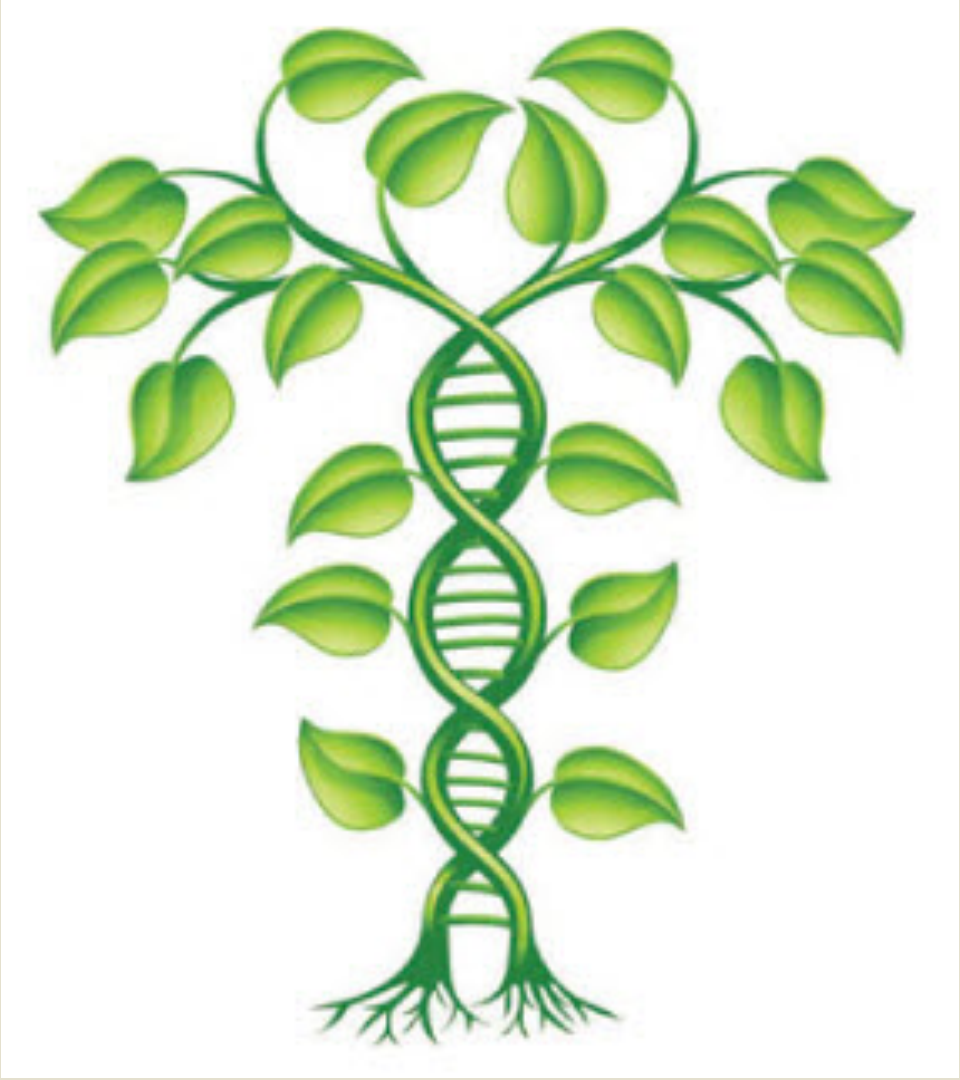
Boston College
Biology Department Retreat



August 22-23, 2016

The Connors Center

Dover, MA

Organizing Committee: *Meg Barry, Diane Butera,*
Dina Goodfriend, Charles Hoffman,
and Welkin Johnson

Welcome the Biology Ph.D. Class of 2016-2017

Matthew Crum
Previous Institution: Ramapo College
Degree: Bachelor's in Bioinformatics
Current Residence: New Jersey
Research Interests: RNA secondary structure prediction and analysis

Melissa Choz
Previous Institution: Regis College
Degree: Bachelor's in Biology
Current Residence: Leicester, MA
Research interests: Function of gene/drug mechanisms and applications to translational medicine

Samantha Dyckman
Previous Institution: Ramapo College of New Jersey
Degree: Bachelor's in Biology
Current Residence: New Jersey
Research interests: Cellular biology

Micaela Lasser
Previous Institutions and Degrees:
Michigan State University, Bachelor's in Psychology
University of Pennsylvania, Master's in Biotechnology
Current Residence: Michigan
Research Interests: Molecular biology, neuroscience, stem cells

Joshua Linnane
Previous Institution: University of New Hampshire
Degree: Bachelor's in Biology
Current Residence: Weare, NH
Research Interests: Microbiology and cell biology

Defne Sururjon
Previous Institution: Amherst College
Degree: Bachelor's in Biochemistry & Mathematics
Current Residence: Brighton, MA
Research Interests: Bioinformatics, sequence analysis, microbiome

Kevin White
Previous Institution: San Diego State University
Degree: Bachelor's in Cellular and Molecular Biology
Current Residence: Brighton, MA
Research Interests: Stem cell biology and regenerative medicine. Immunology and the study of infectious diseases

BIOLOGY DEPARTMENT RETREAT
August 22-23, 2016

The Connors Center: 20 Glen Street Drive, Dover, MA

Day 1: Monday, August 22nd

9:00	Continental Breakfast	
9:15	Opening Remarks—Welkin Johnson	
9:30	Guest Speaker: Abhishek Chaterjee, Ph.D. (Chemistry)	
10:15	*Break*	
10:45	**Session I:**	
	Session Chair:	*Douglas Warner*
	10:45-11:15	Eric Folker
	11:15-11:30	Brigitte Lawhorn – Johnson lab
	11:30-11:45	Torrey Mandigo – Folker lab
	11:45-12:00	Karen Zhu – van Opijnen lab
12:00	*Lunch and afternoon break*	
2:00	**Session II**	
	Session Chair:	*Danielle Taghian*
	2:00-2:45	David Burgess
	2:45-3:00	Jeff DaCosta
	3:00-3:15	Mary Ann Collins – Folker lab
3:30	*Group photo and break*	
4:00	Poster Session and Cocktail Reception	
5:30	*Break*	
6:00	Dinner	
7:30	Documentary Film: *Naturally Obsessed*	

BIOLOGY DEPARTMENT RETREAT
August 22-23, 2016

The Connors Center: 20 Glen Street Drive, Dover, MA

Day 2: Tuesday, August 23rd

8:00	Continental Breakfast	
9:00	**Session III**	
	Session Chair:	*Christopher P. Kenaley*
	9:00-9:30	Laura Anne Lowery
	9:30-9:45	Arianne Babina – Meyer Lab
	9:45-10:00	Andrea Kirmaier – Johnson Lab
	10:00-10:15	Jaclyn Mallard – Williams Lab
	10:15-10:30	Alex Auld – Folker Lab
10:30	*Break*	
10:45	Award Presentations—*Tony Annunziato & Charlie Hoffman*	
	Closing Remarks—*Welkin Johnson*	
11:15	Departures	

Guest Speaker: Abhishek Chaterjee, Ph.D.
Chemistry Department, Boston College

A Passion for Synthesis: At the Interface of Chemistry and Biology

We are broadly interested in the synthesis of new biological function – by engineering existing biological machinery using an interdisciplinary approach – to generate tools that enable teasing apart complex biological riddles, or biologics that target diseases with high precision. Specifically, we use tools from mammalian genetic code expansion to site-specifically incorporate novel unnatural amino acids into the capsids of human viruses (using adeno-associated virus as the model system) to probe and engineer their cellular entry pathways in exciting new ways that offer unprecedented precision and maneuverability. We also use virus based tools to improve the performance of the machinery that enable site-specific incorporation of unnatural amino acids into proteins in mammalian cells, creating a synergistic symbiosis of these two traditionally distinct fields of research.

Session I
Talks

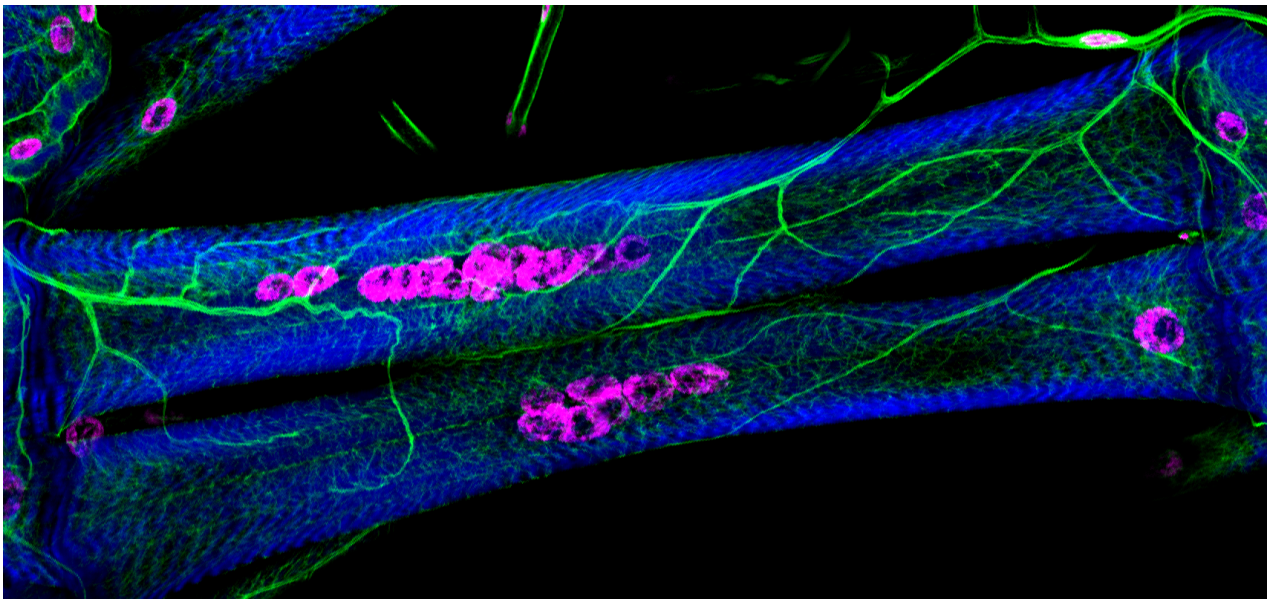


Image Credit: Torrey Mandigo, Folker Lab & Bret Judson, Higgins Imaging Facility
Larval muscle from a *Drosophila* model for Emery-Dreifuss Muscular Dystrophy stained for F-actin (blue), Tubulin (green), and nuclei (pink).

