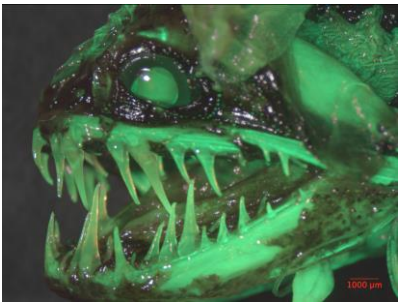

Image Credit: John Psaltis. Full head overlay image of photophores on Echiostoma barbatum. An overlay of a transmitted light image and a fluorescent image obtained using a GFP filter is shown. Scalre bar 1,000µm. This image was taken as part of Bret Judson's BI545 Advanced Lab in Cell Imaging

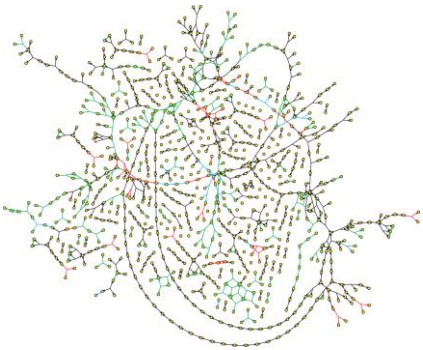

Image Credit: Karen Zhu. This image depicts the first curated, genome-scale metabolic network of the pathogenic bacterium *Streptococcus pneumoniae.* Each node represents a metabolite; each branch represents a metabolic gene. Integration of genome-wide data reveals that the phenotypic stress network (red) and the transcriptional stress network (green) consist of distinct gene-sets, undermining the widely held assumption that genes that change in expression upon environmental disturbance must also phenotypically matter.