Neisseria gonorrhoeae Escape from Cervical Epithelial Cells to Promote Bacterial Persistence

Samuel Bish, Wenxia Song, Daniel Stein

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

The human pathogen *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhea. In the female reproductive tract, gonococci can breach the mucosal epithelial barrier to initiate invasive disease, yet most women remain asymptomatic. Most of the important gonococci-female reproductive tract interactions that allow gonococci to suppress symptoms and persist to cause disease are not defined. The purpose of this study is to determine how gonococci interact with cervical epithelial cells to avoid innate immune defenses and maintain infection.

The main hypothesis tested is that a subpopulation of gonococci manipulate distinct cellular processes to cycle in and out of CEC, thereby evading innate immune defenses. To demonstrate that gonococci invade cervical epithelial cells, we cloned and expressed beta-lactamase on the gonococcal cell surface. Confocal microscopy revealed that beta-lactamase expressing gonococci invade cervical epithelial cells loaded with a fluorescent beta-lactamase cleavable substrate. Using modified gentamicin protection assays, we show that, once inside cervical epithelial cells, most of the invading gonococcal population is cleared by intracellular killing mechanisms, but some viable gonococci escape into the extracellular milieu. Treatment of cervical epithelial cells with tannic acid after invasion inhibits gonococcal escape suggesting that gonococci utilize the host exocytosis pathways to escape cervical epithelial cells.

In the model of female gonococcal disease, persistent gonococci may undergo transient invasion-escape cycles without triggering a robust immune response. Invasion allows gonococci to avoid extracellular host defenses and escape lets gonococci evade intracellular killing and infect new susceptible host cells. In this investigation, we have uncovered a possible escape mechanism utilized by a subpopulation of gonococci to avoid the innate immune response and establish long-term, low-level infection in the female reproductive tract.

*Presenter: Samuel E. Bish (sambish@mail.umd.edu)

Regulation of Bacterial Cell Envelope by a Novel Second Messenger Molecule c-di-GMP

Vincent Lee*[1]

Deptarment of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742
[1] Harvard Medical School, Boston, MA 02115

Pseudomonas aeruginosa can cause both acute and chronic infection utilizing distinct sets of virulence factors. Acute infection requires both activation of the host inflammatory response as well as neutralization of the host defenses by secreted toxins and type III secretion delivered effector molecules. In addition to transcriptional regulation of these factors, a novel secondary messanger molecule c-di-GMP can modulate the activity of several of these virulence factors both transcriptionally and post-translationally. The P. aeruginosa genome contains 40 genes that encode diguanylate cyclase and phosphodiesterase that regulate the production and degradation of c-di-GMP. We have now identified a number of binding proteins to c-di-GMP that activate the production of alginate and exopolysaccharide. These findings indicate another potential regulatory mechanism that mediates the transition of acute to chronic infections.

1. Kulasakara, H., Lee, V., Brencic, A., Liberati, N., Urbach, J., Miyata, S., Lee, D. G., Neely, A. N., Hyodo, M., Hayakawa, Y., Ausubel, F. M., and Lory, S. (2006) *Proc Natl Acad Sci U S A* **103**(8), 2839-2844
2. Romling, U., Gomelsky, M., and Galperin, M. Y. (2005) *Mol Microbiol* **57**(3), 629-639

Work was supported by funding from NIH and Cystic Fibrosis Foundation

*Presenter. vlee@hms.harvard.edu

The memory B cell response to TLR4 and TLR9 ligands

Wenxia Song[1], Katharina Richard[1] and Susan K. Pierce[2].
[1]*Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742; and* [2]*Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852.*

Humoral immune memory is composed of both long-lived plasma cells (PC) responsible for maintaining serum antibody levels in the absence of antigen and memory B cells (mB cells) capable of responding to challenges with antigen to give rise to new PCs. At present the cellular and molecular basis of the generation and maintenance of humoral memory is poorly understood. Here we explore the role of toll like receptors (TLRs) in the activation of mB cells to differentiate to PCs using NP-KLH immunized mice as a source of mB cells. Sorted splenic mB cells ($B220^+$ IgM^- IgD^- $CD138^-$ NP-binding cells) contained no detectable PC but were induced by the TLR9 ligand, CpG, to proliferate and differentiation into high affinity, NP-specific, IgG-secreting PC, in contrast to IgM^+ IgD^+ unswitched B cells ($B220^+$ IgM^+ IgD^+ $CD138^-$ NP-binding cells) that gave rise to predominantly low affinity, NP-specific, IgM-secreting PC. Both populations expressed equivalent levels of TLR9. Thus, the ability to give rise to high affinity PC secreting isotype-switched versus low affinity IgM antibodies in response to TLR9 ligand provided a useful tool to identify mB cells versus non-memory B cells. By this criterion both mB cells and unswitched B cells responded to the TLR4 ligand, LPS, *in vitro*, with the unswitched B cells yielding 10 fold more PC. Reflecting this response pattern the expression level of TLR4 was lower on mB cells as compared to unswitched B cells. The response to CpG and LPS was also analyzed *in vivo* and after adoptive transfer of B cells from NP-KLH immunized mice to $Rag1^{-/-}$ mice and showed that mB cells responded to both CpG and LPS *in vivo* albeit less efficiently as compared to the response of mB cells to the specific antigen. Taken together, these results suggest a potential role for TLRs, in particular TLR9, in maintaining humoral immunity by directly activating mB cells to differentiate into plasma cells.
This work was supported by NIH grant to W.S.