Session Chair: Douglas Warner

Eric Folker, Ph.D.

Mechanisms and functions of myonuclear movement

Nuclear movement is conserved throughout the eukaryotic cell lineage. Yet, the mechanisms that drive nuclear movement are poorly understood. Furthermore, although cell function is dependent on the movement and position of the nucleus in many cell types, the mechanisms by which the nucleus can influence cell function have not been described. We use muscle developments a model cell type to investigate both the mechanisms and functions of nuclear movement. Muscle is an ideal tissue for this analysis because aberrantly positioned nuclei strongly correlate with muscle disease. In fact, mispositioned nuclei is a diagnostic hallmark of disparate muscle diseases. However, many consider this hallmark of the disease to be a secondary effect due to ongoing muscle repair. To test this hypothesis we investigated nuclear position in embryonic and larval *Drosophila* muscles, which do not undergo repair. We have identified several factors that are necessary for the proper positioning of nuclei including cytoskeletal proteins (Dynein and Kinesin), nuclear envelope proteins that have been linked to Emery-Dreifuss Muscular Dystrophy (nesprins, Emerin, SUN proteins), and membrane shaping proteins linked to Centronuclear myopathy (Amphiphysin and myotubularin). Furthermore, we have demonstrated that properly positioned nuclei are essential for the organization of the contractile myofibril network that allows the muscle to produce force. Inhibition of the ability of the nuclei to interact with the myofibril proteins resulted in a disorganized myofibril network. Similarly, disruption of nuclear position without impacting the ability of the nucleus to interact with the myofibril network also resulted in a disorganized myofibril network. Together these data indicated that mispositioned nuclei are a bona fide phenotype of muscle disease and that one function of nuclear position is to stabilize the contractile myofibril network.

Brigitte Lawhorn, Johnson Lab

Impact of Host and Viral evolution on Lentiviral Nuclear Import Mechanisms

Lentiviruses are a genus of retroviruses that infect a diverse range of mammalian species. Included in this group are the human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2, respectively) and the simian immunodeficiency viruses (SIVs) of over forty non-human primate species. Following entry into the host cell, lentiviruses proceed to reverse transcribe the viral RNA to DNA, translocate into the nucleus, and subsequently integrate the viral DNA into the host chromatin. The capsid core which encases the viral genome has a critical role in these early events, ensuring efficient reverse transcription as well as mediating translocation through nuclear pore complexes (NPC) embedded in the nuclear envelope. The ability of lentiviral capsids to mediate transport across NPCs enables these viruses to replicate in non-dividing cells such as macrophages and resting CD4+ T cells – critical cell types in both HIV-1 persistence and pathogenesis.

While HIV-1 capsid appears to interact with several host proteins identified as critical cofactors in HIV-1 nuclear import, the precise mechanisms underlying HIV-1 nuclear import are poorly understood. Furthermore, it is unclear to what degree nuclear import mechanisms are conserved among lentiviruses as well as what impact selective pressures from host genetic variation have on lentiviral nuclear import mechanisms during cross-species transmission and emergence within a new host species. The nucleoporin Nup358 was previously identified as an important host factor in HIV-1 nuclear import and HIV-1 capsid was found to bind the C-terminal cyclophilin-like domain of Nup358 (Nup358-Cyp). Here, we examine the mechanism through which HIV-1 utilizes Nup358 to mediate nuclear import, the conservation of this mechanism among primate lentiviruses, the potential impact of primate Nup358 genetic variation on capsid binding, and the influence this genetic variation on lentiviral cross-species transmission and emergence. Preliminary results suggest that: 1) interaction between capsid and Nup358-Cyp was lost during emergence of SIV of sooty mangabeys (SIVsm) within rhesus macaques as SIVmac; 2) interaction with Nup358 is host-species specific for several non-human primate lentiviruses; 3) SIVsm adapted to better interact with the human ortholog of Nup358 during emergence within humans as HIV-2; and 4) the Ran-binding domain 4 of Nup358 (Nup358-RBD4) – which immediately precedes the Cyp domain – influences interaction between capsid and Nup358-Cyp. Based on these results, we hypothesize that the capsid-Nup358 interaction has an important role in lentiviral nuclear import. We are currently working on identifying the residues in both capsid and Nup358 responsible for the host-species specificity in interaction.

Torrey Mandigo, Folker Lab

Coordination of nuclear movement by bocksbeutel (Drosophila emerin)

Collaborators: Michael R. Hussey, Eric S. Folker

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.

Karen Zhu, van Opijnen Lab

How to Deal With Your Stressful Surroundings When You're a Bacterial Pathogen with a Large Pan-Genome: A Multi-Omics Approach

Collaborators: Paul Jensen, Tim van Opijnen

The increasing availability of fully sequenced genomes has demonstrated a distinction between a species core genome (the set of genes shared by all strains of the species) and its pan-genome (the species global gene repertoire) in many bacterial species. Such genomic fluidity is remarkable, since genes are embedded in complex gene networks; the gain or loss of a gene can thus rewire an existing network and affect associated phenotypes. However, functional characterization of pan-genomes is lacking, partially due to the technical difficulties with conducting genome-wide experiments on a species-wide scale. Here we present an integrated systems biology approach to contextualize differences in growth profiles and nutrient requirements that we found among three strains of the bacterial pathogen *Streptococcus pneumoniae*. We employ two genome-wide tools, Tn-seq and RNA-seq, to profile genome-wide responses in the three strains to nutrient depletion at both phenotypic and transcriptomic levels. These profiles are integrated into strain-specific flux balance analyses models with a goal to computationally characterize the inter-strain differences. Over 10% of the core genome, mostly genes encoding metabolic enzymes, transporters and regulators, display either strain-dependent fitness or expression level. Surprisingly, by simultaneous analyses of gene fitness and expression datasets we found a conserved pattern that fitness and expression changes rarely occur on the same gene, meaning that differentially expressed genes are not per definition genes that matter phenotypically. By network topological analyses of the datasets, we re-established the relationship between phenotypic importance and expression status-genes with fitness changes are located in close proximity with differentially expressed genes. Moreover, genes with changes in fitness are interspersed among essential genes, whereas differentially expressed genes are located at points distant from essential genes. Overall, our results provide a systems-level understanding of how a bacterium rewires phenotypic importance and transcriptional regulation on different but closely related genes in the context of the cellular metabolic network. More importantly, our results imply that optimal antibiotic targets may be genes with phenotypic importance, which are a few steps away from transcriptional changes and often overlooked by transcriptomic analyses alone. Finally, we present a hybrid computational/experimental approach that may be critical and easily transferrable to reconciling differences in other multi-omic datasets.

Session II Talks

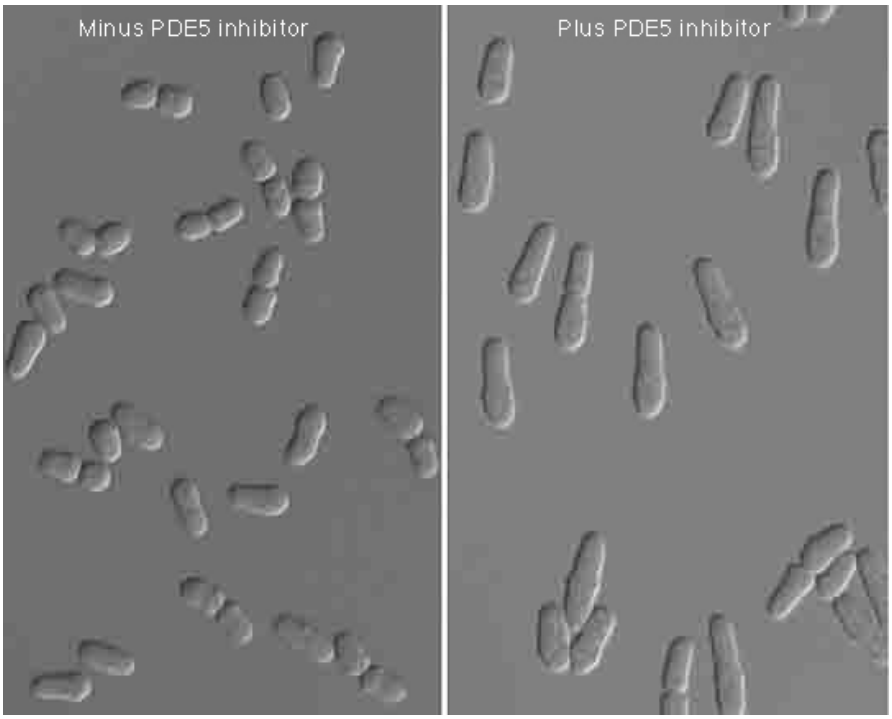


Image Credit: Charles Hoffman
S. pombe on a PDE5 inhibitor

Session Chair: Danielle Taghian

David Burgess, Ph.D.

Retrospective

I will tell a story of being able to maintain funding and enjoy doing science for over 40 years as a faculty member. Using examples of the questions asked and tools used over this period leads to some lessons learned with the hope that some of these lessons may help in your career.

Jeff DaCosta, Ph.D.

