Eck Institute for Global Health Research Retreat

Monday, January 13, 2014

Poster Presentations

List of Poster Presentations (in alphabetical order by first author)
Class D β-lactamases: are they all carbapenemases?

Nuno T. Antunes, Toni L. Lamoureux, Marta Toth, Nichole K. Stewart, Hilary Frase, Sergei B. Vakulenko
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

Class D β-lactamases have been known for several years and have been described in the chromosome and plasmids of several bacterial species. During the last years their clinical importance has arisen as they became major determinants of resistant to carbapenems, antibiotics of last resort for the treatment of various life-threatening infections. The most relevant clinically are predominantly found in Acinetobacter baumannii (OXA-23, -24/40, -58) but also in Pseudomonas aeruginosa (OXA-2, -10) and Enterobacteriaceae (OXA-48). While all the enzymes found in Acinetobacter and Enterobacteriaceae are classified as carbapenem-hydrolyzing class D β-lactamases (CHDLs), OXA-2 and -10 are currently classified as narrow-spectrum class D β-lactamases. Here we demonstrate that OXA-2 and -10 produce a narrow-spectrum antibiotic pattern when expressed in E. coli, although they behave as extended-spectrum β-lactamases and confer resistance to carbapenem antibiotics when expressed in A. baumannii. We purified and performed steady state kinetics against carbapenems with the six enzymes described above. OXA-2 and -10 present similar, and in some cases better, kinetic parameters against carbapenems to those of enzymes classified as carbapenemases. The results here obtained show that OXA-2 and -10 are CHDLs and suggest that the classification of other class D β-lactamases may need to be rethought.

Suppression of Chikungunya virus (CHIKV) replication in Vero cells by hammerhead ribozymes targeting the nonstructural genes

Velmurugan Balaraman, Tresa Fraser, Priya Mishra, James R Carter, Pruska Nawataisong, and Malcolm J Fraser
Department of Biological Sciences, University of Notre Dame, IN-46556

Chikungunya (CHIKV) virus is an alphavirus, transmitted to the humans by the bite of the infected Aedes mosquitoes. Transgenic mosquitoes with resistance to CHIKV replication could control the spread of the virus among human population. We designed 8 hammerhead ribozymes (hRz#1, 2, 3, 4, 5, 6, 7&8) against conserved regions within the nonstructural genes (nsp1, nsp2, nsp3 and nsp4) of the CHIKV viral genome. In-vitro cleavage assays demonstrate cleavage activity of these hRzs against artificial CHIKV target. These hRzs were cloned into a pantrophic retrovirus vector with Aedes tRNA valine pol III driving expression of the hRzs and a poly A tail sequence to recruit RNA helicase to disrupt the secondary structure of the viral RNA. The cloned plasmids were transfected into Vero cells and selected for hygromycin resistance for 6-8 weeks. Reverse transcriptase PCR confirmed expression of all hRzs in the transformed cells. The transformed cell lines were challenged with CHIKV 181/25 strain at a MOI of 0.0001 and the viral titer of the supernatants was determined at 3 days post infection by using TCID50 IFA assay. hRz#1 and hRz#8 targeting the nsp1 and nsp4 genes respectively, showed 2-3logs suppression of CHIKV replication as compared to wild type infected Vero cells. Homogenous cell populations were obtained using single cell limited dilution method, and were challenged with CHIKV at a MOI of 0.0001. The viral titers of supernatants determined at 3 dpi using TCID50 IFA showed much greater levels of suppression of CHIKV replication with the homogenous cell populations. These hRzs will be cloned into pXLBacII 3xp3 ECFP between the transposon elements. Based on these in vitro studies we anticipate that
transgenic mosquitoes expressing these hammerhead ribozymes will be refractory to CHIKV infection and transmission, and may be useful in effective suppression of CHIKV in natural populations.

Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito Anopheles gambiae

Nathaniel J. Balmert, Samuel S.C. Rund, John P. Ghazi, Peng Zhou, and Giles E. Duffield

Department of Biological Sciences and Eck Institute for Global Health, Galvin Life Science Center, University of Notre Dame, Notre Dame, IN 46556

Mosquitoes exhibit ~24 hour rhythms in physiology and behavior, regulated by the cooperative action of an endogenous circadian clock and the environmental light:dark (LD) cycle. We proposed to characterize diel rhythms (observed under LD conditions) in metabolic detoxification and resistance to insecticide challenge in Anopheles gambiae mosquitoes. A better understanding mosquito chronobiology will yield insights into developing novel control strategies for this important disease vector. We have previously identified >2000 rhythmically expressed An. gambiae genes. These include metabolic detoxification enzymes peaking at various times throughout the day. Especially interesting was the identification of rhythmic genes encoding enzymes responsible for pyrethroid and/or DDT metabolism (CYP6M2, CYP6P3, CYP6Z1, and GSTE2). We hypothesized that these temporal changes in gene expression would confer time-of-day specific changes in metabolic detoxification and responses to insecticide challenge. An. gambiae mosquitoes (adult female Pimperena and Mali-NIH strains) were tested by gene expression analysis for diel rhythms in key genes associated with insecticidal resistance. Biochemical assays for total GST, esterase, and oxidase enzymatic activities were undertaken on time-specific mosquito head and body protein lysates. To determine for rhythmic susceptibility to insecticides by survivorship, mosquitoes were exposed to DDT and deltamethrin across the diel cycle. We report the occurrence of temporal changes in GST activity in samples extracted from the body and head with a single peak at late night to dawn, but no rhythms were detected in oxidase or esterase activity. The Pimperena strain was found to be resistant to insecticidal challenge, and subsequent genomic analysis revealed the presence of the resistance-conferring kdr mutation. We observed diel rhythmicity in key insecticide detoxification genes in the Mali-NIH strain, with peak phases as previously reported in the Pimperena strain. The insecticide sensitive Mali-NIH strain mosquitoes exhibited a diel rhythm in survivorship to DDT exposure and ultradian rhythmicity to deltamethrin challenge. Our results demonstrate rhythms in detoxification in An. gambiae mosquitoes; this knowledge could be incorporated into mosquito control and experimental design strategies, and contributes to our basic understanding of mosquito biology.

Supported by the Eck Institute for Global Health.

Climate change and the distribution of two invasive mosquito species in the eastern US: a climate envelope model

Bethany Blakely

Aedes japonicus and Aedes albopictus are two invasive mosquito species introduced to the United States from Asia in the late 20th century. Both species have the potential to vector medically important viruses
(e.g. Dengue, West Nile). Using Maximum Entropy (MaxEnt) climate envelope modeling, the possible future distributions of both species were projected for two climate change scenarios. Under climate change, A. albopictus continues to occupy the southeastern US but moves out of the southern half of the Midwest and into the Great Lakes region, putting Chicago, Toronto, and other major cities at risk. In contrast, A. japonicas moves dramatically northward, occupying the southeastern coast of Canada and a narrow band of latitude at 50° - 55° N, reducing risk to most heavily populated areas (with the notable exception of Quebec).

Regulation of Expression of the β-Lactam Antibiotic-Resistance Determinants in Methicillin-Resistant Staphylococcus aureus (MRSA)

Blas Blázquez¹, Leticia I. Llarrulli², Juan R. Luque-Ortega³, Carlos Alfonso³ and Shahriar Mobashery¹
¹ Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA
² Institute of Molecular and Cell Biology of Rosario (IBR), CONICET-UNR; Department of Biological Chemistry, Biophysics Area, Universidad Nacional de Rosario, Rosario, 2000, Argentina
³ Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, CSIC, SPAIN

β-Lactam antibiotics are becoming less effective as therapeutics in treatment of staphylococcal infections as resistance to them increases. Resistance is mediated by a β-lactamase (encoded by blaZ) that hydrolyzes penicillins and a penicillin-binding protein (PBP2a, encoded by mecA), which is not modified effectively by these antibiotics. The 14.8-kDa MecI protein represses transcription of mecA and its three-dimensional structure reveals a dimer of two intimately intertwining dimerization domains, held together by a hydrophobic core, and two independent winged helix domains, each of which binds a palindromic DNA-operator half site. The mec operator consists of a single 30-bp palindrome with two 15-bp half-sites. We provide insight into the interactions of the MecI protein and the mec operator by a gel retardation assay. Equilibrium sedimentation studies provided the dissociation constant for the monomer-dimer equilibrium. Fluorescence anisotropy experiments have shown that binding of MecI to mec promoter is different depending on protein concentration. The anisotropy data has been fit using equations derived for different binding models, taking into account the possibility of binding of MecI as a monomer and/or dimer, taking into account the in vivo MecI concentrations, which were evaluated.