Parasite-derived murine MCP-1 enhances the Recruitment of a Restrictive Population of CCR2+ Macrophages

Sean M. Conrad, Dalit Strauss-Ayali, Anni Field and David M. Mosser

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Leishmania major parasites expressing a transgene that encodes the murine chemokine, MCP-1, were generated. These parasites transcribe MCP-1 mRNA and secrete MCP-1 protein. Infection of BALB/c, C57BL/6, or MCP-1 KO mice with these transgenic parasites resulted in minimal lesion development with fewer parasites in the infected foot, lymph node, and spleen, compared to mice infected with wild type *Leishmania major* parasites. In contrast to these mice, transgenic parasites cause substantial lesion development with relatively high numbers of parasites in CCR2KO mice indicating that the parasites were viable and healthy. Prior infection of mice with transgenic parasites offered no protection to subsequent wild type *Leishmania major* infection suggesting that the transgenic parasites are controlled by an early innate immune response. The sequential enumeration of transgenic parasites in lesions confirmed that the parasite numbers in infected ears were significantly reduced as early as day 7 post-infection. Flow cytometry on cells from transgenic infected mouse ears revealed an increase in the number of CCR2-positive macrophages, which coincided with parasite resolution. These CCR2-positive macrophages isolated from ears of mice infected with transgenic parasites contained fewer parasites relative to CCR2-positive macrophages from wild type infected ears. This appeared to be a CCR2-dependent phenomenon because macrophages from CCR2 KO mice were heavily infected with both transgenic and wild-type parasites. The lack of parasite survival in mice infected with MCP-1-transgenic parasites suggests that the parasite-derived MCP-1 is recruiting a population of CCR2-positive macrophages that is responsible for controlling *Leishmania* infection.

conrad033@hotmail.com
http://www.life.umd.edu/CBMG/faculty/mosser/mosser2.html

A plant pathway for the biological production of astaxanthin

Francis X. Cunningham, Jr.* and Elisabeth Gantt

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

The ketocarotenoid astaxanthin (3,3'-dihydroxy-4,4'-diketo-β,β-carotene) imparts an attractive orange-red color to the flesh of wild salmon and other fish and a blue hue, changing to orange-red upon boiling as the proteins that bind astaxanthin are denatured, to the carapace of lobster and of other crustaceans. The diets of fish and crustaceans that are raised in captivity must be supplemented with astaxanthin in order for these organisms to acquire the appropriate coloration. The substantial and rapidly expanding market for astaxanthin is currently supplied largely by chemical synthesis, but biological production could well provide a much less costly source of this valuable carotenoid. Astaxanthin is synthesized in certain bacteria, in some green algae, in a few fungi, and in the flowers of certain species in the genus *Adonis* (*Ranunculaceae*). We have identified two genes of the green plant *Adonis aestivalis*, the products of which are necessary and sufficient to convert the ubiquitous plant pigment β-carotene into astaxanthin in a simple bacterial cell (*Escherichia coli*). The biochemical route to astaxanthin in *Adonis* is unexpected and markedly different from those in bacteria and green algae. In *Adonis*, each ring of β-carotene is first desaturated at the 3,4 position, a reaction catalyzed by an enzyme described previously (F.X. Cunningham, Jr. and E. Gantt, 2005, *Plant J.* **41:** 478-492). A second enzyme then leads to the introduction of both a 3-hydroxyl and a 4-keto group *via* a route that remains uncertain but likely involves a terminal keto-enol tautomerization step. Because the *Adonis* enzymes function efficiently in the context of a plant pathway of carotenoid biosynthesis, they provide what may be the most appropriate technology for development of an economical, plant-based biological production process for astaxanthin.

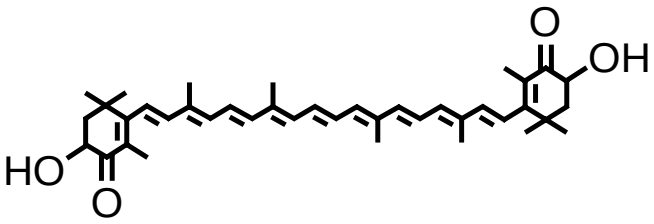

Figure 1. Chemical structure of astaxanthin.

Figure 2. The flower petals of *Adonis aestivalis* are an especially plentiful source of astaxanthin.

This work was supported, in part, by grants from Quest International and from the National Science Foundation (MCB-0316448) to F. X. Cunningham Jr. and E. Gantt.

*Presenter: email BuddyX@umd.edu

Breaking down the walls with a diversity of plant cellulases

Elena del Campillo*, and Stephanie Cole

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

The utility of plant tissue as source of biomass for fuel production, carbon sequestration, and chemical feedstocks depends critically on the composition of the plant cell wall. The primary wall is a network of cellulose microfibrils cross-linked by xyloglucans that are embedded in another network of glycoproteins, and pectins. In the secondary cell wall, cellulose microfibrils form layers of distinct orientation that later become more rigid by lignification. The structure of the assembly is modified during and after cell expansion in order to establish the wall's unique features according to cell type, location, faces, differences between inner and outer layers, and presence, number and type of plasmodesmata (PD). These features require remodeling of the wall to a varying degree and some changes target only one face of the cell. For example, the formation of root hairs and the formation of secondary PD both require the thinning, loosening and, ultimately, the perforation of existing cell wall in a precise location.