Comparative genomics of a species radiation: Sequencing the apple tribe

Species radiations are a striking feature of evolution that are often preceded by the emergence of a key innovation, and may be responsible for much of life's diversity. Yet understanding the evolution of species radiations and how genome evolution progresses in this context remains a central problem in biology. Members of the rose family (Rosaceae) include familiar domesticates such as roses, apples, peaches, almonds, strawberries, and cherries. An intriguing and important radiation within the rose family is the Maleae tribe, which produced the apple, pear, and quince genera, along with several valuable ornamentals. This clade consists of about 750 woody species distributed in 35 genera and is characterized by a whole genome duplication (WGD) event in its common ancestor. Early diverging Maleae have dry fruits and few species. But after the evolution of the pome, the fleshy fruit found in most Maleae and the basis for their economic importance, they diversified into 32 genera, sorting into lineages with distinctive phenotypic, life history, and ecological traits. Here I discuss a comparative genomic analysis of Maleae and its closest relative pre-dating the WGD, which includes de novo genome assembly, gene annotation, and exploration of the evolutionary history of duplicated genes.

Mary Ann Collins, Folker Lab

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

Collaborators: **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.

Session III Talks

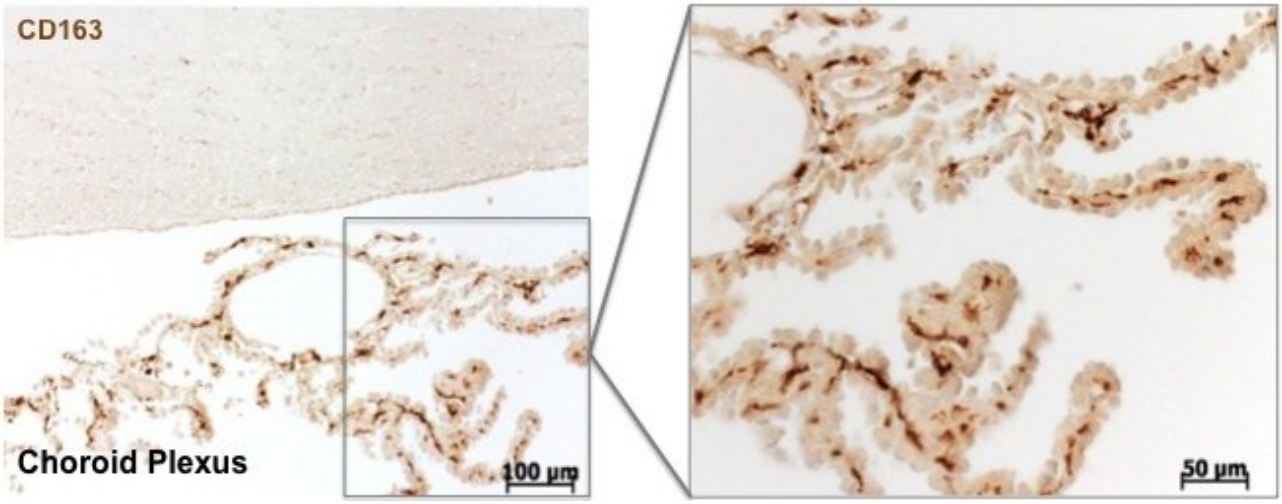


Image Credit: Jaclyn Mallard, Williams Lab
CD163+ Macrophages in the Choroid Plexus

Session Chair: Christopher P. Kenaley

Laura Anne Lowery, Ph.D.

Regulation of microtubule dynamics by TACC proteins during growth cone guidance and cell migration

Microtubule plus-end tracking proteins (+TIPs) play key roles in the regulation of microtubule dynamics. Despite their importance, it is still unclear how various +TIPs interact with each other and with plus-ends to control microtubule behaviors, particularly during embryonic development. My lab studies +TIP function by quantitative analysis of microtubule behaviors using high-resolution live-imaging data of cultured embryonic Xenopus laevis cells and embryos. Previously, we demonstrated that the transforming acidic coiled-coil (TACC) domain family member, TACC3, can function as a +TIP to promote microtubule polymerization as well as axon outgrowth. More recently, we determined that TACC1 and TACC2 can also act as +TIPs. However, each +TIP displays distinct localization profiles on the microtubule plus-end, differential cell-type-specific effects on microtubule dynamics, and embryonic cell type-specific expression patterns. In sum, our work highlights the unique and collective functions of TACC family members and how they interact to regulate microtubule plus-end behaviors in different embryonic cell types during development.

Arianne M. Babina, Meyer Lab

It takes two: Biological importance of the glycine riboswitch dual ligand-binding domains in *Bacillus subtilis*

Collaborators: **Arianne M. Babina**, Nicholas Lea, and Michelle M. Meyer

Riboswitches are structured domains of mRNA transcripts that regulate gene expression in response to specific interactions with small molecules. Bacterial genomes employ riboswitches to control a number of essential processes, including amino acid and nucleic acid biosynthesis, biofilm formation, motility, and virulence. In most bacteria, the glycine riboswitch consists of two tandem ligand-binding domains (aptamers), and allows expression of the *gcvT* operon upon glycine binding. The *gcvT* operon encodes components of the glycine cleavage system, which catalyze the use of glycine as a carbon source and maintain optimal intracellular glycine concentrations. In *Bacillus subtilis*, these tandem aptamers are believed to demonstrate cooperative binding. While the structure and molecular dynamics of the glycine riboswitch have been the subject of numerous biophysical studies, no work has explored the importance of these interactions *in vivo.* Additionally, recent findings suggest that the cooperativity observed is an artifact of *in vitro* experimental conditions. To elucidate the function and structural dynamics of the glycine riboswitch in a biologically relevant context, we introduced point mutations into the riboswitch within the *B. subtilis* genome to disrupt glycine binding and aptamer cooperativity and assayed the mutant strains for fitness defects. Mutations to the glycine-binding domains of both aptamers inhibit biofilm formation, motility, and overall growth in media with increasing glycine concentrations. This suggests that the *B. subtilis* glycine riboswitch plays a key role in glycine detoxification and the function of both aptamers is imperative for optimal cell fitness in high glycine environments.

Andrea Kirmaier, Johnson Lab

Neutralization resistance as a determinant of viral transmission, pathogenicity and escape

Andrea Kirmaier[1], Fan Wu[2], Laura Hall[1], Vanessa Hirsch[2], Welkin Johnson[1]

[1] Biology Department, Boston College, Chestnut Hill, MA 02467
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In HIV and SIV infection, viral escape from the host's antibody response is associated with accelerated and more severe disease outcome. Neutralizing antibodies (Nab) eliminate the momentarily circulating virus in a host but the high mutation rates of retroviruses cause rapid escape, resulting in an endless game of catch-up between host and virus. Different viral strains tend to exhibit differential susceptibility to Nabs, and heterologous viruses tend to be particularly vulnerable to neutralization. A 4-tier system is used to categorize viral susceptibility to Nabs: tier 1A viruses are highly susceptible to Nabs, tier 1B viruses demonstrate above-average sensibility to neutralization, tier 2 viruses are moderately susceptible, and tier 3 viruses display low sensitivity to Nab.

In the present study, we investigated whether long-term disease progression in rhesus macaques infected with a heterologous virus, SIV from sooty mangabeys (SIVsm), is affected by the virus' initial susceptibility to Nabs, the development of the Nab response over time, and if/how viral escape affects these dynamics. Three representative isogenic SIVsmE660 variants with tier 1A, tier 2 and tier 3 envelopes were used to infect six animals, respectively. We evaluated number of inoculations to infection, plasma viremia, antibody titers, development of Nab, and survival times, as well as evolution of the viral *env* genes using illumina NGS over a 1.5 year period.

Animals in the tier-1A group took significantly more inoculations to become infected, and animals infected with the neutralization-resistant tier 3 virus succumbed to AIDS-like disease significantly faster than tier-1A-infected animals, even though there were no significant differences in acute and set-point plasma viremia between any of the three groups. Interestingly, the development of both autologous and broadly neutralizing antibodies in all animals appeared to be independent of the inoculating virus, indicating that neutralization resistance of the inoculating virus had no impact on the potency of the antibody development and response.

Viral residues in the variable regions of the *env* genes of all three inoculating viruses tended to be maintained or to diversify, whereas viral residues in the constant regions converged on the residues found in the tier 3 envelope genes, indicating a fitness advantage conferred by higher resistance to neutralization over the course of infection.

Overall, our results suggest that neutralization resistance of the inoculating virus affects pathogenicity and mortality, but not plasma viremia and Nab response. Viral convergence on residues facilitating neutralization resistance do suggest a selective advantage of a higher degree of resistance to neutralization but other viral adaptations may instead be the main drivers of the long-term outcome.