Colorimetric Detection of Chikungunya Viruses

James R. Carter, Cheryl A. Kucharski, Velmurugan Balaraman Tresa S. Fraser, and Malcolm J. Fraser, Jr.
Department of Biological Sciences, Eck Institute of Global Health, University of Notre Dame, Notre Dame, Indiana 46556, USA

DNAzymes (DNA-based enzymes; DZs) have been used to target a number of viruses including HPV, HCV and HIV in a sequence specific manner without the need for native cellular cofactors. The value of these DNA-based enzymes in nanotechnology has, until recently, been untested. Current viral detection methods require specialized training, are expensive, and can take hours to days before results can be obtained. In light of this we sought to develop a novel, rapid and sensitive chikungunya virus (CHIKV) diagnostic approach that couples the activity of CHIKV-specific DNAzymes (CDZ) to the aggregation properties of gold nanoparticles (AuNP). In vitro cleavage analysis of CHIKV RNAs confirmed CDZ
targeting and catalytic activities. RT-PCR analysis was performed to confirm the presence of CHIKV in the reaction mixture. Serial dilutions of $10^9$/ml, originating from C6/36 cell culture fluids, were produced and analyzed in a buffered mixture containing 0.5% SDS. CDZ targeting of CHIKV RNA lead to destabilization and aggregation of CDZ-tethered AuNPs, resulting in a red to clear color transition of sample tubes in the presence of 1.5M NaCl. This color change was detected visually and spectrophotometrically to signify the presence and, thus positive detection of CHIKV. The inclusion of SDS in the reaction mixture allowed detection of CHIKV directly from culture fluids without additional sample processing or RNA extraction procedures. Specificity assays demonstrated detection occurs in a CHIKV-specific manner, while sensitivity assays display a sensitivity of $1 \times 10^3$ TCID50 units. Future analysis will be performed to validate this method as a viable candidate for CHIKV detection in mosquito populations for epidemiologic surveillance. Our results demonstrate a unique, rapid, simple, and sensitive approach towards coupling effective DNAzyme targeting of CHIKV RNA genomes with AuNP aggregation for positive detection of CHIKV directly from C6/36 cell supernatants without sample processing.

Simultaneous suppression of the Arboviruses Dengue and Chikungunya through group-I intron activation coupled with conditional expression of the Bax C-terminal domain.

James R. Carter, Samantha Taylor, Tresa S. Fraser, Malcolm J Fraser Jr.

Department of Biological Sciences, Eck Institute of Global Health, University of Notre Dame, Notre Dame, Indiana 46556, USA.

An increase in the number of regions that are co-endemic to dengue (DENV) and chikungunya (CHIKV) viruses has been observed in recent years. In light of this development we engineered an antiviral approach that simultaneously suppressed the replication of both arboviruses in cultured mosquito cells. We examined the effectiveness of coupled expression of a proapoptotic effector, ΔN Bax, to the trans-splicing anti-DENV/CHIKV group I intron to simultaneously suppress infections of DENV and CHIKV. RT-PCR demonstrated the utility of these introns in trans-splicing the ΔN Bax sequence downstream of either the DENV or CHIKV target site in transformed Aedes albopictus C6/36 cells independent of the order virus specific targeting sequences were inserted into the construct. This trans-splicing reaction forms DENV or CHIKV capsid-ΔN Bax RNA fusions that lead to apoptotic cell death as shown by annexin V staining. TCID50-IFA analysis demonstrates the targeted suppression of DENV and CHIKV by our anti-arbovirus group I intron approach. The suppression of these arboviruses is further enhanced by clonal cell populations expressing our antiviral effectors due to the exclusion of untransformed cells. These results demonstrate we can simultaneously target DENV and CHIKV RNAs in a sequence specific manner with our dual anti-DENV/CHIKV group I intron, leading to the inhibition of replication, and providing a promising single antiviral for the transgenic suppression of multiple arboviruses.

Systematic and fair evaluation of global network aligners

Joseph Crawford¹, Yihan Sun¹,², and Tijana Milenković³

¹ Computer Science and Engineering, University of Notre Dame, USA
² Computer Science and Technology, Tsinghua University

Biological network alignment (NA) identifies topologically and functionally conserved regions between networks of different species. Then, biological function can be transferred from well- to poorly-
annotated species between aligned network regions. NA consists of two algorithmic components, a node cost function (NCF) and an alignment strategy (AS). Since different existing NA methods use both different NCFs and ASs, it is not clear whether the superiority of a method comes from its NCF, AS, or both. Thus, here we fairly evaluate state-of-the-art NA approaches by mixing and matching their NCFs and ASs.

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**Functional studies of ebolavirus matrix protein VP40**

Kathryn Del Vecchio

Filoviruses are filamentous viruses and include Ebola (EBOV) and Marburg (MARV), which are morphologically identical but antigenically distinct. These remarkable viruses can vary in length from ~1 to 14 um and are pleomorphic in shape. Mortality rates can be as high as 90% and to date there are no FDA approved vaccines or small molecules for treatment. EBOV harbors a genome of 7 proteins, the most abundantly expressed of which is Viral Protein 40 (VP40) also known as the matrix protein. VP40 is required for the assembly and budding of EBOV and virus like particles (VLPs) from the plasma membrane of host cells and is a viable target for pharmacological development. Recent work by the Stahelin and Ollmann-Saphire (Scripps Research Institute) labs indicates that VP40 adopts two different structures to elicit different functions in the viral life cycle. Cellular data demonstrates that each structure adopts a specific function, one for budding form the plasma membrane of human cells and one for regulation of viral transcription. This work will investigate how distinct VP40 structures assemble in the presence of synthetic lipid vesicles and at the inner leaflet of the plasma membrane in live cells. This project aims to determine lipid composition requirements for functionality of VP40 mutants vs. wild-type VP40, and to elucidate the function of VP40 oligomerization with site-specific mutants.

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**The Influence of Calcium on Pseudomonas aeruginosa Surface Motility**

Callan M. Driscoll$^{1,2}$, Nydia Morales-Soto$^{1,2}$, and Joshua D. Shrout$^{1,2,3}$

1 *Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame*
2 *Eck Institute for Global Health, University of Notre Dame*
3 *Department of Biological Sciences, University of Notre Dame*

The bacterium *Pseudomonas aeruginosa* is ubiquitous in the environment and is also an opportunistic human pathogen. A hallmark characteristic of *P. aeruginosa* is its ability to readily develop attached-growth biofilms on many surfaces under many growth conditions. It is known that both type IV pili (TFP) - and flagellar-mediated behaviors affect *P. aeruginosa* biofilm development. On surfaces, *P. aeruginosa* displays at least four distinct surface motility patterns after attachment, two of which are pili mediated: horizontally oriented "crawling", and vertically oriented "walking." We have investigated calcium as a possible nutrient cue for *P. aeruginosa* surface motility as calcium has been linked previously with increased biofilm formation. The extent as to the effects of calcium on pili is not quite clear. Single *P. aeruginosa* cells were tracked using time-lapse microscopy of early stage biofilms growing in flow cells. It was observed that low calcium conditions lead to an increase in TFP motility. Specifically, horizontal crawling increases approximately 5-fold for *P. aeruginosa* growing in either calcium-deficient medium (i.e., no added calcium) or calcium-chelated medium (i.e., calcium-deficient medium with EGTA). When
biofilms were allowed to develop and mature for 24-48 hours, the calcium-deficient conditions yielded numerous tower and mushroom structures, while the biofilms developing under normal calcium conditions were flat and homogeneous. Such phenotypic differences in biofilm structure are in agreement with previous biofilm studies that have noted the importance of TFP function in developing mushroom-shaped structures. Calcium starvation promotes TFP motility and subsequently more structured biofilms.

This calcium-dependent TFP motility did not extend to experiments conducted with “twitch” plate agar assays. There were no differences in twitch zones between calcium-deficient, normal calcium, or high calcium media conditions. Yet, in parallel as a control, an iron-starvation effect that promotes TFP twitching was observed. Thus, this calcium-TFP effect is conditional to some, as yet uncharacterized, aspect of a flow-cell growth environment. Future work on this project will research both the regulation of this calcium-dependent phenotype and the conditional growth conditions responsible.