Genes that remodel the wall at specific location are critical for understanding how plants generate and assemble components. Specifically, we are interested in genes that can change the cellulose of the wall. Most plants, monocot and dicot, contain in their genome multiple sequences coding endo b-1,4 glucanase (cellulase) genes. Our research goals are to understand the role and function of these genes in plants using Arabidopsis as a model system. There are 25 cellulases and three structurally distinct groups in the Arabidopsis genome. The largest group encompasses short and secreted proteins with predicted signal peptides. A second group encompasses proteins containing an amino acid extension at the C-terminus that resembles a carbohydrate-binding site (3 members). The third group encompasses membrane proteins having a short hydrophobic trans-membrane segment at the N-terminus (also only 3 members).

We are using expression studies, lines containing single or multiple cellulase mutations, determination of cellulose content and manipulation of stress conditions to probe the function of these genes. Mutations in KORRIGAN (KOR), one of the three membrane cellulases and KOR-GUS analysis support a role for KOR in expanding cells through its association with cellulose production. The role of many other members of the family remains unknown and is likely to be different from that of the KOR cellulase. We have found that two secreted cellulases, AtCel2 and AtCel4 play a role in secondary cell wall but their activities are redundant. Also, we are currently analyzing the role of AtCel6, a gene with an extension at the C-terminal of unknown function. We determined the tissue and cell specific expression of AtCel6, with promoter-reporter fusions, RT-PCR, characterized two insertional mutants and response to TMV infection. Our results suggest that AtCel6 plays a role in PD modification.

*Elena del Campillo. edelcamp@umd.edu URL: http://www.life.umd.edu/labs/ATRIUM//delCampillolab.html

Regulation of Amino Acid Metabolism during T cell Activation: Identification of Pathways Regulated by Glutamine

Erikka L. Carr* and Kenneth Frauwirth

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Activation of a naïve T cell is a high energy consuming event which leads to an increase in metabolism. Upon stimulation, T cells must increase in size, rapidly proliferate and differentiate, all of which lead to a high demand for energetic and biosynthetic precursors. Even though amino acids are the basic building blocks of protein biosynthesis, the role of amino acid metabolism in this process has not been well characterized. We began investigating this by stimulating T cells in media lacking individual amino acids. Glutamine, in particular, was found to be important for proliferation as well as for cytokine production. Glutamate, a precursor of glutamine could not compensate for glutamine deprivation when equal concentrations were supplemented. We also focused on transport of glutamine in activated T cells. SN1, a glutamine transporter as well as XCT, a glutamate transporter were both upregulated during T cell activation, which further strengthens the idea that glutamine is necessary for T cell activation. We have now also begun studying signaling pathways that are affected by glutamine depletion. We have examined mTOR signaling, a pathway known to be involved in amino acid sensing, in T cells grown in the absence of glutamine. Our data suggest that glutamine does not regulate mTOR signaling, however, we are currently examining other pathways such as the stress activated kinases GCN2 pathway and the MAPK cascade in which glutamine may be important. A better understanding of glutamine utilization and transport into T cells is important because of its future therapeutic implications in diseases caused by malfunctioning of T cell activation.

This work was partially supported by NIH grant K01CA092156.

*Erikka Carr. email address: ecarr2@umd.edu

Integrating Transport with Plant Reproduction, Growth & Survival

Senthilkumar Padmanaban[1], Kevin Bock[1], Xiyan Li[1], Yongxian Lu[1], Salil Chanroj[1], John M Ward[2], Kendal Hirchi[3] & Heven Sze[1]
[1]Dept. Cell Biology & Molecular Genetics, University of Maryland, College Park, MD 20742; [2]Univ. Minnesota, St. Paul, MN; [3]Baylor College of Medicine, Houston, TX

Transport directly impacts all life processes, including nutrition, reproduction, signal transduction, movement and adaptation to the environment. Although transporter genes make up nearly 5% of the total genome in Arabidopsis, the functions of most transporters are still unknown. One major goal of this project is to determine the roles of a large family of putative cation/proton exchangers (CPA), including genes of the CHX, KEA, NHX and CAX families. Approaches include determining (i) tissue expression using promoter::GUS, and analyses of whole-genome transcriptomics, (ii) membrane localization using GFP-tagged proteins, (iii) biochemical function by expression in a suitable yeast mutant, and (iv) biological function through analyses of mutants. To facilitate the characterization of plant genes, the Cre-*loxP* site-specific recombination system was adapted to make reporter vectors for plant expression studies. This system allows promoter fragments to be cloned into a small vector (univector) and subsequently recombined *in vitro* with binary vectors containing different reporter genes precisely at near-perfect efficiency. These new vectors are efficient and economical alternatives to the other plant reporter vectors currently available. To integrate transport with the plant life cycle, the first genome-wide analysis of transporters showed that 67% of all classified transporters are expressed in the male gametophyte. Some genes are developmentally regulated during microsporogenesis, and others, including 14 CHXs, are preferentially expressed in pollen. CHXs are also expressed in roots, hydathodes, root tips, and guard cells. Transiently expressed CHXs tagged with GFP were localized mostly to endomembranes of onion epidermal cells. Unlike NHXs, CHX did not restore salt-tolerance in yeast mutants, but improved yeast growth at low K^+ and alkaline pH, suggesting CHX has a role in K^+ uptake under certain conditions. Single mutants of many CHX showed no obvious phenotype; however, mutant lacking a guard cell CHX was impaired in light-induced stomatal opening. Our results suggest that CHXs are functionally distinct from NHXs, and that members of the CHX family have roles in osmoregulation. Future studies are directed at testing the roles of CHX in pollen and guard cells function.