Jaclyn Mallard, Williams Lab

SIV-infected monocyte/macrophages in the choroid plexus and brain are the source of CNS viral reservoirs

Jaclyn Mallard[1], Brittany Rife[2], Emily Papazian[1], Arianna Noggle[1], David J Nolan[2], Marco Salemi[2], Kenneth C. Williams[1]

[1]Department of Biology, Boston College, Chestnut Hill, MA
[2]Emerging Pathogens Inst., University of Florida, Gainesville, FL

HIV-associated neurocognitive disorders (HAND) and SIV-associated encephalitis (SIVE) are characterized by accumulation of monocyte/macrophages (Mo/MΦ) and virus in central nervous system (CNS). It is thought that Mo/MΦ traffic is responsible for CNS parenchymal viral reservoirs. T cells and Mo/MΦ enter the CNS from either the cerebrospinal fluid (CSF) *via* the choroid plexus (CP) or the blood-brain barrier at the meninges. In live humans, the CSF provides the only method to sample CNS virus. While both T cells and Mo/MΦ can be SIV+, CNS perivascular Mo/MΦ, not T cells are the predominantly infected cell population in HAND and SIVE lesions. Therefore, while SIV+ T cells remain in the CSF, SIV+ Mo/MΦ from the CP may seed CNS virus in HAND and SIVE. We hypothesized that both CP and CSF virus are a mixture of CNS, plasma, Mo/MΦ, T cell derived sequences and that only CP virus is an indicator of CNS viral reservoirs. Brain, CP, CSF, plasma, and T cells and Mo/MΦ from blood from from 19 SIVmac251-infected and 2 uninfected rhesus macaques (11 CD8-depleted and 10 non-depleted) were assessed. SIV+ animals included: 4 without AIDS, 6 AIDS with SIVE and 9 with AIDS without SIVE (SIVnoE). We used double label *in situ* hybridization and immunohistochemistry to count the number of infected T cells and Mo/MΦ in paraffin-embedded CP tissue sections. There were higher numbers of Mo/MΦ, but not T cells in CP from SIVE compared to SIVnoE animals. Both Mo/MΦ and T cells in CP contained SIV-RNA+ populations. Phylogenetic analysis of SIV *gp120* cDNA sequences was used to compare CSF, CP and CNS parenchymal virus with virus from CD3+ T cells and CD14+ Mo/MΦ in blood. CSF viral sequences were dispersed between CNS and peripheral (plasma, bone marrow, Mo/MΦ, T cells) sequences. In SIVE animals, CP and CNS sequences most often clustered together and were highly compartmentalized, potentially indicating viral reservoirs. The detection of SIV-RNA+ T cells and Mo/MΦ in CP underscores the CP as a source of CSF virus. The dispersed phylogeny of CSF viral sequences among peripheral and CNS sequences indicates that the CSF is not a viral reservoir. Mo/MΦ accumulation and compartmentalization of viral sequences in the CP and CNS suggests infected Mo/MΦ in these tissues are the source of CNS viral reservoirs.

Alexander Auld, Folker Lab

Nucleus-dependent sarcomere assembly is mediated by the LINC complex

Two defining characteristics of muscle cells are the many precisely positioned nuclei, and the linearly arranged sarcomeres, yet the relationship between these two features is not known. We have demonstrated that nuclear positioning precedes sarcomere formation. Furthermore, Zasp-GFP, a Z-line protein, colocalized with F-actin in puncta at the cytoplasmic face of nuclei before sarcomere assembly. In embryos with mispositioned nuclei, Zasp-GFP was still recruited to the nuclei prior to its incorporation into sarcomeres. Furthermore, the first sarcomeres appeared in positions in close proximity to the nuclei, regardless of nuclear position. These data suggest that the interaction between sarcomere proteins and nuclei is not dependent on properly positioned nuclei. Mechanistically, Zasp-GFP localization to the cytoplasmic face of the nucleus did require the Linker of Nucleoskeleton and Cytoskeleton (LINC) Complex. Muscle-specific depletion of klarsicht (nesprin) or klariod (SUN) blocked the recruitment of Zasp-GFP to the nucleus during the early stages of sarcomere assembly. As a result, sarcomeres were poorly formed and the general myofibril network that was less stable, incomplete, and/or torn. These data suggest that the nucleus, through the LINC complex is crucial for the proper assembly and stability of the sarcomere network.

Posters

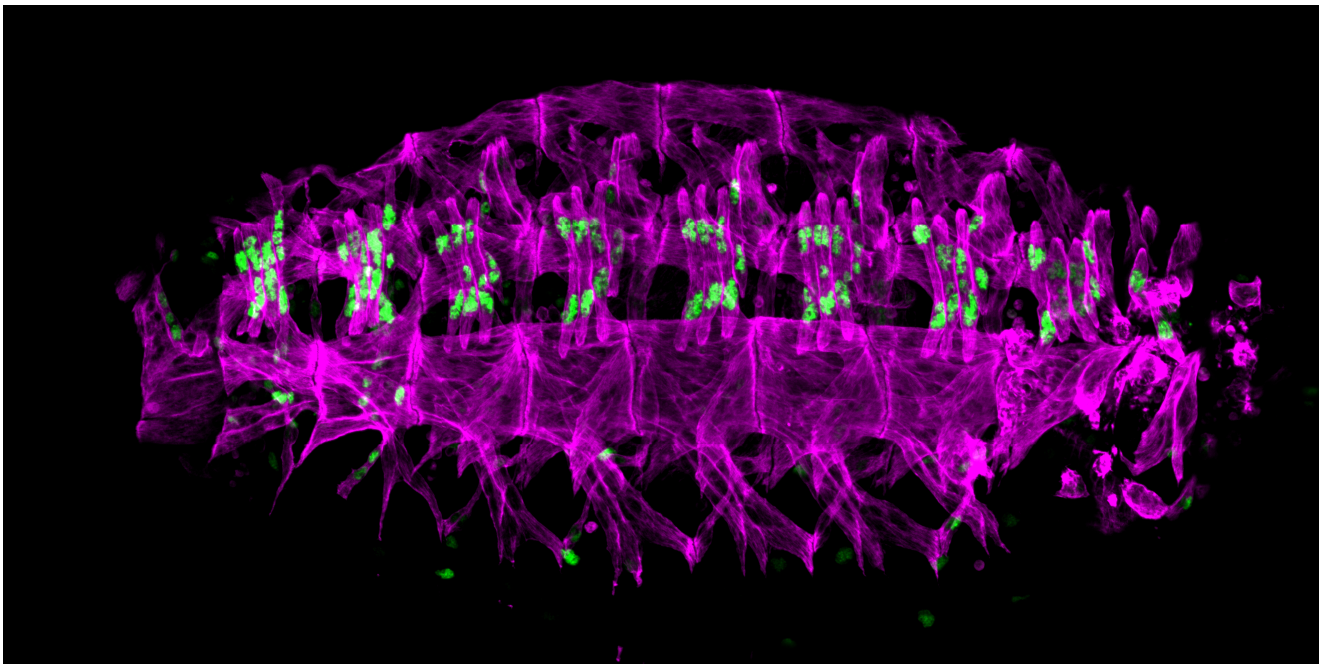

Image Credit: Torrey Mandigo, Folker Lab & Bret Judson, Higgins Imaging Facility
Drosophila embryo stained for the muscles (magenta) and the nuclei in a subset of muscles (green)

Poster 1

Nutrient availability regulates growth and proliferation responses during B lymphocyte activation

Shannon Argueta, *Timothy Connolly, Cheryl Doughty, Thomas Chiles*

Nutrient availability regulates growth and proliferation responses during B lymphocyte activation B cell activation is an energetically demanding process during which B lymphocytes undergo reprogramming and shifts from a resting state to a highly proliferative, metabolically active state. Little is known about the metabolic reprogramming process or the role extracellular nutrients play in the activation response. Here we demonstrate that there are distinct requirements for the nutrients L-glutamine and glucose during activation. We show that cells activated in glucose-depleted conditions are still able to undergo growth and signaling events. In contrast, we show that extracellular L-glutamine is essential for all but the earliest activation events, and cells cultured in L-glutamine-deprived conditions are unable to enter the cell cycle. Consistently, we show that extracellular supplementation of the cell-permeable derivative of α-ketoglutarate (α-KG), a glutaminolytic product, is able to rescue cell activation in the absence of glutamine. We also show the induction of the high affinity amino acid transporter ASCT2 is required for glutamine uptake following BCR (B cell receptor) crosslinking. Specifically, we found that halting glutamine uptake or processing by inhibiting ASCT2 or the glutaminolytic enzyme glutaminase causes activation defects that parallel those observed in glutamine deprived conditions, indicating a requirement for glutaminolysis during the very early stages of activation. Finally, we found that a-KG does not contribute to epigenetic remodeling, but is necessary for mammalian target of rapamycin complex 1 (mTORC1) activation. In turn, mTORC1 activity is partially required for upregulation of the glucose transporter Glut1 during the initial 24 hours of activation, as well as increased glucose uptake. These findings indicate a distinct metabolic profile that begins with glutamine uptake, and acts through mTORC1 signaling to later promote glucose uptake.

Poster 2

New tools to analyze overlapping coding regions

Amir Bayegan, *Juan Antonio Garcia-Martin, Peter Clote*

Retroviruses transcribe messenger RNA for the overlapping Gag and Gag-Pol polyproteins, by using a programmed -1 ribosomal frameshift which requires a slippery sequence and an immediate downstream stem-loop secondary structure, together called frameshift stimulating signal (FSS). It follows that the molecular evolution of this genomic region of HIV-1 is highly constrained, since the retroviral genome must contain a slippery sequence (sequence constraint), code appropriate peptides in reading frames 0 and 1 (coding requirements), and form a thermodynamically stable stem-loop secondary structure (structure requirement). We describe a unique computational tool, RNAsampleCDS, designed to compute the number of RNA sequences that code up to six user-specified peptides, possibly of various lengths (where optional IUPAC constraints on RNA sequences and GC-content may be stipulated), in six overlapping reading frames. Subsequently, RNAsampleCDS can sample a user-specified number of RNAs that correctly code the stipulated peptides, and/or compute the exact position-specific scoring matrix. We generalize the notion of codon preference index (CPI) to overlapping reading frames, and use RNAsampleCDS to generate control sequences required in the computation of CPI. Moreover, by applying RNAsampleCDS, we are able to quantify the extent to which the overlapping coding requirement in HIV-1 [resp. HCV] contribute to the formation of the stem-loop [resp. double stem-loop] secondary structure known as the frameshift stimulating signal. Using our software, we confirm that certain experimentally determined deleterious HCV mutations occur in positions for which our software RNAsampleCDS and RNAiFold both indicate a single possible nucleotide. These applications show that RNAsampleCDS constitutes a unique tool in the software arsenal now available to evolutionary biologists.

Availability: Source code for the programs and additional data are available at http:// bioinformatics.bc.edu/clotelab/RNAsampleCDS.