RADtag Mapping of QTL for Reproductive Diapause in Culex pipiens

Roberta Engel, David Severson

*Culex pipiens* is a broadly distributed species complex. Human diseases are transmitted by this complex. *Cx. p. pipiens*, one member of the complex, is found across temperate zones of the world while *Cx. p. quinquefasciatus* is restricted to subtropical and tropical regions. One physiological trait that distinguishes *Cx. p. pipiens* from its sister taxon is its ability to enter reproductive diapause. Photoperiod, and possibly other environmental cues, is thought to trigger the expression of this complex life history trait. Females that enter reproductive diapause have not taken a bloodmeal thus ovarian follicle development is arrested. Previous work mapped quantitative trait loci in a F2 mapping population using markers developed with traditional methods. Our ability to generate informative SNPs and infer QTL has increased dramatically with the advent of massively parallel sequence technology (e.g., Illumina HiSeq2000). In addition, a published reference genome for *Cx. p. quinquefasciatus* is available. Phenotypic extremes were sampled from the F6 generation of an advanced intercross line (*Cx. p. quinquefasciatus* Johannesburg x *Cx. p. pipiens* South Bend) and a reduced representation paired-end library was built using the RADtag approach. Linkage groups were generated using SNPs inferred in silico. Future work will include fine scale mapping of the identified QTL regions.

Global Network Alignment In The Context Of Aging

Fazle Faisal and Tijana Milenkovic

Department of Computer Science and Engineering, University of Notre Dame, IN 46556

Analogous to sequence alignment, network alignment (NA) can be used to transfer biological knowledge across species between conserved network regions. NA faces two algorithmic challenges: 1) Which node cost function (NCF) to use to capture “similarities” between nodes in different networks? 2) Which alignment strategy (AS) to use to rapidly identify “high-scoring” alignments from all possible alignments? We “break down” existing state-of-the-art methods that use both different NCFs and different ASs to evaluate each combination of their NCFs and ASs. We find that a combination of the NCF of one method and the AS of another method beats the existing methods. Hence, we propose this combination as a
novel superior NA method. Then, since human aging is hard to study experimentally due to long lifespan, we use NA to transfer aging-related knowledge from well annotated model species to poorly annotated human between aligned network regions. By doing so, we produce novel aging-related information, which complements currently available information about aging that has been obtained mainly by sequence alignment, especially in human. To our knowledge, we are the first to use NA to study aging.

Fluorescent Labeling of *Staphylococcus aureus* Penicillin-Binding Protein 2a to Probe Active-Site Conformational Changes

Jennifer Fishovitz1, Negin Taghizadeh1, Matthew Dawley1, Juan Hermoso2, Mayland Chang1, and Shahriar Mobashery1
1 Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556
2 Department of Crystallography and Structural Biology, Instituto Quimica-Fisica “Rocasolano”, CSIC, Spain

One mechanism of resistance of *Staphylococcus aureus* is the presence of a gene that encodes for the penicillin-binding protein PBP2a. Like other PBPs, PBP2a is responsible for the biosynthesis of bacterial cell wall by catalyzing a transpeptidation reaction in its active site. In contrast to other PBPs, the transpeptidase activity of which can be inhibited by beta-lactam antibiotics by the irreversible acylation of the catalytic serine, PBP2a does not experience modification by beta-lactam antibiotics readily. Therefore, the presence of PBP2a is a key factor in resistance in *S. aureus*, as it is able to catalyze cell-wall biosynthesis despite the challenge by beta-lactam antibiotics. The crystal structure of PBP2a has been determined, identifying two mobile loops that cover the active site in a closed conformation and an allosteric site 60 Å away. Upon binding of substrate in the allosteric site, signal propagation takes place to cause the active site to open by movement of these two loops, allowing access for the transpeptidation reaction and ultimate biosynthesis of cell wall. In order to probe the conformational change around the active site, we have mutated an amino acid on each loop to a cysteine residue and labeled with the environmentally sensitive fluorescent coumarin probe, MDCC. Monitoring of the fluorescence of this labeled protein in the absence and presence of several antibiotics provides a convenient assay to probe the conformational changes. We have also introduced mutations in proximity to the allosteric site to determine the effect of allosteric substrate binding on signal propagation and active-site accessibility.

Leishmania Influence Differential microRNA Expression Profiles with Immunologically Critical Target Transcript Networks among Human Host Dendritic Cells.

Nicholas Geraci

*Leishmania donovani (Ld)* and *L. major (Lm)* are vector-borne intracellular protozoan parasites and two Old World causative agents of visceral and cutaneous leishmaniasis respectively. These pathogens avoid innate immune destruction when parasitizing host dendritic cells (DC), in part, by eliciting unique host cell type-specific transcriptional profiles. We explored the role of translational regulation by host microRNAs (miRNAs) in a *Leishmania* species specific manner via RNAseq, microarray, and quantitative proteomics analyses. Argonaute 2 (AGO2)-loaded mature miRNA profiles were explored through next
generation sequencing of small RNAs (<30nt) isolated from a time course of DC in situ infections with Lm and Ld separately. Expression profiles of large coding RNAs were assessed using microarray and protein expression determined via quantitative mass spectrometry of matched donor samples. Results of integrative multi-dimensional dataset analyses revealed differential miRNA expression profiles which were host cell type and infecting parasite species specific. Most AGO2-associated miRNAs were downregulated across infection conditions compared to uninfected controls. Correlative target prediction analysis identified negative key immune pathway regulator transcripts as the primary targets of upregulated miRNAs, with a divergent role of miR-155 in Lm versus Ld infection contexts. Functional studies are underway to confirm these target predictions and assess the potential for pathway dysregulation by Leishmania induced miRNAs as a permissive mechanism for parasite survival.

The Role of Streptolysin S in Streptococcus pyogenes Host-Pathogen Interactions

Rebecca Hyatt

One of the primary weapons in the arsenal of S. pyogenes is the ribosomally produced peptide toxin Streptolysin S (SLS). In addition to its well-recognized role as a cytolysin, recent publications have indicated that SLS may also influence host cell signal transduction. Our lab has utilized several SLS-deficient mutants of S.pyogenes to gain a better understanding of the host cell signaling cascades initiated by Streptolysin S. Our studies suggest that S. pyogenes utilizes SLS to induce a shift in host signal transduction that results in the down-regulation of a major survival pathway, AKT, with consequent induction of apoptosis in host epithelial cells. Furthermore, addition of wild-type (WT) forms of SLS toxin to host cells results in sustained activation of a key inflammatory pathway, NFκB. These changes in host signaling correlate with increasing cellular damage in cells treated with WT SLS toxin but not inactive SLS isoforms. Comparing the responses of host cells to WT and SLS-deficient S. pyogenes infection will allow us to directly evaluate the role of SLS on both a cellular and organismal level. Further elucidation of the means by which SLS manipulates host cell signaling during infection may prove instrumental in identifying new treatments and preventative measures to combat severe GAS disease.

Exploring the role of the Streptolysin-associated gene (sag) cluster in Staphylococcus aureus

Trevor Kane, Clayton Thomas, Abe Yu, Matthew Champion, Shaun Lee

Methicillin antibiotic resistant Staphylococcus aureus (MRSA) is a human pathogen responsible for about 15,000 deaths a year in the United States. MRSA causes a range of human infections including necrotizing fasciitis, scalded skin syndrome, and endocarditis. Recently, the streptolysin-associated gene (sag) cluster has been discovered in a subset of human S. aureus strains. The sag cluster was previously identified in Streptococcus pyogenes and is responsible for the biosynthesis of Streptolysin-S (SLS). SLS is a potent cytotoxin and virulence factor of Group A Streptococcus. This work attempts to better characterize the potential SLS-like toxin produced by the sag cluster in S. aureus. We would like to determine whether the SLS-like toxin serves as an antimicrobial peptide or virulence factor against host cells. Preliminary data suggest that the S. aureus (USA300) strain containing the sag cluster exhibits greater hemolytic activity, and bacteriostatic activity as compared to strains that lack the sag cluster (MW2, MRSA252). Future work will focus on creating isogenic mutants in the sag cluster and in-vitro
reconstitution of the active SLS-like toxin produced by *S. aureus*.