(Supported by National Science Foundation to HS)

Ref: (1) Sze, H. et al (2004) Expression pattern of a novel gene family, AtCHX, highlights their potential roles in osmotic adjustment and K+ homeostasis in pollen biology. Plant Physiol. 136:2532-47.

(2) Shigaki, T et al (2005) The Cre-loxP recombination-based reporter system for plant transcriptional expression studies. Plant Mol Biol. 58(1):65-73.

(3) Bock, KW et al. (2006) Integrating membrane transport with male gametophyte development and function through transcriptomics. Plant Physiol. 140:1151-68

Presenter: hsze@umd.edu

CBMG Core Laboratory Facility

Amy E. Beaven*

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

The CBMG Core Laboratory Facility provides faculty and students with a centralized, shared resource where multiple users have the opportunity to access sophisticated instrumentation whose initial cost and continued maintenance fees would likely exceed the budgets of individual investigators. The college provides support staffing for the facility and maintenance costs are subsidized by CBMG, the Biology department and by the college, thus allowing faculty and students cost-effective access to state-of-the-art equipment. The minimal fees charged to users help the departments and college cover the cost of service contracts on the instruments, as well any consumables and/or materials needed to maintain the machines (Table 1). The facility is accessible 24 hours a day and a staff member is available during normal business hours to train new users, provide technical support and ensure the instrumentation is kept in optimal condition.

Table 1

Equipment	Description	Cost
Applied Biosystems 3730xl DNA Analyzer	96-capillary DNA Analyzer. Suitable for DNA sequencing and fragment analysis applications	$35.00 per run (1 full plate)
Applied Biosystems 3100 (North) Genetic Analyzer	16-capillary DNA Analyzer. Set up for sequencing analysis applications.	$12.50 per run (16 wells)
Applied Biosystems 3100 (West) Genetic Analyzer	Fully automated 16-capillary DNA Analyzer. Set up for fragment analysis applications.	$10.00 per run (16 wells)
Applied Biosystems 7700 Sequencer Detector	96-well Real Time PCR machine.	$10.00 per run
Zeiss LSM 510 Confocal Microscope	Laser scanning confocal microscope equipped with 458, 488, 543 and 633nm laser lines.	$10.00 per hour
DeltaVision Deconvolution Microscope	Inverted Nikon fluorescent microscope and accompanying software capable of collecting & deconvolving fluorescent images.	$7.50 per hour
Olympus Fluorescent Microscope	Equipped with fluoroscein & rhodamine filters	$2.00 per hour
Konica SRX101 Developer	Automated film processor	No Cost
Mastercycler	Thermocycler	No Cost

*Amy Beaven: abeaven@umd.edu

Microbial genome evolution in hydrothermal environments

Jocelyne DiRuggiero and Patricia Escobar-Paramo

Microbial genomics has increasingly demonstrated the remarkable diversity of natural microbial communities and has significantly impacted our understanding of microbial evolution and ecology. We, and others, have shown that microorganisms can exhibit biogeographic patterns, which argues against the hypothesis that "microorganisms are everywhere and the environment selects" - the so-called Bass-Becking hypothesis (Escobar-Paramo et al., 2005). These studies and the high degree of genome mosaicism observed from environmental DNA sequencing raise the questions of what is the extent of genetic diversity in natural microbial populations and what generates and maintains that diversity?

In a first step towards testing the hypothesis that adaptive genomic properties such as recombination, genome rearrangements and lateral gene transfer events are responsible for microbial genetic heterogeneity in the environment, we determined the genotypic diversity in a *Pyrococcus* population isolated from a marine hydrothermal system in Vulcano Island, Italy. Multilocus sequence analysis of 5 loci and 19 Vulcano isolates revealed that recombination events likely occurred within the population. The extent of chromosome rearrangement was evaluated by comparative genomic hybridization and uncovered 8 highly variable chromosomal regions between *P. furiosus* and 7 isolates from Vulcano Island. One of those regions, a 16-kb fragment, was flanked by insertion sequence (IS) elements and was only found in *P. furiosus* and a closely related hyperthermophile, *Thermococcus litoralis* (DiRuggiero et al., 2000). Analysis of IS elements for 36 isolates from different hydrothermal systems around the world exposed their presence only in the isolates from Vulcano Island in the Mediterranean Sea. The extensive rearrangements associated with IS elements, as the result of both transposition and intragenic recombination events, suggests that these regions might be hot spots for genome shuffling. Finally, the occurrence of horizontal gene transfer was investigated using suppressive subtractive hybridization and revealed several possible target sequences. Taken together, these data suggest that the *Pyrococcus* population from Vulcano Island is undergoing rapid genomic evolution that might be the result of a highly dynamic environment.

Escobar-Paramo P., S. Gosh and J. DiRuggiero. 2005. Evidence for genetic drift in the diversification of a geographically isolated population of the hyperthermophilic archaeon *Pyrococcus*. Mol. Biol. Evol. 22:2297–2303

DiRuggiero J., Dunn D., Maeder D.L., Holley-Shanks R., Chatard J., Horlacher R., Robb F.T., Boos W. and R.B. Weiss. 2000. Evidence of recent lateral gene transfer among hyperthermophilic archaea. Mol. Microbiol. 38:684-693

Programmed -1 Ribosomal Frameshifting: It's Not Just For Viruses Anymore.