Poster 3

Kinetic analysis of synthetically designed tandem theophylline aptamer hammerhead riboswitch-ribozymes

Daniel Beringer, *Juan Antonio Garcia-Martin, Amir Bayegan, Peter Clote, Michelle M. Meyer*

The theophylline aptamer is an in vitro selected RNA structure with high affinity for its ligand and low affinity for a structural analog, caffeine. By combining this aptamer in tandem with a series of synthetically designed trans-cleaving hammerhead ribozymes, we expect to create riboswitch-ribozymes (RRs) where theophylline stabilizes an RNA conformation that allows cleavage of a target RNA. We performed a series of assays using theophylline and caffeine to test the ability of several RRs to effectively cleave their target RNAs in the presence of theophylline while remaining inactive with caffeine. Interestingly, all of the RRs tested to date cleave in the presence of both theophylline and caffeine. Kinetic analysis, however, uncovered differences in the maximum fraction cleaved (Fmax) and single RR turnover rate (kcat) (13.1-59.0% and 0.00465-0.160 min-1 for theophylline and 39.6-53.7% and 0.00218-0.0530 min-1 for caffeine, respectively). In all cases, theophylline caused cleavage to occur at a faster rate with kcat's 2-8 times higher than caffeine. We expect that caffeine does not bind to the aptamer and that the activity observed in the caffeine condition should be the same as the activity observed without ligand. To confirm this hypothesis, we are repeating assays in the absence of ligand.

Poster 4

ExoY Identification

Brett Bukowski, *Charles Hoffman*

Pseudomonas aeruginosa is an opportunistic pathogen that is a growing health concern for immunocompromised individuals, such as the elderly inhabitants of the intensive care unit. Exoenzyme Y (ExoY), a promiscuous cyclase, is a Type 3 secretion system (T3SS) effector protein that is unique to P. aeruginosa and found in approximately 90% of isolates. It is a protein that is known to play a role in a wide array of biological functions including host cell cytoskeletal disruption, host cell Tau phosphorylation, and inhibition of P. aeruginosa cell invasion, all of which increase the virulence of P. aeruginosa. Work is being done to identify novel inhibitors, via high throughput screening, in order to give researchers more tools to use in elucidating the best treatment for P. aeruginosa infection. Furthermore, immunofluorescence is being used in order to localize ExoY in the cell and test a hypothesis on cofactors.

Poster 5

Exploring a mechanistic function for TACC3 at the plus end of the microtubule

Garrett Cammarata, *Burcu Erdogan, Patrick Ebbert, Andrew Francl, Laura Anne Lowery*

Developing neurons actively extend their growing axons out into their environment to achieve proper neuronal connections in the brain. Underlying these processes are microtubules and their associated +TIP (Plus-end tracking proteins) which assist in translating external guidance cues into mechanical changes in the cytoskeleton. One such +TIP, TACC3 (transforming acidic coiled coil 3), has shown great promise in its ability to influence the dynamic plus end of the microtubule. It is at this plus end that TACC3 is known to interact with microtubule polymerase XMAP215 to promote axon outgrowth. Our experiments show that TACC3 likely brings XMAP215 to the plus end where it functions as a polymerase. Effects on axon length in vivo may be partially described through a synergistic like effect that is seen between TACC3 and XMAP215 interaction. Although this interaction has been attributed to TACC3's coiled coil domain, recent experiments show that this characteristic binding region is not sufficient to track the plus end. Furthermore, while TACC3 has been explored by the lab in an in vivo environment, uncovering the role of TACC3 without other confounding factors and +TIPs has not been determined. In vitro TIRF experiments show that TACC3 may promote microtubule polymerization independently, although further work will have to be done to conclude how this interaction is affected by XMAP215 in vitro.

Poster 6

One is the loneliest number: The combined effect of multiple intercellular interaction mediators

Sandra Dedrick, *Lori Niehaus, Babak Momeni*

In many natural communities, microorganisms interact with one another through the production and consumption of compounds known as mediators. These mediators can be products of either biosynthesis or catabolism, and can act as either enhancers or inhibitors of bacterial growth. Within a multispecies community, interaction networks are intrinsically complex; thus, community structure and composition is rarely a product of a single mediator acting in isolation. In order to develop models that accurately illustrate interactions amongst microbes, the effect of multiple mediators must be characterized. An ecological null model hypothesizes that the presence of multiple mediators affects target cell fitness in an additive manner. However, experimental validation of this assumption is scarce.

We characterize the combined effect of mediators on bacterial growth by analyzing the growth rate of *Escherichia coli* in the presence of multiple enhancers and inhibitors. Our experiments comparing *E. coli* growth rate on single and multiple carbon sources have demonstrated that growth rates may differ from the predictions of the null model (which assumes co-utilization) because of diauxic growth. We are also conducting experiments to determine the effect of mechanistically diverse inhibitors such as fermentation byproducts (ethanol, acetic acid, lactic acid, etc.), antibiotics, cations, and other organic weak acids. These studies will not only enhance our understanding of microbial community interactions, but will also establish some general principles that can improve current *in silico* models.

Poster 7

Refining the *Toxoplasma gondii* basal complex proteome by BioID reveals an expanded hierarchy and suggests a function beyond cell division

Klemens Engelberg, *Sudeshna Saha, Yani Zhou, Eranthie Weerapana, Marc-Jan Gubbels*

Apicomplexan parasites replicate by several cell division modes with two well-characterized examples being *Plasmodium* schizogony and *Toxoplasma gondii* endodyogeny. These division modes give rise to between two and up to thousands of newly formed parasites, respectively. *Toxoplasma* endodyogeny is arguably the simplest form of apicomplexan division generating two daughter cells as the offspring. Daughter cells are formed by an internal budding mechanism, which is concluded by separation and basal closure of the nascent cells. These processes are poorly understood but differ substantially from the array of cytokinesis modes known today. *Toxoplasma* cytokinesis is independent of an actinomyosin motor and microtubules but relies on a structure, functionally equivalent to the cytokinesis-related contractile ring, called the basal complex. To understand this unusual cytokinesis apparatus, we have started to dissect its proteomic composition by use of proximity-dependent biotin identification (BioID). Our results show that BioID is very suitable to reveal new basal complex components as indicated by our identification of several novel proteins localizing to this distinct structure. We furthermore uncover a set of kinases and phosphatases that indicate a potential tight regulation of basal complex functions.

Surprisingly, we also identify a group of proteins only associated with the mature basal complex following completion of cell division. These candidates are dispensable for proliferation but might be involved in basal complex actions beyond cytokinesis, a previously underappreciated feature of this elusive compartment. Collectively, our work highlights a multifunctional basal complex in *Toxoplasma* with an expanding proteomic complexity and hierarchy in assembly.

Poster 8

Regulation of microtubule plus-end dynamics by TACC3 during axon outgrowth and guidance

Burcu Erdogan, *Garrett Cammarata, Andrew Francl, Jessica Tiber, Eric Lee, Ben Pratt, Laura Anne Lowery*

Precise neuronal connection requires proper axon guidance. Microtubules (MTs) of neuronal growth cones are the driving force to navigate the growing ends of axons. Pioneering microtubules and their plus-end resident proteins, +TIPs, play integrative roles during this navigation. Recently, we introduced the protein TACC3 as a member of the +TIP family that regulates microtubule dynamics in growth cones, and we showed that manipulation of TACC3 levels affects axon outgrowth. Knowing that dynamic MT regulation is key to the growth cone's directional motility during axon outgrowth and guidance, we sought to further investigate the mechanism of TACC3-mediated MT regulation and the impact of this regulation in axon outgrowth and guidance. Here, we use quantitative analysis of high-resolution live imaging and show that TACC3 is required to promote axon outgrowth and prevent spontaneous retractions in cultured embryonic Xenopus laevis neurons. Furthermore, we find that TACC3 regulates the stability of microtubules within the growth cone. Additionally, we demonstrate that manipulation of TACC3 levels interferes with the growth cone response to axon guidance cues ex vivo. We also show that ablation of TACC3 cause pathfinding defects in axons of developing spinal cord motor neurons and retinal ganglion cells in Xenopus laevis in vivo. Together, our results suggest that by regulating MT behavior, the +TIP TACC3 is involved in axon outgrowth and pathfinding decision of neurons during embryonic development.

Poster 9

Ig gene repertoire analysis of SIV Envelope-specific antibodies selected via phage display

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We have constructed a single chain Fv (scFv) phage display library from an SIV-infected rhesus macaque, which developed unusual high titer neutralizing antibody against SIVmac239. We have also cloned VH-VL antibody fragments from 7 rhesus macaque B-cell lines (BLCL) that produce SIV gp120-specific monoclonal antibodies (mAbs). The library was screened using trimeric (gp140) and monomeric (gp120) forms of SIVmac239 envelope (Env) glycoprotein. 32 gp140-specific mAbs were selected along with 20 gp120-specific ones. gp140-specific mAbs as well as BLCL's belonged primarily to the major antibody families, VH1, VH4 and VH3. Phage display selected gp120-specific mAbs were only from VH4 family. A preferential VH combination with Vλ light chain was observed with SIV Env-specific mAbs (gp120 and gp140) but not with BLCL's or the un-panned library. None of the tested antibodies had detectable neutralizing activities against tier-1 SIVmac239. The majority of gp120-specifc mAbs potently neutralized tier-3 SIVmac316 with IC50 below 1μg/ml. For gp140-specific antibodies, which were in fact all gp41-specific, only 2 out of 11 tested, neutralized SIVmac316 with IC50 of 7 and 5μg/ml. These data evidence a pattern of preferential antibody VH segment usage in non-immunized SIV infected rhesus macaques. A comparative analysis with animals presenting low viral load or complete protection will be of great importance.

Poster 10

Identifying the mechanisms of microbial cell-to-cell interactions using Tn-Seq

***Michael Gates**, Babak Momeni*

Polymicrobial communities exhibit "community-level" functions that are the product of cell-to-cell interactions between community members. The genotypes of these members, in turn, are the basis of these interactions. However, ascertaining the details of these interactions is often challenging. Developing methodologies to identify cell-to-cell interactions is thus an important step in enhancing our understanding of microbial communities.