**Virtual Screening of Anopheles gambiae Octopamine Receptor**

Kevin Kastner¹, Guillermina Estiu², Douglas Shoue³, Jesus Izaguirre¹,*, Mary Ann McDowell³,*
¹ Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN 46556
² Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556,
³ Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

Octopamine receptors perform key operations in the biological pathways of invertebrates only, making this class of G-Protein Coupled Receptors (GPCRs) a potentially good target for insecticides. The lack of structural and experimental data for this insect-essential GPCR class has prompted the development of homology models that are good representations of their biological equivalents useful for insecticide development. We report here the discovery of new octopamine receptor agonists and antagonists based on virtual screening and Molecular Dynamics (MD) simulations. We generated Anopheles gambiae octopamine receptor AGAP000045B (45B) homology models refined via MD simulations and computationally screened the ZINC library.

Supporting prior GPCR studies, Asp100 in the TM3 region, and Ser206 and Ser210 in the TM5 region were found to be important to the activation of the protein. The current combined computational and experimental approach seems appropriate for creating and refining homology models of the octopamine receptor and in turn aid in the discovery of new and effective insecticides.

* THESE AUTHORS CONTRIBUTED EQUALLY TO THIS WORK

**The Identification of EspM, a Novel Component Required for Mycobacterial ESX-1 Protein Secretion**

George M. Kennedy, Matthew M. Champion, Scott J. Emrich, and Patricia A. Champion

The ESX-1 protein secretion system is a critical virulence factor for mycobacterial and Gram-positive pathogens including *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Bacillus anthracis*. Known components and substrates of the mycobacterial ESX-1 secretion system are encoded by three distinct genetic loci; the extended RD1 region, espACD, and espR. To uncover additional genetic loci required for ESX-1 secretion in mycobacteria, we screened a *Mycobacterium marinum* transposon-insertion library. We identified a *M. marinum* transposon insertion strain with a smooth colony phenotype that is defective for ESX-1 protein substrate secretion *in vitro*. The transposon inserted into a novel gene, espM, not previously associated with ESX-1 secretion. Consistent with a defect in ESX-1 export, disruption of *espM* leads to loss of hemolytic activity and attenuation of *M. marinum ex vivo*. Yeast-two-hybrid analysis found that EspMₜₘₜₜ directly interacts with EccAₜₘₜₜ, a known component of the ESX-1 secretion system. Given the integral role protein-protein interaction plays in protein secretion systems, we hypothesize EspM is required for the stability or localization of EccA to facilitate ESX-1 mediated secretion.
Mechanisms of Genetic Uncoupling of ESX-1 secretion in Mycobacteria

M. Kuspa, and P. Champion

Bacterial pathogens use secretion systems to export proteins that mediate host interaction. The ESX-1 secretion system is a virulence determinant in Gram-positive pathogens and pathogenic mycobacteria. The functions of specific ESX-1 substrates are unclear because secretion of substrates is “co-dependent”. Generally, the secretion of each ESX-1 substrate is required for secretion of all other substrates, making it difficult to assign functions to each substrate using genetic knockouts. Our goal is to understand the basic mechanisms of the ESX-1 protein secretion system and its role in mycobacterial pathogenesis by determining how the association of ESX-1 ATPases promotes substrate secretion. We previously demonstrated that expression of a hybrid substrate, FL-EC7 (FLAG-EspC bearing the 7 C-terminal amino acids of CFP-10), in Mycobacterium uncouples ESX-1 secretion, allowing for secretion of the known substrates except EspA. We hypothesize that FL-EC7 associates two ESX-1 ATPases directly, bypassing the need for EspA. To test this hypothesis, we have identified specific residues in EspC required for the interaction with the EccA ATPase. We found several classes of EspC variants which do not interact with EccA. Interestingly, disruption of the interaction does not always prevent EspC export. Using these tools, we measured interaction of EspC with ESX-1 associated ATPases and substrates in Mycobacterium. In these studies we have identified an EspC variant that shows increased secretion and increased interaction with the EccA ATPase and other ESX-1 components in vivo. Finally, we are using FL-EC7 to test the role of EspA secretion in macrophage infections. The knowledge gained from this project will advance our understanding of the basic molecular biology of this virulence pathway. We hope to construct additional individual Mycobacterium strains that are phenotypically “null” for each ESX-1 substrate to clarify the role of these substrates in virulence.

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Increased light sensitivity under low-light conditions associated with extensive Aaop1 redistribution in Aedes aegypti

Matthew T. Leming, Alexander J. Metoxen, Xiaobang Hu, Michelle A. Whaley, Joseph E. O'Tousa

We are evaluating the role of vision and circadian cues in the activity and behavior of the disease vector Aedes aegypti. At night, a time period for which Ae. aegypti is not typically active, low levels of light exposure triggered robust mosquito activity, indicating that light acts outside the realm of circadian input to influence mosquito behavior. The Ae. aegypti compound eye is composed of approximately 300 ommatidia, each containing a bundle of eight (R1-R8) photoreceptor cells. The blue light-sensitive Aaop1 is a major rhodopsin of the retina, being expressed in the R1-6 photoreceptors cells. The photosensitive organelle in these cells, known as the rhabdomere, has a microvillar structure that allows a large plasma membrane surface to concentrate rhodopsins and other proteins that function in phototransduction. The Aaop1 rhodopsin is localized to vesicles in the cytoplasm during daylight and absent from the photosensitive membranes of the rhabdomere. At dusk, Aaop1 moves from cytoplasmic multi-vesicular bodies and becomes localized within the rhabdomeres. We examined the influence of circadian rhythms on Aaop1 behavior by exposing the animals to sustained light or precocious dusk. These experiments showed that light itself, not circadian signals, is responsible for the movement of Aaop1. We also carried out protein blot analysis to examine the levels of Aaop1 at different times in the day/night cycle. The
results showed that Aaop1 levels gradually increase during the morning period but then declines during the afternoon and evening periods. It is likely that a circadian input influences the change in Aaop1 levels because the decrease was still observed even when light cycles were altered. We also examined the effect of rhodopsin translocation on light sensitivity. There is a correlation between Aaop1 translocation and increased light sensitivity when measured by electroretinogram. The results show there is approximately a 1.5 log unit increase in sensitivity upon translocation of Aaop1 into the rhabdomeres, establishing a correlation between Aaop1 translocation at dusk and increased light sensitivity.

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**Using amoeba to identify a novel component of ESX-1 secretion system**

Felix Mba Medie and Patricia A. DiGiuseppe Champion

*Department of Biological Sciences, Center for Rare and Neglected Diseases, Eck Institute for Global Health, University of Notre Dame, 232 Galvin Life Sciences Building, Notre Dame, IN, 46556*

The ESX-1 secretion system is required for mycobacterial and Gram-positive pathogenesis. ESX-1 functions to export several substrates from the mycobacterial cell, including ESAT-6 and CFP-10. (Stanley et al. 2003; Guinn et al. 2004; Burts et al. 2005; Garufi et al. 2008). The ESX-1 system is encoded by three genomic loci, including the extended RD1 region, the espA locus, and the espR locus. We sought to identify additional genes required for ESX-1 export in *M. marinum*, an established model for *M. tuberculosis* pathogenesis. We previously demonstrated that the ESX-1 secretion system was required for cytotoxicity of amoebae (Kennedy et al. 2012). Therefore, we exploited this model to screen for non-cytotoxic *M. marinum* strains. We constructed a non-saturating, arrayed, 4x-coverage transposon insertion library in *M. marinum* (Champion et al. 2012). We conducted a pilot screen of 1000 *M. marinum* colonies and identified seven strains that were defective for cytolysis of amoebae. The focus of this study is the 59F6 *M. marinum* strain. As compared to the wild-type strain, the 59F6 strain is non-cytotoxic to amoeba and macrophages. Furthermore, 59F6 is attenuated in macrophages. Although the transposon insertion in the 59F6 strain maps to a gene that is outside of the three known ESX-1 loci, this strain is also deficient for the secretion of known ESX-1 substrates, and is non-hemolytic. We are currently determining how this novel gene promotes ESX-1 export.

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**Complete suppression of chikungunya virus replication in Vero cells using clonal hammerhead ribozymes**

Priya Mishra, Velmurugan Balaraman, Tresa Fraser, Malcolm Fraser Jr.