Jonathan L. Jacobs, Ashton T. Belew*, Rasa Rakauskaite, and Jonathan D. Dinman

Department of Cell Biology & Molecular Genetics, University of Maryland, College Park MD 20742,

Abstract. *Cis*-acting mRNA elements that promote programmed -1 ribosomal frameshifting (-1 PRF) redirect a fraction of translating ribosomes into a new reading frame. In viruses, these signals typically direct the translation of alternative protein products from a single mRNA. However, endogenous programmed frameshifts could also direct elongating ribosomes to premature termination codons, in which case the mRNAs could become targets for degradation by the nonsense mediated mRNA decay pathway (NMD). Computational analyses revealed the presence of 10,340 consensus -1 PRF signals in the *Saccharomyces cerevisiae* genome. Of the 6,353 yeast open reading frames (ORFs) included in this study, 1,275 are predicted to have at least one strong and statistically significant -1 PRF signal. In contrast to viral frameshifting, the predicted outcomes of nearly all of these genomic frameshift signals would direct ribosomes to premature termination codons, in theory making these mRNAs substrates for NMD. Nine of these predicted -1 PRF signals were tested empirically, eight of which promoted efficient levels of PRF *in vivo*. Furthermore, several of these were shown to act as mRNA destabilizing elements and were derepressed in a *upf3*Δ strain background. Importantly, these signals are found in genes whose mRNAs are known to be natural targets for NMD. These findings support the hypothesis that PRF may be used by cellular mRNAs to initiate "mRNA suicide". We present a model that programmed frameshifting may act as a general post-transcriptional mechanism to control gene expression by regulating mRNA abundance.

*Presenter. email address: abelew@umd.edu

Frequency of Recombination Between Diverse HIV-1 Subtypes: Estimating Selection Pressure From Reverse Transcription to Virus Replication.

Reshma M. Anthony[1], Heather A. Baird[2,3], Yong Gao[2], Román Galetto[4], Matthew Lalonde[2,5], Véronique Giacomoni[4], Measho Abreha[2], Matteo Negroni[4], Eric J. Arts[2,3], and Jeffrey J. Destefano[1*]

[1]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, [2]Division of Infectious Diseases, Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106 USA, [3]Department of Pharmacology, Case Western Reserve University, [4]Unité des Regulation Enzymatique et Activités Cellulaires, Institut Pasteur, Paris, Cedex 15, 75724 France, [5]Department of Biochemistry, Case Western Reserve University.

HIV-1 recombination between different subtypes has a major impact on the global epidemic. The generation of these intersubtype recombinants follows a defined set of events starting with dual infection of a host cell, heterodiploid virus production, strand transfers (recombination) during reverse transcription, and then selection. The goals of this collaboration are to measure the distribution of recombination breakpoints in single cycle virus infection with different subtypes, then use *in vitro* reconstituted strand transfer assays to understand what factors (homology, reverse transcriptase pausing, specific genome structures) determine breakpoints, and finally use multiple cycle cell infections to select for replication competent viruses. In this study, recombination frequencies were measured in the C1-C4 regions of the envelope. Virus isolates from Ugandan subtypes A and D HIV-1 env sequences (115-A, 120-A, 89-D, 122-D, 126-D) were employed in all three assay systems. These subtypes co-circulate in East Africa and frequently recombine in this human population. Increased sequence identity between viruses or RNA templates resulted in increased recombination frequencies, with the exception of the 115-A isolate. Clear recombination "hotspots" within the conserved regions (C1, C2, and C3) of *env* were identified in single cycle assays using the various intersubtypes. Based on a mathematical model that accounts for differential fitness and single-cycle recombination frequencies, we calculate that 75% of intra- or intersubtype A/D HIV-1 recombinants generated after each single round of infections were not replication-competent and did not survive in the multiple-cycle system. Analyses of the recombination breakpoints and mechanistic studies revealed that the presence of a recombination hotspot in the C3 region, unique to 115-A as donor RNA, could account for the higher recombination frequencies with the 115-A template. Using donor and acceptor templates from the various subtypes corresponding to the hotspot region of C3, a potential mechanism to explain why this hotspot was unique to 115-A was uncovered and will be discussed.

*Presenter: Jeffrey J. DeStefano, jdestefa@umd.edu,
http://www.life.umd.edu/cbmg/faculty/destefano/destefano.html

This work was funded in part by NIH NIGM grant number GM051140

Arrangement of the Minor Structural Protein L2 Within the Papillomavirus Virion

Christopher B. Buck[1,2]*, Naiqian Cheng[3], Cynthia D. Thompson[1], Douglas R. Lowy[1], Alasdair C. Steven[3], John T. Schiller[1] and Benes L. Trus[3,4]

[1]Laboratory of Cellular Oncology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, 20892
[2]Deptarment of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742
[3]Laboratory of Structural Biology Research, National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH, Bethesda, MD 20892
[4]Imaging Sciences Laboratory, Center for Information Technology, National Institute of Arthritis Musculoskeletal and Skin Diseases, NIH, Bethesda, MD 20892