We plan to optimize transposon-sequencing (Tn-Seq) to uncover mechanisms of interaction between microbes. Tn-Seq is a recently developed technique that allows high-throughput screen of mutant libraries using next generation sequencing. It has proven useful in genotype-phenotype mapping, for instance to examine response of pathogens to antibiotics.

To establish the methodology, we construct two-species synthetic communities with defined interactions using E. coli. The engineered interactions will be either inhibitory – via contact dependent inhibition (CDI), or facilitating – via auxotrophic dependence. Previous studies defining the molecular aspects of CDI and auxotrophic dependence should allow us to assess the power of Tn-Seq in identifying interaction mechanisms. Performing the experiments in E. coli also gives us the opportunity to validate the Tn-Seq results directly by constructing candidate mutants.

In our constructed communities, each interaction is based off a producer-recipient relationship. We use Tn-Seq to probe the recipient population in cocultures along with the producers, as well as in monocultures. Identifying mutations with rising or falling frequencies in coculture directs us to the genes or gene networks that contribute to the interaction. Growth in monoculture helps us exclude mutations not involved in the interaction, while further highlighting those that do in coculture. This study will serve as a proof-of principle for establishing Tn-Seq as a methodology for identifying intercellular interaction mechanisms. Even though here we are using engineered lab strains to establish the methodology, the exciting next step is to expand to clinically relevant microbes from mouth, gut, and skin microbiomes.

Poster 11

Expression and Functional Characterization of Ancient ERV-Fc ENV Proteins Encoded in Primate Genomes

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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 proviral sequences 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[1]. We have identified ERV-Fc-related env sequences with intact ORFs in seven additional mammalian species: aardvark, dolphin, grey mouse lemur, squirrel monkey, marmoset, dog, and panda. Investigation of these ORFs has the potential to reveal whether ERV-Fc Env proteins have functional roles for the host, and to give insight into the evolutionary history of the ERV-Fc lineage. To characterize these proteins, codon-optimized expression constructs were synthesized encoding a human (HERV-Fc1) and a baboon (ERV-Fc-Bab) Env protein. Both constructs express full-length envelope protein (Env), but are not cleaved into the predicted surface (SU) and transmembrane (TM) subunits. Since HERV-Fc1 Env retains a canonical furin cleavage site, it is not clear why the protein fails to mature in transfected cells. In contrast, ERV-Fc-Bab lacks a canonical cleavage site, but when one is recreated by site-directed mutagenesis (I-Q-K-Q to R-Q-K-R), functional Env protein was produced. Furthermore, removal of 22 residues from the C-terminus of the cytoplasmic tail of ERV-Fc-Bab enhanced both syncytia formation (in cell-cell fusion assays) and the ability of ENV-Bab-Fc pseudotyped MLV particles to infect 293T cells, suggesting the presence of an R-peptide cleavage site like that of murine leukemia virus (MLV). In a superinfection assay ectopic expression of ERV-Fc-Bab Env can inhibit infection of 293T cells by MLV particles pseudotyped with Bab-ERV-Fc-env, raising the possibility that the endogenous glycoprotein encoded in the baboon genome could function as an entry inhibitor. A survey of a small panel of cells revealed that only human cell lines were infected by Baboon ERV-Fc-env pseudotyped MLV particles, 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 still expressed on human cells.

1. Benit L., et al.; Virology; 2003; (312) p159-168.

Poster 12

An intact retroviral gene conserved in spiny-rayed fishes for over 100 million years

Jamie Henzy, *Robert J. Gifford, Christopher P. Kenaley, Welkin E. Johnson*

We have identified a retroviral envelope gene with a complete, intact open reading frame (ORF) in 20 species of spiny-rayed fishes (Acanthomorpha). The taxonomic distribution of the gene, "percomORF", indicates that it inserted into the ancestral lineage between 110 and 180 million years ago, making it the oldest known conserved gene of viral origin. Underscoring its ancient provenence, percomORF exists as an isolated ORF within the intron of a widely conserved host gene, with no discernible proviral sequence nearby. Despite its remarkable age, percomORF retains canonical features of a retroviral glycoprotein, and tests for selection strongly suggest cooption for a host function. Retroviral envelope genes have been coopted for a role in placentogenesis by numerous lineages of mammals, including eutherians and marsupials, representing a variety of placental structures. Therefore percomORF's presence within the group Percomorpha – unique among spiny-finned fishes in having evolved placentation and live birth – is especially intriguing.

Poster 13

Higgins Imaging Facility

Bret Judson

Facility description: The shared Boston College imaging facility has 712 ft^2 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 14 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 (BIOL 5450) id offered every Fall/Spring by the facility manager for advanced undergraduate and graduate students.

Poster 14

Dissecting unexpected functional cooperation between two histone-modifying complexes

***David Layman**, Hugh Cam*

Transcriptional regulation in Eukaryotic systems is accomplished partly through the tightly orchestrated pattern of residue-specific histone posttranslational modifications. Methylation of histone H3 Lysine 4 (H3K4me) is typically associated with active gene transcription. In S. pombe, all H3K4me is achieved solely through the activity of the Set1/COMPASS (Set1C) complex, leading to its well-documented role in the maintenance of euchromatic regions of the genome. Interestingly, we observe a unique genetic interaction between an HDAC-containing Set3C complex and specific subunits of Set1C, namely Ash2 and Sdc1. Double deletion strains result in impaired growth phenotype and significant genetic derepression, as seen in microarray screens. This interaction of chromatin-modifying complexes represents an uncharacterized pathway between histone methylation and deacetylation and illustrates the nuanced coordination mechanisms underlying transcriptional control. Future studies aim to assess involvement of catalytic and chromatin-binding domains of Set3C and the participation of H2B-ubiquintination in the Set1C-SetC connection.

Poster 15

TACC3 Mitigates the effects of Nocodazole and Slit2 at low concentrations

Eric Jinsuk Lee, *Burcu Erdogan, Benjamin Pratt, Laura Anne Lowery*

Proper navigation of the developing axon necessitates the complex interactions between the microtubules (MT's) of the growth cone, associated proteins, and extracellular cues. Specifically, +TIPs, proteins that reside at the plus-end of microtubules, play a crucial role in the development of embryonic neurites. We have previously demonstrated that TACC3 functions as one of these +TIPs and binds plus-ends of MTs in Xenopus laevis embryonic growth cones. Here, using quantitative analysis of high-resolution live imaging, we also show that TACC3 mitigates reduction in MT dynamics parameters -growth speed, length and lifetime- in response to Nocodazole exposure at low concentrations and limits neurite retraction under low concentrations of Slit2. Our results illustrate that TACC3 may play a role in altering a developing axon's response to external guidance cues.

Poster 16

Network Properties of Enriched Communities

Lori Niehaus, Minghao Liu, *Babak Momeni*

In microbial ecology, it is widely understood that in nature, interactions among microorganisms of diverse intrinsic fitness can stably coexist through. Some of the mechanisms through which interspecies interactions can lead to coexistence have been studied in simple communities, but those mechanisms remain elusive in more complex communities. Studying coexistence mechanisms experimentally poses several challenges, including the inability to completely control the environment and adequately characterize the interactions between different species. To address this problem, we use a computer-based algorithm to observe the formation of stable, in silica communities in a controlled environment. Here, we focus on enriched communities as one of the major routes for establishing species coexistence. Using a mechanistic model in which species interact through chemical mediators, we identify common network properties of stable microbial communities. We find that, for instance, facilitation interactions (i.e. promoting the growth of self or other microorganisms) is favored in enriched communities. Identification of these properties offers vital insight for engineering stable industrial communities or managing persistent infections, which have a myriad of biomedical, environmental, and industrial applications.

Poster 17

Impact of coronavirus accessory proteins on the host antiviral immune response

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Our work focuses on the interplay between viruses and the individual host cell. Specifically, we are interested in accessory proteins of emerging viruses and their role in undermining the antiviral immune response. For our research we use proximity-based and interaction-based assays to isolate host cell proteins associated with viral accessory proteins. Once isolated, such complexes are analyzed by Mass Spectrometry in collaboration with Eranthie Weerapana's laboratory in the Chemistry Department. Combined with our assays this enables us to analyze individual interactions and to model global interaction maps of viral accessory proteins and host cell proteins (interactomes). This will provide insight into how accessory proteins of newly emerging viruses fit into the known network of the antiviral immune response and will identify new hubs of viral interference.

Our current study focusses on the newly emerging Middle East respiratory syndrome coronavirus (MERS CoV). This RNA virus is the etiological agent for an ongoing epidemic outbreak of severe respiratory illness centered in the Middle East with a mortality rate of ~36%. Pathogenesis following infection is characterized by aberrant cytokine expression and delayed induction of interferon beta (IFNβ). These effects are likely to involve immune antagonistic mechanisms of MERS CoV accessory proteins, such as "open reading frame 4a" (ORF4a).

Applying the proximity-based BioID Assay, we identified TRIM25 as novel interaction partner of MERS CoV ORF4a. TRIM25 is a key regulator for activation of the RIG-I-mediated immune response to RNA viruses. Lys-63 linked polyubiquitination of the receptor RIG-I by TRIM25 facilitates relay of signals, resulting in IFNβ expression. Viral counteraction of this mechanism was described for the NS1 protein of Influenza A virus. Binding of the viral protein to TRIM25 prevents RIG-I ubiquitination/activation and results in reduced IFNβ levels.

Counterintuitive, our results show that interaction between TRIM25 and MERS CoV ORF4a results in enhanced TRIM25-mediated RIG-I ubiquitination and elevated IFNβ levels. Interestingly, ORF4a proteins from the closely related bat viruses HKU4 and HKU5 display the opposite phenotype. Since they also bind TRIM25, we are currently investigating how the differences in these three viral proteins contribute to such differential effects on the antiviral immune response and in which way the interaction with TRIM25 plays a role. Moreover, we are interested in identifying if and how binding of TRIM25 contributes to cross-species transmission of MERS CoV from bats to humans.