Chikungunya virus (CHIKV) is a positive sense RNA Alphavirus. The virus is endemic in Asia and parts of Africa and is propagated by the bite of female mosquito, mainly *Aedes aegypti*, with disease characterized by abrupt onset of fever, muscle pain, nausea, headache, fatigue, rashes and debilitating joint pain. Our objective in this study is to develop mosquito vectors refractory for Chikungunya virus by transgenic introduction of expressed antiviral molecules into the mosquito genome. In this study we designed and tested a set of antiviral Hammerhead ribozymes. Hammerhead ribozymes are small catalytic RNA molecules that can cleave their target RNA in a sequence specific manner. They cleave at the 3’ end of NUH triplet on the target RNA where H can be any nucleotide except G, and N is any nucleotide. Hammerhead ribozymes had been used successfully to effectively inhibit the replication of
many viruses, including Hepatitis (1), HIV (2,3), and Dengue (4). We designed our Hammerhead ribozymes by aligning 100 of Chikungunya genomes using the ClustalX program and looked for NUH triplets in the middle of 16-19 nucleotides conserved sequences. Based on the alignment studies, we designed 7 ribozymes targeting structural proteins of the virus. We analyzed these ribozymes for cleavage activity against artificial targets in in vitro assays, and then for effective suppression of Chikungunya virus in infected cell assays. The results of the in vitro cleavage assays demonstrate all seven ribozymes are catalytically active, while suppression assays demonstrate that all seven ribozyme are effective in inhibiting the Chikungunya virus replication in Vero cells.

Assembly of Aedes aegypti odorant receptor regulatory matrix

Mysore, Keshava; Li, Ping; Andrews, Emily; Duman-Scheel, Molly
Indiana University School of Medicine, Department of Medical and Molecular Genetics, South Bend, IN, 46617 and University of Notre Dame, Eck Institute for Global Health, Notre Dame, IN, 46556.

Though the socioeconomic and medical importance of mosquitoes is profound, surprisingly very little is known about developmental mechanisms in the mosquito sensory system, including the olfactory system, a tissue of vector importance. Knowledge about the mechanism that specifies mosquito olfactory receptor neurons (ORNs) to express a particular odorant receptor (OR) from a large OR pool, an important step for odor detection and discrimination, is lacking. Here, we investigate this process in Aedes aegypti, the dengue and yellow fever vector mosquito. Studies in Drosophila melanogaster have suggested that the combination and levels of expression of various cis-regulators of transcription in ORNs generates the OR regulatory matrix, a code governing which particular OR gene is expressed and which are repressed in any given ORN. Although OR sequences have rapidly evolved in insects, our preliminary studies suggest that mosquito OR expression is regulated in a comparable manner. Here, we begin to examine the functions of eight transcription factors (TFs) that are hypothesized to function in the Ae. aegypti OR regulatory matrix. The TFs are expressed in a subset of Ae. aegypti antennal ORNs, and expression levels of each TF varies from neuron to neuron within this subset. Searches for consensus binding site sequences for the TFs in the 1 kb 5’ flanking sequences of 115 Ae. aegypti OR genes known to be expressed in the adult antenna uncovered multiple consensus binding sites for each TF in the 5’ flanking regions of Ae. aegypti OR genes. These data strongly suggest that the TFs will function in the Ae. aegypti OR regulatory matrix. To functionally test this hypothesis, we used chitosan/siRNA nanoparticles to target one of the transcription factors, Ae. aegypti single-minded (sim), during olfactory development. The results of this investigation suggest that Sim regulates expression of a subset of OR genes and functions in the Ae. aegypti OR regulatory matrix. Our ongoing efforts will continue to assemble this OR regulatory matrix which is critical to the adult mosquito sense of smell.

LmaPA2G4, a downstream target of MAPK7, is an essential gene and is implicated in translation control in L. major

Brianna Norris-Mullins, Kaitlin VanderKolk, Paola Vacchina, Michelle V. Joyce and Miguel A. Morales

Downstream targets of MAPKs are largely uncharacterized in trypanosomatids. Here we report the identification of a downstream target of the L. major MPK7 pathway, LmaPA2G4. LmaPA2G4 is a homolog of human proliferation-associated 2G4 protein (PA2G4, also termed Ebp1). We show that it is
an essential gene in \textit{L. major} and a gain-of-function approach allowed us to implicate LmaPA2G4 in translation and subsequent protein synthesis reduction, growth defects and virulence attenuation. This work highlights the essential role of MAPK targets in the biology of the parasite and thus make them attractive targets for drug development.

\begin{center}
\textbf{Identifying GPCRs in the Genome of the Sand Fly \textit{P. papatasi} using Ensemble*}
\end{center}

RJ Nowling, M Wadsworth, JL Abrudan, DA Shoue, B Abdul-Wahid, GM Stayback, FH Collins, MA McDowell, and JA Izaguirre

G-protein Coupled Receptors (GPCRs) are a class of seven transmembrane (7TM) proteins involved in signal transduction that respond to a diverse range of stimuli. A sign of their importance in regulating many physiological processes, GPCRs are relatively abundant in metazoan genomes (1% of the \textit{Drosophila melanogaster} and 1.6% of the \textit{Anopheles gambiae} genomes). Due to their physiological importance, abundance, and specificity, GPCRs are attractive targets for the development insecticides, repellents, and other products for the control of vector populations.

In our previous work, we evaluated existing GPCR classifiers on vector peptide sequences, showing that their accuracy and sensitivity are less than desired. In response, we developed Ensemble*, a novel GPCR classifier tuned for arthropod genomes. Ensemble* was validated on test sets of known GPCRs and applied to the vector species \textit{Aedes aegypti}, \textit{An. gambiae}, and \textit{Pediculus humanus}, resulting in 52 novel hits. Validation of the hits confirmed 19 of the predictions as GPCRs and gave evidence that another 11 hits were putative GPCRs.

The genome of the sand fly \textit{Phlebotomus papatasi}, a vector of leishmaniasis and pappataci fever, has recently been sequenced and assembled. Ensemble* was run on the \textit{P. papatasi} genome peptide translations, resulting in 142 hits. Subsequent validation with BLAST against the NCBI nr-database and ScanPROSITE resulted in the identification of 97 confirmed and 7 hypothetical GPCRs.

\begin{center}
\textbf{Daily rhythms in antennal protein and olfactory sensitivity in the malaria mosquito \textit{Anopheles gambiae}}
\end{center}

\textit{Department of Biological Sciences and Eck Institute for Global Health, Galvin Life Science Center, University of Notre Dame, Notre Dame, IN 46556;}
\textit{* Department of Chemistry and Biochemistry and Eck Institute for Global Health, Nieuwland Science Hall, University of Notre Dame, Notre Dame, IN 46556.}

We recently characterized 24-hr daily rhythmic patterns of gene expression in \textit{Anopheles gambiae} mosquitoes. These include numerous odorant binding proteins (OBPs), soluble odorant carrying proteins enriched in olfactory organs. Here we demonstrate that multiple rhythmically expressed genes including OBPs and takeout proteins, involved in regulating blood feeding behavior, have corresponding rhythmic protein levels as measured by quantitative proteomics. This includes AgamOBP1, previously shown as important to \textit{An. gambiae} odorant sensing. Further, electrophysiologocal investigations demonstrate
time-of-day specific differences in olfactory sensitivity of antennae to major host-derived odorants. The pre-dusk/dusk peaks in OBPs and takeout gene expression correspond with peak protein abundance at night, and in turn coincide with the time of increased olfactory sensitivity to odorants requiring OBPs and times of increased blood-feeding behavior. This suggests an important role for OBPs in modulating temporal changes in odorant sensitivity, enabling the olfactory system to coordinate with the circadian niche of An. gambiae. Supported by the Eck Institute for Global Health, Center for Rare and Neglected Disease, and the Indiana CTSI.

A novel non-ribosomal peptide synthetase required for ESX-1 export in M. marinum

Rachel E. Schluttenhofer and Patricia A. Champion

The ESX-1 (ESAT-6 System 1) secretion system is required for virulence in pathogenic mycobacteria. We designed and performed a genetic screen to identify novel genes required for ESX-1 export in M. marinum, a pathogenic mycobacterial species related to M. tuberculosis. Although M. marinum is a popular model for the ESX-1 system in M. tb, M. marinum resides in a different environmental niche than the human pathogen and likely uses the ESX-1 system in ways that M. tb does not. We identified an M. marinum transposon insertion strain exhibiting smooth colony morphology (strain 212A1). Smooth colony morphology has been previously linked to a loss of ESX-1 function. Therefore, we hypothesized that the 212A1 strain may be ESX-1 deficient. We found the 212A1 strain phenotypically similar to the ΔRD1 M. marinum strain, which bears a deletion in several genes required for ESX-1 secretion. Specifically, the 212A1 strain was non-hemolytic. We measured export of ESX-1 substrates in vitro and found that the 212A1 strain failed to export ESX-1 substrates. We examined virulence using an amoeba cytotoxicity assay. Our preliminary data indicate that the strain is attenuated compared to wild-type M. marinum. We have determined that the transposon insertion resides in a non-ribosomal peptide synthetase (NRPS) that is not conserved in the M. tuberculosis genome. This indicates that this gene may contribute specifically to ESX-1 export in M. marinum. Using the sequence of the NRPS, we used bioinformatics to predict the potential peptide product of this gene cluster. We are currently trying to elucidate how this cluster of NRPS genes and the resulting peptide product promote ESX-1 export and virulence of M. marinum.