Human papillomaviruses (HPVs) are a diverse group of oncogenic non-enveloped DNA viruses that infect skin and mucosal tissues. HPV-induced cervical cancer causes nearly 300,000 deaths per year worldwide. The HPV major capsid protein L1 can spontaneously assemble into 72-pentamer icosahedral particles with an exterior surface that closely resembles authentic virions. Empty HPV capsids assembled from purified recombinant L1 are the basis of a successful group of vaccines targeting several sexually-transmitted HPV types. Although the minor capsid protein L2 is not required for capsid formation, L2 is thought to participate in packaging of the viral genome, and plays a number of essential roles in the viral infectious entry pathway. The relative abundance of L2 and its arrangement within the virion remain unclear. To address these issues, we developed methods for serial propagation of HPV16 pseudoviruses in cultured human cell lines. Biochemical analysis of these synthetic HPV virions revealed that the ratio of L1 to L2 is variable, but can be as high as 72 molecules of L2 per virion (i.e., up to one L2 molecule per L1 pentamer). Computerized image analysis of electron micrographs revealed an icosahedrally-ordered L2-specific density beneath the center of each L1 capsomer. Lower density filaments of L2-specific density also extend radially along the floor of the capsid, raising the possibility of homotypic L2 interactions within assembled virions. Further analysis using bi-molecular fluorescence, or "split GFP" L2 chimeras supported the conclusion that the N- and C-termini of neighboring L2 molecules can interact within the capsid. We therefore conclude that L2 may form a sub-network of L2-L2 or L2-L1 contacts. This structural information should facilitate investigation of several aspects of the virus life cycle, including the processes of virion assembly and uncoating, as well as L2's roles in assembly and entry.

*Presenter. email address: buckc@nih.gov

Molecular mechanism and importance for bacterial virulence of *M.tuberculosis* mediated inhibition of host cell apoptosis.

Kamalakannan Velmurugan, Amro Bohsali, Jessica Miller, Serdar Gurses and Volker Briken*

Deptarment of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), claims the lives of 2-3 million people annually. The emergence of multi-drug resistant Mtb strains and the deadly synergism between the HIV epidemic and tuberculosis due to reactivation of persisting bacteria reinforces the need for the discovery of efficient drugs and vaccines. Our research seeks to test the hypothesis that the capacity of Mtb to inhibit infection-induced apoptosis of macrophages is a major pathway of the bacteria to avoid the host's innate and adaptive immune response. Furthermore we propose that the discovery of mycobacterial genes involved in the inhibition of host cell apoptosis will lead to new potential drug targets for resolving persistent bacterial infections and to new improved attenuated vaccine strains. There are a multitude of reports linking apoptosis inhibition to virulence of mycobacteria which are all presently based only correlative data due to the lack of defined bacterial mutants. One important aim of our research targets to fill that gap in our knowledge by identifying mycobacterial genes important for apoptosis inhibition using a gain-of-function genetic screen. To date one anti-apoptotic gene, nuoG, has thus been identified. Furthermore we investigate the mechanisms by which the mycobacterial gene manipulate host cell apoptosis by identifying interacting host cell proteins and pathways using genomics, proteomics and lipidomics approaches. Finally, we use the bacterial mutants to address the importance of apoptosis inhibition for the escape from the host's innate and acquired IR using immunodeficient (SCID) and immunocompetent (BALB/c) mice. The capacity of the current vaccine strain (BCG) will be analyzed after deletion of the anti-apoptosis gene in order to test our hypothesis that such a mutant would be a better vaccine.

*vbriken@umd.edu

From Genomics to Cellular Dynamics: Genetic dissection of ABA and calcium signaling in Arabidopsis guard cells

Aprajita Garg, Daeshik Cho, Jungmin Choi, Julie Keating, Peter Lee, Adrienne Moraff, Arpit Partel, Jenny Seo, Charlotte Song, and June M. Kwak

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Guard cells are a well-suited model for dissecting early signal transduction mechanisms. Relatively few signal transduction components have been identified from recessive ABA insensitive disruption mutants known to function during early ABA signal transduction upstream of transcription. The limited number of genetically identified positive ABA transducers is most likely due to redundancy in genes encoding ABA signaling components. To overcome this limitation and to dissect redundant signal transduction proteins, I have developed an alternative "single cell-type genomics" approach. This approach includes gene chip experiments performed with *Arabidopsis* guard cell RNA and degenerate oligo-based PCR screening of *Arabidopsis* guard cell cDNA libraries. The single cell-type expression study and molecular genetic and cell biological analyses enabled us to demonstrate that two guard cell-expressed NADPH oxidase catalytic subunit genes play central roles as positive signal transducers in guard cell ABA signal transduction. Furthermore, our preliminary results indicate that MAPK cascade(s) functions downstream of ROS in guard cell ABA signal transduction. MAPK cascade genes that are preferentially and highly expressed in guard cells are currently being characterized. In addition, the microarray results allow us to identify genes that are highly and preferentially expressed in guard cells. Promoters of guard cell-specific genes should provide a basis for cell type-specific gene disruption, development and signal transduction studies, and molecular engineering of plants.

It has long been a question how universal Ca^{2+} signal elicits the specific cellular activities in response to various stimuli. In stomatal guard cells, cytosolic Ca^{2+} has been shown to regulate stomatal movements. For example, ABA induces increases in the cytosolic Ca^{2+} concentration which result in stomatal closure, and Ca^{2+} oscillations encode necessary information for stomatal movements. Furthermore, it was shown that Ca^{2+} oscillation amplitude and frequency control gene expression in mammalian cells. However, molecular components mediating Ca^{2+} oscillation-regulated cellular responses including gene expression remain largely unknown in both kingdoms. Our efforts to genetically dissect Ca^{2+} signal transduction mechanisms using *Arabidopsis* guard cells will be presented.