Poster 18

A different perspective on neuromuscular junction development: The role of myonuclei

Margherita Perillo, *Juan Pablo Forero, Eric Folker*

Neuromuscular junctions (NMJs) are highly plastic synapses that form between motor neurons and skeletal muscles. Molecules from both neurons and muscles are known to play an important role during NMJ development. In particular well documented are the roles of signaling molecules, receptors and neurotransmitters. During muscle development, myonuclei are actively moved in the myofiber in order to maximize their distance, while a specific subset of myonuclei cluster right at the NMJ. Although this structural arrangement is remarkable, how synaptic nuclei contribute to NMJ development is not known. Since the overall NMJ architecture is conserved across animals, we use Drosophila melanogaster larvae, a well-known versatile tool for genetic and cell biological studies to determine the impact of muscle nuclei on NMJ development. Our approach is to initially evaluate changes in the NMJ structure in muscles that have been depleted of either cytoskeletal proteins or proteins of the nuclear envelope. Using the Gal4-UAS system, we depleted specifically from muscle, the cytoskeletal proteins kinesin heavy chain (KHC), dynein heavy chain (DHC) and several of their partners, including ensconsin, bicaudal D, and proteins of the dynactin complex (dnctp62, glued). With the only exception being glued, the internuclear distances in all mutants are reduced compared to controls. In all the mutants, NMJ development is also disrupted - branches are shorter, less complex and with fewer boutons than controls. Additionally, the microtubule associated protein ensconsin blocked bouton formation. Finally, ensconsin was unique in that it was required in muscle for the proper orientation of neuronal branches. Because kinesin and dynein will impact NMJ formation by many mechanisms, we tested the effects of the nuclear envelope proteins Klar and MSP300 (SYNE1) on NMJ structure. While Klar does not have an effect on NMJ growth, it does affect branching and bouton maturation, suggesting a later role in NMJ development. In contrast, a dramatic phenotype is observed when MSP300 is depleted in muscles. Although myonuclei are clustered together and more synaptic nuclei are close to the NMJ, branches are less complex and with fewer boutons. Together, these data suggest that the position of the nucleus is crucial for NMJ development.

Poster 19

Mapping the genetic basis of Toxoplasma gondii virulence traits through in vitro lab adaptation

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

Background

The apicomplexan parasite, *Toxoplasma gondii*, is an obligate intracellular parasite that causes the disease toxoplasmosis, a leading cause of foodborne-related death in the US. The three dominant North American genotypes display very little variation. Here we focus on two distinct Type I strains: GT1, a primary isolate of limited in vitro passage, and RH, which has been continuously passaged in vitro for nearly 40 years. In vitro adaptation of RH resulted in enhanced host independent virulence traits such as extracellular survival time, doubling time, and motility. The genetic divergence between RH and GT1 is only 0.002%, comprising 133 SNPs leading to non-synonymous amino acid changes. However, linking these SNPs to virulence traits through gene-by-gene allele swaps did only identify weak genotype-phenotype correlations. Therefore, it appears that epistatic interactions are critical for this correlation.

Methods

To decipher epistatic interactions we are subjecting the GT1 strain to in vitro lab adaptation and evaluate the development of virulence traits. We already have passaged GT1 and RH parasites for >50 passages. Furthermore, whole genome shotgun sequencing (WGSS) of P4 and P44 parasite populations was performed on Illumina's NextSeq500 platform to identify the accumulation of genetic changes over time.

Results

Low passage GT1-P12 parasites exhibited relatively short extracellular survival capacity compared to RH, with a significant difference at 3, 4, and 5 hours extracellular. When passaged to P49, GT1parasites showed remarkable increase in extracellular survival closely mirroring RH parasites. Furthermore, viability assays revealed a significant increase in GT1 viability from 15% at P12 to 27% by P49, compared to 43% for RH. Plaque size is an integrated read out of various lab adaptive traits. Going from GT1-P12 to P54 shows a 32% increase in plaque size, however plaques of either GT1 passage are still a factor ten smaller than observed for the lab adapted RH. WGSS on GT1-P4 and GT1-P44 populations revealed 3 SNPs over the course of ~167 generations, thereby estimating *T. gondiia's* substitution rate to be 2.74 E-10.

Conclusions

Our results demonstrate the concept of lab adaptation in *T. gondii.* We have shown that extracellular survival and viability are indeed lab adaptive traits in GT1. Intertingly, different traits evolve at different rates. The only modest increase in plaque size compared to RH indicates the major contributor to lab adaptation is an as yet an untested trait, such as motility or cell cycle length. We also demonstrated the accumulation of mutations, however we have not yet validated their relevance for the phenotypic changes. We are pursuing additional passaging, phenotypes assessment, and genome and transcriptome sequencing to further resolve the genetic basis of host independent virulence traits.

Poster 20

THE GENETIC BACKGROUND EFFECT: Predicting species-wide virulence for a bacterial pathogen with a large pan-genome

Federico Rosconi, *Lauren Havens, Matt Phillippo, Tim van Opijnen*

Streptococcus pneumoniae is a human nasopharyngeal commensal and respiratory pathogen that triggers pneumonia, meningitis, and septicemia. It is one of the most important bacterial pathogens worldwide, resulting in ~1 million deaths annually among children <5 years of age, and another ~0.5 million deaths among the immunocompromised and the elderly. By sampling 2018 strains from all over the world the estimated size of the core genome (the pool of genes shared by all members of a species) was ~1300 genes and the pan genome (a species' global gene repertoire) was ~4400 genes. With an average size of 2216 genes per genome, two strains differ on average in 234 genes. This difference on the genetic background may thus potentially affect important phenotypes for disease onset and control, as drug tolerance, nasopharynx colonization, virulence or the potential to evolve an increased pathogenicity. This means that a gene can be very important for virulence or drug tolerance in one strain while completely dispensable in another. As a consequence, drug or vaccine therapies designed according on functional analyses of one single strain would probably fail at the species-wide level.

Due to difficulties associated with performing both genome-wide as well as species-wide experiments, comprehensive studies have so far been completely neglected. However, with the introduction of Tn-seq tool has now become feasible to untangle the influence of the genetic-background on a genome-wide scale and a species-wide level for a bacterial pathogen. Tn-seq is a combination of transposon mutagenesis with massively parallel sequencing that enables the exact calculation of the growth rate (fitness) of a pool of mutants both in vitro as well as in vivo.

The hypothesis of this project is that a diverse set of genes are involved in S. pneumonia virulence and due to differences in genetic-background these components and the roles they play are only partially conserved across strains. Using Tn-seq, I propose to determine in detail the virulence potential for 30 S. pneumoniae strains, covering 92% of the pan-genome, thereby unraveling the genomic-patterns that are most important for host-colonization and disease induction. With the results obtained, I will be able to predict: 1) the virulence-level of a strain, and 2) a strains' likelihood to evolve a higher virulence-level. Although focus on S. pneumoniae, one of the most important bacterial pathogens in the world, this approach will serve as a roadmap to design similar studies in other bacterial species that are characterized by significant pan-genomes.

Poster 21

Plus-end tracking proteins of the TACC family regulate microtubule dynamics during embryonic development

Erin Rutherford, *Laura Anne Lowery*

Early embryonic morphogenesis relies on the successful orchestration of several dynamic processes dependent on cell motility. The early nervous system in particular undergoes numerous developmental changes involving the coordinated movement and guidance of cells, notably including early neurite outgrowth and neural crest cell migration. In addition to the well-established roles of the actin cytoskeleton, important roles for microtubules (MTs) in these motile processes have recently received more attention: MTs help transport elements of adhesion complexes to the cell periphery, and are also involved in the adhesion turnover necessary to allow directional translocation. Plus-end tracking proteins (+TIPs), a conserved family of proteins which localize to the growing ends of polymerizing microtubules, are known to regulate microtubule dynamics and functionality in a variety of ways and thus are significant players in the dynamic process of embryogenesis. We have shown that transforming acidic coiled coil (TACC) proteins TACC1 and TACC3 are +TIPs which localize to the distal-most plus ends of MTs, in front of EB1, where they influence MT dynamics in multiple Xenopus embryonic cell types. Here, we demonstrate the plus-end localization of TACC2, the final and least-studied member of the TACC family. Additionally, we assess the mRNA expression patterns of the three TACCs along a developmental time course, as a first step in determining precisely which processes each TACC protein may help facilitate during early development. Differential overexpression phenotypes obtained from quantification of MT dynamics in cultured Xenopus cells provide evidence of both functional redundancy and functional divergence within the TACC family. We finally demonstrate that TACC3 manipulation impacts pharyngeal morphology, a finding which supports others' claims that TACC3 plays a role in cell motility, and suggests that this may be true in the context of embryonic neural crest migration. Collectively, our data support an emerging role for the TACC family in cytoskeletal regulatory processes that are crucial for proper embryogenesis.

Poster 22

A Toxoplasma ferlin confers Ca2+-regulation to rhoptry secretion

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Sequential release of materials from the secretory organelles is known to play significant role in Toxoplasma gondii invasion, a process critically dependent on Ca2+ concentration. The role of microneme secretion in this process is much studied; however the exact molecular mechanism behind the release of rhoptries, the second organelle to be secreted, still remains obscure. Our work on Toxoplasma family of multiple C2 domain containing ferlin proteins enlightens a unique aspect of calcium dependence in rhoptry secretion. A genome wide search identified three ferlin proteins that we named TgFER1, 2, and 3, of which we investigate the more conserved TgFER2 in detail. Conditional knockdown of TgFER2 appears detrimental; leading to the significant loss of invasion and attachment of the mutants. However unlike TgDOC2, another essential C2 domain protein, loss of TgFER2 does not affect the microneme secretion but instead rhoptry secretion is abrogated. Thus our work brings into light a completely unexplored aspect of calcium dependence of rhoptry secretion in Toxoplasma as well as apicomplexans in general. Furthermore, our work also provides the first mechanistic insight in rhoptry secretion of Toxoplasma.