Light-regulated blood-feeding behavior and a light phase response curve for the Anopheles gambiae malaria mosquito

Aaron D. Sheppard, Gary George, Hannah Yu, Samuel S.C. Rund, and Giles E. Duffield

Department of Biological Sciences and Eck Institute for Global Health, Galvin Life Science Center, University of Notre Dame, Notre Dame, IN 46556

BACKGROUND: Biting behaviors in anopheline mosquitoes are shown to be time-of-day specific, with the greater abundance of biting occurring during the dark phase of their photoperiod. As such, we investigated whether a single pulse of light administered during the dark phase could inhibit biting behavior. Equally so, Anopheles gambiae locomotion/flight activity has a distinct circadian rhythm, with activity bouts demonstrating a clear 24-hour nocturnal pattern. This circadian control is mediated, in part, through entrainment by the light-dark (LD) cycle. Therefore, we additionally investigated how
METHOD: To investigate biting inhibition through light manipulation, two incipient species of An. *gambiae* mosquitoes (the molecular S and M forms) were treated with white light (10 min, 150-800 lux) at the onset of dark phase of the LD cycle (i.e. Zeitgeber time [ZT] 12), and the percentage taking a blood meal was recorded every two hours up to 8 hrs. To produce the PRC, S form mosquitoes received a single 30 min treatment of white light (300 lux) at various times during the immediate 24-hrs transitioning from a LD photoperiod to constant darkness. The displacement of the onset of the mosquito nocturnal activity bout from where it was predicted to onset (phase shift) was then measured for each time-specific pulse that was administered (at ZT 2, 4, 6, 8, 12, 14, 16, 18, and 22).

RESULTS: The light pulse significantly reduced (one factor ANOVA, p < 0.05) the biting tendency in the S form mosquito for two hours after the light pulse was administered, specifically at 0.20 hr and 2 hrs, with variable responses observed at 4hr, and no differences detected thereafter at 6 and 8 hrs.

Conversely, the M form mosquitoes were unresponsive to the light treatment, i.e. their biting tendency did not change (n.s.) and always remained high. For the PRC analysis, as seen in most other examined species, such as *Drosophila*, mouse and human, *An. gambiae* (S form) mosquitoes demonstrated distinct delays and advances in circadian phase when light was presented during the early and late subjective night, respectively.

CONCLUSION: Our data reveal a strain-specific effect of acute light treatment on biting behavior that is both immediate and sustained (up to 2 hrs but not 6 hrs). The *An. gambiae* (S form) PRC is qualitatively similar to several model insect and vertebrate organisms, but our further investigations will focus on quantitative differences. At present, insecticidal treated bed nets designed to prevent mosquito-human contact and also kill mosquitoes are heavily relied upon to prevent malaria transmission; however, as mosquitoes are becoming increasingly resistant to insecticidal and drug treatments, there is a necessity for the ongoing develop of novel and innovative control strategies. The inhibitory and phase shifting effects of light may prove to be an effective tool in assisting with these control strategies.

Characterization of Exosomal RNA from *M. tuberculosis* infected macrophages: potential use as molecular biomarkers for tuberculosis

Prachi P. Singh*, Li Li and Jeffrey S. Shoresy

*Department of Biological Sciences, Eck Institute for Global Health and Center for Rare and Neglected Diseases, University of Notre Dame, Notre Dame, IN, 46556.*

Exosomes are extracellular vesicles of endocytic origin (30-150 nm) that are released by cells of hematopoietic and non hematopoietic origin and are involved in intercellular communication. Recently it has been found that exosomes contain microRNAs and messenger RNAs that can be transferred between cells affecting their transcription response. Moreover, the presence of exosomal RNA in body fluids such as serum, urine and saliva, has suggested their potential use as biomarkers for various diseases. However, exosome’s capability to transport genetic material in context of a TB infection has not been studied. Therefore we have characterized the exosomes released from *M. tuberculosis*-infected or uninfected macrophages for the presence of mRNA and microRNA with a hypothesis that the exosomes from infected macrophages might carry a unique subset of RNA molecules. We identified a cohort of mouse microRNAs in exosomes, however, quantitative analysis showed an overall down-regulation of microRNAs in exosomes released from infected macrophages compared to uninfected cells. We also identified subsets of mRNA transcripts that differed between exosomes derived from *M.*
"tuberculosis"-infected and uninfected macrophages. Most interestingly, we have identified mycobacterial transcripts not only in exosomes released from infected macrophages but also in exosomes isolated from the serum of TB patients. Our results suggest that characterizing exosomal RNA in TB patients may lead to new biomarkers of disease.

Predicting Drug Selectivity from Host-Pathogen Drug Networks

Geoffrey H. Siwo, Roger Smith, Asako Tan, Lisa Checkley and Michael T. Ferdig

Thousands of small molecules possess cytotoxic activity against infectious agents in vitro, but the successful development of therapeutic agents also demands that those molecules be safe for use in the human host. Host-pathogen drug selectivity remains a key barrier to the effective deployment of new therapeutic agents. Computational methods that predict selectivity could greatly enhance the pace of drug discovery. Here, we test the idea that relationships among drugs within and between the human host and an infectious agent can be used to predict selectivity of individual drugs and their combinations. To determine these drug relationships between the human host and the malaria parasite Plasmodium falciparum, we computed pairwise correlations among 10 drugs in both host cell lines and in parasite cultures using data from the connectivity map and our own transcriptional perturbation studies, respectively. Comparing drug pairs across the 2 species, we find strong positive correlations between a drug’s set of relationships in the host and pathogen, demonstrating conservation in drug response patterns and mechanisms of action. For example, methotrexate’s correlations to other drugs in the host is highly positively correlated to its relationships in the parasite \( r = 0.74 \) compared to \( r = 0.01 \) in randomized data, an observation consistent with its high cytotoxicity in both species. However, we also find that some drugs have distinct patterns in the host and parasite, highlighting potential drug selectivity. We further explore the idea of applying pairwise drug correlations to predict selective drug combinations. The anti-malarial drug artemisinin is highly negatively correlated to chloramphenicol in the parasite \( r = -0.83 \) compared to the human host \( r = -0.04 \). We propose that differential drug correlations in host versus parasite could indicate the value of precisely deployed drug combinations against a pathogen at concentrations that are safe for host cells but cytotoxic to the pathogen (selective synergism). Our study demonstrates high conservation of transcriptional responses to drugs across 2 species and challenges the dogma that the P. falciparum transcriptome is hard-wired. In addition, we demonstrate the value of integrating data from infectious agents with the connectivity map of host transcriptional responses to facilitate the in silico discovery of selective chemical agents.

Mycobacterial proteins found in the context of exosomes are ubiquitinated

Victoria L. Smith*, Liam T. Jackson, and Jeffery S. Schorey

Department of Biological Sciences, Eck Institute for Global Health and Center for Rare and Neglected Diseases, University of Notre Dame, Notre Dame, IN, 46556

Exosomes, membrane-bound vesicles of endocytic origin that are 30-100nm in sizes, have been shown to function in intercellular communication, immune cell activation, and serve as a biomarker for disease states. Exosomes characterized in the context of a Mycobacterium tuberculosis infection contain mycobacterial proteins and can either promote or inhibit a pro-inflammatory response depending on the exosome’s origin and the biological readout. The mechanism through which mycobacterial proteins
are trafficked to the multivesicular body (MVB) for release on exosomes is not well understood. We hypothesize that some mycobacterial proteins utilize the host ESCRT-ubiquitin pathway to traffic to the MVB. Our initial studies were to determine if the ESCRT machinery were required for exosome biogenesis in macrophages as this had not been previously determined. We identified that ESCRT-0 and ESCRT-1 were required for exosome biogenesis in the macrophage. Furthermore, ubiquitinated proteins could be found on exosomes derived from *M. tuberculosis* infected cells. Immunoprecipitation experiments demonstrated that mycobacterial proteins are pulled-down with ubiquitin. Finally, mutation of lysine residues in HspX resulted in its inability to be loaded onto exosomes. Overall, our data suggests that mycobacterial proteins may utilize the host ubiquitination pathway for trafficking to the MVB for subsequent release on exosomes.