*Presenter email address: aprajita@umd.edu
URL: http://www.life.umd.edu/cbmg/faculty/kwak/lab/index.html
Research in Kwak lab is supported by grants from NSF and USDA.

RTE1*, a novel membrane protein that is highly conserved in a wide range of organisms is important for ethylene receptor function in *Arabidopsis thaliana

Josephine S. Resnick*, Chi-Kuang Wen, Jason A. Shockey and Caren Chang

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Arabidopsis thaliana is an important plant model organism that has five ethylene receptors, which bind ethylene to elicit important responses for growth and development. *REVERSION-TO-ETHYLENE-SENSITIVITY1* (*RTE1*) is a novel predicted membrane protein that is required for the action of at least one of the ethylene receptors in *Arabidopsis* (1). *RTE1* was identified in a screen based on the ability to suppress the insensitivity of a weak gain-of-function receptor mutant, *etr1-2*. *rte1* was found to be unable to suppress insensitive mutations in the other four receptors, and was also unable to suppress the strong ethylene gain-of-function mutation *etr1-1*. However, *rte1* mutants were able to suppress *etr1* transgenes that confer insensitivity in wild-type plants, indicating that suppression by *rte1* is not entirely allele-specific. *rte1* loss-of-function mutants strongly resemble *etr1* null mutants, indicating that RTE1 may be required for ETR1 function. Double mutant analysis indicates that both *RTE1* and *ETR1* act in the same pathway, with RTE1 likely acting at or upstream of the ETR1 receptor. *RTE1* has homologues in a wide variety of organisms, including plants, animals and protists, although it is absent from prokaryotes and fungi. *Arabidopsis* has one RTE1 homologue, *RTH*, which thus far has not been implied in the ethylene-signaling pathway, and which also encodes a novel and predicted membrane-bound protein. RTE1 has no known function in any organism, and contains no known protein motifs, although conserved regions within the protein sequence contain highly conserved Cys and His residues. Since such residues are commonly found in metal binding proteins, and since ethylene receptors require the presence of a copper cofactor in order to bind ethylene, it raises the possibility that RTE1 may be involved in aiding of copper binding to ETR1. At least one of the conserved Cys residues is required for RTE1 functionality, since a Cys-Tyr mutation at this residue results in loss of function. Cell biological and biochemical experiments are being undertaken to help ascertain the function of RTE1 and how it acts to regulate ETR1.

Reference:

1. Resnick JS, Wen CK, Shockey JA, and Chang C (2006) *REVERSION-TO-ETHYLENE SENSITIVITY1*, a conserved gene that regulates ethylene receptor function in *Arabidopsis*, *Proc. Natl. Acad. Sci. USA* 103: 7917-7922.

Supported by grants from the Department of Energy (DE-FG02-99ER20329) and National Institutes of Health (1R01GM071855) to C.Chang.

*Presenter. email address: Stockton@umd.edu
URL: http://www.life.umd.edu/CBMG/faculty/chang/lab.html

Mago nashi and the regulation of spermatogenesis in *Marsilea*

Department of Cell Biology & Molecular Genetics, University of Maryland, College Park, MD 20742

Corine M. van der Weele and Stephen M. Wolniak.

Spermatogenesis in the water fern *Marsilea vestita* is a rapid process that relies on mRNA and proteins stored in the dry microspore. The microspore consists of one cell, which when placed in water, undergoes nine division cycles to produce 7 somatic cells and 32 spermatids. After the divisions are completed, each spermatid undergoes a complex differentiation process, which includes nuclear elongation, cell coiling, cytoskeletal formation and ciliogenesis. Mago nashi is a highly conserved protein that is involved in multiple facets of eukaryotic development. In *M. vestita*, *Mv*-mago is important both in establishing cytoplasmic domains for later cell fate specification and for determining the planes of divisions that define sterile and spermatogenous cells. The morphology of RNAi-mago treated spores and the localizations of proteins involved in spermiogenesis (centrin, β-tubulin) and transcripts encoding proteins involved in RNA processing (*Mv*-eIF4AIII, *Mv*-Prp19) combine to show that cell fate specification is disrupted or completely lost after knockdowns of Mv-*mago*. Even with mild knockdowns where gametophyte development appears largely normal, cell division planes are incorrect and jacket cells exhibit anomalous basal body-like staining with anti centrin antibody, showing that *Mv*-mago affects the cytoskeleton or motor proteins. Mago nashi is known as a component of the exon-exon junction complex (EJC) for pre-mRNA splicing. In this process Mago nashi becomes associated with other EJC components and a pre-mRNA to function in the nonsense-mediated mRNA decay pathway, enhancing translation and transport/localization of mRNAs for axis formation. RNAi treatments, using dsRNAs from the EJC components *Mv*-Y14 and *Mv*-eIF4AIII both closely resemble developmental anomalies observed with *Mv*-Mago dsRNA. Thus, *Mv*-Mago acts as part of the EJC in *M. vestita*, and affects patterns of translation through the regulation of mRNA splicing. *Mv*-mago protein becomes localized in dots in the cytoplasm of mainly spermatogenous cells after 4 hours of development, at the end of the division cycles, up to 11 hours, right before release of the gametes from the spore. These 'Mago-dots' are undetectable or absent after treatment with dsRNAs made from *Mv*-mago, *Mv*-Y14, *Mv*-eIF4AIII or *Mv*-Prp-19, and proper development of the gametophyte requires these complexes to be present. Are Mago-dots the EJCs? Anti-Mago antibody staining patterns are distinctly different from those of the decapping enzyme hDcp2 leading us to suspect that the Mago-dots are not the same as p-bodies. As an alternative to EJCs, Mago-dots may be silencing particles, where Bruno dependent mRNA oligomerization exerts translational control for gametophyte development. (Supported by NSF grant MCB 0234423 to SMW).