Poster 23

Mason-Pfizer Monkey Virus envelope glycoprotein antagonizes the antiviral activity of tetherin/BST2 in a species-specific manner

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Host intrinsic defense mechanism comprises of some cellular proteins called restriction factors (RFs) that are either constitutively expressed in certain cell types or induced by interferons during viral infections. RFs are important because they directly interact with viral components and block essential viral replication processes and hence, serve as a barrier in cross-species transmission. BST2/ Tetherin is one such ancient host antiviral factor that has been shown to restrict a wide range of enveloped viruses by inhibiting virion release from the plasma membrane in a non-specific manner. Consequently, in order to infect and successfully replicate in the host cells, several unrelated enveloped viruses have evolved to encode countermeasures to evade restriction by BST2. For example, HIV-1 encodes VpU and SIV encodes Nef to counteract restriction by BST2.

We are specifically interested to know how simple retroviruses, which lack accessory genes like VpU of HIV-1 overcome restriction by BST2. Therefore, to address the above-mentioned question in our current study, we took advantage of Mason-Pfizer Monkey virus (M-PMV), an exogenous recombinant betaretrovirus that causes an AIDS-like syndrome (SAIDS) in rhesus macaques. A recent study showed that human tetherin expression can inhibit M-PMV release[1]. We found that M-PMV release is restricted by human and other non-human primate BST2 orthologues in a species-specific and dose dependent manner. In particular, we found that M-PMV release was strongly inhibited by human and African green monkey BST2 orthologues, but to a much lesser extent by rhesus macaque and pig-tailed macaque BST2. To further investigate the domain of BST2 that determines species-specific differences in restriction, we tested several rhesus and human BST2 chimeras against M-PMV. Our findings suggest that the transmembrane domain of BST2 determines species-specific restriction against the release of M-PMV.

Differential sensitivity to tetherin orthologues also suggested that M-PMV may have a mechanism to actively evade BST2. Therefore, to test for the presence of an anti-BST2 domain in M-PMV, we performed a series of trans- complementation assays, using either M-PMV or SIVmac239 with the Nef gene deleted or SIVmac239 with both Nef and envelope gene deleted. The results of all the assays suggested that the envelope glycoprotein of M-PMV actively overcomes restriction by rhesus BST2. Currently, work is under progress to understand the mechanism of interaction between BST2 and the viral envelope glycoprotein.

1. Jouvenet, N. et al. J. Virol. 83:1837-1844

Poster 24

A Genome Assembly Tool for Bacterial Sequences

***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, which results in ~1 million deaths annually among children <5 years of age, and ~0.5 million among groups including the immunocompromised and the elderly (>65 yrs.), making it one of the most important bacterial pathogens worldwide. *S. pneumoniae* is one of several species for which the availability of complete bacterial genomes has demonstrated that a distinction can be made between its core-genome (the pool of genes shared by all members of a species) and pan-genome (a species' global gene repertoire) and this variation is probably enabled due to its receptiveness to take-up DNA. With an average genome size of 2Mbp, any given *S. pneumoniae* strain has about 2100 genes, of which 1400 genes are shared across all strains (the core), while the pan-genome is predicted to have about 3500 genes [Donati et al., 2010; based on the analysis of 44 strains].

The acquisition of novel clinical isolates and the increase in time and cost effectiveness of next-generation sequencing methods has resulted in a wealth of unprocessed information regarding the pan-genome of *S. pneumoniae*. We aim to validate previous predictions regarding the sizes of the core and pan-genomes through the analysis of 1000s of newly sequenced strains, and make available genomes for use in experiments regarding the phenotypic characterization of these novel strains with respect to virulence and antibiotic resistance.

Here we present a suite of tools for the assembly, organization and summary of new *S. pneumoniae* genomes. All tools are available on the Van Opijnen Lab Galaxy server (prince.bc.edu:8080) or as standalone scripts on Github (https://github.com/dsurujon/genome-assembly). This set of tools are used in the assembly of closed genomes from a set of contigs in a biologically meaningful manner through the use of a set of well-characterized genomes as reference. The complete genomes can then be annotated using the RAST server, and re-organized so that the resulting genome file adheres to the existing sequence file conventions.

Poster 25

Applications of droplet-based microfluidics to identify genetic mechanisms behind antibiotic stress responses in bacterial pathogens

Derek Thibault, *Paul Jensen, Tim van Opijnen*

It has become clear that besides the interaction of an antibiotic with its direct bacterial target, the drug induces stress that can trigger a wave of secondary responses that resonates throughout the bacterium. However, there is a lack of understanding of the genes and pathways involved in these indirect responses. Transposon insertion sequencing (Tn-seq), a technique developed by our lab, utilizes pooled libraries of transposon insertion mutants to understand the complexity of these indirect responses by accurately calculating mutant growth rates at a genome-wide scale. The sensitivity and high-throughput nature of Tn-seq, however, has a few drawbacks: 1) It is unclear how mutant interactions during pooled mutant culture influence the fitness of some independent mutants through, for instance, growth compensation or competition; 2) the 'pooled' approach hampers investigation of how higher order bacterial structures, such as biofilms or microcolonies, influence bacterial fitness. To overcome these challenges we combined Tn-seq with a droplet microfluidics-based technique that encapsulates and cultures single mutants on a genome-wide scale. Specifically, we adapted a droplet microfluidics system to generate monodisperse liquid and agarose droplets. This approach generates planktonic and microcolony modes of growth, which we characterized with several different species of bacterial pathogens, including Streptococcus pneumoniae. Additionally, we developed an accompanying Illumina library preparation protocol for Tn-seq that works with low input amounts of DNA in the nanogram range, which will allow us to profile single-cell genome-wide fitness of mutants that are individually cultured in droplets.

Notes

Notes

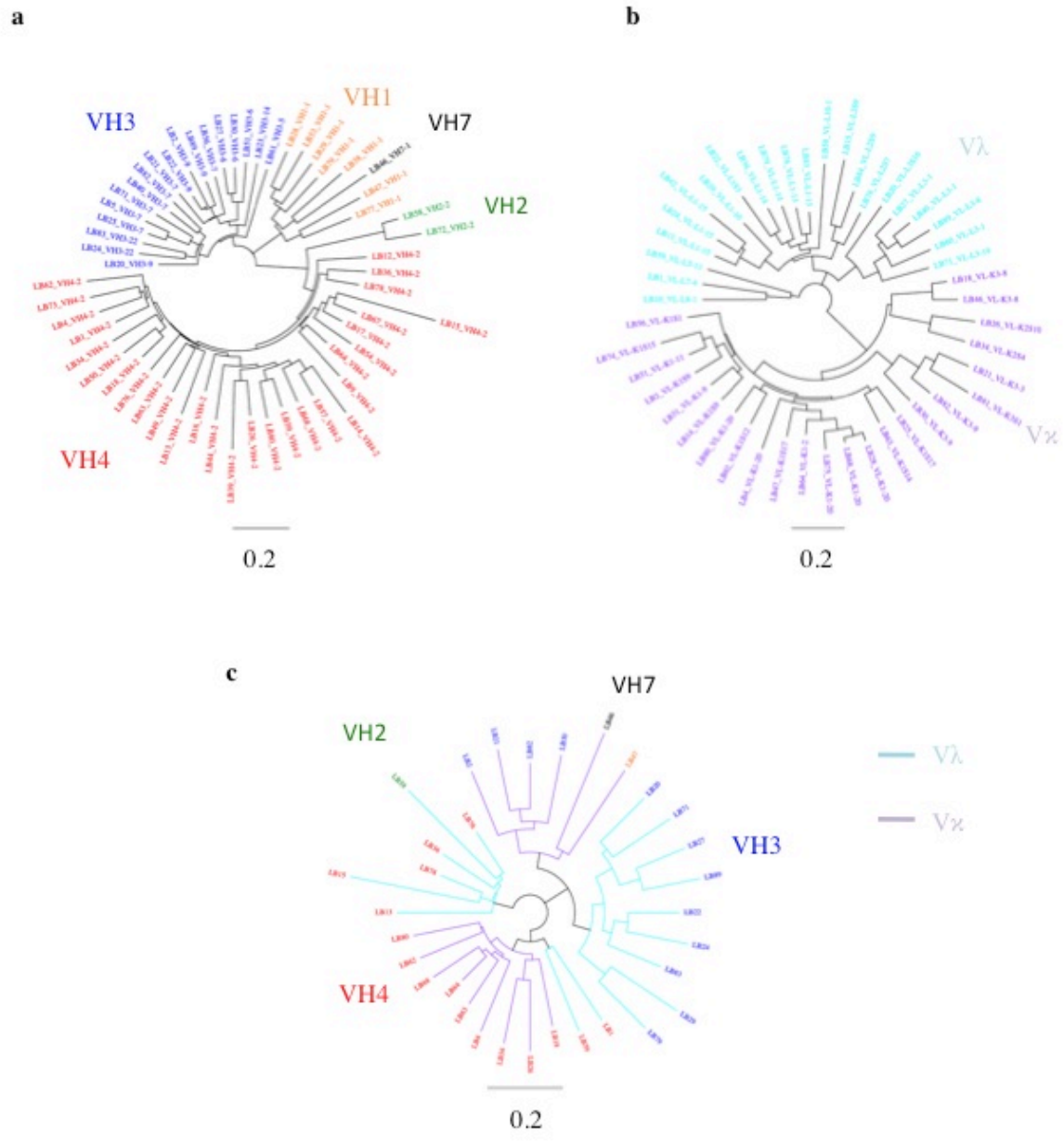

Image Credit: Ismael Ben F. Fofana
Phylogenetic relationship of SIV-specific antibodies
Antibody nucleotide sequences were analyzed using IMGT/V-Quest