**MEMBRANE PENETRATING ABILITY OF EBOLA MATRIX PROTEIN, VP40.**

Smita P. Soni, Emmanuel Adu-Gyamfi, Sylvia S. Yong and Robert Stahelin

Department of Biochemistry and Molecular Biology, School of Medicine, Indiana University, South Bend, IN.

Department of Chemistry and Biochemistry, University of Notre Dame, South Bend, IN.

Ebola from the *filoviridae* family of viruses causes severe and mostly fatal hemorrhagic fevers in primates and has been listed as a category IV pathogen by the NIH. Viral Protein 40 (VP40), the major matrix protein of Ebola virus, regulates the assembly and budding of the virus and alone harbors the ability to form virus-like particles (VLPs) from human cells. We hypothesize that VP40 is a high affinity lipid binding and membrane curvature-inducing protein with specificity for plasma membrane (PM) lipids. This specificity leads to localization of VP40 to the PS-rich inner leaflet of the PM and formation of VLPs. We have employed fluorescence spectroscopy to investigate VP40 binding and insertion within lipid vesicles (PC:PS, POPC:POPS, 80:20) containing 20% brominated lipids (Br-POPC). Because the fluorescence of the tryptophan is variably quenched depending on its distance from the bromine atoms on the lipid acyl chain, a tryptophan introduced into the membrane binding interface was utilized as a probe to detect the depth of membrane penetration of VP40. Results were indicative of VP40’s high affinity and specificity for PS in a PS-concentration dependent manner demonstrating the robust ability of VP40 to penetrate membranes. Further analysis of VP40 membrane insertion revealed a depth of penetration more than halfway into one monolayer of the membrane. In addition, data also confirmed that VP40 binds with nanomolar affinity to vesicles that recapitulated the PM in comparison to the nuclear membrane (NM). In addition VP40-mutants, which inhibits membrane binding and penetration, obstructs VLP formation and release. Cellular assays confirmed the lipid-binding specificity of VP40 in the PM of different cell lines and also demonstrated that deep membrane penetration is essential for VLP formation and release of VLPs from cells. We predict that these results will elucidate the molecular basis of VP40 induced membrane curvature changes, a prerequisite to the PM deformation required for VLP production.

**Dengue Fever Dynamics: role of seasonality in model selection and disease control**

Quirine A. ten Bosch, Dave D. Chadee, Brajendra K. Singh, Edwin Michael

Dengue poses an increasing threat to about two thirds of the world population. Epidemics of this
mosquito borne disease are highly unpredictable due to a complex interplay between environmental factors affecting the mosquito population and immune interactions between the 4 serotypes of the virus. To prevent health systems from getting overwhelmed by unforeseen large outbreaks and facilitate a better allocation of resources, an improved understanding of the temporal dynamics of dengue and its sensitivity to mosquito control is required.

To examine what mechanisms are most likely to underlie dengue epidemiology in Trinidad & Tobago, we performed model selection and calibration based on observed dengue dynamics using a model-data pattern-matching approach. The SIR-type models examined differed in assumptions on the role of cross-immunity between serotypes (CI) and antigen dependent enhancement (ADE: modelled as increased susceptibility and/or transmissibility to a new serotype after a primary infection by another serotype). We examined the parameter regions that allow the models to replicate the dynamics and demonstrate the calibration’s sensitivity to model assumptions. Further, we investigate the influence of model assumptions on the projected vulnerability to interruptions in the transmission rate, such as caused by vector control.

We demonstrate that model selection is largely driven by the seasonal variation in the transmission rate (a proxy for seasonal fluctuations of the mosquito population), with CI being a preferred mechanism in low seasonal and ADE in highly seasonal systems. The resistance to control efforts is found to be increased in systems with CI and positively correlated to the level of seasonal forcing. However, since models with CI are found to be less proficient in high seasonal areas, model selection shifts towards more vulnerable models with increasing seasonality.

We conclude that taking account of seasonality is crucial in effectively explaining regional specific dengue dynamics and projecting the impact of mosquito control.

Identifying novel mosquito attractants by exploiting the evolutionary dynamics

Identification of novel mosquito attractants by exploiting the evolutionary dynamics

Jennifer Topolski1, Madhu Siddappaji1, Julien Pelletier2 and Zainulabeuddin Syed1
1 Department of Biological Sciences and Eck Institute of Global Health, University of Notre Dame, Notre Dame, IN 46556.
2 School of Life Sciences, Keele University, ST5 5BG, UK

Mosquitoes transmit various life threatening diseases while feeding on vertebrates, and historically some of the most successful campaigns against vector-borne diseases have been those targeted against the vectors. They rely heavily on the sense of smell to locate suitable hosts, habitats, oviposition sites and conspecifics. A distinct and limited range of volatiles from these sources are parsimoniously used in various contexts eliciting strong olfactory behaviors. These volatile chemicals are detected and discriminated by large and divergent family of proteins, the olfactory receptor (ORs). Comparison of mosquito OR families among the three sequenced mosquitoes species, Anopheles gambiae, Aedes aegypti and Culex quinquefasciatus reveal interspecific divergence and species specific expansion of particular genes. We aimed to understand the significance of such variations in the olfactory genome in the context of evolution. We functionally characterized a set of highly conserved and divergent ORs by employing a unique ligand-receptor de-orphaning system wherein each receptor was exposed to myriad of odorants collected from the natural host-habits-conspecific environments as they eluted from the high resolution chromatographic column. This unique approach of employing ORs as sensing elements
to isolate and identify novel natural ligands not only provides unique understanding of how evolutionary forces shape ligand-receptor interactions, but those ligands can be exploited for the development of novel baits for odor baited mosquito traps (OBTs).

Expression Variation in *Plasmodium falciparum*: Implications for Parasite Adaptation

Lindsey Turnbull

Phenotypic plasticity is crucial in biological systems because it allows populations to adapt to constantly changing environments. Phenotypic heterogeneity within populations of genetically identical cells is an important but often overlooked source of phenotypic plasticity. Most investigations currently focus on the average of quantitative traits; however, several recent studies in model organisms have demonstrated that variation in expression is an adaptive genetically controlled phenotype that can be repeatedly measured. Thus, we anticipate expression variation will be important for adaptation in *Plasmodium falciparum*. Even small variations in transcriptional abundance among the millions of parasitized red blood cells within a single malaria infection may provide a selective advantage to a subset of the parasite population, thereby enhancing parasite fitness. To study the innate biological expression variation in the malaria parasite, we compared the gene expression variance within five sub-cloned lines of HB3, Dd2, and select progeny from the cross. For each parasite sub-clone, genome-wide gene expression levels were obtained using a custom designed high density exon array. We observed that parasite sub-clones from the same line have diverging gene expression profiles when measured at the same developmental stage and environmental conditions. For example, gene expression variation within HB3 sub-clones was much higher than within Dd2 sub-clones. We also observed differing ranges of expression variance in select progeny of this cross, suggesting that clonal expression variance may be a heritable and segregating phenotype. Therefore, our extended study including QTL mapping could lead to the discovery of genetic determinants of expression plasticity, a potentially important factor in the adaptability of the parasite to drugs.