Presenter: Corine M. van der Weele: cvdweele@wam.umd.edu

Regulation and function of autophagy in cell survival and death

Deborah L. Berry[1] and Eric H. Baehrecke[1,2,*], [1]Center for Biosystems Research, University of Maryland Biotechnology Institute, [2]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742

Autophagy is an important catabolic process that is used to degrade components of the cytoplasm, and is conserved between yeast, plants and higher animals including humans. While autophagy is known to play an important role in cell survival by facilitating nutrient utilization in yeast and mammals, little is known about how this degradation process is regulated and functions in dying animal cells. We are studying the regulation and function of autophagy in the context of development and disease using *Drosophila* models (also see abstracts by CBMG PhD candidates Yakup Batlevi, Jahda Batton and Christina Kary). Apoptosis and autophagic cell death are the two most prominent morphological forms of programmed cell death that occur during animal development. Here we show that *Drosophila* salivary gland cells die in the absence of caspase function using loss-of-function mutants, and these data suggest that non-caspase degradation mechanisms are involved in autophagic cell death. The presence of autophagic vacuoles in dying cells, and the induction of autophagy (Atg) genes just prior to cell death, indicates that autophagy is involved in the death of salivary glands. Autophagy is used to degrade bulk components of the cytoplasm for nutrient utilization, and this suggests the possibility that the death of salivary glands, and other cells during development, occurs to promote growth and development of the organism. The class I PI3K pathway regulates both growth and autophagy, and perturbation of genes in this pathway alters the fate of salivary glands. Expression of Dp110 and Akt in salivary glands induces growth, inhibits autophagy, and inhibits the destruction of salivary glands even though caspase activation occurs in these cells. By contrast, expression of TSC 1 and 2, PTEN, and a dominant negative form of Dp110 induces autophagy and premature death of salivary glands. Altered salivary gland cell death by these growth regulators requires TOR function, but induction of cell growth by the TOR target S6 Kinase does not prevent the death of salivary glands. Combined, these data indicate that autophagy is required for salivary gland cell death. These data also provide an interesting mechanistic link between growth, nutrient utilization and the regulation of cell death during development.

*Presenter. email address: baehreck@umbi.umd.edu
URL: http://www.umbi.umd.edu/~cbr/baehrecke/lab1.htm

Stress Signaling in Filamentous Fungi in the Induction of Resistance to Anti-Fungal Agents

David Straney*, Viji Vydeeswaran [1] and Seema Madhaven

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742
[1]Molecular and Cell Biology Graduate Program, University of Maryland, College Park, MD 20742

Microbes become resistant to antimicrobial compounds in several ways: by changing a target enzyme, by transporting the compound out of the cells, or by degrading the compound. Of the eukaryotic microbes, filamentous fungi display a great diversity in degrading both synthetic and natural antifungal compounds. A general means for detoxifying these compounds is through the activity of cytochrome P450 monooxygenases which make them less lipophyllic. Sequenced genomes of filamentous fungi indicate that fungi possess around 100 genes encoding cytochrome P450s. Since only a minority of these genes are represented in EST databases, those related to detoxification may be like those in metazoan systems in that they are only expressed when challenged with a xenobiotic stimulus. We are studying the regulation of the PDA1 gene encoding the cytochrome P450 pisatin demethylase in *Fusarium solani* as a model of fungal cytochrome P450 gene regulation. Strains of this fungus are pathogenic to humans and to specific plants. *F. solani* is also naturally resistant to many anti-fungal compounds. The PDA1 gene has a defined function in detoxification of the host plant defense compound, pisatin, it possesses a distinct ecological function in pathogenesis, and displays strong induction upon treatment with pisatin. How does the fungus sense this host defense compound to induce PDA1? Is it similar to metazoans that use nuclear receptors to recognize xenobiotic compounds and regulate subfamilies of detoxification-associated CYP genes? We have identified PRF, a binuclear zinc transcription factor from *F. solani* that binds a 40 bp pisatin-responsive element from PDA1. Manipulation of the gene in *F. solani* and of related genes in the genetic model system *Neurospora crassa* show that the transcription factor responds to the physiological stress caused by pisatin. In fact, the transcription factor also responds to synthetic antifungal agents amphotericin B and azoles that are the main treatments for fungal infection of humans. The signaling pathway appears to respond to membrane-active compounds that would directly or indirectly cause membrane leakage. Sequence and functional analysis in both fungi show multiple related transcription factors are active in this pathway. Dissection in yeast has defined specific regions of the transcription factor involved in activation and regulation. Understanding the regulation of this pathway should allow us to determine how the remaining cytochrome P450 encoding genes are regulated and their role in resistance.

This research is funded by a grant from the National Science Foundation
*Presenter. email address: Straney@umd.edu
URL: http://www.life.umd.edu/cbmg/faculty/straney/straney2.html