Mechanisms of miltefosine drug resistance in *Leishmania major*

Kimbra Turner, Paola Vacchina, Michelle V. Joyce and Miguel A. Morales

Trypanosomatid parasites of the *Leishmania* species are the causative agents of leishmaniasis, a disease with several clinical manifestations. *L. major* causes cutaneous leishmaniasis (CL), which is largely characterized by ulcerative lesions appearing on the skin. Current treatments of leishmaniasis include pentavalent antimonials and amphotericin B, however, the toxic side effects of these drugs and difficulty with distribution of the treatments makes these options less than ideal. Miltefosine (MF) is the first oral treatment available for treatment of leishmaniasis. Originally developed for cancer treatments as an antitumor agent, the mechanism of MF in *Leishmania* spp. is largely unknown. While treatment with MF has proven effective and no clinical resistance to the drug has been observed, resistance is easily developed in an *in vitro* environment. Utilizing stepwise selection in generation of MF-resistant cultures of *L. major*, proteomics analysis can reveal differences between wild-type (WT) and MF-resistant cultures for inference of molecular causes of resistance mechanisms.
**Stress response and drug resistance in *Leishmania donovani***

Paola Vacchina, Karmela Marie Dalisay, Michelle V. Joyce and Miguel A. Morales

Leishmaniasis is an emerging disease for which no vaccine and no efficient, safe, and affordable treatment is available. In the absence of vaccination, chemotherapy, together with vector control, remains one of the most important elements in the control of leishmaniasis. Pentavalent antimonials have been the primary first-line treatment for all type of leishmaniasis in most parts of the world, although they are toxic and expensive. Despite this treatment option, the incidence of leishmaniasis has shown a significant increase over the past decades due to the failure of therapeutic measures in disease endemic countries. A new drug, miltefosine, was developed as an anti-cancer agent, and is now used as the only oral treatment for fatal visceral leishmaniasis caused by *Leishmania donovani*. Unfortunately, widespread over-the-counter drug sales and irrational drug prescription contribute to the emergence of drug resistance.

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**Development of a Paper-based Pharmaceutical Screening Tools**

Abigail Weaver, Sara Andria*, Nico Ranieri*, Mark Witkowski*, Cheryl Flurer*, Sarah Halweg, Marya Lieberman and Holly Goodson

*University of Notre Dame, Notre Dame, Indiana and *US Food and Drug Administration*

Paper-based analytical tests, such as glucose strips and pregnancy tests, have become a mainstay in routine medical testing. These offer a user-friendly and inexpensive platform that can take analytical testing out of the lab. Paper-based pharmaceutical test cards that act as screening tools could supplement current supply chain testing and identify poor quality pharmaceuticals that pose the greatest health threat to individuals. Test cards, developed at the University of Notre Dame, contain a library of color producing reagents that build a chemical profile and have been shown to identify beta-lactams and anti-tuberculosis medications with a greater than 90% success. To test the ability of these devices to identify poor quality pharmaceutical dosage forms, authentic and poor quality samples of six pharmaceutical types were tested side by side. The poor quality samples were previously shown by US Food and Drug Administration (US FDA) analysis to contain little to no active ingredient and/or a substituted ingredient. The test card identified poor quality samples with 100% success for 4 of these pharmaceutical types and with 83% and 67% success in the remaining types. We are investigating the range of pharmaceuticals that our current test card can identify and developing new tests to continue to expand this range. This chemical testing is complemented by an investigation into paper-based tests using whole-cell yeast biosensors, engineered to respond to tetracycline drugs. This biologically-based system takes advantage of the highly specific recognition machinery of biological systems and eliminates the need for isolation and stabilization of cellular components. The tetracycline biologically-based test card has the ability to detect doxycycline at concentrations of 1μg/mL, a 1,000-fold lower concentration than can be detected using the chemical test card and has been shown to be a reliable diagnostic for doxycycline at concentrations of 1mg/mL.
A novel anti-Gre transcription factor is required for mycobacterial ESX-1 secretion

Emily A. Williams, Matthew M. Champion, and Patricia A. Champion

The ESX-1 protein secretion system is a major virulence determinant in both mycobacterial and Gram-positive pathogens. Using a modified form of whole-colony MALDI-TOF mass spectrometry to directly detect protein secretion from intact bacterial colonies, we screened a near-saturating Mycobacterium marinum transposon insertion library for strains that fail to export ESX-1 substrates. We have identified several M. marinum strains deficient for ESX-1 secretion. One such strain bears a transposon in a novel genetic locus not previously linked to ESX-1 export. The gene affected by the transposon insertion, which we have renamed espO, is conserved in M. tuberculosis. The orthologous M. tb gene product is characterized as an anti-Gre transcription factor that binds RNA Polymerase. This anti-Gre factor lacks the transcript cleavage activity of Gre factors but still inhibits transcription. This probable anti-Gre factor has not been previously connected to ESX-1 secretion. Interestingly, we found that EspO is an ESX-1 substrate in M. marinum. By characterizing the espO locus, we can further our understanding of how ESX-1 secretion system is regulated at the molecular level.

Selection For Resistance To Oxadiazole Antibiotics in Staphylococcus aureus

Qiaobin Xiao, Sergei Vakulenko, Mayland Chang and Shahriar Mobashery
Department of Chemistry and Biochemistry and Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN 46556 USA

Bacterial resistance to antibiotics poses a serious threat to human health and is currently a global concern. Staphylococcus aureus, a Gram-positive bacterium frequently found on human skin, is responsible for many illnesses ranging from minor skin infections to life-threatening diseases such as meningitis, pneumonia and endocarditis. Methicillin-resistant S. aureus (MRSA) is resistant to most antibiotics currently available. Previous work from our laboratories led to the discovery of a novel oxadiazole class of antibiotics with both in vitro and in vivo activities against MRSA. Emergence of resistance to this class of antibiotics has not been documented and we set out to explore if mutational alteration of the S. aureus genome could result in resistance. We serially passaged S. aureus COL (an MRSA strain) in LB medium supplemented with increasing levels of POD-71-01 (an oxadiazole antibiotic from our laboratories) and generated two strains capable of growing in LB with increased concentrations of POD-71-01. MIC of the antibiotic against one strain (designated S. aureus COL1) increased two-fold (to 4 μg/mL) when compared to the parental strain, while against the other (designated S. aureus COL8) more than four-fold (to >8 μg/mL). Whole-genome sequencing revealed 31 mutations in the S. aureus COL8. Using PCR sequencing, we found that S. aureus COL1 and S. aureus COL8 share 29 of these mutations. Two unique mutations in S. aureus COL8 were the P70Q substitution in the thioredoxin (SACOL1794) and T172I substitution in the MmpL efflux pump (SACOL2566). We will present our analyses of these findings.
Ablation of the Id2 gene results in altered circadian feeding behavior, and sex-specific enhancement of insulin sensitivity and elevated glucose uptake in skeletal muscle and brown adipose tissue

Peng Zhou¹, Deepa Mathew¹, Cameron M. Pywell¹, Daan R. van der Veen¹, Jinping Shao¹, Yang Xi¹, Nicolle A. Bonar¹, Alyssa D. Hummel¹, Sarah Chapman², W. Matthew Levey¹,², Giles E. Duffield¹,³

¹ Department of Biological Sciences
² Notre Dame Integrated Imaging Facility
³ Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN 46556

Inhibitor of DNA binding 2 (ID2) is a helix-loop-helix transcriptional repressor rhythmically expressed in many adult tissues. Our earlier studies have demonstrated a role for ID2 in the input pathway, core clock function and output pathways of the mouse circadian system. We have also reported that Id2 null (id2-/-) mice are lean with low gonadal white adipose tissue deposits and lower lipid content in the liver. These results coincided with altered or disrupted circadian expression profiles of liver genes including those involved in lipid metabolism. In the present phenotypic study we intended to decipher, on a sex-specific basis, the role of ID2 in glucose metabolism and in the circadian regulation of activity, important components of energy balance. We find that id2-/- mice exhibited altered daily and circadian rhythms of feeding and locomotor activity; activity profiles extended further into the late night/dark phase of the 24-hr cycle, despite mice showing reduced total locomotor activity. Also, male id2-/- mice consumed a greater amount of food relative to body mass, and displayed less weight gain. id2-/- females had smaller adipocytes, suggesting sexual-dimorphic programing of adipogenesis. We observed increased glucose tolerance and insulin sensitivity in male id2-/- mice, which was exacerbated in older animals. FDG-PET analysis revealed increased glucose uptake by skeletal muscle and brown adipose tissue of male id2-/- mice, suggesting increased glucose metabolism and thermogenesis in these tissues. Reductions in intramuscular triacylglycerol and diacylglycerol were detected in male id2-/- mice, highlighting its possible mechanistic role in enhanced insulin sensitivity in these mice. Our findings indicate a role for ID2 as a regulator of glucose and lipid metabolism, and in the circadian control of feeding/locomotor behavior; and contribute to the understanding of the development of obesity and diabetes, particularly in shift work personnel among whom incidence of such metabolic disorders is elevated. Supported by the NIGMS and the American Heart Association.
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